Technical Editor comments: "Thank you for the response to the referees. The response looks good but you have not addressed this comment adequately and it is important: "In Table 1 the carbon data should be presented on a volumetric basis. You should also determine the total carbon storage over the soil profile by accounting for bulk density. Express this on an area basis."

If you read recent soil carbon papers, data are corrected for bulk density and expressed as a per area basis. You have the data to do this easily. You can also integrate over the entire profile depth to determine total carbon.

Response: We have converted %C and %N to g $m^{-1}cm^{-1}$ and provided the profile sums in Table 1 as requested. We have modified the stats (page 9 lines 20-26) and results (page 10 lines 4-7) accordingly.

Author responses to reviewer comments on manuscript are included below in bold italic font.

Reviewer(s)' Comments to Author(s):

Reviewer: RC1

Comments to the Author
General Comments This paper describes differences in switchgrass ecotypes with respect to biomass production, rooting structure, and soil microbial biomass and community structure plus its uptake of labeled exudates. The study is scientifically sound, using proper methods and suitable replication. A prodigious amount of work is behind the data.

Specific Comments 1. P2, Lines 8, 15. Substitute "biomass" for "abundance". PLFA is measured in ng of lipid biomarker per mass of soil and is commonly converted to biomass. It is not known to be directly related to cell abundances. For individual groups, it is measured as mole percent of the total and thus is a proportion of the total biomass.

Response: Change was made in the Abstract as suggested. See track changes, Page 2, Lines 10, 18.

2. P2, Lines 16-19. Please provide P values.

Response: We have added the p-value as requested. See track changes, Page 2, Lines 17-19.

3. P2, Line 19. Insert "in" after "excess"

Response: We have inserted the change as suggested. See track changes, Page 2, Line 22.

4. P2, Lines 29, 30 and P3 line 1. I think this statement is reversed and should be "greater productivity in lowland....".

Response: We have reversed the statement as suggested. See track changes, Page 3, Line 4.

5. Introduction. This study appears to be examining a potential mechanism for varying soil carbon sequestration according to ecotype, i.e., root amount, structure, and exudates that may differentially affect soil microbial communities could alter the amount of soil C sequestered. What would be good to know at the onset is: What defensible data are there on the influence of switchgrass ecotypes (maybe even these same ones) on SOC at depths?

Response: There is a surprising lack of data about cultivar effects on root biomass and SOC sequestration. Garten, who examined different lowland varieties, found no cultivar effect. We have added text to the introduction to emphasize the lack of data. See track changes, Page 3, Lines 8-14.

6. P3, lines 8, 12. There are many studies preceding Fierer et al (2003) that examine distributions of soil microbes with depth. A few examples are Federle et al., 1986, Wood et al., 1993, Dodds et al., 1996, Bone and Balkwill, 1988. Surely there are many in earlier decades. Maybe a review, a text, or earlier reference would be better for these general statements.

Response: We have added in earlier references concerning the statements in question as suggested. See track changes, Page 3, Lines 16-17, 21.

7. P4. Lines 13-17. Have these properties been previously measured in these specific ecotypes? This should be clarified in Introduction.

Response: To our knowledge, this is the first use of stable-isotope probing to determine active microbial communities in-situ using these switchgrass cultivars. We have specified this per your suggestion. See track changes, Page 4, Lines 17-22.

8. P4, line 29. Please clarify the timing of the burn with respect to the plant and soil sampling which follows. After May?

Response: The plots were burned in early April. See track changes, Page 5, Line 13.

9. P8, line 1. No mention of neutral fractionation was made in the methods.

Response: See correction in track changes Page 7, Lines 13-14.

10. P10, line 6. Define SRL before use.

Response: See correction in track changes, Page 10, Line 19.

11. P10, lines 19-20. Please clarify that this was a transient increase in an otherwise downward trend. This statement is confusing after line 18.

Response: We have clarified the text as suggested. See track changes, Page 11, Lines 6-7.

12. P11, lines 1-4. The wording around these P values is objectionable to some readers who reject entirely "marginally significant". One option is to always state the P value and not provide an acceptable alpha in the methods and let the reader decide for themselves. A very sticky area; although, I personally have sufficient confidence in these effects.

Response: We have removed the word marginally, altered the method section, and kept the p-values. We understand that some reviewers may not accept the interpretation of the stats. See track changes.

13. P15, lines 9-16. Much of this text is verbatim from p13. Please re-write.

Response: This appears to be an editing error as a result of a previous re-write. We thank you for bringing it to our attention and have removed the text from Page 15. See track changes.

14. P15, line 18. Endophytes don't appear to be targeted by the sampling scheme. Re-write accordingly.

Response: The reviewer is correct that we did not sample endophytes. We have tempered the comment by adding "could have" to the text. See track changes, Page 16, Lines 7-9.

15. P15, lines 24-30. This paragraph switche between AMF and all fungi and is confusing as written. Please re-write.

Response: We have modified the text to emphasize the point that although we observed relatively modest differences between cultivars in fungal C uptake, these could be important in C cycling. See track changes, Page 16, Lines 14-21.

16. P15, line 24. AMF biomarkers can be difficult to reliably use, especially PLFA 16:1w5cis. Please see Sharma and Buyer. Appl Soil Ecol. 2015. My recommendation is to downplay conclusions regarding AMF in this study. You have already shown that fungal biomarkers preferentially took up labeled exudates under Summer which is a nice finding and can be linked to C sequestration processes.

Response: We have rewritten the paragraph to also include the saprotrophic fungi. See track changes, Page 16, Lines 14-21.

17. P16 lines 13-16. This info might be good in the Intro, especially if joined by other studies on ecotypes x SOC interaction.

Response: We have edited the text as suggested however there is a surprising lack of data about cultivar effects and SOC sequestration. Garten, who examined different lowland varieties, found no cultivar effect. Ma et al. looked at SOC, but only one cultivar at two sites. See track changes, Page 3, Lines 8-14.

18. P16, line 24. Was aggregation measured in this study? Maybe insert "and therefore may promote..."

Response: No, unfortunately aggregation wasn't measured in this study. See track changes, Page 17, Line 22.

Reviewer: RC2

This is a very interesting, novel and well-written paper. The significance of the study is fairly well articulated, with robust methods used to evaluate Switchgrass ecotype impacts on microbial communities. There are a few specific suggestions provided below that will help improved the manuscript. More could be made earlier on in the Abstract about the relevance of the research so that readers are drawn in. In places, particularly in the Introduction, the flow of text could be improved.

In Table 1 the carbon data should be presented on a volumetric basis. You should also determine the total carbon storage over the soil profile by accounting for bulk density. Express this on an area basis. If you have similar C contents over the profile after 3 years, yet vastly different root biomass, the result is highly significant to understanding how microbial decomposition versus root deposition of carbon affects carbon dynamics in soils.

Response: Unfortunately, we do not have soil C data from the beginning of the experiment, so we cannot calculate a change over the 3 years. We agree that would be very useful data to have.

Abstract Overall very well written and easy to follow.

Around line 5 the practical relevance of the altered microbial community would be useful to mention to broaden readership.

Response: We added the practical relevance as suggested. See track changes, Page 2, lines 5-8.

Line 11 - it is not known if biomass is per plant or per area. For this study I would argue that per soil area is of greater interest as plant density could vary. You probably state this later (I've not read the paper yet) but this should be clear in the Abstract.

Response: We added units to the measurements referenced as suggested. See track changes. Page 2, Lines 14-15.

Line 15 - Summer soils etc. - this is confusing to the reader as it implies a different soil rather than a soil planted with a different ecotype.

Response: We have changed the phrasing to be clearer for the reader. See track changes, Page 2, Lines 17-19.

Introduction
This described the background to the research very well. Overall the flow is very good, but please review to see if you can make it a bit clearer. One suggestion is provided below. Hypotheses are clear.

page 4 - lines 2-3: You need to link these paragraphs more clearly. A bit of a jump at present.

Response: We have made changes to the text to help the flow of the paper as suggested. See track changes, Page 4, Lines 11-12.

Materials and Methods. Very well described. The experiment could be repeated with the information provided.

page 4, line 28 - include the planting density.

Response: The plants were planted at a rate of 12 per 1m^{-2}. See track changes, Page 5, Lines 10-11.

page 8, line 1 - the fungi information is a Result and should be moved.

Response: We see the reviewer's point of view, but choose to keep the NLFA sentence where it is in the method section. It is crucial to justifying our interpretation of the AMF data without adding yet more data to the manuscript.

Results Again, well written and it describes the results well.

It would be easier to read if general categories of statistical significance were included:
P<0.001, P<0.01, P<0.05, n.s.), e.g. page 9, line 20 - P<0.001 will suffice.

Response: We believe that giving the actual p-value enables the reader to come to their own conclusion about the relative strength of the statistical relationships and have chosen not to group p-values as suggested.

Discussion page 12, line 28 - change IL to 'Illinois in the US Midwest', so international readers can follow.

Response: We have made this change as suggested. See track changes, Page 13, Line 17.

page 13, line 8 - there are plenty of studies on root traits versus soil properties so the finding for lettuce was a bid odd to include when other studies exist for grasses grown in more similar conditions. From your data it appears that you can infer below-ground biomass from above-ground, so is yield not a simple measure to determine optimal ecotypes?

Response: There are actually only a few studies that can document differences in root traits across different cultivars. We have removed the preceding sentence, which was confusing. The paragraph now focuses on cultivar effects. Yes, belowground biomass appears to be proportional to aboveground biomass. However, optimal ecotype determination may also take into account freeze-tolerance not just yield.

page 13, line 28 - change 'higher' to 'greater' to avoid confusing with depth. Check this throughout the paper as it appears in other places.

Response: We have made the suggested change here and throughout the manuscript. See track changes.

page 15, line 17 - you use 'cultivars' here and 'ecotypes' elsewhere. You are best to stick to one term, likely ecotype given the considerable phenotypic differences between the plant treatments.

Response: The terminology between ecotype and cultivar was confusing. We have modified the usage throughout the manuscript to specify ecotype in this manuscript (i.e. the difference between Summer and Kanlow), but cultivar when referring to other studies. This should clarify how our study differs from previous cultivar-specific studies. See track changes throughout.

page 15, line 29 and references - 'Rillig'

Response: We have made the change as requested. See track changes Page 16, Line 23 and References.

Reviewer: EC1

Please see the 2 sets of referee comments provided for your manuscript. Both are favourable but suggest minor edits that would improve presentation. Please address these comments online and prepare a revised version of your paper. Best regards, Paul Hallett Technical Editor

Response: Comments have been addressed and a revised manuscript provided.

Switchgrass ecotypes alter microbial contribution to deep soil C

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Abstract

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

1 Introduction

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

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

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

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

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

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

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

2 Materials and Methods

2.1 Experimental site and treatments

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

2.2 ^{13}C labeling

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

2.3 Plant and soil sampling

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

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

2.4 Root separations

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

2.5 Plant and soil analyses

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

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

2.6 PLFA extraction and quantification

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

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

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

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

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

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

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

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

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

$Bacteria_{total}$ = Gram-negative bacteria + Gram-positive bacteria + General bacteria

and

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

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

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

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

Label uptake by microbial functional group is then defined as:

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

for *n* functional group-specific biomarkers.

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

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

where:

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

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

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

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

2.7 Statistical Analyses

A 2-way ANOVA with switchgrass ecotypes and soil depth as main factors and plot as a random effect was run for belowground plant biomass, soil ~~%~~C, ~~%~~N, bulk density, total PLFA-C for each individual PLFA biomarker (ng PLFA C/g soil) and microbial group, and APE_{rel} for microbial groups using SAS v. 9.3 (SAS Institute, Cary, North Carolina, USA). Aboveground biomass and plant biomass APE was run as a 1-way ANOVA with ecotype as the main effect and plot as a random effect. Where necessary, data were log transformed to meet assumptions of normality and equal variance. P-values are noted in the text after Bonferroni adjustment.

3 Results

3.1 Soil Properties

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

3.2 Switchgrass Biomass

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

3.3 Root Characteristics

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

3.4 Soil microbial biomass and community composition

Differences in soil microbial biomass between ecotypes reflected differences in plant productivity. The soils under Kanlow had greater PLFA-C (6.2 ± 0.2 µg PLFA-C g^{-1} soil)

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

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

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

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

3.5 Plant ^{13}C uptake

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

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

3.6 ^{13}C incorporation into microbial PLFAs

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

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

4 Discussion

4.1 Ecotype root characteristics

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

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

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

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

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

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

4.3 Effect of depth on soil microbial community abundance and composition

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

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

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

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

4.4 Microbial rhizodeposit-C utilization

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

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

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

4.5 Impacts for bioenergy production & C sequestration

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

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

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

5 Conclusions

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

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

Cultivar	Soil Depth (cm)	SOC ($g\ C\ m^{-2}cm^{-1}$)	Total N ($g\ N\ m^{-2}cm^{-1}$)	Texture†	pH
Kanlow	0-10	199.0 (32.3)	17.7 (2.9)	silty clay loam	6.24 (0.21)
	10-30	153.7 (5.4)	13 (0.5)	silty clay loam	6.32 (0.24)
	30-60	112.4 (33.7)	9.7 (3.1)	silty clay loam	6.48 (0.15)
	60-90	56.5 (11)	5.2 (1.3)	silty clay loam	6.60 (0.12)
	90-120	33.5 (3.5)	3.9 (0.5)	silty clay loam/silt loam	6.66 (0.15)
	120-150	20.5 (4.2)	2.5 (0.4)	silt loam	6.90 (0.12)
	0-150	575.5 (48.6)	52.0 (4.5)		
Summer	0-10	188.2 (15.2)	17.0 (1.1)	silty clay loam	5.92 (0.60)
	10-30	188.7 (43.7)	16.2 (4)	silty clay loam	6.19 (0.57)
	30-60	110.7 (20.9)	9.2 (1.8)	silty clay loam	6.64 (0.29)
	60-90	57.1 (9.2)	5 (0.9)	silty clay loam	6.61 (0.19)
	90-120	33.2 (3.2)	3.7 (1.1)	silty clay loam/silt loam	6.70 (0.19)
	120-150	24.4 (1.8)	3.7 (0.1)	silt loam	6.83 (0.01)
	0-150	602.3 (51.7)	54.6 (4.7)		

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

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

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

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

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

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

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

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

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

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

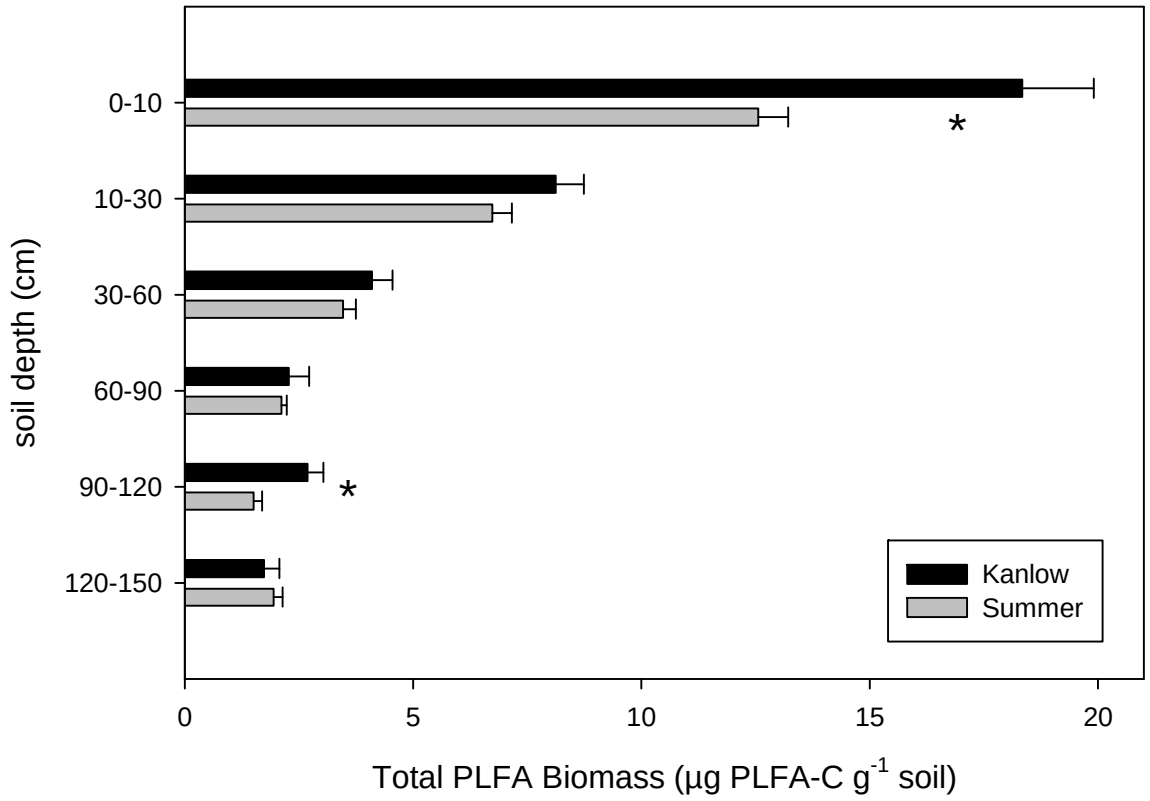

Figure 1. PLFA-derived C (µg PLFA-C g^{-1} soil) for switchgrass cultivars Kanlow and Summer by depth. Error bars represent standard deviations (n=3). * indicates a significance difference between cultivars within depth.

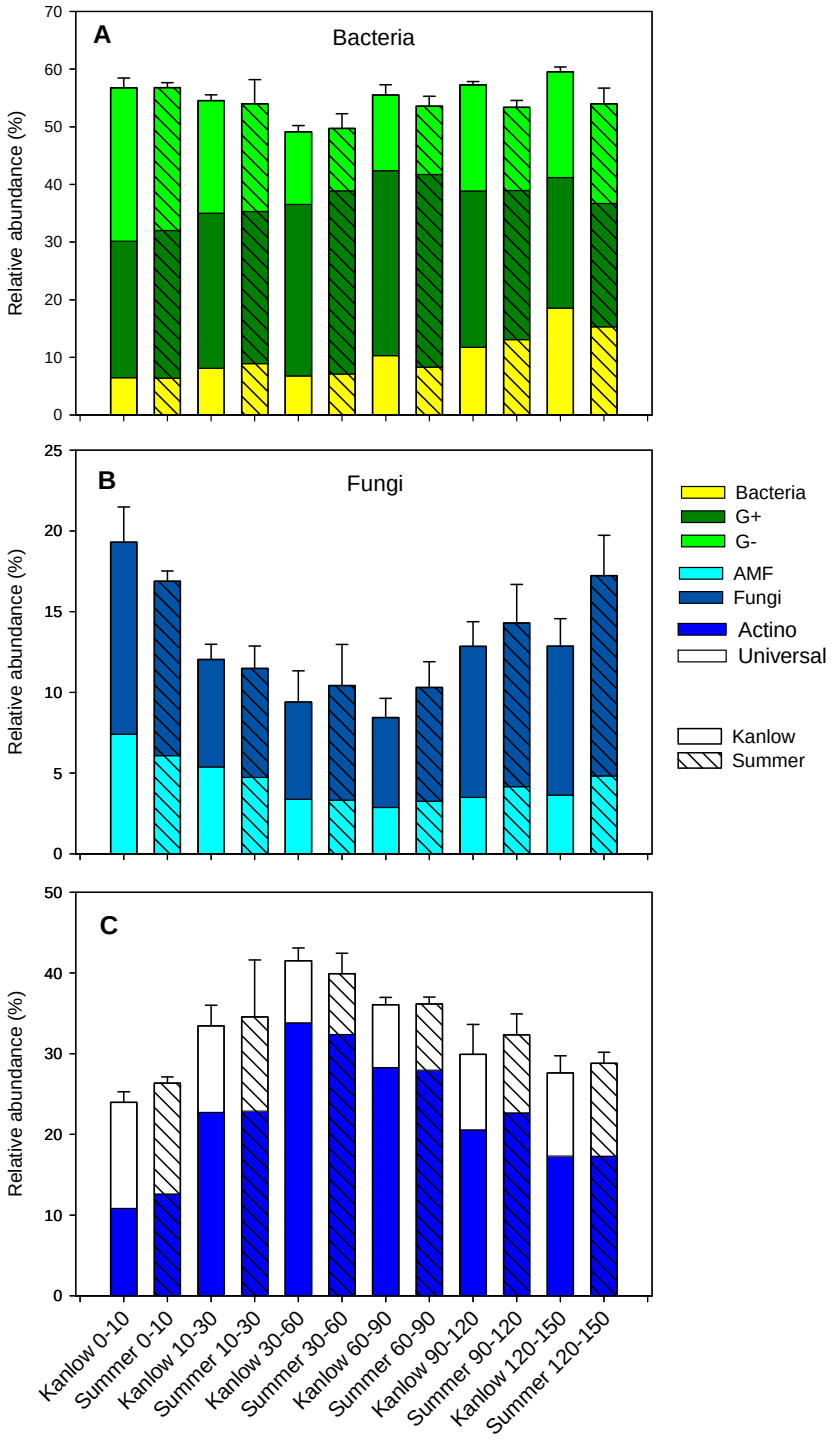

Figure 2. Soil microbial community composition (relative abundance, mol%) for switchgrass cultivars Kanlow and Summer from 0-150 cm for A) bacterial groups, B) fungal groups and C) actinomycetes and universal microbial groups. Error bars represent standard deviations (n=3).

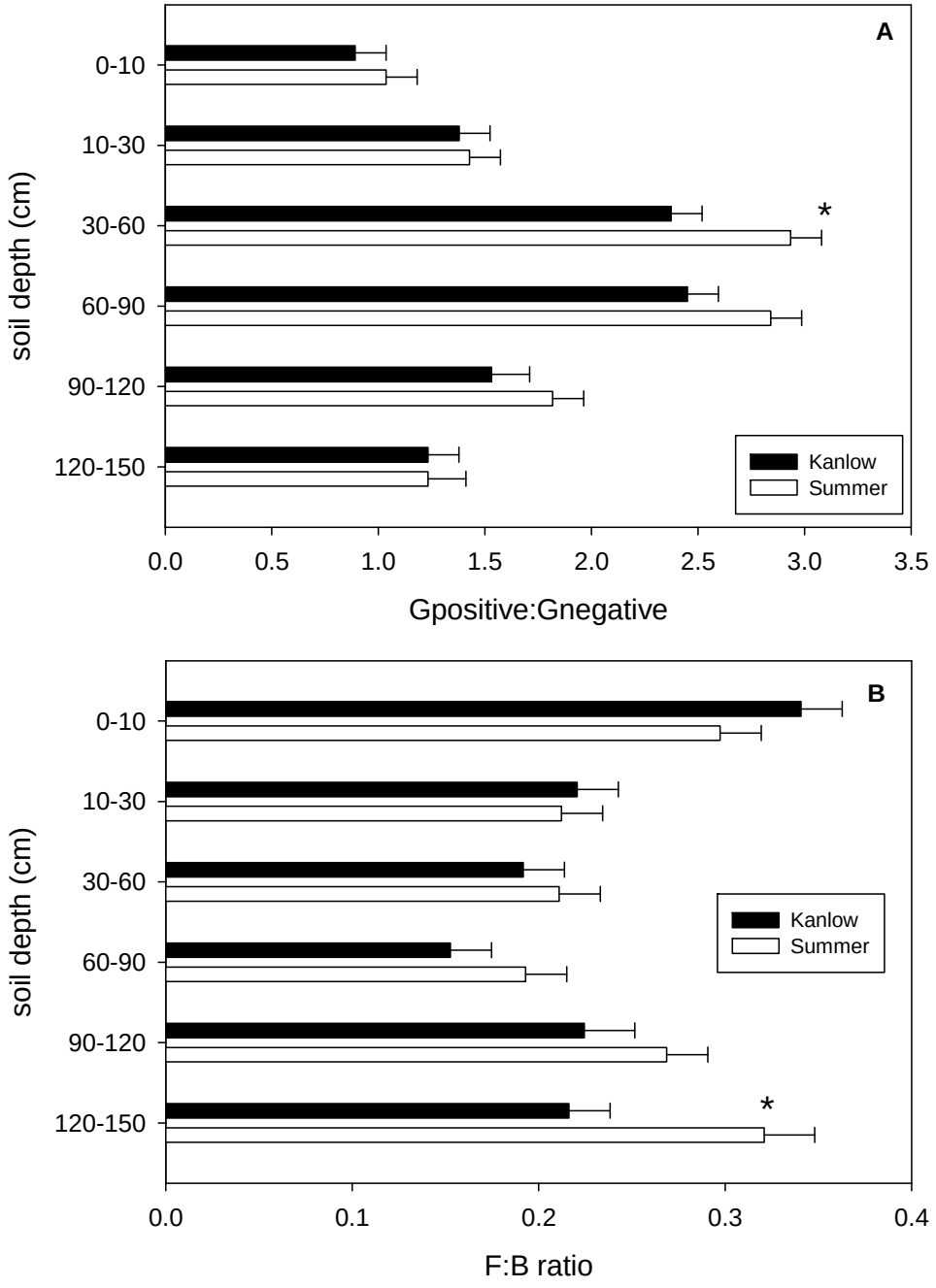

Figure 3. Gram-positive:gram-negative ratios (A) and fungal:bacterial ratios B) for switchgrass cultivars Kanlow and Summer by depth. * indicates a significant difference between cultivars within depth.

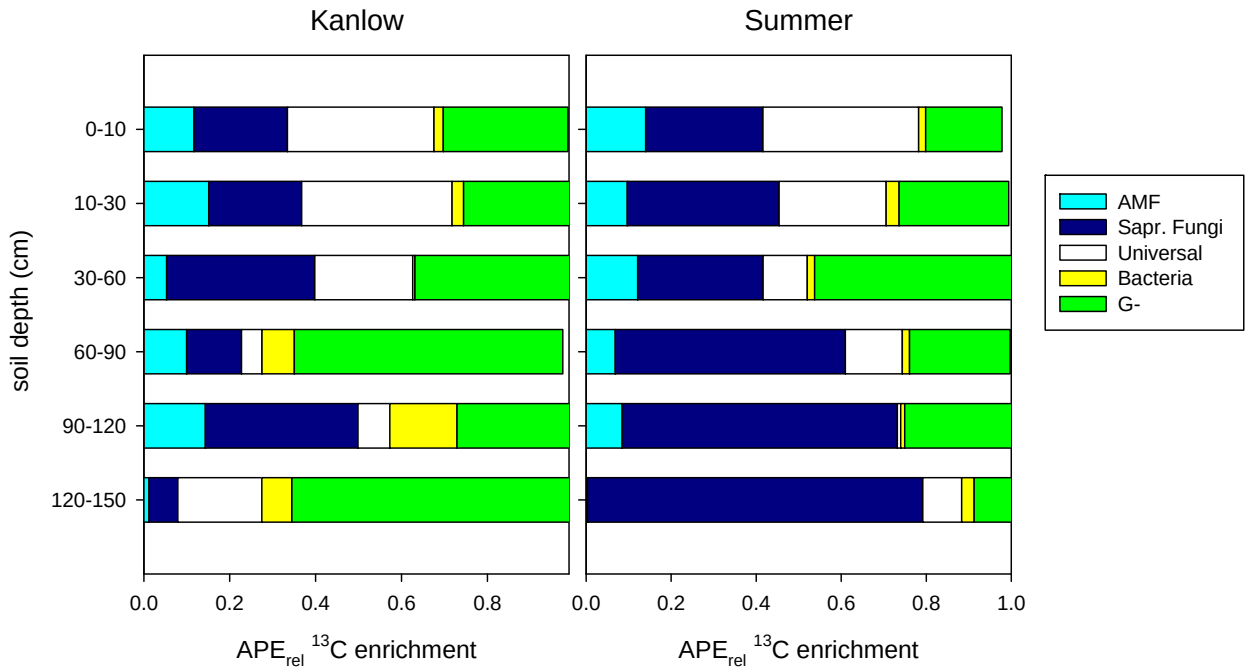

Figure 4. Relative rhizodeposit uptake (PLFA APE_{rel} enrichment), for switchgrass cultivars Kanlow and Summer at all sampled depths 48 hours after ^{13}C labeling. Functional groups actinomycetes and gram positive bacteria not included because ^{13}C enrichment was not obtained in those groups (Supplementary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

Switchgrass ecotypes alter microbial contribution to deep soil C

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Abstract

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

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1 Introduction

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

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

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

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

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

One technique for observing microbial biomass and the soil microbial community composition is m~~M~~icrobial phospholipid fatty acid (PLFA) analysis, ~~is~~ a biochemical profiling technique, designed to evaluate soil microbial abundance and functional group composition (Vestal and White, 1989). In addition, stable isotope probing of PLFAs following $^{13}CO_2$ pulse-labeling of plants can determine which microbial groups are metabolizing recently produced rhizosphere-substrate (Denef et al., 2007, Jin and Evans, 2010) as root exudates cycle through microbial biomass quickly (de Graaff et al., 2014). PLFAs have been used to characterize microbial biomass and composition under bioenergy crops such as switchgrass and corn (Liang et al. 2012), and PLFA-stable isotope probing in grazed perennial grasslands (Denef et al. 2007) ~~Fierer et al., 2003; Aliasgharzad et al., 2010; ., 2012~~However, to our knowledge, stable-isotope probing has not been used to characterize rhizodeposit uptake in the field under different switchgrass ecotypes.

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

2 Materials and Methods

2.1 Experimental site and treatments

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

2.2 ^{13}C labeling

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

2.3 Plant and soil sampling

Plants and soils for single, randomly selected individual switchgrass plants from each plot were harvested two days following ^{13}C pulse-chase labeling. The aboveground biomass was

removed by clipping at the soil surface. Plant samples were separated into tillers, stems, leaves, and oven dried at 55°C and ground for further analysis. Soil samples were then collected through the crown of the plant using a 10.16 cm diameter core attached to a hydraulic soil probe. Soil cores were divided in increments of 0-10, 10-30, 30-60, 60-90, 90-120, and 120-150 cm. Each depth increment was split in half length-wise, packed on ice, transported to the USDA-ARS laboratory in Ft. Collins Colorado, and refrigerated at 4°C until further processing. Soils were weighed, and a subsample was oven-dried at 110°C for 24 hours for determination of soil moisture content and soil bulk density. The half core for root separations was immediately frozen (-22°C). Samples for PLFA extraction and analysis were handpicked to remove all identifiable plant material, frozen at -22 °C and freeze-dried (Labconco FreeZone 77530, Kansas City, MO).

2.4 Root separations

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

2.5 Plant and soil analyses

For the other half of the soil core, the crowns were separated from the roots, the soil was sieved to 2 mm and all large roots and non-soil materials removed prior to soil characterization and microbial analysis. Soil pH was determined with a Beckman PHI 45 pH meter using a 1:1 soil:water ratio. Total organic C, total N, and $\delta^{13}C$ in both plant and soil samples were determined in duplicate by a continuous flow Europa Scientific 20-20 Stable Isotope Analyzer

interfaced with Europa Scientific ANCA-NT system Solid/Liquid Preparation Module (Europa Scientific, Crewe Cheshire, UK-Sercon Ltd.) Soil subsamples for PLFA analysis were handpicked to remove all identifiable plant material, frozen at -22°C, then freeze-dried (Labconco FreeZone 77530, Kansas City, MO) and stored at room temperature until lipid extraction.

2.6 PLFA extraction and quantification

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

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

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

°C with a 2 min hold, 3 °C min^{-1} to 220 °C, 2 °C min^{-1} to 255 °C, and 10 °C min^{-1} to 280 °C with a final hold of 1 min. The FAME $\delta^{13}C$ values were calibrated using working standards (C12:0 and C19:0) calibrated on an elemental analyzer-IRMS (Carbo Eba NA 1500 coupled to a VG Isochrom continuous flow IRMS, Isoprime Inc.). To obtain $\delta^{13}C$ values of the PLFAs, measured $\delta^{13}C$ FAMEs values were corrected individually for the addition of the methyl group during transesterification by simple mass balance (Denef et al., 2007; Jin and Evans, 2010).

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

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

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

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

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

$Bacteria_{total}$ = Gram-negative bacteria + Gram-positive bacteria + General bacteria

and

$Fungi_{total}$ = AMF + Saprophytic fungi

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

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

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

Label uptake by microbial functional group is then defined as:

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

for *n* functional group-specific biomarkers.

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

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

where:

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

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

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

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

2.7 Statistical Analyses

A 2-way ANOVA with switchgrass ~~cultivar~~ ecotypes and soil depth as main factors and plot as a random effect was run for belowground plant biomass, soil %C, %N, bulk density, total PLFA-C for each individual PLFA biomarker (ng PLFA C/g soil) and microbial group, and APE_{rel} for microbial groups using SAS v. 9.3 (SAS Institute, Cary, North Carolina, USA). Aboveground biomass and plant biomass APE was run as a 1-way ANOVA with ~~cultivar~~ ecotype as the main effect and plot as a random effect. Where necessary, data were log

transformed to meet assumptions of normality and equal variance. ~~Treatments were considered significantly different for~~P-values are noted in the text ~~P ≤ 0.05~~ after Bonferroni adjustment.

3 Results

3.1 Soil Properties

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

3.2 Switchgrass Biomass

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

3.3 Root Characteristics

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

3.4 Soil microbial biomass and community composition

Differences in soil microbial biomass between ecotypes reflected differences in plant productivity. The soils under Kanlow had greater PLFA-C (6.2 ± 0.2 µg PLFA-C g^{-1} soil) compared to Summer (4.7 ± 0.2 µg PLFA-C g^{-1} soil) averaged across all depths (P = 0.0035, Figure 1). Total microbial biomass decreased with soil depth under both ~~cultivars~~ ecotypes (P < 0.0001, Figure 1) and the ecotype by depth interaction was also significant (P = 0.0019). Total PLFA-C decreased with depth under Summer, ~~but increased~~with a transient increase in the 90-120 cm depth under Kanlow and continued decrease in the 120-150cm depth. Despite the decreasing total PLFAs with depth, over half of the total observed PLFA biomass was below 10 cm (Figure 1).

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

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

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

surface and deeper depths with a minimum at 30-60 cm or 60-90 cm depths ($P < 0.0001$, Figure 2A and 2B).

3.5 Plant ^{13}C uptake

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

3.6 ^{13}C incorporation into microbial PLFAs

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

Relative rhizodeposit C uptake (APE_{rel}) under Kanlow was greatest in gram-negative bacteria (44.1 ± 2.3% APE_{rel}, 16:1ω7, 17:0cy, 18:1ω7) and in saprotrophic fungi (48.5 ± 2.2% APE_{rel}, c18:1ω9, 18:2ω9,12) under Summer (Figure 4) averaged over all depths. These community differences became more pronounced through the soil profile, particularly in depths deeper than 60 cm. Microbial communities in Kanlow soils had greater rhizodeposit uptake in non-specific PLFAs (24.0 ± 1.7%, P= 0.006, 16:0) than Summer soils averaged over all soil depths, and took up 32% of the rhizodeposited ^{13}C label in the top two soil depths ($P < 0.0001$).

Rhizodeposit uptake in the AMF was dominant in biomarker 16:1ω5, did not differ between the two ~~cultivars~~ecotypes, and decreased from 13.1 ± 1.3% relative enrichment in surface soils to 1.4 ± 2.4% relative enrichment in the deepest soil layer (120-150 cm).

4 Discussion

4.1 Ecotype root characteristics

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

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

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

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

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

4.3 Effect of depth on soil microbial community abundance and composition

There was an overall decrease in the total microbial biomass (μg PLFA-C g^{-1} soil) with depth (Figure 1) which corresponds to previous studies (Fierer et al., 2003; Kramer and Gleixner, 2008; Aliasgharzad et al., 2010). Because soil microbes primarily use C from root exudates as

their energy source and C availability decreases with soil depth (Table 2), microbial biomass is also expected to decline (Chaudhary et al., 2012).

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

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

4.4 Microbial rhizodeposit-C utilization

Microbial uptake of rhizodeposit ^{13}C was observed in PLFAs throughout the soil profile to 150 cm depth 48 hrs post-labeling and illustrated distinct microbial community uptake patterns between switchgrass ecotypes, particularly deeper than 60 cm. The majority of labeled rhizodeposit uptake under Kanlow was by gram-negative bacteria which took up 44.1 ± 2.3% of the total ^{13}C label recovered from all biomarkers whereas under Summer the rhizodeposit uptake was predominantly by the saprotrophic fungi (48.5 ± 2.2% relative enrichment) (Figure 4). ~~These microbial community differences could be a function either of microbial community modification by the plant from root exudation (Broeckling et al., 2008; Gschwendtner et al.~~

~~2010) or root litter turnover and decomposition (DeGraaff et al., 2013, 2014).~~ Although we did not measure root exudation here, other studies have documented that cultivar differences in root exudation influence microbial community structure (Gschwendtner et al., 2010; Marschner et al., 2001).

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

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

4.5 Impacts for bioenergy production & C sequestration

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

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

5 Conclusions

The two switchgrass ecotypes had distinct differences in root biomass and morphology that resulted in differences in the associated soil microbial biomass, microbial community composition and rhizodeposit C uptake. The lowland ecotype had significantly greater RMD but similar RLD due to having shorter SRL compared to the upland ecotype, Summer. Kanlow had more microbial biomass and a more bacterial dominated microbial community than Summer. Although the differences between ~~cultivar~~ ecotype microbial communities was modest,

rhizodeposit uptake was quite different between ecotypes. The rhizodeposit C was processed primarily by gram negative bacteria under Kanlow and saprotrophic fungi under Summer. Variation in microbial community composition as well as rhizodeposit C uptake could result in different C sequestration dynamics. For bioenergy production systems, variation between switchgrass ecotypes could impact C sequestration and storage as well as potentially other belowground processes by altering microbial communities and their role in C processing.

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

Cultivar	Soil Depth (cm)	SOC (%)	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)

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

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

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

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

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

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

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

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

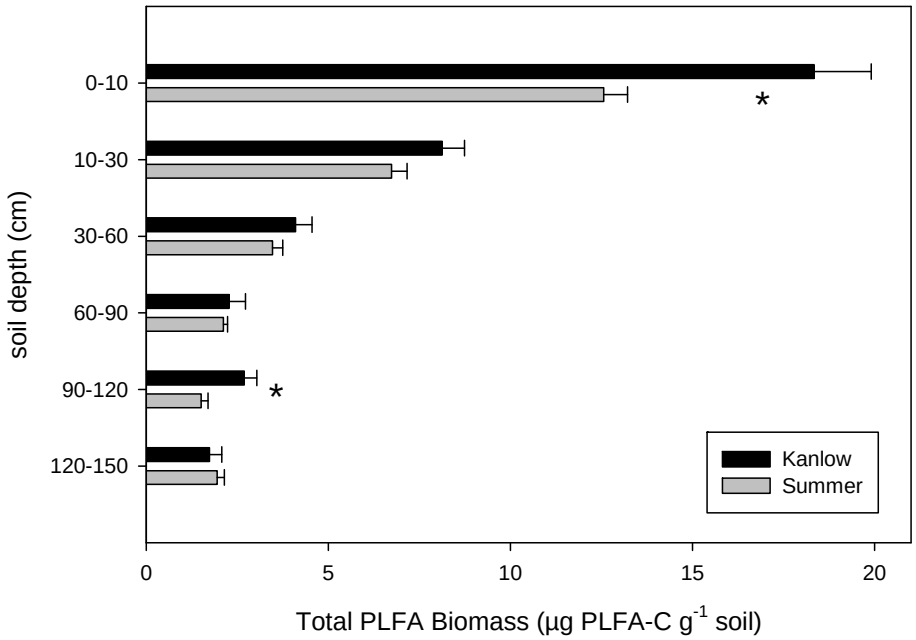

Figure 1. PLFA-derived C (µg PLFA-C g^{-1} soil) for switchgrass cultivars Kanlow and Summer by depth. Error bars represent standard deviations (n=3). * indicates a significance difference between cultivars within depth.

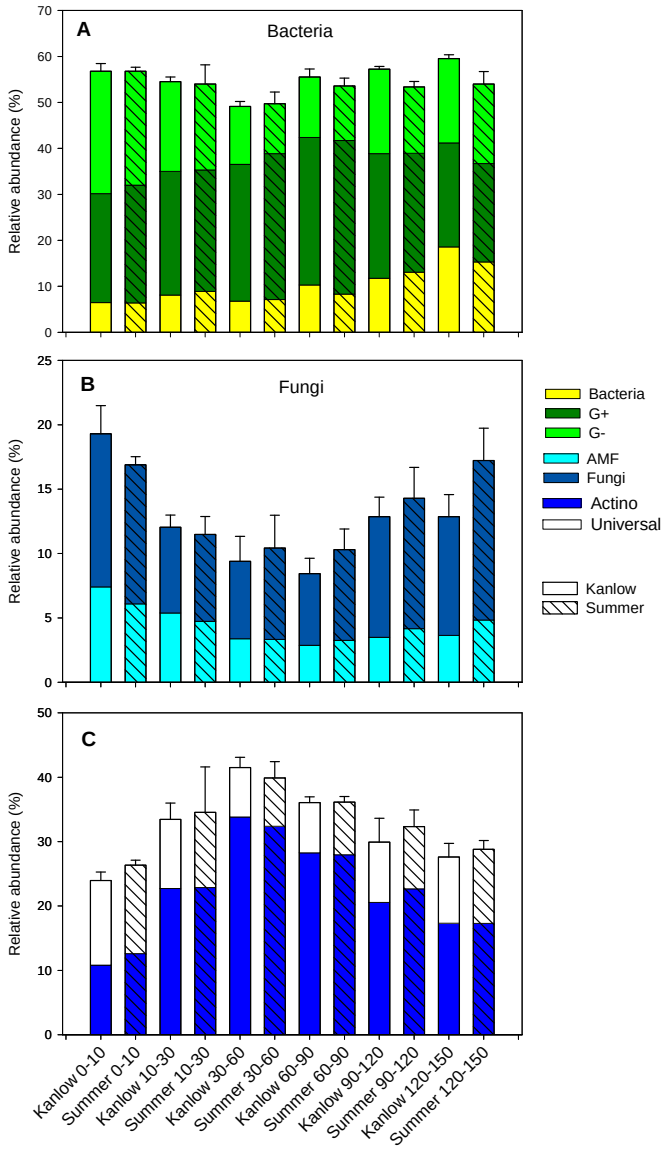

Figure 2. Soil microbial community composition (relative abundance, mol%) for switchgrass cultivars Kanlow and Summer from 0-150 cm for A) bacterial groups, B) fungal groups and C) actinomycetes and universal microbial groups. Error bars represent standard deviations (n=3).

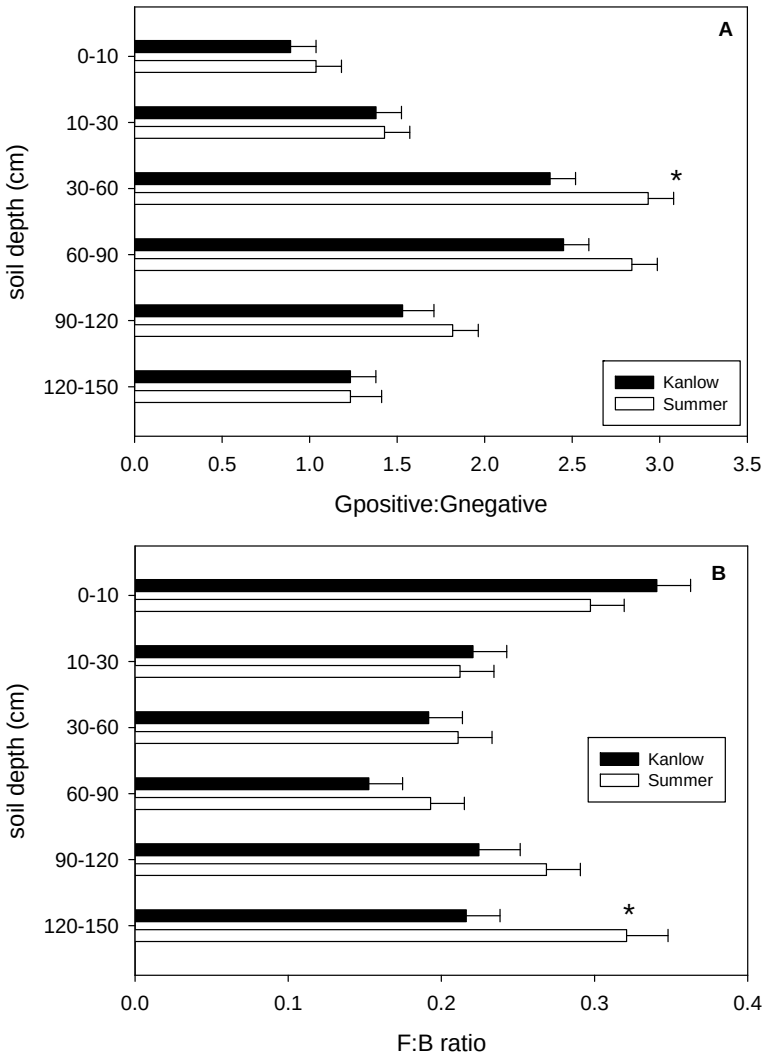

Figure 3. Gram-positive:gram-negative ratios (A) and fungal:bacterial ratios B) for switchgrass cultivars Kanlow and Summer by depth. * indicates a significant difference between cultivars within depth.

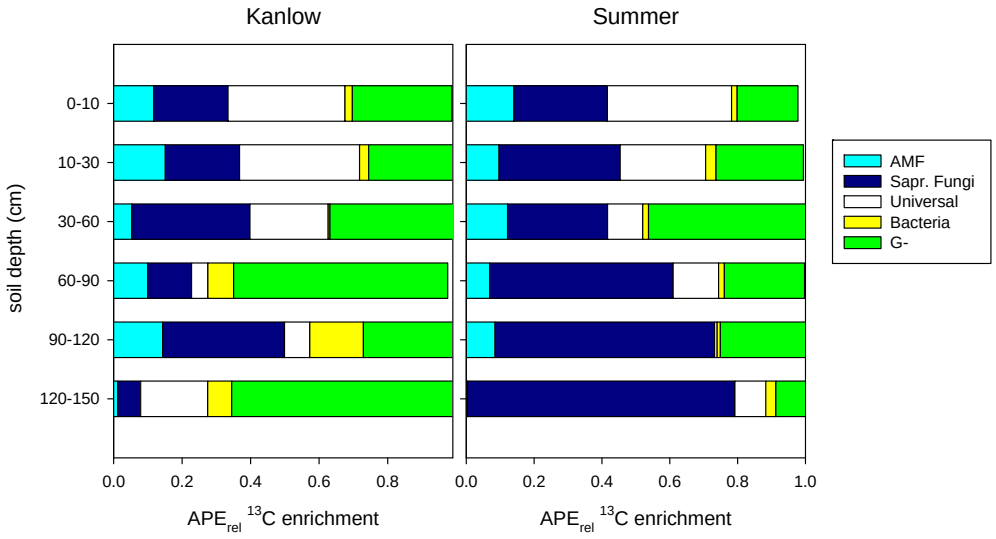

Figure 4. Relative rhizodeposit uptake (PLFA APE$_{rel}$ enrichment), for switchgrass cultivars Kanlow and Summer at all sampled depths 48 hours after ^{13}C labeling. Functional groups actinomycetes and gram positive bacteria not included because ^{13}C enrichment was not obtained in those groups (Supplementary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