



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. Differences
6 in root biomass and rooting characteristics of switchgrass ecotypes could lead to distinct
7 differences in belowground microbial biomass and microbial community composition. We
8 quantified root biomass and root architecture and the associated microbial abundance,
9 composition and rhizodeposit C uptake for two switchgrass cultivars using stable isotope probing
10 of microbial phospholipid fatty acids (PLFA) after ¹³CO₂ pulse-chase labeling. Kanlow, a
11 lowland cultivar with thicker roots, had greater plant biomass above- and belowground, greater
12 root mass density, and lower specific root length compared to Summer, an upland cultivar with
13 finer root architecture. The relative abundance of bacterial biomarkers dominated microbial
14 PLFA profiles for both Kanlow and Summer soils (55.4% and 53.5%, respectively), with
15 differences attributable to a greater relative abundance of gram-negative bacteria in Kanlow soils
16 (18.1%) compared to Summer soils (16.3%). The two ecotypes also had distinctly different
17 microbial communities process rhizodeposit C; greater relative atom % ¹³C excess in gram-
18 negative bacteria (44.1 ± 2.3%) under the thicker roots of Kanlow and greater relative atom %
19 ¹³C excess saprotrophic fungi under the thinner roots of Summer (48.5 ± 2.2%). For bioenergy
20 production systems, variation between switchgrass ecotypes could alter microbial communities
21 and impact C sequestration and storage as well as potentially other belowground processes.

22

23 1 Introduction

24 Switchgrass cultivars have been developed from ecotypes adapted to northern vs southern
25 latitudes and reflect trade-offs between plant productivity and stress resistance. Upland ecotypes
26 are lower yielding with greater resistance to drought and freezing and lowland ecotypes are
27 higher yielding with poorer freeze tolerance traits (Fike et al., 2006; Garten et al., 2010; Monti,
28 2012). Since switchgrass belowground biomass is proportional to or greater than aboveground
29 biomass in many switchgrass cultivars (Frank et al., 2004; Garten et al., 2010), greater
30 aboveground productivity in upland compared to lowland ecotypes may result in more root



1 biomass and thus more carbon (C) available as an energy substrate for belowground microbial
2 communities. Because most of the aboveground biomass is removed at harvest, the production
3 and dynamics of belowground biomass are important for potential soil C storage (De Deyn et al.,
4 2008; Garten et al., 2010). Very few switchgrass studies, however, examine if and how cultivar
5 influences soil microbial community abundance and composition by affecting rhizodeposit C,
6 particularly in deeper soil depths.

7 Surface soils are studied most intensely because the densities of soil microorganisms are
8 highest within organic matter and nutrient-rich surface soils (Fierer et al., 2003). Only limited
9 information is available for soil microbial communities deeper than 25 cm despite evidence that
10 more than half of the entire microbial community resides in subsurface soils (Van Gestel et al.,
11 1992; Dodds et al., 1996; Fritze et al., 2000; Blume et al., 2002). Because microorganisms are
12 involved in soil formation, ecosystem biogeochemistry, and groundwater quality (Fierer et al.,
13 2003), microbial dynamics in deeper soils are likely to exert considerable control on ecosystem
14 services, including C and nutrient cycles (De Deyn et al., 2008; Liang et al., 2012).

15 Soil C sequestration potential is determined by multiple factors such as topography,
16 mineralogy, and texture. Although microbial biomass represents a very small fraction of the total
17 soil C pool (Wardle, 1992), microbial metabolites stabilize soil organic carbon (SOC) and
18 provide plant nutrients, effectively driving plant C inputs into soils (De Deyn et al., 2008).
19 Intraspecific variability in switchgrass rooting architecture, structure, and root tissue could
20 produce differences in ecosystem C dynamics by affecting belowground C cycling and C
21 stabilization (de Graff et al., 2013) through both direct and indirect mechanisms on root
22 exudation and microbial community structure. While there is much uncertainty about the direct
23 impact of fine roots on soil C cycling, fine roots are one of the most important sources of soil C
24 input (Rasse et al., 2005; Joslin et al., 2006). Greater root exudation has been found in fast
25 growing plant species with branched, fine root systems (Personeni and Loiseau, 2004; De Deyn
26 et al., 2008). However, species with thicker roots may have a thicker cortical layer to support
27 more arbuscular mycorrhizal (AM) fungi (Brundrett, 2002; Comas et al., 2012; Comas et al.,
28 2014). Previous switchgrass studies report that root architecture varies by cultivar or plant
29 genotype (Jackson, 1995; Fischer et al., 2006) and that upland switchgrass ecotypes have longer
30 specific root length (SRL) and finer root systems compared to coarser rooted lowland ecotypes



1 (de Graaff et al., 2013). What is less clear is if differences in root traits alter overall microbial
2 biomass and soil microbial community composition in the field.

3 Microbial phospholipid fatty acid (PLFA) analysis is a biochemical profiling technique to
4 evaluate soil microbial abundance and functional group composition (Vestal and White, 1989).
5 In addition, stable isotope probing of PLFAs following $^{13}\text{CO}_2$ pulse-labeling of plants can
6 determine which microbial groups are metabolizing recently produced rhizosphere-substrate
7 (Denef et al., 2007, Jin and Evans, 2010) as root exudates cycle through microbial biomass
8 quickly (de Graaff et al., 2014).

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

18

19 **2 Materials and Methods**

20 **2.1 Experimental site and treatments**

21 The study site is located on the University of Nebraska-Lincoln's Agricultural Research
22 and Development Center (ARDC), Ithaca, Nebraska, USA (41.151°N, 96.401°W). Soils are
23 classified as Yutan silty clay loam (fine-silty, mixed, superactive, mesic Mollic Hapludalf) and
24 Tomek silt loam (fine, smectitic, mesic Pachic Argiudoll). The study is a randomized complete
25 experimental design with three field replicates of two switchgrass cultivars Summer and Kanlow.
26 Each plot consisted of twelve switchgrass plants of the same cultivar arranged in a 4 x 3 plant
27 grid. Switchgrass plants represent genetic individuals that were hand planted in summer 2009. At
28 the time of sampling for the current study, switchgrass was well-established and 3 years old.
29 Prior to the 2012 growing season, the plots were burned to remove aboveground biomass.



1 **2.2 ¹³C labeling**

2 All 12 switchgrass plants in each plot were labeled in May 2012 using a customized
3 portable ¹³CO₂ pulse-chase labeling system consisting of a 1.0 m³ clear polymethyl methacrylate
4 (PMMA) chamber with an open bottom for placement over the entire plot and interior fans to
5 provide air circulation (Saathoff et al., 2014). This chamber was attached to a Portable
6 Photosynthesis System Model LI-6200 (Li-cor, Lincoln, NE) to monitor CO₂ concentration, air
7 temperature and relative humidity within the chamber headspace. Isotopically enriched CO₂ label
8 (99 atom% ¹³C (Sigma-Aldrich Co. St. Louis, MO)) was introduced into the chamber by opening
9 the gas regulator for approximately 15 seconds. Label was added to raise chamber CO₂
10 concentrations between 1000 to 2000 ppm above atmospheric CO₂ concentration (420 ppm).
11 Once the label was introduced, plants were allowed to take up labeled CO₂ until headspace
12 concentrations were at least 100 ppm below ambient CO₂ levels.

13 **2.3 Plant and soil sampling**

14 Plants and soils for single, randomly selected individual switchgrass plants from each
15 plot were harvested two days following ¹³C pulse-chase labeling. The aboveground biomass was
16 removed by clipping at the soil surface. Plant samples were separated into tillers, stems, leaves,
17 and oven dried at 55°C and ground for further analysis. Soil samples were then collected through
18 the crown of the plant using a 10.16 cm diameter core attached to a hydraulic soil probe. Soil
19 cores were divided in increments of 0-10, 10-30, 30-60, 60-90, 90-120, and 120-150 cm. Each
20 depth increment was split in half length-wise, packed on ice, transported to the USDA-ARS
21 laboratory in Ft. Collins Colorado, and refrigerated at 4°C until further processing. Soils were
22 weighed, and a subsample was oven-dried at 110°C for 24 hours for determination of soil
23 moisture content and soil bulk density. The half core for root separations was immediately frozen
24 (-22°C). Samples for PLFA extraction and analysis were handpicked to remove all identifiable
25 plant material, frozen at -22 °C and freeze-dried (Labconco FreeZone 77530, Kansas City, MO).

26 **2.4 Root separations**

27 The frozen half soil core was thawed to room temperature and the remaining plant crown
28 was separated from roots and root samples were hand-washed. Specifically, roots were gently
29 washed from the entire half core over a 1 mm (#20) soil sieve set over a second screen or sieve to
30 capture all roots. Roots were picked off of the sieves and separated by hand into fine (1- 2



1 branches), 3rd order coarse roots, and coarse roots (4-5 order). Fresh root subsamples were
2 scanned with a desktop scanner to quantify morphological and architectural features (Comas and
3 Eissenstat, 2009). DT-SCAN software (Regent Instruments, Inc., Quebec, Canada) generated
4 length, average diameter, and volume of roots in each image, which were used to calculate root
5 length density (root length per soil volume, m cm⁻³), specific root length (root length per root
6 mass, m g⁻¹), and root mass density (root mass per soil volume mg cm⁻³). After scanning, root
7 samples were freeze-dried and then weighed. Root length and mass were scaled to the whole
8 core on a soil mass base using the weight of the ½ cores and the volume of the whole core.
9 Weight averages for the whole profile were scaled by depth increment using soil volume.

10 **2.5 Plant and soil analyses**

11 For the other half of the soil core, the crowns were separated from the roots, the soil was
12 sieved to 2 mm and all large roots and non-soil materials removed prior to soil characterization
13 and microbial analysis. Soil pH was determined with a Beckman PHI 45 pH meter using a 1:1
14 soil:water ratio. Total organic C, total N, and δ¹³C in both plant and soil samples were
15 determined in duplicate by a continuous flow Europa Scientific 20-20 Stable Isotope Analyzer
16 interfaced with Europa Scientific ANCA-NT system Solid/Liquid Preparation Module (Europa
17 Scientific, Crewe Cheshire, UK-Sercon Ltd.) Soil subsamples for PLFA analysis were
18 handpicked to remove all identifiable plant material, frozen at -22°C, then freeze-dried
19 (Labconco FreeZone 77530, Kansas City, MO) and stored at room temperature until lipid
20 extraction.

21 **2.6 PLFA extraction and quantification**

22 The extraction and derivatization of PLFAs was adapted from Bossio and Scow (1995)
23 and modified by Deneff et al. (2007). Briefly, 6 g of soil from the surface depth increments (0-30
24 cm) and 8 g of soil from each subsoil depth increment (30-120 cm) were extracted using
25 phosphate buffer:chloroform:methanol in a 1:1:2 ratio. Total lipids were collected in the
26 chloroform phase, and fractionated on silica gel solid-phase extraction (SPE) columns
27 (Chromabond, Macherey-Nagel Inc., Bethlehem, PA) using chloroform, acetone, and methanol
28 as eluents. Polar lipid fractions representing PLFAs were collected from the methanol extractant
29 by mild alkaline transesterification using methanolic KOH to form fatty acid methyl esters
30 (FAMES).



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

10 The $\delta^{13}\text{C}$ signature of individual FAMEs was measured by capillary gas chromatography-
11 combustion-isotope ratio mass spectrometry (GC-c-IRMS) (Trace GC Ultra, GC Isolink and
12 Delta V IRMS, Thermo Scientific). A capillary GC column type DB-5 was used for FAME
13 separation (30 m length x 0.25 mm ID x 0.25 μm film thickness; Agilent). The temperature
14 program started at 60 $^{\circ}\text{C}$ with a 0.10 min hold, followed by a heating rate of 10 $^{\circ}\text{C min}^{-1}$ to 150
15 $^{\circ}\text{C}$ with a 2 min hold, 3 $^{\circ}\text{C min}^{-1}$ to 220 $^{\circ}\text{C}$, 2 $^{\circ}\text{C min}^{-1}$ to 255 $^{\circ}\text{C}$, and 10 $^{\circ}\text{C min}^{-1}$ to 280 $^{\circ}\text{C}$ with
16 a final hold of 1 min. The FAME $\delta^{13}\text{C}$ values were calibrated using working standards (C12:0
17 and C19:0) calibrated on an elemental analyzer-IRMS (Carbo Eba NA 1500 coupled to a VG
18 Isochrom continuous flow IRMS, Isoprime Inc.). To obtain $\delta^{13}\text{C}$ values of the PLFAs, measured
19 $\delta^{13}\text{C}$ FAMEs values were corrected individually for the addition of the methyl group during
20 transesterification by simple mass balance (Denef et al., 2007; Jin and Evans, 2010).

21 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,
22 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,
23 and a-17:0 are classified as gram-positive bacteria, (Zelles, 1999). The 3-OH 12:0, 14:0, 15:0, 3-
24 OH 14:0, 17:0, and 18:0 are used as general bacterial indicators (Fröstegard and Bååth, 1996;
25 Zelles, 1999). The 16:0 fatty acid is classified as a universal PLFA (Zelles, 1999). The
26 10ME16:0, 10ME17:0 and 10ME18:0 are classified as actinomycete biomarkers. The 16:1 ω 5,
27 20:4 ω 6, 20:4 ω 3, and 20:1 are biomarkers for arbuscular mycorrhizal fungi (AMF) (Graham et
28 al, 1995), and 18:3 ω 3, c18:2 ω 9,12, and c18:1 ω 9 are biomarkers for saprotrophic fungi (Zelles,
29 1997). Although 16:1 ω 5 can also be a gram-negative biomarker (Nichols, et al., 1986), in this



1 study the neutral lipid fatty acid (NLFA) fraction had high amounts of 16:1 ω 5, indicating
2 significant contribution from fungi (data not shown).

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

$$8 \quad \text{mol\%PLFA-C} = \frac{(\text{PLFA-C})_i}{\sum_{i=1}^n (\text{PLFA-C})_i} \times 100 \quad (1)$$

9 where (PLFA-C)_i is the concentration of PLFA-C in solution (mol L⁻¹) and n is the total number
10 of identified biomarkers. Relative abundance values were then summed across all individual
11 biomarkers previously defined for each microbial functional group.

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

$$15 \quad \text{Bacteria}_{\text{total}} = \text{Gram-negative bacteria} + \text{Gram-positive bacteria} + \text{General bacteria}$$

16 and

$$17 \quad \text{Fungi}_{\text{total}} = \text{AMF} + \text{Saprophytic fungi}$$

18 Isotopic ¹³C enrichment in plant tissues and in soil microbial PLFAs were calculated as
19 atom percent enrichment (APE)

$$20 \quad \text{APE } ^{13}\text{C}_i = \text{atom\% } ^{13}\text{C}_{\text{labeled}} - \text{atom\% } ^{13}\text{C}_{\text{unlabeled}} \quad (2)$$

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

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

$$23 \quad \text{APE } ^{13}\text{C}_{\text{group}} = \sum_{i=1}^n \text{APE } ^{13}\text{C}_i \quad (3)$$

24 for n functional group-specific biomarkers.

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



1 Relative recovery_{group} = APE ¹³C_{group} / APE ¹³C_{total} x 100, (4)

2 where:

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

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

5 Due to differing ¹³C label uptake between the two cultivars (Table 2), we express ¹³C enrichment
6 on a relative APE base (APE_{rel} (Balasooriya et al. 2013)):

7 APE_{rel} = $\frac{APE^{13}C_i}{APE^{13}C_{total}} \times 100$ (6)

8 **2.7 Statistical Analyses**

9 A 2-way ANOVA with switchgrass cultivar and soil depth as main factors and plot as a
10 random effect was run for belowground plant biomass, soil %C, %N, bulk density, total PLFA-C
11 for each individual PLFA biomarker (ng PLFA C/g soil) and microbial group, and APE_{rel} for
12 microbial groups using SAS v. 9.3 (SAS Institute, Cary, North Carolina, USA). Aboveground
13 biomass and plant biomass APE was run as a 1-way ANOVA with cultivar as the main effect
14 and plot as a random effect. Where necessary, data were log transformed to meet assumptions of
15 normality and equal variance. Treatments were considered significantly different for $P \leq 0.05$
16 after Bonferroni adjustment.

17

18 **3 Results**

19 **3.1 Soil Properties**

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

25 **3.2 Switchgrass Biomass**

26 The lowland cultivar Kanlow had more aboveground biomass ($4886 \pm 1220 \text{ g m}^{-2}$)
27 compared to Summer ($1778 \pm 660 \text{ g m}^{-2}$, $P = 0.0153$, Table 2). Total belowground root biomass



1 down to 150 cm was also greater in Kanlow ($6633 \pm 2165 \text{ g m}^{-2}$) compared to Summer ($2271 \pm$
2 694 g m^{-2} , $P = 0.029$). This difference was driven by the top two depths (0-10 and 10- 30 cm),
3 which comprised 91% and 85% of root biomass for Kanlow and Summer, respectively.

4 **3.3 Root Characteristics**

5 Kanlow had significantly coarser, denser roots compared to Summer, resulting in a
6 shorter SRL throughout the soil profile, despite having similar root length densities (RLD)
7 (Table 3). Root mass density (RMD) was 2.8 to 6 times greater in Kanlow compared to Summer
8 in the first three soil depths and decreased with depth (Table 3). Weight averaged over the 0-150
9 cm profile, RMD was $5.48 \pm 1.59 \text{ mg cm}^{-3}$ for Kanlow and $1.92 \pm 0.69 \text{ mg cm}^{-3}$ for Summer ($P =$
10 0.001). However, the cultivars had similar root length densities (RLD) because the greater RMD
11 in Kanlow was comprised of roots with shorter SRL (Table 3). Kanlow's SRL averaged over the
12 soil profile was lower ($25.96 \pm 1.73 \text{ m g}^{-1}$ root) compared to Summer ($52.66 \pm 12.08 \text{ m g}^{-1}$ root,
13 $P = 0.001$). The SRL for both ecotypes increased with depth as a result of lower RMD.

14 **3.4 Soil microbial biomass and community composition**

15 Differences in soil microbial biomass between ecotypes reflected differences in plant
16 productivity. The soils under Kanlow had greater PLFA-C ($6.2 \pm 0.2 \text{ } \mu\text{g PLFA-C g}^{-1}$ soil)
17 compared to Summer ($4.7 \pm 0.2 \text{ } \mu\text{g PLFA-C g}^{-1}$ soil) averaged across all depths ($P = 0.0035$,
18 Figure 1). Total microbial biomass decreased with soil depth under both cultivars ($P < 0.0001$,
19 Figure 1) and the ecotype by depth interaction was also significant ($P = 0.0019$). Total PLFA-C
20 decreased with depth under Summer, but increased in the 90-120 cm depth under Kanlow.
21 Despite the decreasing total PLFAs with depth, over half of the total observed PLFA biomass
22 was below 10 cm (Figure 1).

23 Soil microbial community composition differed between switchgrass ecotypes and
24 through the soil profile due to differences in bacteria (Figure 2). Kanlow had relatively more
25 total bacterial PLFAs (55.4 vs. 53.5 % relative abundance, $P = 0.0367$), particularly more gram-
26 negative bacteria (18.1 % relative abundance) compared to Summer (16.3% relative abundance,
27 $P = 0.0455$) (Figure 2A). This resulted in the Kanlow soil microbial community having a
28 significantly lower gram-positive to gram-negative ratio (1.64) compared to Summer (1.88)
29 averaged over depths ($P = 0.0165$, Figure 3A).



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

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

15 **3.5 Plant ^{13}C uptake**

16 The ^{13}C enrichment was detected in plant and root biomass throughout the soil profile 48
17 hours after labeling (Table 4). Enrichment was greater throughout the plant in Summer compared
18 to Kanlow with leaves 630 ± 113 vs. 474 ± 10 ng excess $^{13}\text{C g}^{-1}$ DM ($P < 0.069$) and tillers (1469
19 ± 252 vs. 756 ± 110 ng excess $^{13}\text{C g}^{-1}$ DM, $P < 0.007$). Enrichment was also evident in labeled
20 roots throughout the soil profile and was generally greater in Summer vs. Kanlow and significant
21 in half the depths sampled (0-10, 10-30, 90-120 cm $P < 0.0198$). The root ^{13}C enrichment was
22 similar within ecotype throughout the soil profile down to the 120-150 cm sample depth (Table
23 4).

24 **3.6 ^{13}C incorporation into microbial PLFAs**

25 Microbial uptake of rhizodeposit C was observed in PLFAs throughout the profile to 150
26 cm after 48 hours. PLFA ^{13}C enrichment for AMF, saprotrophic fungi, general bacteria, gram-
27 negative bacteria, gram-positive bacteria and universal microbial biomarkers was greater in the
28 pulse-labeled samples compared to the control (non-labeled) samples (Supplementary Tables 1
29 and 2). The two deepest depths (90-120 and 120-150 cm) should be interpreted with caution due
30 to large variation in the labeled PLFAs. Although total PLFA APE (ng excess $^{13}\text{C g}^{-1}$) was 1.78



1 times greater under Summer (10.97 ng excess ^{13}C g^{-1}) compared to Kanlow (6.18 ng excess ^{13}C
2 g^{-1}), it was not significant due to variability in individual plant and microbial ^{13}C uptake (data not
3 shown). To normalize for these differences in ^{13}C uptake, we express PLFA ^{13}C enrichment as
4 relative atom % ^{13}C excess (APE_{rel}) to compare between the two cultivars.

5 Relative rhizodeposit C uptake (APE_{rel}) under Kanlow was greatest in gram-negative
6 bacteria ($44.1 \pm 2.3\%$ APE_{rel} , 16:1 ω 7, 17:0cy, 18:1 ω 7) and in saprotrophic fungi ($50.6 \pm 2.7\%$
7 APE_{rel} , c18:1 ω 9, 18:2 ω 9,12) under Summer (Figure 4) averaged over all depths. These
8 community differences became more pronounced through the soil profile, particularly in depths
9 deeper than 60 cm. Microbial communities in Kanlow soils had greater rhizodeposit uptake in
10 non-specific PLFAs ($24.0 \pm 1.7\%$, $P= 0.006$, 16:0) than Summer soils averaged over all soil
11 depths, and took up 32% of the rhizodeposited ^{13}C label in the top two soil depths ($P < 0.0001$).
12 Rhizodeposit uptake in the AMF was dominant in biomarker 16:1 ω 5, did not differ between the
13 two cultivars, and decreased from $13.1 \pm 1.3\%$ relative enrichment in surface soils to $1.4 \pm 2.4\%$
14 relative enrichment in the deepest soil layer (120-150 cm).

15

16 **4 Discussion**

17 **4.1 Ecotype root characteristics**

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



1 Root mass density was two times greater under the lowland ecotype Kanlow than the
2 upland ecotype, Summer. This is the opposite relationship found by Ma et al. (2000), who found
3 that the upland ecotype Cave-in-Rock had significantly greater RMD compared to the lowland
4 ecotypes Alamo and Kanlow in 7 year old switchgrass stands on a sandy loam in Alabama.
5 Variation between specific cultivars, soil nutrient status, soil texture, as well as climate
6 contributes to switchgrass rooting variability across sites and studies (Ma et al., 2000). Other
7 studies document cultivar-specific differences in root architecture between genotypes. Jackson
8 (1995) found root biomass cultivation and allocation were similar for lettuce (*Lactuca spp.*)
9 genotypes but their root architecture differed. Likewise, fine root morphology and architecture
10 are found to vary among species, apparently genetically determined and less plastic, while root
11 physiology appears to vary depending on current, whole plant metabolic activity (Comas et al.,
12 2004; Fischer et al., 2006).

13 **4.2 Effect of switchgrass cultivar on soil microbial community biomass and** 14 **composition**

15 These differences in rooting characteristics resulted in different microbial biomass and
16 microbial community structure. In contrast to our hypothesis that Summer would have greater
17 microbial biomass, we found greater soil microbial biomass (PLFA-C) in Kanlow reflecting
18 greater belowground root biomass in Kanlow (Table 2 & Figure 1). The communities of the two
19 ecotypes also differed, with the lowland ecotype, Kanlow associated with a slightly more
20 bacterially-dominated soil microbial community than Summer. These community differences
21 could be a function either of microbial community modification by the plant from root exudation
22 (Broeckling et al., 2008; Gschwendtner et al. 2010) or root litter turnover and decomposition
23 (DeGraaff et al., 2013, 2014). Plant cultivars have been shown to develop different microbial
24 rhizosphere communities (Broeckling et al., 2008; Gschwendtner et al. 2010) through root
25 exudation patterns (Broeckling et al., 2008). To our knowledge, this may be the first illustration
26 of switchgrass cultivar-specific impacts on soil communities in the field.

27 We observed greater fungal:bacterial ratios under the fine-rooted upland ecotype,
28 Summer, compared to the coarser rooted Kanlow over the profile, and the highest
29 fungal:bacterial ratio was found in the 120-150 cm depth. This was in contrast to our hypothesis
30 that Kanlow would have a more fungal community, particularly AMF. The finer rooting



1 architecture of Summer may promote greater root turnover and, in turn, promote a more
2 saprotrophic fungal community. It is interesting to note that there was no difference in the AMF
3 communities between the two cultivars, which may be a function of the thinner roots of Summer
4 having less cortex to support AM (Comas et al. 2014), or abundant N in this agronomic setting.
5 However, the presence of AM communities has been shown to stimulate root litter
6 decomposition, plant N uptake, and saprotrophic fungal abundance without altering AM
7 abundance (Herman et al. 2012).

8 **4.3 Effect of depth on soil microbial community abundance and composition**

9 There was an overall decrease in the total microbial biomass ($\mu\text{g PLFA-C g}^{-1}$ soil) with
10 depth (Figure 1) which corresponds to previous studies (Fierer et al., 2003; Aliasghar zad et al.,
11 2010; Kramer and Gleixner, 2008). Because soil microbes primarily use C from root exudates as
12 their energy source and C availability decreases with soil depth (Table 2), microbial biomass is
13 also expected to decline (Chaudhary et al., 2012).

14 Microbial community structure also changed with depth. Our results for 0-60 cm soils
15 agree with those of Fierer et al. (2003), who found gram-positive bacteria and actinomycetes
16 increased in proportional abundance with increasing soil depth and that gram-negative bacteria
17 and fungi were highest in surface soils. In the current study, the proportion of total PLFAs
18 attributable to fungi (saprotrophic fungi and AMF) was generally higher in surface soils than
19 deeper soils and that fungi and gram-negative biomarkers decreased with depth (0-60 cm). More
20 specifically, fungi and gram-negative PLFAs decreased in proportional abundance down through
21 60 to 90 cm in depth and subsequently increased through the 120 cm depth profile while gram-
22 positive and actinomycetes PLFAs showed the opposite trend, increasing in proportional
23 abundance through 60 to 90 cm in depth and decreasing through the remainder of the 120 cm
24 depth profile.

25 Previous studies have shown that higher available C or rates of C addition to soil tend to
26 have greater proportional abundance of fungi and gram-negative bacteria while gram-positive
27 and actinomycetes are proportionately lower under the same conditions (Griffiths et al., 1999;
28 Fierer et al., 2003). Thus in depths that are C-rich we should expect higher proportions of fungi
29 and gram-negative bacteria and in areas of C limitation we should expect higher proportions of
30 gram-positive and actinomycetes. This suggests more microbial C-limitation at the middle of the



1 depth profile, perhaps reflecting the high soil C content near the surface and active plant root
2 exudation deeper in the profile.

3 **4.4 Microbial rhizodeposit-C utilization**

4 Microbial uptake of rhizodeposit ^{13}C was observed in PLFAs throughout the soil profile
5 to 150 cm depth 48 hrs post-labeling and illustrated distinct microbial community uptake
6 patterns between switchgrass ecotypes, particularly deeper than 60 cm. The majority of labeled
7 rhizodeposit uptake under Kanlow was by gram-negative bacteria which took up $44.1 \pm 2.3\%$ of
8 the total ^{13}C label recovered from all biomarkers whereas under Summer the rhizodeposit uptake
9 was predominantly by the saprotrophic fungi ($48.5 \pm 2.2\%$ relative enrichment) (Figure 4). These
10 microbial community differences could be a function either of microbial community
11 modification by the plant from root exudation (Broeckling et al., 2008; Gschwendtner et al.
12 2010) or root litter turnover and decomposition (DeGraaff et al., 2013, 2014). Although we did
13 not measure root exudation here, other studies have documented that cultivar differences in root
14 exudation influence microbial community structure (Gschwendtner et al., 2010; Marschner et al.,
15 2001).

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

24 Although rhizodeposit uptake by AMF biomarkers did not differ between the two
25 switchgrass cultivars and only comprised 13% of total enrichment in the 0-10 cm soil depth, soil
26 fungi have the potential to strongly affect soil C sequestration. Fungal mycelia are comprised of
27 complex, nutrient-poor carbon forms like chitin and melanin, allowing fungal metabolites to
28 reside longer in soil than bacteria whose membranes mainly consist of phospholipids that are
29 quickly reincorporated by soil biota (Rilling and Mummey, 2006; Six et al., 2006; De Deyn et
30 al., 2008). By immobilizing C in their mycelium, extending root lifespan, and improving C



1 sequestration in soil aggregates mycorrhizal fungi can reduce soil C loss (Langley et al., 2006;
2 Rillig and Mummey, 2006; De Deyn et al., 2008).

3

4 **4.5 Impacts for bioenergy production & C sequestration**

5 Switchgrass is a strong candidate for soil C sequestration due to its fibrous root system
6 that can extend through a depth of 3 m (Ma et al., 2000; Liebig et al., 2005; Schmer et al., 2011).
7 Previous studies have shown that switchgrass has the capacity to increase SOC, mitigate
8 greenhouse gas emissions, and improve soil quality (Sanderson et al., 1999; Garten et al., 2000;
9 Frank et al., 2004; Liebig et al., 2005; Stewart et al., 2014). Furthermore, results from previous
10 studies indicate that switchgrass is effective at storing SOC below depths of 30 cm, not just near
11 the soil surface (Sanderson et al., 1999; Garten et al., 2000; Follett et al. 2012; Liebig et al.,
12 2005).

13 Garten et al. (2010) studied differences in above and belowground biomass in addition to
14 soil C stocks and N stocks for varying 3 year-old switchgrass plant cultivars. They found no
15 significant difference among lowland cultivars for total aboveground or belowground biomass, C
16 stocks, or N stocks in the 0-90 cm soils sampled in their study. In contrast to their observations,
17 our results indicate cultivar differences in root production and soil microbial communities in
18 only 3 year-old switchgrass plants through a soil depth profile of 150 cm for the two cultivars
19 Kanlow and Summer. It should be noted that the cultivars within the study done by Garten et al.
20 (2010) contained only lowland ecotypes whereas our study is comparing a lowland ecotype
21 (Kanlow) to an upland ecotype (Summer). Our results suggest Kanlow as higher yielding for
22 aboveground biomass, belowground root biomass and promoting total soil microbial biomass
23 (Table 2, Figure 1), but Summer may have a greater potential for soil C sequestration due to
24 greater C transfer to the soil fungal community and promotion of soil aggregation.

25

26 **5 Conclusions**

27 The two switchgrass ecotypes had distinct differences in root biomass and morphology
28 that resulted in differences in the associated soil microbial biomass, microbial community
29 composition and rhizodeposit C uptake. The lowland ecotype had significantly greater RMD but



1 similar RLD due to having shorter SRL compared to the upland ecotype, Summer. Kanlow had
2 more microbial biomass and a more bacterial dominated microbial community than Summer.
3 Although the differences between cultivar microbial communities was modest, rhizodeposit
4 uptake was quite different between ecotypes. The rhizodeposit C was processed primarily by
5 gram negative bacteria under Kanlow and saprotrophic fungi under Summer. Variation in
6 microbial community composition as well as rhizodeposit C uptake could result in different C
7 sequestration dynamics. For bioenergy production systems, variation between switchgrass
8 ecotypes could impact C sequestration and storage as well as potentially other belowground
9 processes by altering microbial communities and their role in C processing.

10

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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 (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)*

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.

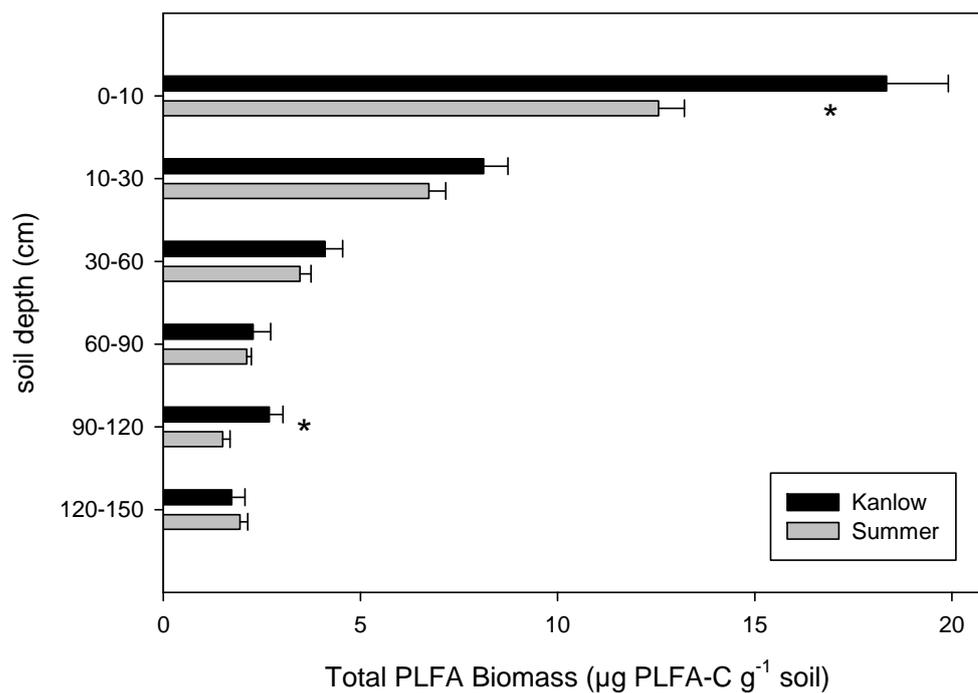


1 Table 4. The ^{13}C enrichment of aboveground plant biomass and belowground root biomass (ng
 2 $^{13}\text{C g}^{-1}$ plant biomass) plus standard deviation (in parenthesis) for both switchgrass cultivars
 3 Kanlow and Summer. P-values equal to or below 0.05 indicates significant difference between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

5

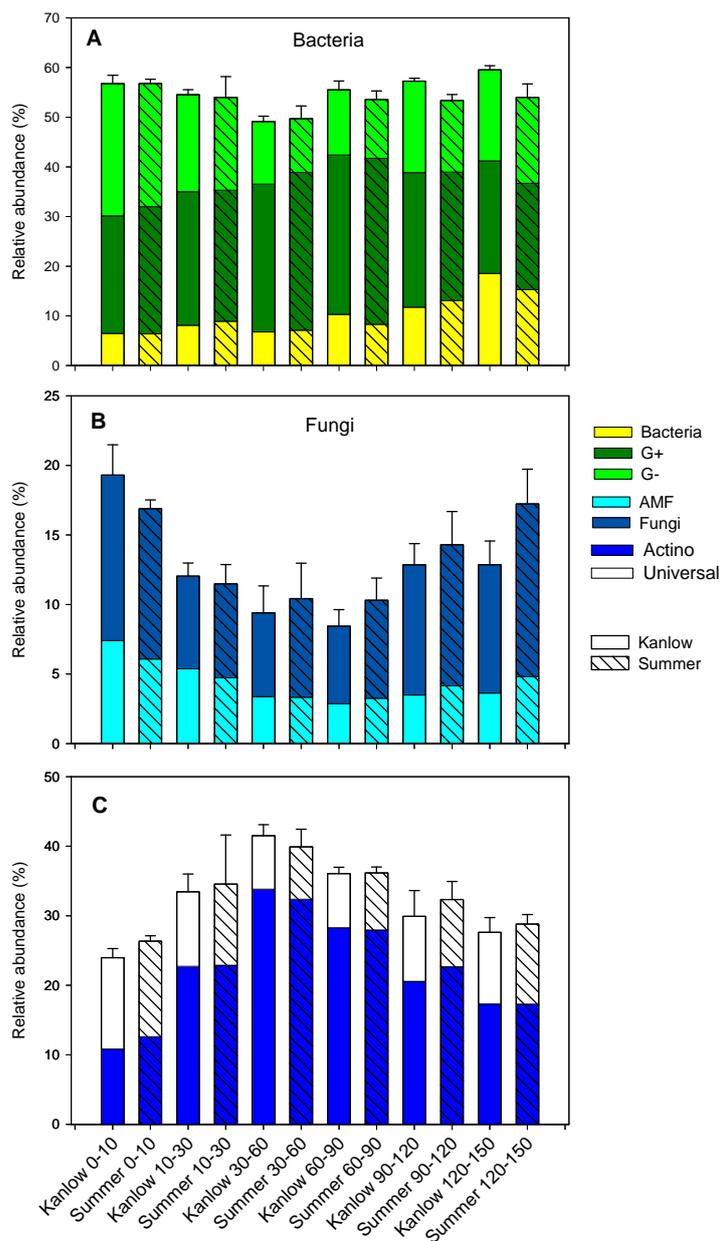
		Kanlow	Summer	
		ng excess $^{13}\text{C g}^{-1}$ DM		P-value
Leaves		474.43 (10.15)	630.47 (113.19)	0.069
Tillers		756.37 (110.11)	1469.93 (252.99)	0.007
Crown		4.69 (1.22)	70.81 (39.38)	0.003
Roots	0-10	9.96 (3.14)	119.88 (54.09)	<0.0001
	10-30	11.04 (1.65)	76.56 (21.01)	0.0002
	30-60	16.21 (4.24)	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

6

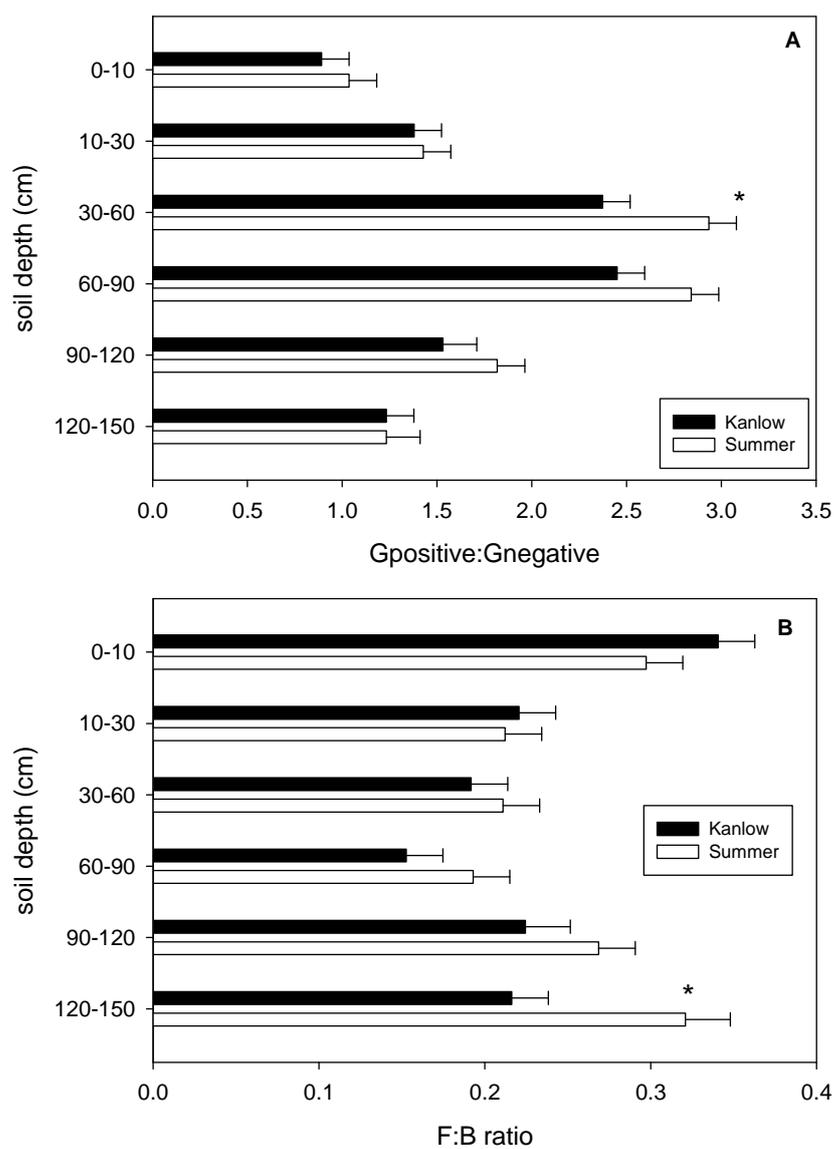


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Figure 1. PLFA-derived C ($\mu\text{g PLFA-C g}^{-1}$ soil) for switchgrass cultivars Kanlow and Summer by depth. Error bars represent standard deviations ($n=3$). * indicates a significance difference between cultivars within depth.



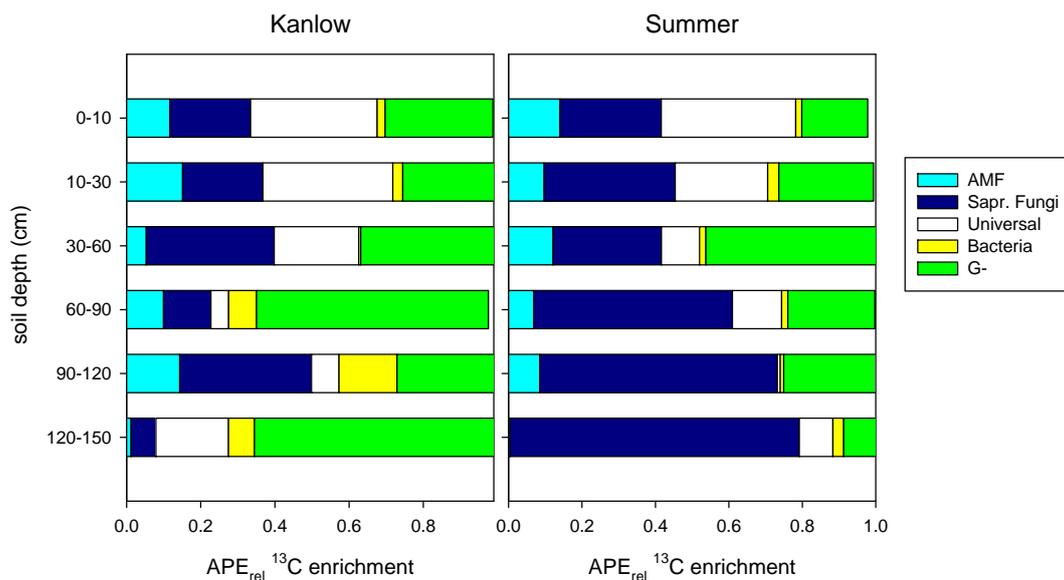
1
 2 Figure 2. Soil microbial community composition (relative abundance, mol%) for switchgrass
 3 cultivars Kanlow and Summer from 0-150 cm for A) bacterial groups, B) fungal groups and C)
 4 actinomycetes and universal microbial groups. Error bars represent standard deviations (n=3).



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



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