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Switchgrass ecotypes alter microbial contribution to deep-soil C

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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 (PLFAs) 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.

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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; Vogel and Mitchell, 2008). 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 soil organic carbon (SOC; Garten et al., 2010, 2011) have not contrasted upland and lowland ecotypes. Although switchgrass generally has been shown to increase soil C below 30 cm (Garten and Wullschleger, 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 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 Graaff 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, 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 whether 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, 2009; 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 arbuscular mycorrhizal fungi (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 in 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 a lowland ecotype, Kanlow. Each plot consisted of 12 switchgrass plants of the same ecotype arranged in a 4×3 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. An 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 s. The label was added to raise chamber CO_2 concentrations between 1000 and 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 2 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, and 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 into increments of 0–10, 10–30, 30–60, 60–90, 90–120, and 120–150 cm. Each depth increment was split in half lengthwise, packed on ice, transported to the USDA-ARS laboratory in Fort Collins Colorado, and refrigerated at 4 °C until further processing. Soils were weighed, and a subsample was oven-dried at 110 °C for 24 h 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 separation

The frozen half soil core was thawed to room temperature, the remaining plant crown was separated from roots, and root samples were handwashed. 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 (one to two branches), third-order coarse, and coarse roots (four to five 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 the 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 half 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 were 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 $\delta^{13}C$ 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 neutral lipid fatty acids (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 × 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 (bacterial acid methyl ester) mixes (Supelco Inc) as well as mass spectral matching with the NIST 2011 mass spectral library.

The δ^{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 × 0.25 mm ID × 0.25 µm 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 δ^{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 δ^{13}C values of the PLFAs, measured δ^{13}C FAME 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 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 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 a molar C percentage (mol %) of each biomarker using the following formula:

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

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:

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

and

$$\text{Fungi}_{\text{total}} = \text{AMF} + \text{Saprophytic fungi}.$$

Isotopic ^{13}C enrichment in plant tissues and in soil microbial PLFAs was calculated as atom percent enrichment (APE),

$$\text{APE}^{13}\text{C}_i = \text{atom \%}^{13}\text{C}_{\text{labeled}} - \text{atom \%}^{13}\text{C}_{\text{unlabeled}}, \quad (2)$$

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

Label uptake by microbial functional group is then defined as

$$\text{APE}^{13}\text{C}_{\text{group}} = \sum_{i=1}^{n} \text{APE}^{13}\text{C}_i \quad (3)$$

for n functional group-specific biomarkers.

The relative distribution (%) of total label taken up that was recovered in each functional group can then be calculated as

$$\text{Relative recovery}_{\text{group}} = \text{APE}^{13}\text{C}_{\text{group}} / \text{APE}^{13}\text{C}_{\text{total}} \times 100, \quad (4)$$

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

Ecotype	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 (Natural Resource and Conservation Service) (https://soilseries.sc.egov.usda.gov/OSD_Docs/Y/YUTAN.html).

where

$$\mathrm{APE}^{13}\mathrm{C}_{\mathrm{total}} = \sum_{i=1}^{m} \mathrm{APE}^{13}\mathrm{C}_i \quad (5)$$

for m total biomarkers identified; other terms are defined above.

Due to differing ^{13}C label uptake between the two ecotypes (Table 4), we express ^{13}C enrichment on a relative APE base (APE_{rel}; Balasooriya et al., 2013):

$$\mathrm{APE}_{\mathrm{rel}} = \frac{\mathrm{APE}^{13}\mathrm{C}_i}{\mathrm{APE}^{13}\mathrm{C}_{\mathrm{total}}} \times 100. \quad (6)$$

2.7 Statistical analyses

A two-way analysis of variance (ANOVA) with switchgrass ecotypes and soil depth as main factors and plot as a random effect was run for belowground plant biomass, soil C, N, bulk density, total PLFA-C for each individual PLFA biomarker ($ng\,PLFA\,C\,g^{-1}$ soil) and microbial group, and APE_{rel} for microbial groups using SAS v. 9.3 (SAS Institute, Cary, North Carolina, USA). Aboveground biomass and plant biomass APE was run as a one-way ANOVA with ecotype as the main effect and plot as a random effect. Where necessary, data were log transformed to meet assumptions of 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 ($4886 \pm 1220\,g\,m^{-2}$) compared to Summer ($1778 \pm 660\,g\,m^{-2}$; $P = 0.0153$; Table 2). Total belowground root biomass down to 150 cm was also greater in Kanlow ($6633 \pm 2165\,g\,m^{-2}$) compared to Summer ($2271 \pm 694\,g\,m^{-2}$; $P = 0.029$). 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 (RLDs) (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). With weight averaged over the 0–150 cm profile, RMD was $5.48 \pm 1.59\,mg\,cm^{-3}$ for Kanlow and

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

	Kanlow ($g\,m^{-2}$)	Summer ($g\,m^{-2}$)	P value
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

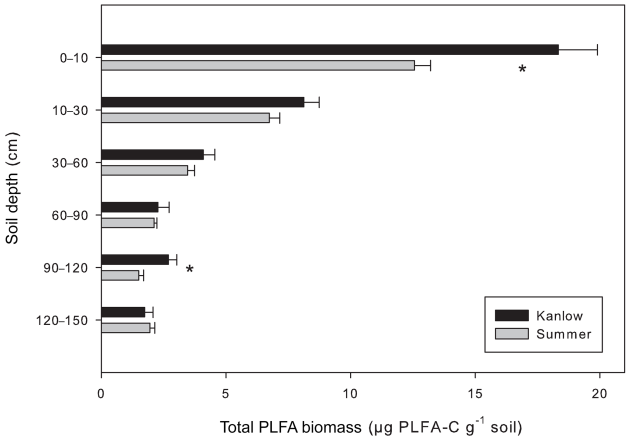

Figure 1. PLFA-derived C (μg PLFA-C g^{-1} soil) for switchgrass ecotypes Kanlow and Summer by depth. Error bars represent standard deviations ($n = 3$). The asterisk indicates a significance difference between ecotypes within depth.

$1.92 \pm 0.69\,mg\,cm^{-3}$ for Summer ($P = 0.001$). However, the two ecotypes had similar RLDs 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 ($25.96 \pm 1.73\,m\,g^{-1}$ root) compared to Summer ($52.66 \pm 12.08\,m\,g^{-1}$ 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 ($P = 0.0035$; Fig. 1). Total microbial biomass decreased with soil depth under both ecotypes ($P < 0.0001$; Fig. 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 120–150 cm depth. Despite the decreasing total PLFAs with depth, over half of the total observed PLFA biomass was below 10 cm (Fig. 1).

Soil microbial community composition differed between switchgrass ecotypes and through the soil profile due to differences in bacteria (Fig. 2). Kanlow had relatively more total bacterial PLFAs (55.4 vs. 53.5 % relative abundance; $P = 0.0367$) and in particular more Gram-negative bacteria (18.1 % relative abundance) compared to Summer (16.3 % relative abundance; $P = 0.0455$) (Fig. 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 ($P = 0.0165$; Fig. 3a).

In contrast, soils under Summer tended to have more fungal biomarkers and nonspecific 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 (Fig. 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 ($P < 0.0001$; Fig. 2) with Gram-positive bacteria and actinomycetes being the most abundant in the 30–90 cm depths. Actinomycetes increased in 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 ($P < 0.0001$; Fig. 2a). Bacteria increased with depth initially, declined at the 30–60 cm depth, and then continued to increase through the 120–150 cm depth ($P < 0.0001$; Fig. 2a). Fungi and Gram-negative bacteria were greatest at the surface and deeper depths with a minimum at 30–60 or 60–90 cm depths ($P < 0.0001$; Fig. 2a and b).

3.5 Plant ^{13}C uptake

The ^{13}C enrichment was detected in plant and root biomass throughout the soil profile 48 h after labeling (Table 4). Enrichment was greater throughout the plant in Summer compared to Kanlow with leaves (630 ± 113 vs. 474 ± 10 ng excess $^{13}C\,g^{-1}$ dry matter (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 enrich-

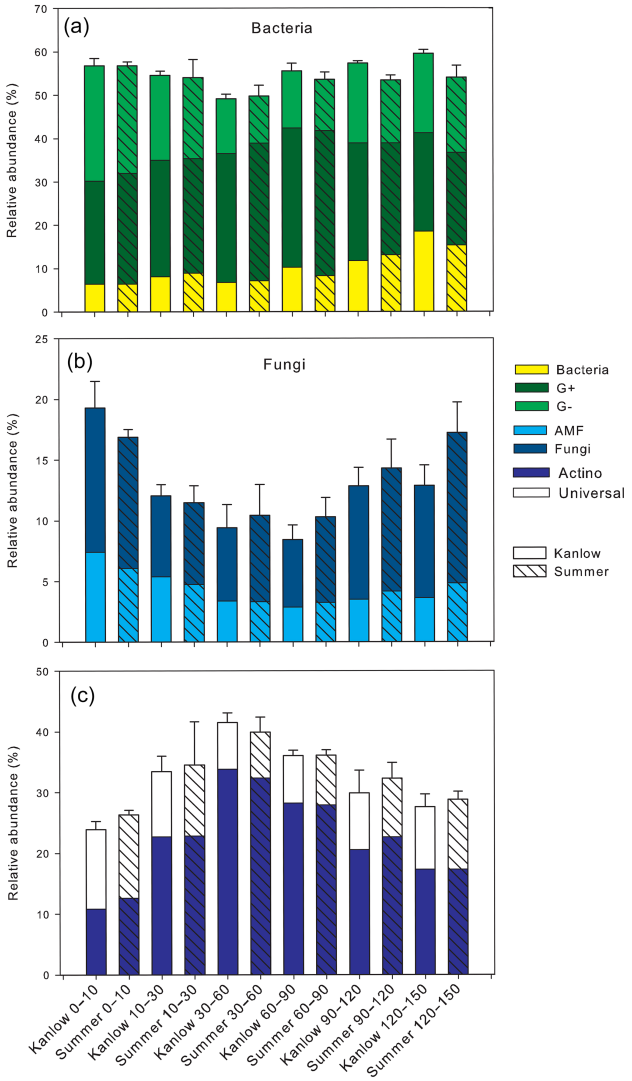

Figure 2. Soil microbial community composition (relative abundance, mol %) for switchgrass ecotypes Kanlow and Summer from 0 to 150 cm for **(a)** bacterial groups, **(b)** fungal groups, and **(c)** actinomycetes and universal microbial groups. Error bars represent standard deviations ($n = 3$).

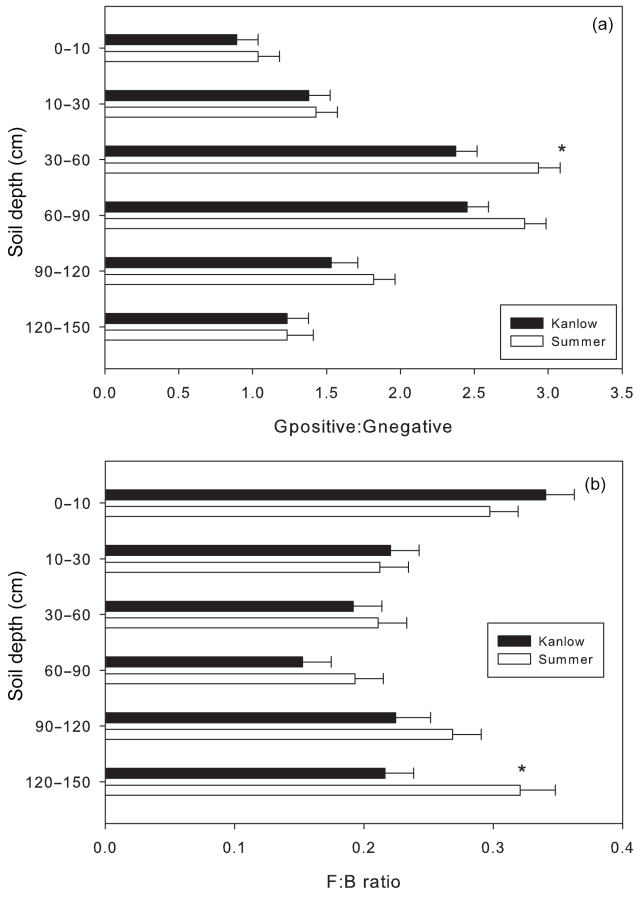

Figure 3. Gram-positive : Gram-negative ratios **(a)** and fungal : bacterial ratios **(b)** for switchgrass ecotypes Kanlow and Summer by depth. The asterisk indicates a significant difference between ecotypes within depth.

ment 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 h. 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 (Tables S1 and S2 in the Supplement). 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 (Fig. 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 nonspecific 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 domi-

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 parentheses for switchgrass lowland ecotype (Kanlow) and upland ecotype (Summer).

Depth (cm)	Root mass density (mg cm^{-3})		Root length density (cm cm^{-3})		Specific root length (m g^{-1} root)	
	Kanlow	Summer	Kanlow	Summer	Kanlow	Summer
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)*

The asterisk indicates a significant difference between Kanlow and Summer at the 0.05 probability level. ** indicates a significant difference between Kanlow and Summer at the 0.01 probability level. *** indicates a significant difference between Kanlow and Summer at the 0.001 probability level.

Table 4. The ^{13}C enrichment of aboveground plant biomass and belowground root biomass (ng ^{13}C g^{-1} plant biomass) plus standard deviation (in parentheses) for both switchgrass ecotypes Kanlow and Summer. *P* values equal to or below 0.05 indicate significant difference between ecotypes withineach soil depth. DM: dry matter biomass (0 % moisture).

		Kanlow ng excess ^{13}C g^{-1} DM	Summer ng excess ^{13}C g^{-1} DM	*P* value
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

nant 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 dry weight – DW) was double that of Kanlow (8.3 m g^{-1} root DW) in the 0–10 cm depth and throughout the soil profile. De Graaff 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 2 times greater under the lowland ecotype, Kanlow, than the upland ecotype, Summer. This is the opposite of the 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, ap-

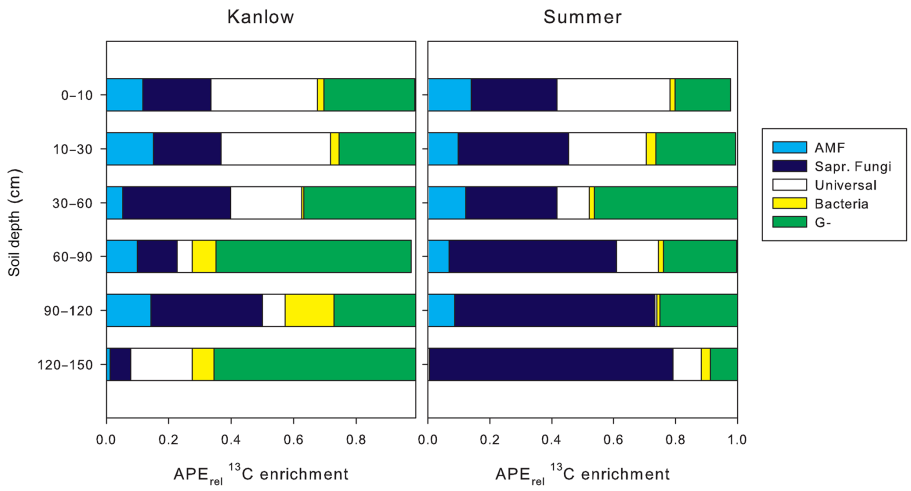

Figure 4. Relative rhizodeposit uptake (PLFA APE$_{rel}$ enrichment), for switchgrass ecotypes Kanlow and Summer at all sampled depths 48 h after ^{13}C labeling. Functional groups actinomycetes and Gram-positive bacteria not included because ^{13}C enrichment was not obtained in those groups (Tables S1 and S2).

parently genetically determined and less plastic, while root physiology appears to vary depending on current, whole-plant metabolic activity (Comas and Eissenstat, 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 and Fig. 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 (De Graaff 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 more coarsely 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 (Fig. 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 that 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 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 actinomycete 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 in 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 h 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) (Fig. 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 (Proteobacteria) have been found to associate with switchgrass and have been shown to increase switchgrass growth (Xia et al., 2013). The finer root system of Summer may have exudation patterns that promote decomposition by saprotrophic fungi as a means of 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 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 and Evans, 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 and 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 and Wullschleger, 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 and Wullschleger, 2000; Follett et al., 2012; Liebig et al., 2005).

Garten and Wullschleger (2000) found no significant difference among 3-year-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 the 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 and Wullschleger (2000) contained only lowland ecotypes, whereas our study compares a lowland ecotype (Kanlow) to an upland ecotype (Summer). Our results suggest that Kanlow yields more in terms of aboveground biomass and belowground root biomass and promotes total soil microbial biomass (Table 2, Fig. 1); however, 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 bacterially dominated microbial community than Summer. Although the differences between ecotype microbial commu-

nities 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.

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