Switchgrass ecotypes alter microbial contribution to deep soil C

Damaris Roosendaal[1], Catherine E. Stewart[1,2], Karolien Denef[3], Ronald F. Follett[1*], Elizabeth Pruessner[1], Louise H. Comas[4], Gary E. Varvel[5*], Aaron Saathoff[6], Nathan Palmer[7], Gautam Sarath[7], Virginia L. Jin[5], Marty Schmer[5], Madhavan Soundararajan[8]

[1]{Soil-Plant-Nutrient Research Unit, United States Department of Agriculture-Agricultural Research Service, Suite 100, 2150 Centre Avenue, Building D, Fort Collins, CO 80526-8119}

[2]{Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523-1499, USA}

[3]{Central Instrument Facility (CIF), Department of Chemistry, Colorado State University, Fort Collins, CO 80523-1872, USA}

[4]{Water Management Research Unit, United States Department of Agriculture-Agricultural Research Service, Suite 100, 2150 Centre Avenue, Building D, Fort Collins, CO 80526-8119}

[5]{Agroecosystems Management Research Unit, USDA-ARS, 251 Filley Hall/Food Ind. Complex, Univ. of Nebraska, Lincoln, NE 68583-0937}

[6]{LI-COR Biosciences, Lincoln, NE 68504, USA}

[7]{Grain, Forage, and Bioenergy Research Unit, USDA-ARS, 251 Filley Hall/Food Ind. Complex, Univ. of Nebraska, Lincoln, NE 68583-0937}

[8]{Department of Biochemistry, University of Nebraska, Lincoln, NE 68588-0664}

[*]{Retired}

Correspondence to: catherine.stewart@colostate.edu

Abstract

Switchgrass (*Panicum virgatum* L.) is a C_4, perennial grass that is being developed as a bioenergy crop for the United States. While aboveground biomass production is well documented for switchgrass ecotypes (lowland, upland), little is known about the impact of plant belowground productivity on microbial communities down deep in the soil profiles. Microbial dynamics in deeper soils are likely to exert considerable control on ecosystem services, including C and nutrient cycles, due to their involvement in such processes as soil formation and ecosystem biogeochemistry. Differences in root biomass and rooting characteristics of switchgrass ecotypes could lead to distinct differences in belowground microbial biomass and microbial community composition. We quantified root abundance and root architecture and the associated microbial abundance, composition and rhizodeposit C uptake for two switchgrass ecotypes using stable isotope probing of microbial phospholipid fatty acids (PLFA) after $^{13}CO_2$ pulse-chase labeling. Kanlow, a lowland ecotype with thicker roots, had greater plant biomass above- and belowground (g m^{-2}), greater root mass density (mg cm^{-3}), and lower specific root length (m g^{-1}) compared to Summer, an upland ecotype with finer root architecture. The relative abundance of bacterial biomarkers dominated microbial PLFA profiles for soils under both Kanlow and Summer (55.4% and 53.5%, respectively, P = 0.0367), with differences attributable to a greater relative abundance of gram-negative bacteria in soils under Kanlow (18.1%) compared to soils under Summer (16.3%, P = 0.0455). The two ecotypes also had distinctly different microbial communities process rhizodeposit C; greater relative atom % ^{13}C excess in gram-negative bacteria (44.1 ± 2.3%) under the thicker roots of Kanlow and greater relative atom % ^{13}C excess in saprotrophic fungi under the thinner roots of Summer (48.5 ± 2.2%). For bioenergy production systems, variation between switchgrass ecotypes could alter microbial communities and impact C sequestration and storage as well as potentially other belowground processes.

1 Introduction

Switchgrass cultivars have been developed from ecotypes adapted to northern vs southern latitudes and reflect trade-offs between plant productivity and stress resistance. Upland ecotypes are lower yielding with greater resistance to drought and freezing and lowland ecotypes are

higher yielding with poorer freeze tolerance traits (Fike et al., 2006; Garten et al., 2010; Hartman et al., 2011; Monti, 2012). Since switchgrass belowground biomass is proportional to or greater than aboveground biomass (Frank et al., 2004; Garten et al., 2010), greater aboveground productivity in lowland compared to upland ecotypes may result in more root biomass and thus more carbon (C) available as an energy substrate for belowground microbial communities. Because most of the aboveground biomass is removed at harvest, the production and dynamics of belowground biomass are important for potential soil C storage (De Deyn et al., 2008; Garten et al., 2010). Switchgrass ecotype could affect soil C differently due to differences in root biomass and architecture (Ma et al. 2000), but the few field studies that investigate cultivar effects on SOC (Garten et al. 2010, 2011) have not contrasted upland and lowland ecotypes. Although switchgrass generally has been shown to increase soil C below 30cm (Garten et al., 2000; Follett et al. 2012), how ecotypes influence soil microbial community abundance and composition by affecting rhizodeposit C, in deeper soil depths is less clear.

Surface soils are studied most intensely because the densities of soil microorganisms are greatest within organic matter and nutrient-rich surface soils (Federle et al., 1986; Bone and Balkwill, 1988; Fierer et al., 2003). Only limited information is available for soil microbial communities deeper than 25 cm despite evidence that more than half of the entire microbial community resides in subsurface soils (Van Gestel et al., 1992; Dodds et al., 1996; Fritze et al., 2000; Blume et al., 2002). Because microorganisms are involved in soil formation, ecosystem biogeochemistry, and groundwater quality (Dodds et al., 1996; Fierer et al., 2003), microbial dynamics in deeper soils are likely to exert considerable control on ecosystem services, including C and nutrient cycles (De Deyn et al., 2008; Liang et al., 2012).

Soil C sequestration potential is determined by multiple factors such as topography, mineralogy, and texture. Although microbial biomass represents a very small fraction of the total soil C pool (Wardle, 1992), microbial metabolites stabilize soil organic carbon (SOC) and provide plant nutrients, effectively driving plant C inputs into soils (De Deyn et al., 2008). Intraspecific variability in switchgrass rooting architecture, structure, and root tissue could produce differences in ecosystem C dynamics by affecting belowground C cycling and C stabilization (de Graff et al., 2013) through both direct and indirect mechanisms on root exudation and microbial community structure. While there is much uncertainty about the direct impact of fine roots on soil C cycling, fine roots are one of the most important sources of soil C

input (Rasse et al., 2005; Joslin et al., 2006). Greater root exudation has been found in fast growing plant species with branched, fine root systems (Personeni and Loiseau, 2004; De Deyn et al., 2008). However, species with thicker roots may have a thicker cortical layer to support more arbuscular mycorrhizal (AM) fungi (Brundrett, 2002; Comas et al., 2012; Comas et al., 2014). Previous switchgrass studies report that root architecture varies by cultivar or plant genotype (Jackson, 1995; Fischer et al., 2006) and that upland switchgrass ecotypes have longer specific root length (SRL) and finer root systems compared to coarser rooted lowland ecotypes (de Graaff et al., 2013). What is less clear is if differences in root traits alter overall microbial biomass and soil microbial community composition in the field.

One technique for observing microbial biomass and the soil microbial community composition is microbial phospholipid fatty acid (PLFA) analysis, a biochemical profiling technique, designed to evaluate soil microbial abundance and functional group composition (Vestal and White, 1989). In addition, stable isotope probing of PLFAs following $^{13}CO_2$ pulse-labeling of plants can determine which microbial groups are metabolizing recently produced rhizosphere-substrate (Denef et al., 2007, Jin and Evans, 2010) as root exudates cycle through microbial biomass quickly (de Graaff et al., 2014). PLFAs have been used to characterize microbial biomass and composition under bioenergy crops such as switchgrass and corn (Liang et al. 2012), and PLFA-stable isotope probing in grazed perennial grasslands (Denef et al. 2007) However, to our knowledge, stable-isotope probing has not been used to characterize rhizodeposit uptake in the field under different switchgrass ecotypes.

The objectives of this study were to determine the effect of differences in root traits between two contrasting switchgrass ecotypes on soil microbial biomass, soil microbial community abundance and functional group composition, and microbial utilization of rhizodeposit-C throughout the soil depth profile following ^{13}C pulse-labeling. We hypothesize that the upland ecotype Summer will have finer roots, longer SRL, and greater specific surface area, and that these traits will be associated with greater microbial biomass throughout the soil profile compared to the lowland ecotype, Kanlow. We also hypothesize that rooting traits in Kanlow will favor a greater relative abundance of soil fungi, particularly AMF, compared to Summer due to lower specific root area.

2 Materials and Methods

2.1 Experimental site and treatments

The study site is located on the University of Nebraska-Lincoln's Agricultural Research and Development Center (ARDC), Ithaca, Nebraska, USA (41.151°N, 96.401°W). Soils are classified as Yutan silty clay loam (fine-silty, mixed, superactive, mesic Mollic Hapludalf) and Tomek silt loam (fine, smectitic, mesic Pachic Argiudoll). The study is a randomized complete block experimental design with three field replicates of two switchgrass ecotypes, an upland ecotype, Summer and lowland ecotype, Kanlow. Each plot consisted of twelve switchgrass plants of the same ecotype arranged in a 4 x 3 plant grid for a planting density of 12 plants m^{-2}. Switchgrass plants represent genetic individuals that were hand planted in summer 2009. At the time of sampling for the current study, switchgrass was well-established and 3 years old. Prior to the 2012 growing season, the plots were burned in early April to remove aboveground biomass.

2.2 ^{13}C labeling

All 12 switchgrass plants in each plot were labeled in May 2012 using a customized portable $^{13}CO_2$ pulse-chase labeling system consisting of a 1.0 m^3 clear polymethyl methacrylate (PMMA) chamber with an open bottom for placement over the entire plot and interior fans to provide air circulation (Saathoff et al., 2014). This chamber was attached to a Portable Photosynthesis System Model LI-6200 (Li-cor, Lincoln, NE) to monitor CO_2 concentration, air temperature and relative humidity within the chamber headspace. Isotopically enriched CO_2 label (99 atom% ^{13}C (Sigma-Aldrich Co. St. Louis, MO)) was introduced into the chamber by opening the gas regulator for approximately 15 seconds. Label was added to raise chamber CO_2 concentrations between 1000 to 2000 ppm above atmospheric CO_2 concentration (420 ppm). Once the label was introduced, plants were allowed to take up labeled CO_2 until headspace concentrations were at least 100 ppm below ambient CO_2 levels.

2.3 Plant and soil sampling

Plants and soils for single, randomly selected individual switchgrass plants from each plot were harvested two days following ^{13}C pulse-chase labeling. The aboveground biomass was removed by clipping at the soil surface. Plant samples were separated into tillers, stems, leaves, and oven dried at 55°C and ground for further analysis. Soil samples were then collected through

the crown of the plant using a 10.16 cm diameter core attached to a hydraulic soil probe. Soil cores were divided in increments of 0-10, 10-30, 30-60, 60-90, 90-120, and 120-150 cm. Each depth increment was split in half length-wise, packed on ice, transported to the USDA-ARS laboratory in Ft. Collins Colorado, and refrigerated at 4°C until further processing. Soils were weighed, and a subsample was oven-dried at 110°C for 24 hours for determination of soil moisture content and soil bulk density. The half core for root separations was immediately frozen (-22°C). Samples for PLFA extraction and analysis were handpicked to remove all identifiable plant material, frozen at -22 °C and freeze-dried (Labconco FreeZone 77530, Kansas City, MO).

2.4 Root separations

The frozen half soil core was thawed to room temperature and the remaining plant crown was separated from roots and root samples were hand-washed. Specifically, roots were gently washed from the entire half core over a 1 mm (#20) soil sieve set over a second screen or sieve to capture all roots. Roots were picked off of the sieves and separated by hand into fine (1- 2 branches), 3rd order coarse roots, and coarse roots (4-5 order). Fresh root subsamples were scanned with a desktop scanner to quantify morphological and architectural features (Comas and Eissenstat, 2009). DT-SCAN software (Regent Instruments, Inc., Quebec, Canada) generated length, average diameter, and volume of roots in each image, which were used to calculate root length density (root length per soil volume, m cm^{-3}), specific root length (root length per root mass, m g^{-1}), and root mass density (root mass per soil volume mg cm^{-3}). After scanning, root samples were freeze-dried and then weighed. Root length and mass were scaled to the whole core on a soil mass base using the weight of the ½ cores and the volume of the whole core. Weight averages for the whole profile were scaled by depth increment using soil volume.

2.5 Plant and soil analyses

For the other half of the soil core, the crowns were separated from the roots, the soil was sieved to 2 mm and all large roots and non-soil materials removed prior to soil characterization and microbial analysis. Soil pH was determined with a Beckman PHI 45 pH meter using a 1:1 soil:water ratio. Total organic C, total N, and δ^{13}C in both plant and soil samples were determined in duplicate by a continuous flow Europa Scientific 20-20 Stable Isotope Analyzer interfaced with Europa Scientific ANCA-NT system Solid/Liquid Preparation Module (Europa Scientific, Crewe Cheshire, UK-Sercon Ltd.)   Soil subsamples for PLFA analysis were

handpicked to remove all identifiable plant material, frozen at -22°C, then freeze-dried (Labconco FreeZone 77530, Kansas City, MO) and stored at room temperature until lipid extraction.

2.6 PLFA extraction and quantification

The extraction and derivatization of PLFAs was adapted from Bossio and Scow (1995) and modified by Denef et al. (2007). Briefly, 6 g of soil from the surface depth increments (0-30 cm) and 8 g of soil from each subsoil depth increment (30-120 cm) were extracted using phosphate buffer:chloroform:methanol in a 1:1:2 ratio. Total lipids were collected in the chloroform phase, and fractionated on silica gel solid-phase extraction (SPE) columns (Chromabond, Macherey-Nagel Inc., Bethlehem, PA) using chloroform, acetone, and methanol as eluents. Neutral lipid fractions representing NLFAs were collected from the chloroform extractant (data not shown) and polar lipid fractions representing PLFAs were collected from the methanol extractant by mild alkaline transesterification using methanolic KOH to form fatty acid methyl esters (FAMEs).

All PLFA samples were analyzed to identify and quantify individual PLFA biomarkers using gas chromatography-mass spectrometry (GC-MS) (Shimadzu QP-20120SE) with a SHRIX-5ms column (30 m length x 0.25 mm ID, 0.25 μm film thickness). The temperature program started at 100 °C followed by a heating rate of 30 °C min^{-1} to 160 °C, followed by a final heating rate of 5 °C min^{-1} to 280 °C. Prior to GC-MS analysis, a mixture of two internal FAME standards (12:0 and 19:0) was added to the FAME extract. Individual fatty acids were identified and quantified using these internal standards in addition to the relative response factors for each of the external standard 37FAME and BAME mixes (Supelco Inc) as well as mass spectral matching with the NIST 2011 mass spectral library.

The $\delta^{13}C$ signature of individual FAMEs was measured by capillary gas chromatography-combustion-isotope ratio mass spectrometry (GC-c-IRMS) (Trace GC Ultra, GC Isolink and Delta V IRMS, Thermo Scientific). A capillary GC column type DB-5 was used for FAME separation (30 m length x 0.25 mm ID x 0.25μm film thickness; Agilent). The temperature program started at 60 °C with a 0.10 min hold, followed by a heating rate of 10 °C min^{-1} to 150 °C with a 2 min hold, 3 °C min^{-1} to 220 °C, 2 °C min^{-1} to 255 °C, and 10 °C min^{-1} to 280 °C with a final hold of 1 min. The FAME $\delta^{13}C$ values were calibrated using working standards (C12:0

and C19:0) calibrated on an elemental analyzer-IRMS (Carbo Eba NA 1500 coupled to a VG Isochrom continuous flow IRMS, Isoprime Inc.). To obtain $\delta^{13}C$ values of the PLFAs, measured $\delta^{13}C$ FAMEs values were corrected individually for the addition of the methyl group during transesterification by simple mass balance (Denef et al., 2007; Jin and Evans, 2010).

Of the identified PLFAs, 2-OH 10:0, 2-OH 12:0, 2-OH 14:0, 16:1ω7, 17:0cy, 2-OH 16:0, c18:1ω7, and 19:0cy are classified as gram-negative bacteria while i-15:0, a-15:0, i-16:0, i-17:0, and a-17:0 are classified as gram-positive bacteria, (Zelles, 1999). The 3-OH 12:0, 14:0, 15:0, 3-OH 14:0, 17:0, and 18:0 are used as general bacterial indicators (Fröstegard and Bååth, 1996; Zelles, 1999). The 16:0 fatty acid is classified as a universal PLFA (Zelles, 1999). The 10ME16:0, 10ME17:0 and 10ME18:0 are classified as actinomycete biomarkers. The 16:1ω5, 20:4ω6, 20:4ω3, and 20:1 are biomarkers for arbuscular mycorrhizal fungi (AMF) (Graham et. al, 1995), and 18:3ω3, c18:2ω9,12, and c18:1ω9 are biomarkers for saprotrophic fungi (Zelles, 1997). Although 16:1ω5 can also be a gram-negative biomarker (Nichols, et al., 1986), in this study the neutral lipid fatty acid (NLFA) fraction had high amounts of 16:1ω5, indicating significant contribution from fungi (data not shown).

The abundance of individual PLFAs was calculated in absolute C amounts (ng PLFA-C g^{-1} dry soil) based on the PLFA-C concentrations in the liquid extracts, and used as a proxy for microbial biomass. Changes in the microbial functional group composition were evaluated based on shifts in PLFA relative abundances calculated and expressed as molar C percentage (mol%) of each biomarker using the following formula:

$$\text{mol\%PLFA-C} = \frac{(\text{PLFA-C})_i}{\sum_{i=1}^{n}(\text{PLFA-C})_i} \times 100 \qquad (1)$$

where $(\text{PLFA-C})_i$ is the concentration of PLFA-C in solution (mol L^{-1}) and n is the total number of identified biomarkers. Relative abundance values were then summed across all individual biomarkers previously defined for each microbial functional group.

The ratio of fungi to bacteria was calculated as total fungal to total bacterial biomass where total bacteria and fungi were determined by the sum of previously defined group biomarkers as follows:

$\text{Bacteria}_{total} = $ Gram-negative bacteria + Gram-positive bacteria + General bacteria

1   and

2          $Fungi_{total} = AMF + Saprophytic\ fungi$

3   Isotopic ^{13}C enrichment in plant tissues and in soil microbial PLFAs were calculated as

4   atom percent enrichment (APE)

5          $APE\ ^{13}C_i = atom\%^{13}C_{labeled} - atom\%^{13}C_{unlabeled}$ (2)

6   for each i plant component (leaves, tillers, roots) or PLFA biomarker.

7   Label uptake by microbial functional group is then defined as:

8          $APE\ ^{13}C_{group} = \sum_{i=1}^{n} APE^{13}C_i$ (3)

9   for *n* functional group-specific biomarkers.

10   The relative distribution (%) of total label taken up that was recovered in each functional group

11   can then be calculated as:

12          $Relative\ recovery_{group} = APE\ ^{13}C_{group} / APE\ ^{13}C_{total}\ x\ 100,$ (4)

13   where:

14          $APE\ ^{13}C_{total} = \sum_{i=1}^{m} APE^{13}C_i$ (5)

15   for *m* total biomarkers identified, and other terms are previously defined.

16   Due to differing ^{13}C label uptake between the two ecotypes (Table 2), we express ^{13}C enrichment

17   on a relative APE base (APE*rel* (Balasooriya et al. 2013)):

18          $APE_{rel} = \dfrac{APE\ 13Ci}{APE\ 13Ctotal} \times 100$ (6)

19   **2.7 Statistical Analyses**

20   A 2-way ANOVA with switchgrass ecotypes and soil depth as main factors and plot as a

21   random effect was run for belowground plant biomass, soil C, N, bulk density, total PLFA-C for

22   each individual PLFA biomarker (ng PLFA C/g soil) and microbial group, and APE_rel for

23   microbial groups using SAS v. 9.3 (SAS Institute, Cary, North Carolina, USA). Aboveground

24   biomass and plant biomass APE was run as a 1-way ANOVA with ecotype as the main effect

25   and plot as a random effect. Where necessary, data were log transformed to meet assumptions of

26   normality and equal variance. P-values are noted in the text after Bonferroni adjustment.

3 Results

3.1 Soil Properties

Soil C and N decreased with soil depth ($P < 0.0001$) and pH increased with soil depth ($P = 0.003$). For each depth increment, the soil characteristics beneath the two ecotypes were similar (soil C, N, bulk density, pH and texture, Table 1). There was no significant effect of ecotype on bulk density ($P = 0.9634$, data not shown).

3.2 Switchgrass Biomass

The lowland ecotype Kanlow had more aboveground biomass (4886 ± 1220 g m^{-2}) compared to Summer (1778 ± 660 g m^{-2}, $P = 0.0153$, Table 2). Total belowground root biomass down to 150 cm was also greater in Kanlow (6633 ± 2165 g m^{-2}) compared to Summer (2271 ± 694 g m^{-2}, $P = 0.029$). This difference was driven by the top two depths (0-10 and 10-30 cm), which comprised 91% and 85% of root biomass for Kanlow and Summer, respectively.

3.3 Root Characteristics

Kanlow had significantly coarser, denser roots compared to Summer, resulting in a shorter specific root length (SRL) throughout the soil profile, despite having similar root length densities (RLD) (Table 3). Root mass density (RMD) was 2.8 to 6 times greater in Kanlow compared to Summer in the first three soil depths and decreased with depth (Table 3). Weight averaged over the 0-150 cm profile, RMD was 5.48 ± 1.59 mg cm^{-3} for Kanlow and 1.92 ± 0.69 mg cm^{-3} for Summer ($P = 0.001$). However, the two ecotypes had similar root length densities (RLD) because the greater RMD in Kanlow was comprised of roots with shorter SRL (Table 3). Kanlow's SRL averaged over the soil profile was lower (25.96 ± 1.73 m g^{-1} root) compared to Summer (52.66 ± 12.08 m g^{-1} root, $P = 0.001$). The SRL for both ecotypes increased with depth as a result of lower RMD.

3.4 Soil microbial biomass and community composition

Differences in soil microbial biomass between ecotypes reflected differences in plant productivity. The soils under Kanlow had greater PLFA-C (6.2 ± 0.2 µg PLFA-C g^{-1} soil) compared to Summer (4.7 ± 0.2 µg PLFA-C g^{-1} soil) averaged across all depths ($P = 0.0035$,

Figure 1). Total microbial biomass decreased with soil depth under both ecotypes (P < 0.0001, Figure 1) and the ecotype by depth interaction was also significant (P = 0.0019). Total PLFA-C decreased with depth under Summer, with a transient increase in the 90-120 cm depth under Kanlow and continued decrease in the 120-150cm depth. Despite the decreasing total PLFAs with depth, over half of the total observed PLFA biomass was below 10 cm (Figure 1).

Soil microbial community composition differed between switchgrass ecotypes and through the soil profile due to differences in bacteria (Figure 2). Kanlow had relatively more total bacterial PLFAs (55.4 vs. 53.5 % relative abundance, P = 0.0367), particularly more gram-negative bacteria (18.1 % relative abundance) compared to Summer (16.3% relative abundance, P = 0.0455) (Figure 2A). This resulted in the Kanlow soil microbial community having a significantly lower gram-positive to gram-negative ratio (1.64) compared to Summer (1.88) averaged over depths (P = 0.0165, Figure 3A).

In contrast, soils under Summer tended to have more fungal biomarkers and non-specific microbial biomass biomarkers averaged over the soil profile compared to Kanlow soils (P = 0.140 and P = 0.0866, respectively). This resulted in greater fungal:bacterial ratios averaged over the profile (P = 0.064), particularly at the deeper depths (Figure 3B). There was no difference between ecotypes in microbial community structure in the 0-10 or 10-30 cm depths.

A depth effect was observed in microbial community structure (P < 0.0001, Figure 2) with gram-positive bacteria and actinomycetes being the most abundant in the 30-90 cm depths. Actinomycetes increased to the 30-60 cm soil depth, then declined through the 150 cm depth under both ecotypes. Gram-positive bacteria followed a similar pattern, but peaked in the 60-90 cm depth increment before declining (P < 0.0001, Figure 2A). Bacteria increased with depth initially, declined at the 30-60 cm depth, and then continued to increase through the 120-150 cm depth (P < 0.0001, Figure 2A). Fungi and gram-negative bacteria were greatest at the surface and deeper depths with a minimum at 30-60 cm or 60-90 cm depths (P < 0.0001, Figure 2A and 2B).

3.5 Plant ^{13}C uptake

The ^{13}C enrichment was detected in plant and root biomass throughout the soil profile 48 hours after labeling (Table 4). Enrichment was greater throughout the plant in Summer compared to Kanlow with leaves 630 ± 113 vs. 474 ± 10 ng excess ^{13}C g^{-1} DM (P < 0.069) and tillers (1469 ± 252 vs. 756 ± 110 ng excess ^{13}C g^{-1} DM, P < 0.007). Enrichment was also evident in labeled

roots throughout the soil profile and was generally greater in Summer vs. Kanlow and significant in half the depths sampled (0-10, 10-30, 90-120 cm P < 0.0198). The root ^{13}C enrichment was similar within ecotype throughout the soil profile down to the 120-150 cm sample depth (Table 4).

3.6 ^{13}C incorporation into microbial PLFAs

Microbial uptake of rhizodeposit C was observed in PLFAs throughout the profile to 150 cm after 48 hours. PLFA ^{13}C enrichment for AMF, saprotrophic fungi, general bacteria, gram-negative bacteria, gram-positive bacteria and universal microbial biomarkers was greater in the pulse-labeled samples compared to the control (non-labeled) samples (Supplementary Tables 1 and 2). The two deepest depths (90-120 and 120-150 cm) should be interpreted with caution due to large variation in the labeled PLFAs. Although total PLFA APE (ng excess ^{13}C g^{-1}) was 1.78 times greater under Summer (10.97 ng excess ^{13}C g^{-1}) compared to Kanlow (6.18 ng excess ^{13}C g^{-1}), it was not significant due to variability in individual plant and microbial ^{13}C uptake (data not shown). To normalize for these differences in ^{13}C uptake, we express PLFA ^{13}C enrichment as relative atom % ^{13}C excess (APE$_{rel}$) to compare between the two ecotypes.

Relative rhizodeposit C uptake (APE$_{rel}$) under Kanlow was greatest in gram-negative bacteria (44.1 ± 2.3% APE$_{rel}$, 16:1ω7, 17:0cy, 18:1ω7) and in saprotrophic fungi (48.5 ± 2.2% APE$_{rel}$, c18:1ω9, 18:2ω9,12) under Summer (Figure 4) averaged over all depths. These community differences became more pronounced through the soil profile, particularly in depths deeper than 60 cm. Microbial communities in Kanlow soils had greater rhizodeposit uptake in non-specific PLFAs (24.0 ± 1.7%, P= 0.006, 16:0) than Summer soils averaged over all soil depths, and took up 32% of the rhizodeposited ^{13}C label in the top two soil depths (P < 0.0001). Rhizodeposit uptake in the AMF was dominant in biomarker 16:1ω5, did not differ between the two ecotypes, and decreased from 13.1 ± 1.3% relative enrichment in surface soils to 1.4 ± 2.4% relative enrichment in the deepest soil layer (120-150 cm).

4 Discussion

4.1 Ecotype root characteristics

Switchgrass ecotypes have a broad range in phenology that reflects their adaptation across a wide geographic area. The lowland ecotype, Kanlow, had 2.7 times more aboveground and 2.9 times more belowground biomass than the upland ecotype, Summer. Although both ecotypes allocated two-thirds of biomass belowground, there was a significant difference in rooting traits throughout the soil profile. Differences between the two switchgrass ecotypes' phenology were evident as the lowland ecotype, Kanlow, had significantly thicker roots with shorter SRL compared to the upland ecotype, Summer. The SRL for Summer (17.2 m g^{-1} root DW) was double that of Kanlow (8.3 m g^{-1} root dry weight (DW)) in the 0-10 cm depth and throughout the soil profile. DeGraaff et al. (2013) also found greater SRL in upland (253 ± 60 cm g^{-1} DW) compared to lowland (170 ± 28 cm g^{-1} DW) cultivars in the 0-15 cm depth across eight switchgrass cultivars grown in Illinois in the US Midwest.

Root mass density was two times greater under the lowland ecotype Kanlow than the upland ecotype, Summer. This is the opposite relationship found by Ma et al. (2000), who found that the upland ecotype Cave-in-Rock had significantly greater RMD compared to the lowland ecotypes Alamo and Kanlow in 7 year old switchgrass stands on a sandy loam in Alabama. Other studies document cultivar-specific differences in root architecture between genotypes. Jackson (1995) found root biomass cultivation and allocation were similar for lettuce (*Lactuca spp.*) genotypes but their root architecture differed. Likewise, fine root morphology and architecture are found to vary among species, apparently genetically determined and less plastic, while root physiology appears to vary depending on current, whole plant metabolic activity (Comas et al., 2004; Fischer et al., 2006).

4.2 Effect of switchgrass ecotype on soil microbial community biomass and composition

These differences in rooting characteristics resulted in different microbial biomass and microbial community structure. In contrast to our hypothesis that Summer would have greater microbial biomass, we found greater soil microbial biomass (PLFA-C) in Kanlow reflecting greater belowground root biomass in Kanlow (Table 2 & Figure 1). The communities of the two ecotypes also differed, with the lowland ecotype, Kanlow associated with a slightly more bacterially-dominated soil microbial community than Summer. These community differences could be a function either of microbial community modification by the plant from root exudation

(Broeckling et al., 2008; Gschwendtner et al. 2010) or root litter turnover and decomposition (DeGraaff et al., 2013, 2014). Plant cultivars have been shown to develop different microbial rhizosphere communities (Broeckling et al., 2008; Gschwendtner et al. 2010) through root exudation patterns (Broeckling et al., 2008). To our knowledge, this may be the first illustration of switchgrass ecotype-specific impacts on soil communities in the field.

We observed greater fungal:bacterial ratios under the fine-rooted upland ecotype, Summer, compared to the coarser rooted Kanlow over the profile, and the greatest fungal:bacterial ratio was found in the 120-150 cm depth. This was in contrast to our hypothesis that Kanlow would have a more fungal community, particularly AMF. The finer rooting architecture of Summer may promote greater root turnover and, in turn, promote a more saprotrophic fungal community. It is interesting to note that there was no difference in the AMF communities between the two ecotypes, which may be a function of the thinner roots of Summer having less cortex to support AM (Comas et al. 2014), or abundant N in this agronomic setting. However, the presence of AM communities has been shown to stimulate root litter decomposition, plant N uptake, and saprotrophic fungal abundance without altering AM abundance (Herman et al. 2012).

4.3 Effect of depth on soil microbial community abundance and composition

There was an overall decrease in the total microbial biomass (μg PLFA-C g^{-1} soil) with depth (Figure 1) which corresponds to previous studies (Fierer et al., 2003; Kramer and Gleixner, 2008; Aliasgharzad et al., 2010). Because soil microbes primarily use C from root exudates as their energy source and C availability decreases with soil depth (Table 2), microbial biomass is also expected to decline (Chaudhary et al., 2012).

Microbial community structure also changed with depth. Our results for 0-60 cm soils agree with those of Fierer et al. (2003), who found gram-positive bacteria and actinomycetes increased in proportional abundance with increasing soil depth and that gram-negative bacteria and fungi were greatest in surface soils. In the current study, the proportion of total PLFAs attributable to fungi (saprotrophic fungi and AMF) was generally greater in surface soils than deeper soils and that fungi and gram-negative biomarkers decreased with depth (0-60 cm). More specifically, fungi and gram-negative PLFAs decreased in proportional abundance down through 60 to 90 cm in depth and subsequently increased through the 120 cm depth profile while gram-

positive and actinomycetes PLFAs showed the opposite trend, increasing in proportional abundance through 60 to 90 cm in depth and decreasing through the remainder of the 120 cm depth profile.

Previous studies have shown that higher available C or rates of C addition to soil tend to have greater proportional abundance of fungi and gram-negative bacteria while gram-positive and actinomycetes are proportionately lower under the same conditions (Griffiths et al., 1999; Fierer et al., 2003). Thus in depths that are C-rich we should expect greater proportions of fungi and gram-negative bacteria and in areas of C limitation we should expect greater proportions of gram-positive and actinomycetes. This suggests more microbial C-limitation at the middle of the depth profile, perhaps reflecting the high soil C content near the surface and active plant root exudation deeper in the profile.

4.4 Microbial rhizodeposit-C utilization

Microbial uptake of rhizodeposit ^{13}C was observed in PLFAs throughout the soil profile to 150 cm depth 48 hrs post-labeling and illustrated distinct microbial community uptake patterns between switchgrass ecotypes, particularly deeper than 60 cm. The majority of labeled rhizodeposit uptake under Kanlow was by gram-negative bacteria which took up 44.1 ± 2.3% of the total ^{13}C label recovered from all biomarkers whereas under Summer the rhizodeposit uptake was predominantly by the saprotrophic fungi (48.5 ± 2.2% relative enrichment) (Figure 4). Although we did not measure root exudation here, other studies have documented that cultivar differences in root exudation influence microbial community structure (Gschwendtner et al., 2010; Marschner et al., 2001).

The differing rhizodeposit uptake patterns in the microbial communities associated with the two ecotypes illustrated differing active plant-microbial associations. Kanlow, with thicker roots, may have greater root exudation and could have promoted more bacterial associations. Gram negative bacterial endophytes (Protobacteria) have been found to associate with switchgrass and have been shown to increase switchgrass growth (Xia et al., 2012). The finer root system of Summer may have exudation patterns that promote decomposition by saprotrophic fungi as a means for recovering nutrients from fine-root turnover. Recent work suggests that plants may promote litter decomposition for nutrient acquisition (Herman et al., 2012).

Fungi have the potential to strongly affect soil C sequestration. Although AMF fungal rhizodeposit uptake comprised a small part (13% of total enrichment in the 0-10 cm soil depth) and uptake by AMF biomarkers did not differ between the two switchgrass ecotypes, rhizodeposit uptake in saprotrophic fungi comprised nearly 49% under Summer soils averaged over all depths. Furthermore, rhizodeposit uptake by saprotrophic fungi increased through the entire Summer soil depth profile to 150 cm. In general, fungal mycelia are comprised of complex, nutrient-poor carbon forms like chitin and melanin, allowing fungal metabolites to reside longer in soil than bacteria whose membranes mainly consist of phospholipids that are quickly reincorporated by soil biota (Rillig and Mummey, 2006; Six et al., 2006; De Deyn et al., 2008; Jin et al., 2010). By immobilizing C in their mycelium, extending root lifespan, and improving C sequestration in soil aggregates mycorrhizal fungi can reduce soil C loss (Langley et al., 2006; Rillig and Mummey, 2006; De Deyn et al., 2008).

4.5 Impacts for bioenergy production & C sequestration

Switchgrass is a strong candidate for soil C sequestration due to its fibrous root system that can extend through a depth of 3 m (Ma et al., 2000; Liebig et al., 2005; Hartman et al., 2011: Schmer et al., 2011). Previous studies have shown that switchgrass has the capacity to increase SOC, mitigate greenhouse gas emissions, and improve soil quality (Sanderson et al., 1999; Garten et al., 2000; Frank et al., 2004; Liebig et al., 2005; Stewart et al., 2014). Furthermore, results from previous studies indicate that switchgrass is effective at storing SOC below depths of 30 cm, not just near the soil surface (Sanderson et al., 1999; Garten et al., 2000; Follett et al. 2012; Liebig et al., 2005).

Garten et al. (2010) found no significant difference among 3-yr old lowland switchgrass ecotypes for total aboveground or belowground biomass, C stocks, or N stocks in the 0-90 cm soils sampled in their study. In contrast to their observations, our results indicate ecotype differences in root production and soil microbial communities under 3 year-old switchgrass lowland ecotype Kanlow and upland ecotype Summer in the 0- 150 cm soil profile. It should be noted that the cultivars within the study done by Garten et al. (2010) contained only lowland ecotypes whereas our study is comparing a lowland ecotype (Kanlow) to an upland ecotype (Summer). Our results suggest Kanlow as greater yielding for aboveground biomass,

belowground root biomass and promoting total soil microbial biomass (Table 2, Figure 1), but Summer may have a greater potential for soil C sequestration due to greater C transfer to the soil fungal community and therefore may promote soil aggregation.

5   Conclusions

The two switchgrass ecotypes had distinct differences in root biomass and morphology that resulted in differences in the associated soil microbial biomass, microbial community composition and rhizodeposit C uptake. The lowland ecotype had significantly greater RMD but similar RLD due to having shorter SRL compared to the upland ecotype, Summer. Kanlow had more microbial biomass and a more bacterial dominated microbial community than Summer. Although the differences between ecotype microbial communities was modest, rhizodeposit uptake was quite different between ecotypes. The rhizodeposit C was processed primarily by gram negative bacteria under Kanlow and saprotrophic fungi under Summer. Variation in microbial community composition as well as rhizodeposit C uptake could result in different C sequestration dynamics. For bioenergy production systems, variation between switchgrass ecotypes could impact C sequestration and storage as well as potentially other belowground processes by altering microbial communities and their role in C processing.

Acknowledgements

The authors acknowledge field assistance from technician Nathan Mellor and support scientist Paul Koerner and laboratory assistance from Tamara Higgs, Erin Grogan Amber Brandt, Jordan Wieger, Philip Korejwo and numerous student workers. This work is part of the USDA-ARS GRACEnet Project: http://www.ars.usda.gov/research/GRACEnet. This work was supported in part by the Office of Science (BER), U. S. Department of Energy Grant Number DE-AI02-09ER64829, and by the USDA-ARS CRIS project 5440-21000-030-00D.   The U.S. Department of Agriculture, Agricultural Research Service, is an equal opportunity/affirmative action employer and all agency services are available without discrimination. Mention of commercial products and organizations in this manuscript is

1   solely to provide specific information. It does not constitute endorsement by USDA-ARS

2   over other products and organizations not mentioned.

References

Aliasgharzad, N., Martensson, L., Olsson, P., 2010. Acidification of a sandy grassland favours bacteria and disfavours fungal saprotrophs as estimated by fatty acid profiling. Soil Biology & Biochemistry, 42, 1058-1064.

Balasooriya, W., Huygens, D., Denef, K., Roobroeck, D., Verhoest, N., Boeckx, P., 2013. Temporal variation of rhizodeposit-D assimilating microbial communities in a natural wetland, Biology and Fertility of Soils, 49, 333-341.

Blume, E., Bischoff, M., Reichart, J., Moorman, T., Konopka, A., Turco, R., 2002. Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. Applied Soil Ecology, 592, 1-11.

Bone, T.L., Balkwill, D.L., 1988. Morphological and cultural comparison of microorganisms in surface soil and subsurface sediments at a pristine study site in Oklahoma. Microbial Ecology, 16, 49-64.

Bossio, D.A., Scow, K.M., 1995. Impact of carbon and flooding on the metabolic diversity of microbial communities in soils. Applied Environmental Microbiology, 61, 4043-4050.

Bouma, T.J., Nielson, K.L., Koutstaal, B.A.S., 2000. Sample preparation and scanning protocol for computerized analysis of root length and diameter. Plant and Soil, 218, 185–196.

Broeckling, C.D., Broz, A.K., Bergelson, J, Manter, D.K., Vivanco, J.M., 2008. Root exudates regulate soil fungal community composition and diversity. Applied and Environmental Microbiology, 74, 738-744.

Brundrett, M., 2002. Coevolution of roots and mycorrhizas of land plants. New Phytologist, 154, 275-304.

Chaudhary, D., Saxena, J., Lorenz, N., Dick, L., Dick, R., 2012. Microbial profiles of rhizosphere and bulk soil microbial communities of biofuel crops switchgrass and jatropha. Applied and Environmental Soil Science, 906864, 1-6.

Comas, L.H., & Eissenstat, D.M., 2004. Linking fine root traits and maximum potential growth rate among 11 mature temperate tree species. Functional Ecology, 18, 388-397.

1   Comas, L.H., Eissenstat, D.M., 2009. Patterns in root trait variation among 25 co-existing North

2   Amercian forest species. New Phytologist, 182, 919-928.

3   Comas, L.H., Mueller, K.E., Taylor, L.L., Midford, P.E., Callahan, H.S., & Beerling, D.J.. 2012.

4   Evolutionary patterns and biogeochemical significance of angiosperm root traits. International

5   Journal of Plant Sciences, 173, 584-595.

6   Comas, L.H., Callahan, H.S., Midford, P.E., 2014. Patterns in root traits of woody species

7   hosting arbuscular and ectomycorrhizas: implications for the evolution of belowground

8   strategies. Ecology and Evolution, 4, 2979-2990.

9   De Deyn, G.B., Comelissen, J.H.C., Bardgett, R.D., 2008. Plant functional traits and soil carbon

10   sequestration in contrasting biomes. Ecology Letters, 11, 516-531.

11   De Graaff, M.-A., Six, J., Jastrow, J.D., Schadt, C.W., Wullschleger, S.D., 2013. Variation in

12   root architecture among switchgrass cultivars impacts root decomposition rates. Soil Biology &

13   Biochemistry, 58, 198-206.

14   De Graaff, M.-A., Jastrow, J.D., Gillette, S., Johns, A., Wullschleger, S.D., 2014. Differential

15   priming of soil carbon driven by soil depth and root impacts on carbon availability. Soil Biology

16   & Biochemistry, 69, 147-156.

17   Denef, K., Bubenheim, H., Lenhart, K., Vermeulen, J., Van Cleemput, O., Boeckx, P., Müller, C,

18   2007. Community shifts and carbon translocation within metabolically-active rhizosphere

19   microorganisms in grasslands under elevated CO_2. Biogeosciences Discussions, 4, 1437-460.

20   Denef, K., Roobroeck, D., Manimel Wadu, M.C.W., Lootens, P., Boeckx, P., 2009. Microbial

21   community composition and rhizodeposit-carbon assimilation in differently managed temperate

22   grassland soils. Soil Biology & Biochemistry, 41, 144-153.

23   Dodds, W.K., Banks, M.K., Clenan, C.S., Rice, C.W., Sotomayor, D., Strauss, E.A., Yu, W.,

24   1996. Biological properties of soil and subsurface sediments under abandoned pasture and

25   cropland. Soil Biology & Biochemistry, 28, 837-846.

26   Federle, T.W., Hullar, M.A., Livingston, R.J., Meeter, D.A., White, D.C., 1986. Spatial

27   distribution of biochemical parameters indicating biomass and community composition of

microbial assemblies in estuarine mud flat sediments, Applied and Environmental Microbiology, 45, 58-63.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biology & Biochemistry, 35,167-76.

Fike, J.H., Parrish, D.J., Wolf, D.D., Balasko, J.A., Green Jr., J.T., Rasnake, M., Reynolds, J.H., 2006. Long-term yield potential of switchgrass-for-biofuel systems. Biomass and Bioenergy, 30, 198-206.

Fischer, D.G., Hart, S.C., Rehill, B.J., Lindroth, R.L., Keim, P., Whithma, T.G., 2006. Do high-tannin leaves require more roots? Oecologia, 149, 668-675.

Follett, R.F., Vogel, K.P., Varvel, G.E., Mitchell, R.B., Kimble, J., 2012. Soil carbon sequestration by switchgrass and no-till maize grown for bioenergy. BioEnergy Research, 5, 866-875.

Frank, A.B., Berdahl, J.D., Hanson, J.D., Liebig, M A., Johnson, H.A., 2004. Biomass and carbon partitioning in switchgrass. Crop Science, 44, 1391-1396.

Fritze, H., Pietikainen, J., Pennanen, T., 2000. Distribution of microbial biomass and phospholipid fatty acids in Podzol profiles under coniferous forest. European Journal of Soil Science, 51, 723-730.

Fröstegard, A., Bååth, E., 2006. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils, 22, 59-65.

Garten, C.T., Wullschleger, S.D., 2000. Soil carbon dynamics beneath switchgrass as indicated by stable isotope analysis. Journal of Environmental Quality, 29, 645-653.

Garten, C.T., Smith, J.L., Tyler, D.D., Amonette, J.E., Bailey, V.L., Brice, D.J., Castro, H.F., Graham, R.L., Gunderson, C.A., Izaurralde, R.C., Jardine, P.M., Jastrow, J.D., Kerley, M.K., Matamala, R., Mayes, M.A., Metting, F.B., Miller, R.M., Moran, K.K., Post, W.M., Sands, R.D., Schadt, C.W., Phillips, J.R., Thomson, A.M., Vugteveen, T., West, T.O., Wullschleger, S.D., 2010. Intra-annual changes in biomass, carbon, and nitrogen dynamics at 4-year old switchgrass field trials in west Tennessee, USA. Agriculture Ecosystems & Environment, 136, 177-184.

1 Graham, J.H., Hodge, N.C., Morton, J.B., 1995. Fatty Acid methyl ester profiles for
2 characterization of glomalean fungi and their endomycorrhizae. Applied and Environmental
3 Microbiology, 61, 58-64.

4 Griffiths, B., Ritz, K., Ebblewhite, N., Dobson, G., 1999. Soil microbial community structure:
5 effects of substrate loading rates. Soil Biology & Biochemistry, 31, 145-153.

6 Gschwendtner, S., Reichmann. M., Müller, M., Radl, V., Munch, J., et al., 2010. Abundance of
7 bacterial genes encoding for proteases and chitinases in the rhizosphere of three different potato
8 cultivars. Biology and Fertility of Soils, 46, 649–652.

9 Hartman, J.C., Nippert, J.B., Orozco, R.A., Springer, C.J., 2011. Potential ecological impacts of
10 switchgrass (*Panicum virgatum* L.) biofuel cultivation in the Central Great Plains, USA.
11 Biomass and Bioenergy, 35, 3415-3421.

12 Jackson, L.E., 1995. Root architecture in cultivated and wild lettuce (*Lactuca* spp.). Plant, Cell
13 and the Environment, 18, 885-894.

14 Jin, V. L., Evans, R. D., 2010. Microbial ^{13}C utilization patterns via stable isotope probing of
15 phospholipid biomarkers in Mojave Desert soils exposed to ambient and elevated atmospheric
16 CO_2. Global Change Biology, 16, 2334-2344.

17 Joslin, J.D., Gaudinski, J.B., Torn, M.S., Riley, W.J., Hanson, P.J., 2006. Fine-root turnover
18 patterns and their relationship to root diameter and soil depth in a 14C-labeled hardwood forest.
19 New Phytologist, 172, 523-535.

20 Kramer, C., Gleixner, G., 2008. Soil organic matter in soil depth profiles: distinct carbon
21 preferences of microbial groups during carbon transformation. Soil Biology & Biochemistry, 40,
22 425–433.

23 Langley, J.A., Chapman, S.K., Hungate, B.A., 2006. Ectomycorrhizal colonization slows root
24 decomposition: the postmortem fungal legacy. Ecology Letters, 9, 955-959.

25 Liang, C., Jesus, E.C., Duncan, D.S., Jackson, R.D., Tiedje, J.M., Balser, T.C., 2012. Soil
26 microbial communities under model biofuel cropping systems in southern Wisconsin, USA:
27 Impact of crop species and soil properties. Applied Soil Ecology, 54, 24-31.

Liebig, M.A., Johnson, H.A., Hanson, J.D., Frank, A.B., 2005. Soil carbon under switchgrass stands and cultivated cropland. Biomass and Bioenergy, 28, 347-354.

Ma, Z., Wood, C.W., Bransby, D.I., 2000. Impacts of soil management on root characteristics of switchgrass. Biomass and Bioenergy, 18, 105-112.

Marschner, P., Yang, C.H., Lieberei, R., Crowley, D.E., 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biology & Biochemistry, 33, 1437-1445.

Monti, A., 2012. Switchgrass: A Valuable Biomass Crop for Energy. London: Springer.

Nichols P., Stulp, B.K., Jones, J.G., White D.C., 1986. Comparison of fatty acid content and DNA homology of the filamentous gliding bacteria Vitreoscilla, Flexibacter, Filibacter. Archives of Microbiology, 146, 1–6.

Personeni, E., Loiseau, P., 2004. How does the nature of living and dead roots affect the residence time of carbon in the root litter continuum? Plant and Soil, 267, 129-141.

Rasse, D.P., Rumpel, C., Dignac, M.F., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilization. Plant and Soil, 269, 341-356.

Rillig, M.C. and Mummey, D.L., 2006. Mycorrhizas and soil structure. New Phytol, 171, 41-53.

Sanderson, M.A., Reed, R. L., Ocumpaugh, W.R., Hussey, M.A., Van Esbroeck, G., Read, J.C., Tischler, C.R., Hons, F.M., 1999. Switchgrass cultivars and germplasm for biomass feedstock production in Texas. Bioresource Technology, 67, 209-219.

Saathoff, A.J. Soundararajan, M., Vogel, K.P., Sarath, G., 2014. A method for field labeling bioenergy crops with $^{13}CO_2$. Australian Journal of Crop Science, 8, 1625-1631.

Schmer, M.R., Liebig, M.A., Vogel, K.P., Mitchell, R.B., 2011. Field-scale soil property changes under switchgrass managed for bioenergy. Global Change Biology Bioenergy, 3, 439-448.

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal, 70, 555-569.

Stewart, C.E., Follett, R.F., Pruessner, E.G., Varvel, G.E., Vogel, K.P. and Mitchell, R.B., 2014. Nitrogen and harvest effects on soil properties under rainfed switchgrass and no-till corn over

9 years: implications for soil quality. Global Change Biology Bioenergy. doi: 10.1111/gcbb.12142

Treonis A.M., Ostle N.J., Stott A.W., Primrose R., Grayston S.J., Ineson P., 2004. Identification of groups of metabolically-active rhizosphere microorganisms by stable isotope probing of PLFAs. Soil Biology & Biochemistry, 36, 533-537.

Van Gestel, M., Ladd, J., Amato, M., 1992. Microbial biomass responses to seasonal change and imposed drying regimes at increasing depths of undisturbed topsoil profiles. Soil Biology & Biochemistry, 24, 103-111.

Vestal, J.R., White, D.C., 1989. Lipid analysis in microbial ecology: quantitative approaches to the study of microbial community. BioScience, 39, 535–541.

Vogel, K.P., Mitchell, R.B., 2008. Heterosis in switchgrass: biomass yield in swards. Crop Science, 48, 2159-2164.

Wardle, D.A., 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biological Reviews of the Cambridge Philosophical Society, 67, 321-358.

Xia Y, Greissworth E, Mucci C, Williams MA, De Bolt S 2013 Characterization of culturable bacterial endophytes of switchgrass (Panicum virgatum L.) and their capacity to influence plant growth. *GCB Bioenergy,* **5,** 674-682.

Zelles, L., 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere, 35, 275-294.

Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. Biology and Fertility of Soils, 29, 111-129.

Table 1. Soil properties (C and N stocks, texture, pH) for switchgrass lowland (cv. Kanlow) ecotype and upland ecotype (cv. Summer) down to 150 cm. Values in parentheses are standard deviations.

Cultivar	Soil Depth (cm)	SOC (g C m^{-2}cm^{-1})	Total N (g N m^{-2}cm^{-1})	Texture[†]	pH
Kanlow	0-10	199.0 (32.3)	17.7 (2.9)	silty clay loam	6.24 (0.21)
	10-30	153.7 (5.4)	13 (0.5)	silty clay loam	6.32 (0.24)
	30-60	112.4 (33.7)	9.7 (3.1)	silty clay loam	6.48 (0.15)
	60-90	56.5 (11.0)	5.2 (1.3)	silty clay loam	6.60 (0.12)
	90-120	33.5 (3.5)	3.9 (0.5)	silty clay loam/silt loam	6.66 (0.15)
	120-150	20.5 (4.2)	2.5 (0.4)	silt loam	6.90 (0.12)
	0-150	575.5 (48.6)	52.0 (4.5)		
Summer	0-10	188.2 (15.2)	17.0 (1.1)	silty clay loam	5.92 (0.60)
	10-30	188.7 (43.7)	16.2 (4)	silty clay loam	6.19 (0.57)
	30-60	110.7 (20.9)	9.2 (1.8)	silty clay loam	6.64 (0.29)
	60-90	57.1 (9.2)	5 (0.9)	silty clay loam	6.61 (0.19)
	90-120	33.2 (3.2)	3.7 (1.1)	silty clay loam/silt loam	6.70 (0.19)
	120-150	24.4 (1.8)	3.7 (0.1)	silt loam	6.83 (0.01)
	0-150	602.3 (51.7)	54.6 (4.7)		

[†]from NRCS (https://soilseries.sc.egov.usda.gov/OSD_Docs/Y/YUTAN.html)

1 Table 2. Aboveground plant biomass (including crowns) and belowground root biomass per ground area (g m^{-2}) and standard
2 deviation (in parenthesis) for switchgrass lowland (cv. Kanlow) ecotype and upland ecotype (cv. Summer). P-values equal to or below
3 0.05 indicates whether the difference in biomass is significantly different between Kanlow and Summer in the aboveground plant
4 sampling, the total root biomass, and at every individual sampling depth.

	Kanlow	Summer	P-value
		(g m^{-2})	
Aboveground Biomass	4886 (1220)	1778 (660)	0.0153
Root Biomass by Depth			
0-10 cm	4212 (1193)	1652 (712)	0.009
10-30 cm	1826 (1059)	272 (108)	<0.0001
30-60 cm	253 (52)	134 (43)	0.068
60-90 cm	110 (14)	105 (45)	0.775
90-120 cm	105 (51)	78 (43)	0.422
120-150 cm	126 (23)	57 (17)	0.044
Total Root Biomass	6633 (2165)	2271 (694)	0.029

Table 3. Root mass density (mg cm^{-3}) root length density (cm cm^{-3} soil), and specific root length (m g^{-1} root) and standard deviation in parenthesis for switchgrass lowland ecotype (cv. Kanlow) and upland ecotype (cv. Summer).

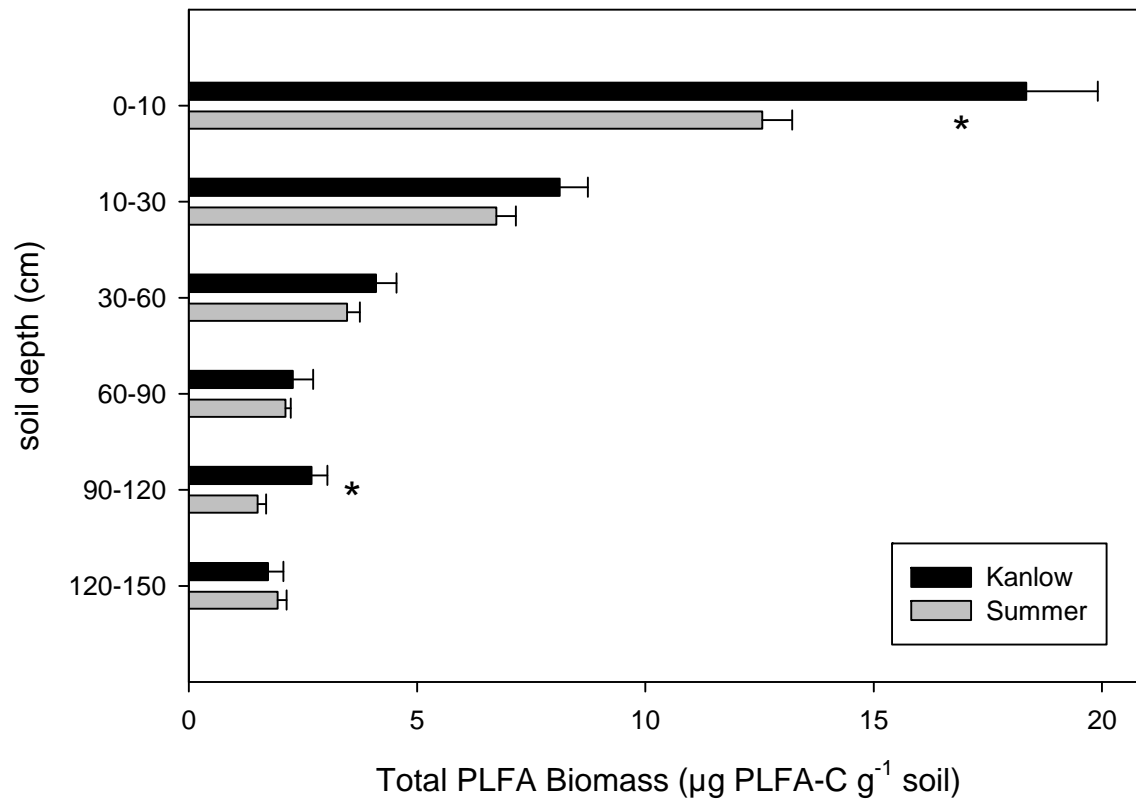
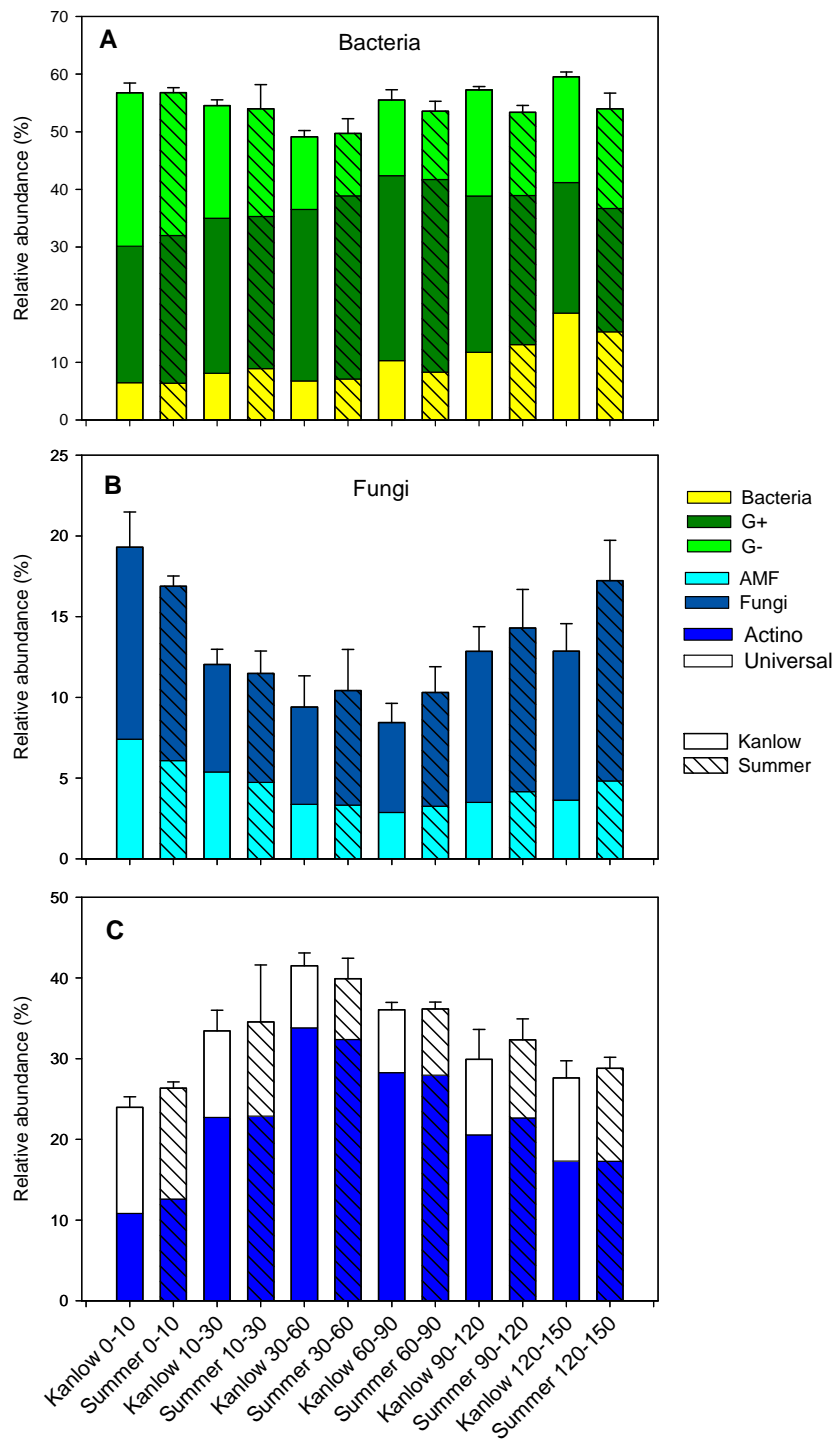
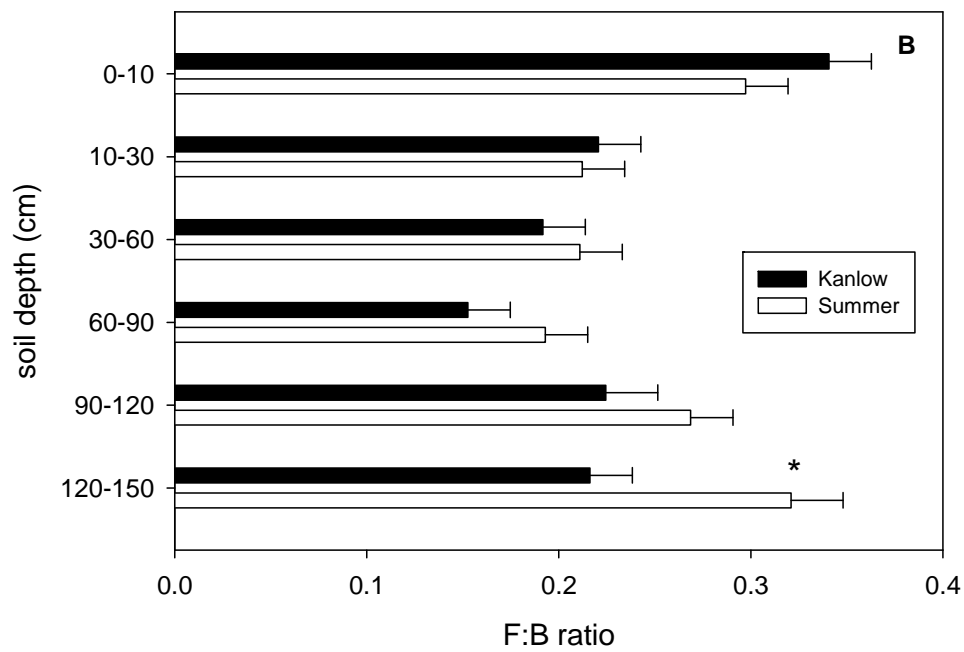
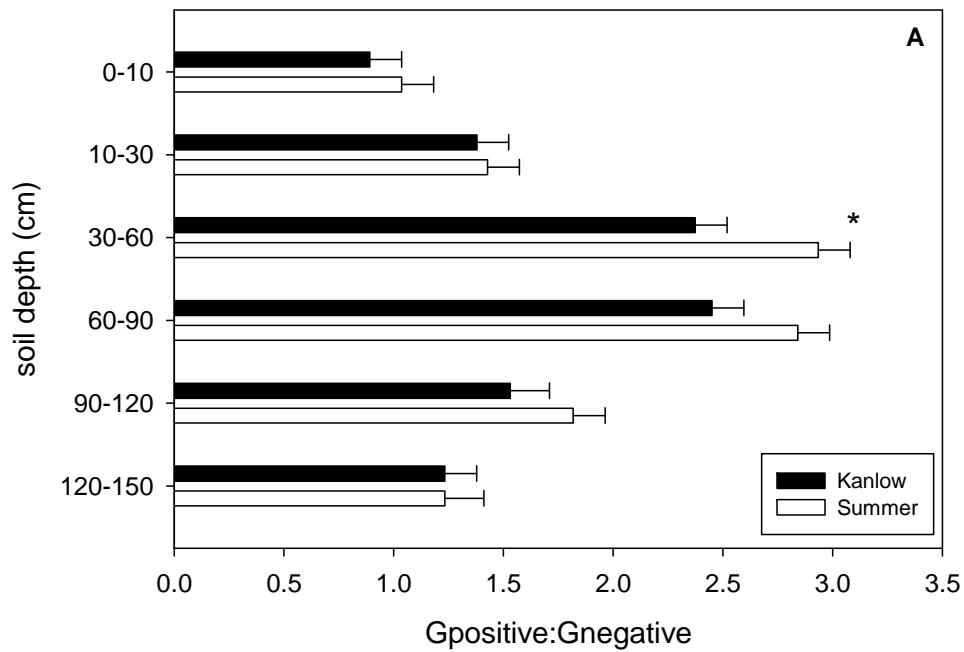
Depth	*Root Mass Density*			*Root Length Density*		*Specific root length*	
	Kanlow	**Summer**		**Kanlow**	**Summer**	**Kanlow**	**Summer**
(cm)	**(mg cm^{-3})**			**(cm cm^{-3})**		**(m g^{-1} root)**	
0-10	21.65 (5.30)	8.26 (3.56)	***	18.00 (4.23)	13.63 (4.02)	8.33 (0.09)	17.22 (2.63)**
10-30	4.89 (2.84)	0.76 (0.34)	***	5.54 (0.17)	2.77 (0.17)*	15.71 (9.26)	39.64 (13.54)***
30-60	0.46 (0.17)	0.24 (0.08)	*	0.97 (0.35)	1.11 (0.15)	21.42 (6.30)	48.40 (8.85)***
60-90	0.19 (0.02)	0.17 (0.06)		0.54 (0.04)	1.46 (0.51)***	31.49 (5.16)	88.12 (1.59)***
90-120	0.19 (0.09)	0.18 (0.09)		0.93 (0.14)	0.99 (0.21)	52.85 (16.00)	69.91 (46.17)***
120-150	0.22 (0.02)	0.11 (0.03)		1.18 (0.35)	1.43 (0.76)	60.83 (13.85)	128.63 (34.72)***
0-150	*5.48 (1.59)*	*1.92 (0.69)*	*	*5.20 (1.59)*	*3.99 (0.76)*	*25.96 (1.73)*	*52.66 (12.08)* *

* indicates a significant difference between the Kanlow and Summer at the 0.05 probability level.

** indicates a significant difference between the Kanlow and Summer at the 0.01 probability level.

*** indicates a significant difference between the Kanlow and Summer at the 0.001 probability level.

1 Table 4. The ^{13}C enrichment of aboveground plant biomass and belowground root biomass (ng
2 ^{13}C g^{-1} plant biomass) plus standard deviation (in parenthesis) for both switchgrass cultivars
3 Kanlow and Summer. P-values equal to or below 0.05 indicates significant difference between
4 cultivars within depth. DM = dry matter biomass (0% moisture).

		Kanlow	Summer	P-value
		ng excess ^{13}C g^{-1} DM		
Leaves		474.43 (10.15)	630.47 (113.19)	0.069
Tillers		756.37 (110.11)	1469.93 (252.99)	0.007
Crown		4.69 (1.22)	70.81 (39.38)	0.003
Roots	0-10	9.96 (3.14)	119.88 (54.09)	<0.0001
	10-30	11.04 (1.65)	76.56 (21.01)	0.0002
	30-60	16.21 (4.24)	36.84 (8.82)	0.0675
	60-90	18.2 (11.04)	29.12 (20.09)	0.3544
	90-120	8.66 (3.29)	33.91 (34.34)	0.0198
	120-150	8.67 (2.48)	26.24 (18.94)	0.0907

2
3 Figure 1. PLFA-derived C (µg PLFA-C g^{-1} soil) for switchgrass cultivars Kanlow and Summer
4 by depth. Error bars represent standard deviations (n=3). * indicates a significance difference
5 between cultivars within depth.

2 Figure 2. Soil microbial community composition (relative abundance, mol%) for switchgrass
3 cultivars Kanlow and Summer from 0-150 cm for A) bacterial groups, B) fungal groups and C)
4 actinomycetes and universal microbial groups. Error bars represent standard deviations (n=3).

2
Figure 3. Gram-positive:gram-negative ratios (A) and fungal:bacterial ratios B) for switchgrass
cultivars Kanlow and Summer by depth. * indicates a significant difference between cultivars
within depth.

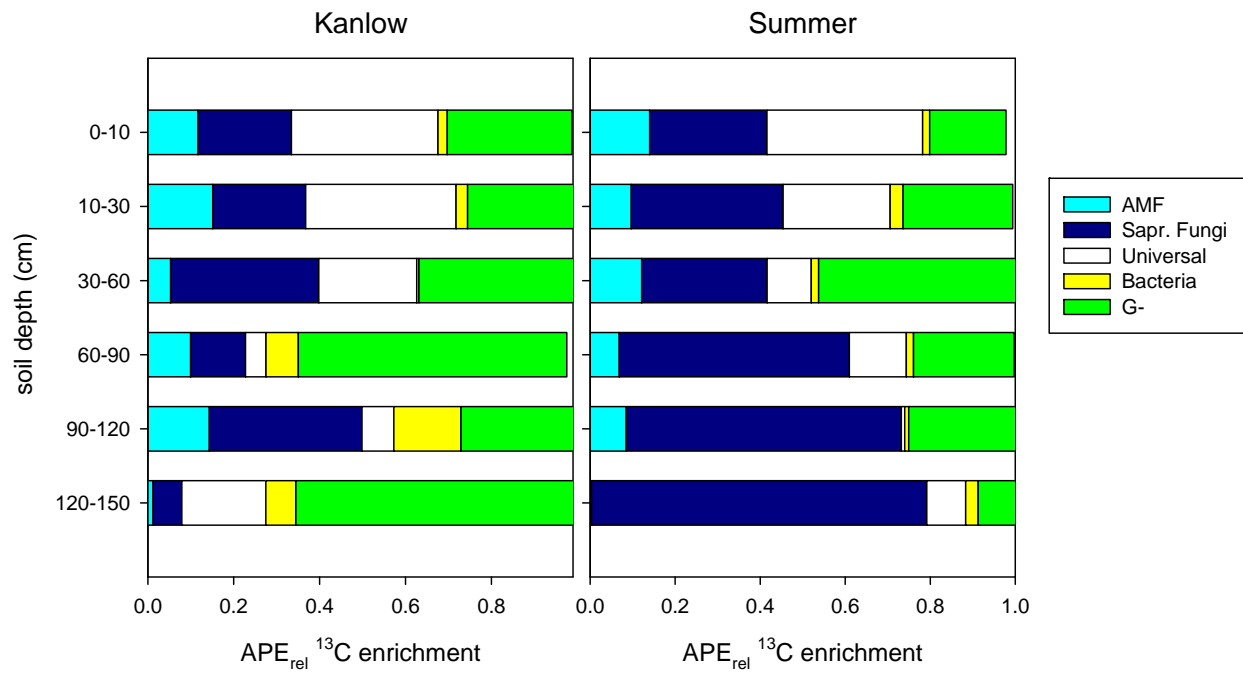



Figure 4. Relative rhizodeposit uptake (PLFA APE_{rel} enrichment), for switchgrass cultivars
Kanlow and Summer at all sampled depths 48 hours after ^{13}C labeling. Functional groups
actinomycetes and gram positive bacteria not included because ^{13}C enrichment was not obtained
in those groups (Supplementary tables 1 and 2).