

1 **Switchgrass ecotypes alter microbial contribution to deep**
2 **soil C**

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1 **Abstract**

2 Switchgrass (*Panicum virgatum* L.) is a C₄, perennial grass that is being developed as a
3 bioenergy crop for the United States. While aboveground biomass production is well
4 documented for switchgrass ecotypes (lowland, upland), little is known about the impact of plant
5 belowground productivity on microbial communities down deep in the soil profiles. Microbial
6 dynamics in deeper soils are likely to exert considerable control on ecosystem services, including
7 C and nutrient cycles, due to their involvement in such processes as soil formation and
8 ecosystem biogeochemistry. Differences in root biomass and rooting characteristics of
9 switchgrass ecotypes could lead to distinct differences in belowground microbial biomass and
10 microbial community composition. We quantified root abundance and root architecture and the
11 associated microbial abundance, composition and rhizodeposit C uptake for two switchgrass
12 ecotypes using stable isotope probing of microbial phospholipid fatty acids (PLFA) after ¹³CO₂
13 pulse-chase labeling. Kanlow, a lowland ecotype with thicker roots, had greater plant biomass
14 above- and belowground (g m⁻²), greater root mass density (mg cm⁻³), and lower specific root
15 length (m g⁻¹) compared to Summer, an upland ecotype with finer root architecture. The relative
16 abundance of bacterial biomarkers dominated microbial PLFA profiles for soils under both
17 Kanlow and Summer (55.4% and 53.5%, respectively, P = 0.0367), with differences attributable
18 to a greater relative abundance of gram-negative bacteria in soils under Kanlow (18.1%)
19 compared to soils under Summer (16.3%, P = 0.0455). The two ecotypes also had distinctly
20 different microbial communities process rhizodeposit C; greater relative atom % ¹³C excess in
21 gram-negative bacteria (44.1 ± 2.3%) under the thicker roots of Kanlow and greater relative atom
22 % ¹³C excess in saprotrophic fungi under the thinner roots of Summer (48.5 ± 2.2%). For
23 bioenergy production systems, variation between switchgrass ecotypes could alter microbial
24 communities and impact C sequestration and storage as well as potentially other belowground
25 processes.

26

27 **1 Introduction**

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

1 higher yielding with poorer freeze tolerance traits (Fike et al., 2006; Garten et al., 2010; Hartman
2 et al., 2011; Monti, 2012). Since switchgrass belowground biomass is proportional to or greater
3 than aboveground biomass (Frank et al., 2004; Garten et al., 2010), greater aboveground
4 productivity in lowland compared to upland ecotypes may result in more root biomass and thus
5 more carbon (C) available as an energy substrate for belowground microbial communities.
6 Because most of the aboveground biomass is removed at harvest, the production and dynamics
7 of belowground biomass are important for potential soil C storage (De Deyn et al., 2008; Garten
8 et al., 2010). Switchgrass ecotype could affect soil C differently due to differences in root
9 biomass and architecture (Ma et al. 2000), but the few field studies that investigate cultivar
10 effects on SOC (Garten et al. 2010, 2011) have not contrasted upland and lowland ecotypes.
11 Although switchgrass generally has been shown to increase soil C below 30cm (Garten et al.,
12 2000; Follett et al. 2012), how ecotypes influence soil microbial community abundance and
13 composition by affecting rhizodeposit C, in deeper soil depths is less clear.

14 Surface soils are studied most intensely because the densities of soil microorganisms are
15 greatest within organic matter and nutrient-rich surface soils (Federle et al., 1986; Bone and
16 Balkwill, 1988; Fierer et al., 2003). Only limited information is available for soil microbial
17 communities deeper than 25 cm despite evidence that more than half of the entire microbial
18 community resides in subsurface soils (Van Gestel et al., 1992; Dodds et al., 1996; Fritze et al.,
19 2000; Blume et al., 2002). Because microorganisms are involved in soil formation, ecosystem
20 biogeochemistry, and groundwater quality (Dodds et al., 1996; Fierer et al., 2003), microbial
21 dynamics in deeper soils are likely to exert considerable control on ecosystem services, including
22 C and nutrient cycles (De Deyn et al., 2008; Liang et al., 2012).

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

1 input (Rasse et al., 2005; Joslin et al., 2006). Greater root exudation has been found in fast
2 growing plant species with branched, fine root systems (Personeni and Loiseau, 2004; De Deyn
3 et al., 2008). However, species with thicker roots may have a thicker cortical layer to support
4 more arbuscular mycorrhizal (AM) fungi (Brundrett, 2002; Comas et al., 2012; Comas et al.,
5 2014). Previous switchgrass studies report that root architecture varies by cultivar or plant
6 genotype (Jackson, 1995; Fischer et al., 2006) and that upland switchgrass ecotypes have longer
7 specific root length (SRL) and finer root systems compared to coarser rooted lowland ecotypes
8 (de Graaff et al., 2013). What is less clear is if differences in root traits alter overall microbial
9 biomass and soil microbial community composition in the field.

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

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

30

1 **2 Materials and Methods**

2 **2.1 Experimental site and treatments**

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

13 **2.2 ¹³C labeling**

14 All 12 switchgrass plants in each plot were labeled in May 2012 using a customized
15 portable ¹³CO₂ pulse-chase labeling system consisting of a 1.0 m³ clear polymethyl methacrylate
16 (PMMA) chamber with an open bottom for placement over the entire plot and interior fans to
17 provide air circulation (Saathoff et al., 2014). This chamber was attached to a Portable
18 Photosynthesis System Model LI-6200 (Li-cor, Lincoln, NE) to monitor CO₂ concentration, air
19 temperature and relative humidity within the chamber headspace. Isotopically enriched CO₂ label
20 (99 atom% ¹³C (Sigma-Aldrich Co. St. Louis, MO)) was introduced into the chamber by opening
21 the gas regulator for approximately 15 seconds. Label was added to raise chamber CO₂
22 concentrations between 1000 to 2000 ppm above atmospheric CO₂ concentration (420 ppm).
23 Once the label was introduced, plants were allowed to take up labeled CO₂ until headspace
24 concentrations were at least 100 ppm below ambient CO₂ levels.

25 **2.3 Plant and soil sampling**

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

1 the crown of the plant using a 10.16 cm diameter core attached to a hydraulic soil probe. Soil
2 cores were divided in increments of 0-10, 10-30, 30-60, 60-90, 90-120, and 120-150 cm. Each
3 depth increment was split in half length-wise, packed on ice, transported to the USDA-ARS
4 laboratory in Ft. Collins Colorado, and refrigerated at 4°C until further processing. Soils were
5 weighed, and a subsample was oven-dried at 110°C for 24 hours for determination of soil
6 moisture content and soil bulk density. The half core for root separations was immediately frozen
7 (-22°C). Samples for PLFA extraction and analysis were handpicked to remove all identifiable
8 plant material, frozen at -22 °C and freeze-dried (Labconco FreeZone 77530, Kansas City, MO).

9 **2.4 Root separations**

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

23 **2.5 Plant and soil analyses**

24 For the other half of the soil core, the crowns were separated from the roots, the soil was
25 sieved to 2 mm and all large roots and non-soil materials removed prior to soil characterization
26 and microbial analysis. Soil pH was determined with a Beckman PHI 45 pH meter using a 1:1
27 soil:water ratio. Total organic C, total N, and δ¹³C in both plant and soil samples were
28 determined in duplicate by a continuous flow Europa Scientific 20-20 Stable Isotope Analyzer
29 interfaced with Europa Scientific ANCA-NT system Solid/Liquid Preparation Module (Europa
30 Scientific, Crewe Cheshire, UK-Sercon Ltd.) Soil subsamples for PLFA analysis were

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

4 **2.6 PLFA extraction and quantification**

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

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

24 The δ¹³C signature of individual FAMES was measured by capillary gas chromatography-
25 combustion-isotope ratio mass spectrometry (GC-c-IRMS) (Trace GC Ultra, GC Isolink and
26 Delta V IRMS, Thermo Scientific). A capillary GC column type DB-5 was used for FAME
27 separation (30 m length x 0.25 mm ID x 0.25µm film thickness; Agilent). The temperature
28 program started at 60 °C with a 0.10 min hold, followed by a heating rate of 10 °C min⁻¹ to 150
29 °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
30 a final hold of 1 min. The FAME δ¹³C values were calibrated using working standards (C12:0

1 and C19:0) calibrated on an elemental analyzer-IRMS (Carbo Eba NA 1500 coupled to a VG
2 Isochrom continuous flow IRMS, Isoprime Inc.). To obtain $\delta^{13}\text{C}$ values of the PLFAs, measured
3 $\delta^{13}\text{C}$ FAMES values were corrected individually for the addition of the methyl group during
4 transesterification by simple mass balance (Denef et al., 2007; Jin and Evans, 2010).

5 Of the identified PLFAs, 2-OH 10:0, 2-OH 12:0, 2-OH 14:0, 16:1 ω 7, 17:0cy, 2-OH 16:0,
6 c18:1 ω 7, and 19:0cy are classified as gram-negative bacteria while i-15:0, a-15:0, i-16:0, i-17:0,
7 and a-17:0 are classified as gram-positive bacteria, (Zelles, 1999). The 3-OH 12:0, 14:0, 15:0, 3-
8 OH 14:0, 17:0, and 18:0 are used as general bacterial indicators (Fröstegard and Bååth, 1996;
9 Zelles, 1999). The 16:0 fatty acid is classified as a universal PLFA (Zelles, 1999). The
10 10ME16:0, 10ME17:0 and 10ME18:0 are classified as actinomycete biomarkers. The 16:1 ω 5,
11 20:4 ω 6, 20:4 ω 3, and 20:1 are biomarkers for arbuscular mycorrhizal fungi (AMF) (Graham et.
12 al, 1995), and 18:3 ω 3, c18:2 ω 9,12, and c18:1 ω 9 are biomarkers for saprotrophic fungi (Zelles,
13 1997). Although 16:1 ω 5 can also be a gram-negative biomarker (Nichols, et al., 1986), in this
14 study the neutral lipid fatty acid (NLFA) fraction had high amounts of 16:1 ω 5, indicating
15 significant contribution from fungi (data not shown).

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

$$21 \quad \text{mol\%PLFA-C} = \frac{(\text{PLFA-C})_i}{\sum_{i=1}^n (\text{PLFA-C})_i} \times 100 \quad (1)$$

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

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

$$28 \quad \text{Bacteria}_{\text{total}} = \text{Gram-negative bacteria} + \text{Gram-positive bacteria} + \text{General bacteria}$$

1 and

$$2 \quad \text{Fungi}_{\text{total}} = \text{AMF} + \text{Saprophytic fungi}$$

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

$$5 \quad \text{APE } ^{13}\text{C}_i = \text{atom}\%^{13}\text{C}_{\text{labeled}} - \text{atom}\%^{13}\text{C}_{\text{unlabeled}} \quad (2)$$

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

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

$$8 \quad \text{APE } ^{13}\text{C}_{\text{group}} = \sum_{i=1}^n \text{APE } ^{13}\text{C}_i \quad (3)$$

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:

$$12 \quad \text{Relative recovery}_{\text{group}} = \text{APE } ^{13}\text{C}_{\text{group}} / \text{APE } ^{13}\text{C}_{\text{total}} \times 100, \quad (4)$$

13 where:

$$14 \quad \text{APE } ^{13}\text{C}_{\text{total}} = \sum_{i=1}^m \text{APE } ^{13}\text{C}_i \quad (5)$$

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)):

$$18 \quad \text{APE}_{\text{rel}} = \frac{\text{APE } ^{13}\text{C}_i}{\text{APE } ^{13}\text{C}_{\text{total}}} \times 100 \quad (6)$$

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
22 for 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.

1

2 **3 Results**

3 **3.1 Soil Properties**

4 Soil %C and %N decreased with soil depth ($P < 0.0001$) and pH increased with soil depth
5 ($P = 0.003$). For each depth increment, the soil characteristics beneath the two ecotypes were
6 generally similar (soil %C, %N, bulk density, pH and texture), except at the 120-150 cm depth
7 where %N was greater under Summer compared to Kanlow ($P = 0.002$, Table 1). There was no
8 significant effect of ecotype on bulk density ($P = 0.9634$, data not shown).

9 **3.2 Switchgrass Biomass**

10 The lowland ecotype Kanlow had more aboveground biomass ($4886 \pm 1220 \text{ g m}^{-2}$)
11 compared to Summer ($1778 \pm 660 \text{ g m}^{-2}$, $P = 0.0153$, Table 2). Total belowground root biomass
12 down to 150 cm was also greater in Kanlow ($6633 \pm 2165 \text{ g m}^{-2}$) compared to Summer ($2271 \pm$
13 694 g m^{-2} , $P = 0.029$). This difference was driven by the top two depths (0-10 and 10- 30 cm),
14 which comprised 91% and 85% of root biomass for Kanlow and Summer, respectively.

15 **3.3 Root Characteristics**

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

26 **3.4 Soil microbial biomass and community composition**

27 Differences in soil microbial biomass between ecotypes reflected differences in plant
28 productivity. The soils under Kanlow had greater PLFA-C ($6.2 \pm 0.2 \text{ } \mu\text{g PLFA-C g}^{-1} \text{ soil}$)

1 compared to Summer ($4.7 \pm 0.2 \mu\text{g PLFA-C g}^{-1}$ soil) averaged across all depths ($P = 0.0035$,
2 Figure 1). Total microbial biomass decreased with soil depth under both ecotypes ($P < 0.0001$,
3 Figure 1) and the ecotype by depth interaction was also significant ($P = 0.0019$). Total PLFA-C
4 decreased with depth under Summer, with a transient increase in the 90-120 cm depth under
5 Kanlow and continued decrease in the 120-150cm depth. Despite the decreasing total PLFAs
6 with depth, over half of the total observed PLFA biomass was below 10 cm (Figure 1).

7 Soil microbial community composition differed between switchgrass ecotypes and
8 through the soil profile due to differences in bacteria (Figure 2). Kanlow had relatively more
9 total bacterial PLFAs (55.4 vs. 53.5 % relative abundance, $P = 0.0367$), particularly more gram-
10 negative bacteria (18.1 % relative abundance) compared to Summer (16.3% relative abundance,
11 $P = 0.0455$) (Figure 2A). This resulted in the Kanlow soil microbial community having a
12 significantly lower gram-positive to gram-negative ratio (1.64) compared to Summer (1.88)
13 averaged over depths ($P = 0.0165$, Figure 3A).

14 In contrast, soils under Summer tended to have more fungal biomarkers and non-specific
15 microbial biomass biomarkers averaged over the soil profile compared to Kanlow soils ($P =$
16 0.140 and $P = 0.0866$, respectively). This resulted in greater fungal:bacterial ratios averaged over
17 the profile ($P = 0.064$), particularly at the deeper depths (Figure 3B). There was no difference
18 between ecotypes in microbial community structure in the 0-10 or 10-30 cm depths.

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

27 **3.5 Plant ^{13}C uptake**

28 The ^{13}C enrichment was detected in plant and root biomass throughout the soil profile 48
29 hours after labeling (Table 4). Enrichment was greater throughout the plant in Summer compared
30 to Kanlow with leaves 630 ± 113 vs. 474 ± 10 ng excess $^{13}\text{C g}^{-1}$ DM ($P < 0.069$) and tillers (1469

1 ± 252 vs. 756 ± 110 ng excess ^{13}C g^{-1} DM, $P < 0.007$). Enrichment was also evident in labeled
2 roots throughout the soil profile and was generally greater in Summer vs. Kanlow and significant
3 in half the depths sampled (0-10, 10-30, 90-120 cm $P < 0.0198$). The root ^{13}C enrichment was
4 similar within ecotype throughout the soil profile down to the 120-150 cm sample depth (Table
5 4).

6 **3.6 ^{13}C incorporation into microbial PLFAs**

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

17 Relative rhizodeposit C uptake (APE_{rel}) under Kanlow was greatest in gram-negative
18 bacteria ($44.1 \pm 2.3\%$ APE_{rel} , 16:1 ω 7, 17:0cy, 18:1 ω 7) and in saprotrophic fungi ($48.5 \pm 2.2\%$
19 APE_{rel} , c18:1 ω 9, 18:2 ω 9,12) under Summer (Figure 4) averaged over all depths. These
20 community differences became more pronounced through the soil profile, particularly in depths
21 deeper than 60 cm. Microbial communities in Kanlow soils had greater rhizodeposit uptake in
22 non-specific PLFAs ($24.0 \pm 1.7\%$, $P = 0.006$, 16:0) than Summer soils averaged over all soil
23 depths, and took up 32% of the rhizodeposited ^{13}C label in the top two soil depths ($P < 0.0001$).
24 Rhizodeposit uptake in the AMF was dominant in biomarker 16:1 ω 5, did not differ between the
25 two ecotypes, and decreased from $13.1 \pm 1.3\%$ relative enrichment in surface soils to $1.4 \pm 2.4\%$
26 relative enrichment in the deepest soil layer (120-150 cm).

27

28 **4 Discussion**

29 **4.1 Ecotype root characteristics**

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

12 Root mass density was two times greater under the lowland ecotype Kanlow than the
13 upland ecotype, Summer. This is the opposite relationship found by Ma et al. (2000), who found
14 that the upland ecotype Cave-in-Rock had significantly greater RMD compared to the lowland
15 ecotypes Alamo and Kanlow in 7 year old switchgrass stands on a sandy loam in Alabama. Other
16 studies document cultivar-specific differences in root architecture between genotypes. Jackson
17 (1995) found root biomass cultivation and allocation were similar for lettuce (*Lactuca spp.*)
18 genotypes but their root architecture differed. Likewise, fine root morphology and architecture
19 are found to vary among species, apparently genetically determined and less plastic, while root
20 physiology appears to vary depending on current, whole plant metabolic activity (Comas et al.,
21 2004; Fischer et al., 2006).

22 **4.2 Effect of switchgrass ecotype on soil microbial community biomass and** 23 **composition**

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

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

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

17 **4.3 Effect of depth on soil microbial community abundance and composition**

18 There was an overall decrease in the total microbial biomass ($\mu\text{g PLFA-C g}^{-1}$ soil) with
19 depth (Figure 1) which corresponds to previous studies (Fierer et al., 2003; Kramer and Gleixner,
20 2008; Aliasghar zad et al., 2010). Because soil microbes primarily use C from root exudates as
21 their energy source and C availability decreases with soil depth (Table 2), microbial biomass is
22 also expected to decline (Chaudhary et al., 2012).

23 Microbial community structure also changed with depth. Our results for 0-60 cm soils
24 agree with those of Fierer et al. (2003), who found gram-positive bacteria and actinomycetes
25 increased in proportional abundance with increasing soil depth and that gram-negative bacteria
26 and fungi were greatest in surface soils. In the current study, the proportion of total PLFAs
27 attributable to fungi (saprotrophic fungi and AMF) was generally greater in surface soils than
28 deeper soils and that fungi and gram-negative biomarkers decreased with depth (0-60 cm). More
29 specifically, fungi and gram-negative PLFAs decreased in proportional abundance down through
30 60 to 90 cm in depth and subsequently increased through the 120 cm depth profile while gram-

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

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

12 **4.4 Microbial rhizodeposit-C utilization**

13 Microbial uptake of rhizodeposit ^{13}C was observed in PLFAs throughout the soil profile
14 to 150 cm depth 48 hrs post-labeling and illustrated distinct microbial community uptake
15 patterns between switchgrass ecotypes, particularly deeper than 60 cm. The majority of labeled
16 rhizodeposit uptake under Kanlow was by gram-negative bacteria which took up $44.1 \pm 2.3\%$ of
17 the total ^{13}C label recovered from all biomarkers whereas under Summer the rhizodeposit uptake
18 was predominantly by the saprotrophic fungi ($48.5 \pm 2.2\%$ relative enrichment) (Figure 4).
19 Although we did not measure root exudation here, other studies have documented that cultivar
20 differences in root exudation influence microbial community structure (Gschwendtner et al.,
21 2010; Marschner et al., 2001).

22 The differing rhizodeposit uptake patterns in the microbial communities associated with
23 the two ecotypes illustrated differing active plant-microbial associations. Kanlow, with thicker
24 roots, may have greater root exudation and could have promoted more bacterial associations.
25 Gram negative bacterial endophytes (Protobacteria) have been found to associate with
26 switchgrass and have been shown to increase switchgrass growth (Xia et al., 2012). The finer
27 root system of Summer may have exudation patterns that promote decomposition by
28 saprotrophic fungi as a means for recovering nutrients from fine-root turnover. Recent work
29 suggests that plants may promote litter decomposition for nutrient acquisition (Herman et al.,
30 2012).

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

13

14 **4.5 Impacts for bioenergy production & C sequestration**

15 Switchgrass is a strong candidate for soil C sequestration due to its fibrous root system
16 that can extend through a depth of 3 m (Ma et al., 2000; Liebig et al., 2005; Hartman et al., 2011;
17 Schmer et al., 2011). Previous studies have shown that switchgrass has the capacity to increase
18 SOC, mitigate greenhouse gas emissions, and improve soil quality (Sanderson et al., 1999;
19 Garten et al., 2000; Frank et al., 2004; Liebig et al., 2005; Stewart et al., 2014). Furthermore,
20 results from previous studies indicate that switchgrass is effective at storing SOC below depths
21 of 30 cm, not just near the soil surface (Sanderson et al., 1999; Garten et al., 2000; Follett et al.
22 2012; Liebig et al., 2005).

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

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

4

5 **5 Conclusions**

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

18

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-
- 1 solely to provide specific information. It does not constitute endorsement by USDA-ARS
 - 2 over other products and organizations not mentioned.

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

3

Cultivar	Soil Depth (cm)	SOC (%)	Total N (%)	Texture [†]	pH
Kanlow	0-10	2.29 (0.05)	0.20 (0.00)	silty clay loam	6.24 (0.21)
	10-30	1.62 (0.05)	0.14 (0.00)	silty clay loam	6.32 (0.24)
	30-60	1.26 (0.05)	0.11 (0.00)	silty clay loam	6.48 (0.15)
	60-90	0.57 (0.05)	0.05 (0.00)	silty clay loam	6.60 (0.12)
	90-120	0.34 (0.06)	0.04 (0.01)	silty clay loam/silt loam	6.66 (0.15)
	120-150	0.22 (0.07)	0.03 (0.01)	silt loam	6.90 (0.12)
Summer	0-10	2.11 (0.05)	0.18 (0.00)	silty clay loam	5.92 (0.60)
	10-30	1.60 (0.05)	0.14 (0.00)	silty clay loam	6.19 (0.57)
	30-60	1.12 (0.05)	0.10 (0.00)	silty clay loam	6.64 (0.29)
	60-90	0.56 (0.05)	0.06 (0.00)	silty clay loam	6.61 (0.19)
	90-120	0.34 (0.05)	0.04 (0.01)	silty clay loam/silt loam	6.70 (0.19)
	120-150	0.25 (0.01)	0.04 (0.01)	silt loam	6.83 (0.01)

4 [†] 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.

5

	Kanlow	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

6

1 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
 2 parenthesis for switchgrass lowland ecotype (cv. Kanlow) and upland ecotype (cv. Summer).
 3

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)*

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

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

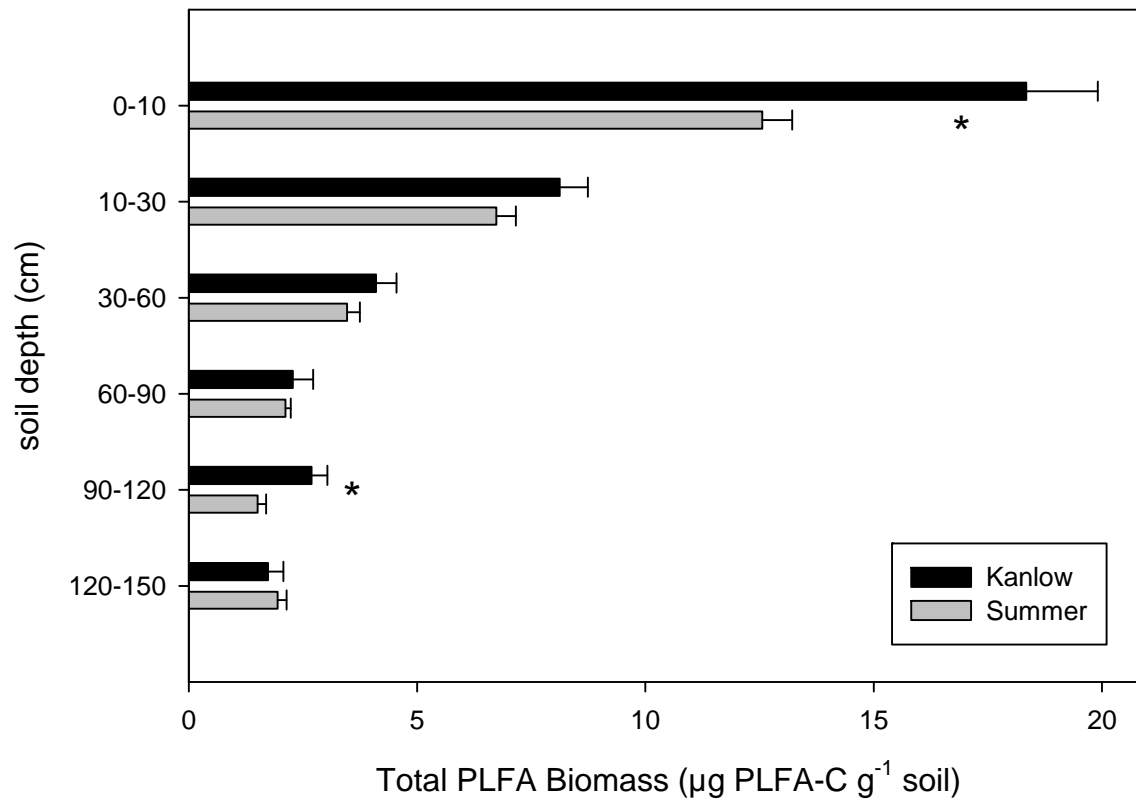
6 *** indicates a significant difference between the Kanlow and Summer at the 0.001 probability level.

1 Table 4. The ^{13}C enrichment of aboveground plant biomass and belowground root biomass (ng
 2 $^{13}\text{C g}^{-1}$ 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).

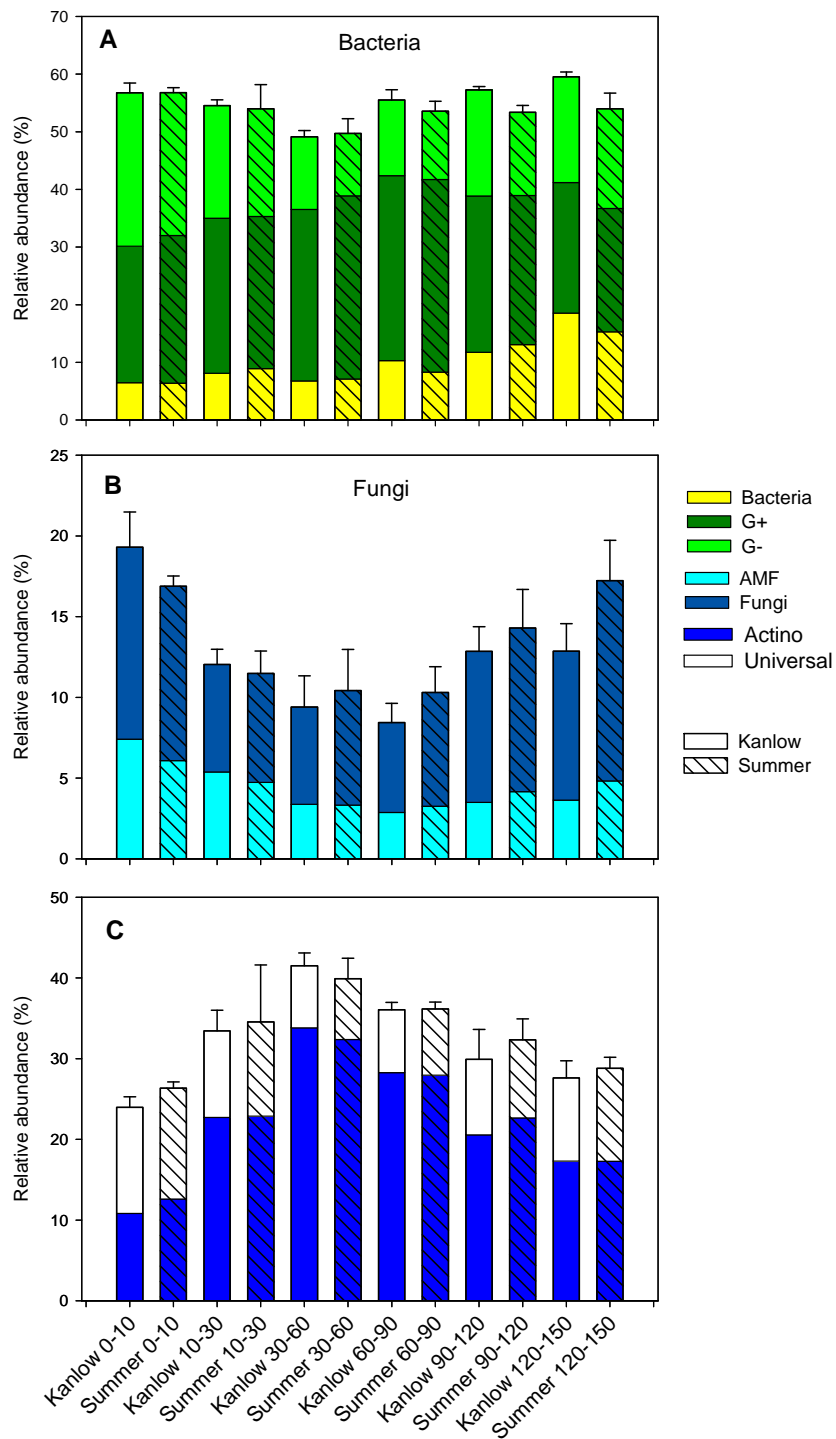
5

		Kanlow	Summer	
		ng excess $^{13}\text{C g}^{-1}$ 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

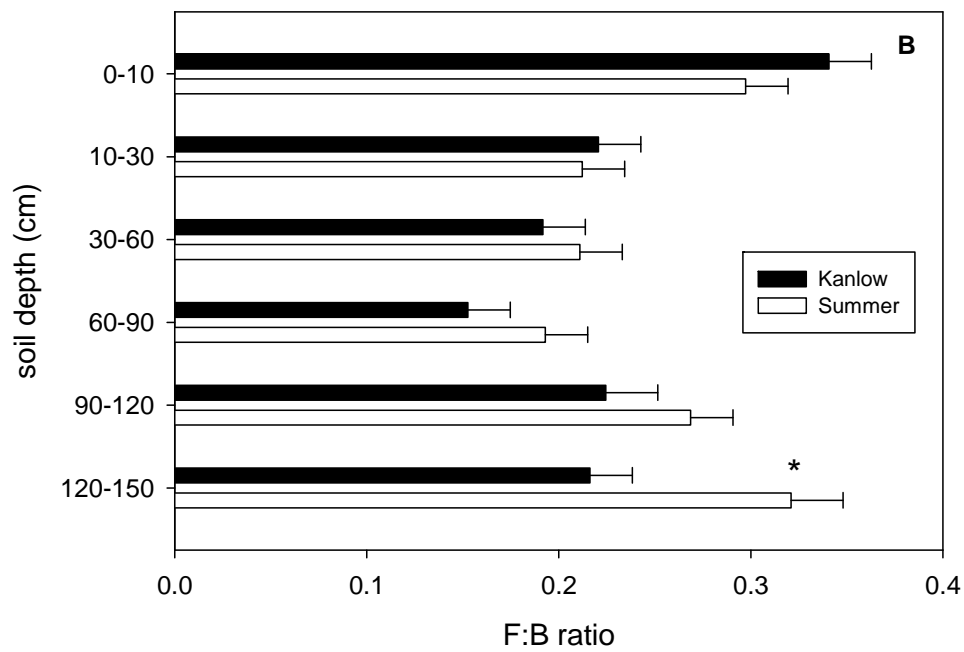
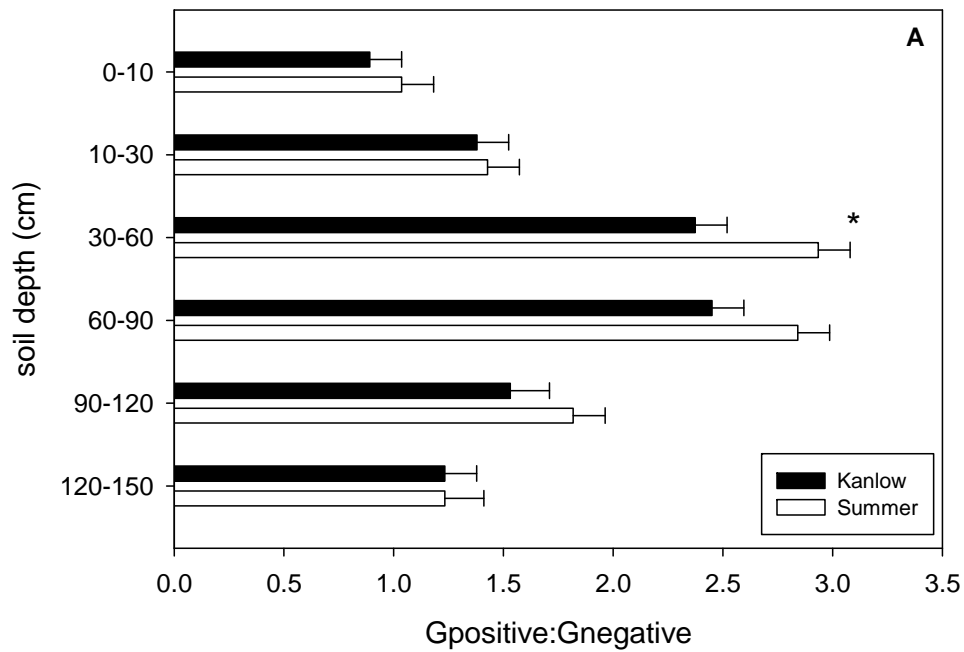
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 3 Figure 1. PLFA-derived C ($\mu\text{g PLFA-C g}^{-1}$ soil) for switchgrass cultivars Kanlow and Summer
 4 by depth. Error bars represent standard deviations ($n=3$). * indicates a significance difference
 5 between cultivars within depth.



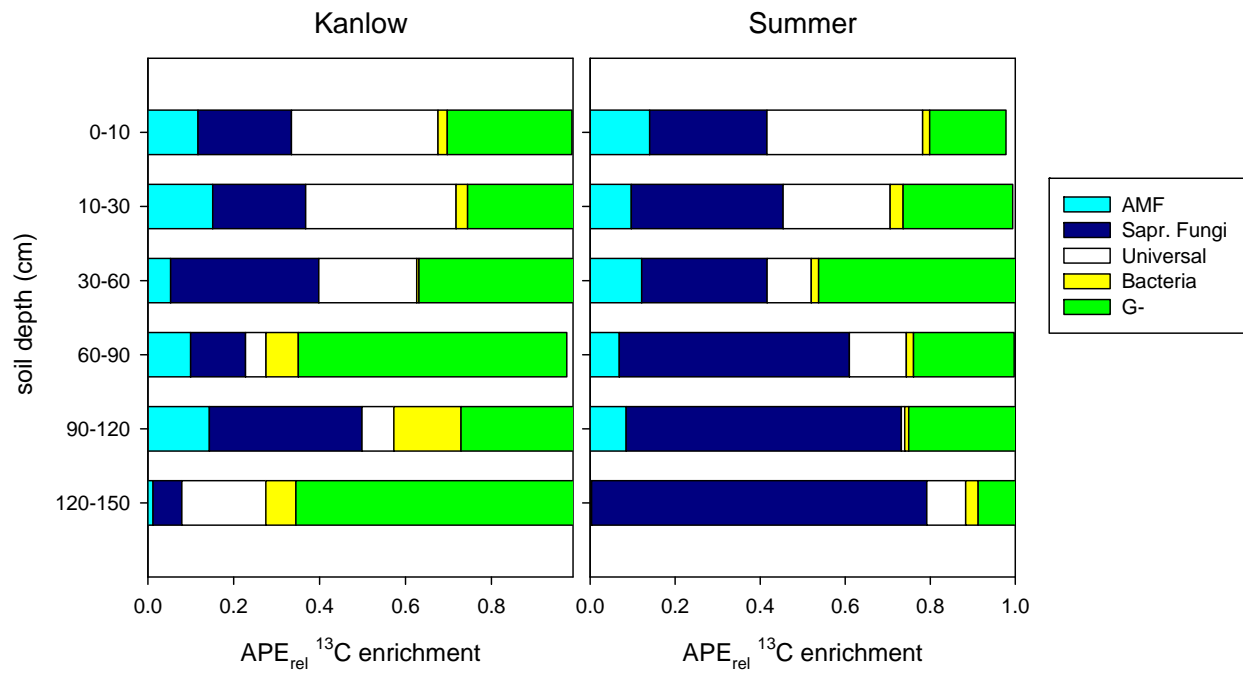
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 2 Figure 2. Soil microbial community composition (relative abundance, mol%) for switchgrass
 3 cultivars Kanlow and Summer from 0-150 cm for A) bacterial groups, B) fungal groups and C)
 4 actinomycetes and universal microbial groups. Error bars represent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



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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.

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