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ABSTRACT

The importance of rhizodeposit C and associated microbial communities in deep soil C stabilization is relatively unknown. Phenotypic variability in plant root biomass could impact C cycling through belowground plant allocation, rooting architecture, and microbial community abundance and composition. We used a pulse-chase ^{13}C labeling experiment with compound-specific stable-isotope probing to investigate the importance of rhizodeposit C to deep soil microbial biomass under two switchgrass ecotypes (*Panicum virgatum* L., Kanlow and Summer) with contrasting root morphology. We quantified root phenology, soil microbial biomass (phospholipid fatty acids, PLFA), and microbial rhizodeposit uptake (^{13}C-PLFAs) to 150 cm over one year during a severe drought. The lowland ecotype, Kanlow, had two times more root biomass with a coarser root system compared to the upland ecotype, Summer. Over the drought, Kanlow lost 78% of its root biomass, while Summer lost only 60%. Rhizosphere microbial communities associated with both ecotypes were similar. However, rhizodeposit uptake under Kanlow had a higher relative abundance of gram-negative bacteria (44.1%), and Summer rhizodeposit uptake was primarily in saprotrophic fungi (48.5%). Both microbial community composition and rhizodeposit uptake shifted over the drought into gram-positive communities. Rhizosphere soil C was greater one year later under Kanlow due to turnover of unlabeled structural root C. Despite a much greater root biomass under Kanlow, rhizosphere δ^{13}C was not significantly different between the two ecotypes, suggesting greater microbial C input under the finer rooted species, Summer, whose microbial associations were predominately saprotrophic fungi. Ecotype specific microbial communities can direct rhizodeposit C flow and C accrual deep in the soil profile and illustrate the importance of the microbial community in plant strategies to survive environmental stress such as drought.

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1. Introduction

Deep soils, defined here as soils below 30 cm soil depth, contain a low SOC concentration but can comprise more than 50% of the total soil C stock (Jobbágy and Jackson, 2000). Despite the abundance of SOC below 30 cm, the mechanisms responsible for subsurface C stock changes, as well as their magnitude, are poorly

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understood (Rumpel and Kögel-Knabner, 2011). Deep-rooted perennial grass species have a high potential to store C at depth where dead root biomass would be protected from decomposition through physico-chemical interactions or microbial inaccessibility. Root biomass turnover plus rhizodeposit-C (i.e., C derived from root exudation and sloughing of root cells), can contribute 2.4 times more to SOC compared to aboveground litter (Rasse et al., 2005, 2006). The relative importance of root morphology, architecture, and interactions with soil microbial community, however, is unclear. In a review comparing *in-situ* root growth experiments to soil incubations with added litter, Rasse et al. (2005) found that root biochemistry accounted for only ¼ of the increase in root-derived C mean residence time compared to shoot-derived C, with other mechanisms such as physico-chemical protection, physical protection through mycorrhiza and root-hair activities, and chemical interactions with metal ions, accounting for the rest. Since rhizodeposit-C is rapidly incorporated into microbial biomass, soil microbial community composition could influence the fate of plant-derived C and its stabilization in the soil (Six et al., 2006).

Plant-derived low molecular weight carbonaceous compounds, like rhizodeposits, may contribute proportionally more to SOC compared to more highly lignified compounds through higher microbial carbon use efficiency (Cotrufo et al., 2013; Parton et al., 2014). Although C sequestration is directly related to the quantity of C inputs to soils, recent work has shown that as more lignified plant residues decompose, they lose proportionally more C as CO_2 and thus, are converted to SOC at a lower rate (Stewart et al., 2015). More 'labile' products (i.e., dissolved organic C, root exudates) promote microbial biomass and are more efficiently converted to SOC due to lower respiration losses and physico-chemical protection through mineral association (Cotrufo et al., 2015). Microbially-processed C comprises the majority of C deep in the soil profile, and plant root morphology and interactions with the microbial community may impact C inputs in deep soils.

Phenotypic variability in plant root biomass could impact C cycling through belowground plant allocation, rooting architecture, and microbial community abundance and composition. Belowground biomass allocation impacts soil C sequestration by determining the overall belowground biomass contribution to SOC. Root architecture (root length versus mass) could impact the relative contribution of rhizodeposit versus root biomass to SOC (Adkins et al., 2016; Roosendaal et al., 2016). Fine roots, defined as the terminal two branches of the root system, contribute more surface area for root exudation (Guo et al., 2004), turn over more quickly than the rest of the root system (Xia et al., 2010), and form intimate associations with mycorrhizal fungi (Smith and Read, 2008).

Switchgrass ecotypes have a wide range in root biomass and architecture, making it an ideal species to test impacts of plant root traits on soil microbial communities and SOC (de Graaff et al., 2013; Roosendaal et al., 2016). Roosendaal et al. (2016) used a pulse-chase ^{13}C labeling experiment with compound-specific stable-isotope probing to investigate the importance of root-derived C to deep soil microbial biomass under two 3-yr old switchgrass ecotypes, Kanlow and Summer. We found that switchgrass ecotypes (*Panicum virgatum* L.) with contrasting root architectures supported different microbial communities that processed rhizodeposit C. Rhizodeposit C uptake in microbial biomass under the fine-rooted upland ecotype ('Summer') was associated with saprotrophic fungi, and the coarser-rooted lowland ecotype ('Kanlow') was associated with gram-negative bacteria. We report here results from sampling later in the season (anthesis and post-frost) when plant-microbial interactions would be expected to change. We measured plant biomass, microbial biomass (phospholipid fatty acids, PLFA) and rhizodeposit transformation (^{13}C-PLFAs), and soil C to a depth of 150 cm during the driest year on record. Due to the finer, smaller root architecture we observed during the initial sampling, we hypothesize that the upland ecotype, Summer, will be more resilient to drought and the associated microbial community to be smaller compared to the lowland ecotype Kanlow. Summer should also have proportionally greater contributions to soil C due to associations with fungal communities compared with Kanlow.

2. Materials and methods

2.1. Experimental site and treatments

The study site was located on the University of Nebraska-Lincoln's Agricultural Research and Development Center (ARDC), Ithaca, Nebraska, USA (41.151°N, 96.401°W) and the experiment is described in detail in Roosendaal et al. (2016). Soils are classified as silt-loam to silty clay loam (Yutan, fine-silty, mixed, superactive, mesic Mollic Hapludalf and Tomek, fine, smectitic, mesic Pachic Argiudoll). Soil C ranged from 199.0 to 20.5 g m^{-2} and N ranged from 17.7 to 2.5 g m^{-2} from the surface to 150 cm. Soil pH increased with depth from 5.9 to 6.9.

Switchgrass (*Panicum virgatum*, L) is a native, perennial C4 grass adapted to a broad geographic range in North America that has resulted in genetic differences in aboveground and below ground productivity, root architecture, and stress resistance (Monti, 2012). Upland ecotypes have a greater stress resistance with lower yields compared to lowland ecotypes which have a low freeze-tolerance, but greater yields. The experimental design was a randomized complete block with three field replicates of two switchgrass cultivars Summer (upland ecotype) and Kanlow (lowland ecotype). Each plot consisted of twelve switchgrass plants of the same ecotype arranged in a 4 × 3 plant grid for a planting density of 12 plants m^{-2}. Switchgrass was well-established and 3 years old when sampled in 2012. Prior to the 2012 growing season, residual biomass from the previous year was burned in the spring before switchgrass growth, as is typically done every year.

2.2. ^{13}C labeling

Switchgrass plants were labeled in May 2012 using a 1.0 m^3 customized portable $^{13}CO_2$ pulse-chase labeling system (Saathoff et al., 2014). Isotopically enriched CO_2 label (99 atom% ^{13}C (Sigma-Aldrich Co. St. Louis, MO)) was introduced into the chamber to raise chamber CO_2 concentrations between 1000 and 2000 ppm above atmospheric CO_2 concentration (420 ppm). Plants took up labeled CO_2 until chamber headspace concentrations decreased to 100 ppm below ambient CO_2 (LI-6200, LI-COR Biosciences, Lincoln, NE).

2.3. Plant and soil sampling

Switchgrass plants and soil from each plot were harvested two days following ^{13}C pulse-chase labeling (31 May 2012), at anthesis (11 July 2012 for Summer and 17 September 2012 for Kanlow), and post-killing frost the following year (2 April 2013). The aboveground biomass was removed by clipping at the soil surface. Plant samples were separated into stems, leaves, tillers and oven dried at 55 °C and ground for further analysis. Soil cores (10.16 cm diam.) were collected through the crown of the plant and divided in increments of 0–10, 10–30, 30–60, 60–90, 90–120, and 120–150 cm (0–15 cm Ap horizon, 15–110 cm Bt horizons, 110–150 cm C horizon). The 120–150 depth was not obtained at the anthesis sampling due to low soil moisture. Each depth increment was split in half length-wise, packed on ice, transported to the USDA-ARS laboratory in Ft. Collins, Colorado. Both half cores were weighed and the one for root separations was immediately

frozen (−22 °C). The other half core was processed for soil chemical and microbiological analyses.

2.4. Root separations

For root separations, the frozen half soil core was thawed at room temperature and the plant crown was separated from roots. Roots were gently washed over a 1 mm (#20) soil sieve and separated by hand into fine (1st and 2nd order branches counting back from root tips), 3rd order coarse roots, and extra coarse roots (4th-5th order). A subsamples of fresh roots were scanned to quantify morphological and architectural features (Comas and Eissenstat, 2009) using DT-SCAN software (Regent Instruments, Inc., Quebec, Canada). Length, average diameter, and volume of roots in each image 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}). Root samples were freeze-dried, weighed, and ground for further analysis. Root length and mass for the whole core were expressed 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, two rhizosphere soil (soil adhering to the root) subsamples were carefully removed; one for PLFA extraction and the other for C, N, $\delta^{13}C$ analysis. To obtain rhizosphere soil, coarse roots were carefully removed from soil clods and only small soil aggregates adhering to the roots were collected. Rhizosphere PLFA soil subsamples 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. The second rhizosphere soil subsample was air-dried for C, N, $\delta^{13}C$ analysis.

Switchgrass crowns were separated from the roots and the remaining (bulk) soil was sieved to 2 mm, removing large roots and rocks. Bulk soil subsamples were oven-dried at 110 °C for 24 h for soil moisture content and soil bulk density. Soil pH was measured with a Beckman PHI 45 pH meter in a 1:1 ratio of soil:water. Total organic C, total N, and $\delta^{13}C$ in both plant and rhizosphere 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.)

2.6. PLFA extraction and quantification

Phospholipid fatty acids (PLFAs) were extracted using the method of Bossio and Scow (1995) modified by Denef et al. (2007) and Roosendaal et al. (2016). Soils (6 g for 0–30 cm, 8 g for 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) into neutral lipid fractions (NLFAs) using acetone, and the polar lipid fraction from the methanol extractant followed by mild alkaline transesterification using methanolic KOH to form fatty acid methyl esters (FAMEs).

The FAMEs were quantified 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) using two internal FAME standards (12:0 and 19:0). 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. Internal standards were used to develop 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 $\delta^{13}C$ of each FAME was quantified using 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 0.25 μm film thickness; Agilent). The temperature program started at 60 °C with a 0.10 min hold, followed by a heating rate of 10 °C min^{-1} to 150 °C with a 2 min hold, 3 °C min^{-1} to 220 °C, 2 °C min^{-1} to 255 °C, and 10 °C min^{-1} to 280 °C with a final hold of 1 min. The FAME $\delta^{13}C$ values were calibrated using working standards (C12:0 and C19:0) calibrated on an elemental analyzer-IRMS (Carbo Erba NA 1500 coupled to a VG Isochrom continuous flow IRMS, Isoprime Inc.). 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, 1997). The 3-OH 12:0, 14:0, 15:0, 3-OH 14:0, 17:0, and 18:0 are used as general bacterial indicators (Frostegard et al., 2011; Zelles, 1997). The 16:0 fatty acid is classified as a universal marker (Zelles, 1997). The 10ME16:0, 10ME17:0 and 10ME18:0 are classified as actinomycete biomarkers. The 16:1ω5, 20:4ω6, 20:4ω3, and 20:1 were used for arbuscular mycorrhizal fungi (AMF) (Graham et al., 1995), and 18:3ω3,6,9, 18:2ω9,12c, and c18:1ω9 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 also contained high amounts of 16:1ω5, indicating significant contribution from fungi (data not shown).

Individual PLFAs are expressed on a C mass base (ng PLFA-C g^{-1} dry soil) and used as a proxy for microbial biomass. Relative PLFA abundance was used to assess changes in the microbial functional group composition and expressed as molar C percentage (mol%) of each biomarker:

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

where $(\text{PLFA-C})_i$ is the concentration of an individual biomarker 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 for each microbial functional group.

The ratio of fungi to bacteria was calculated as total fungal to total bacterial biomass:

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

and

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

The isotopic ^{13}C enrichment in plant tissues and in soil microbial PLFAs were expressed as atom percent enrichment (APE):

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

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

Label uptake by microbial functional group is then defined as:

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

for n functional group-specific biomarkers.

The relative distribution (%) of ^{13}C recovered in each functional group can then be calculated as:

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

where:

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

for m total biomarkers identified.

Due to differing ^{13}C label uptake between the two ecotypes, we express ^{13}C enrichment on a relative APE base (APE_{rel} (Roosendaal et al., 2016)):

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

2.7. Statistical analyses

A repeated measures analysis with switchgrass ecotypes, sampling time, and soil depth as main factors and plot*ecotype and sampling*plot*ecotype as random effects was run for root biomass, root architecture (root length density, root mass density, specific root length), soil C, N, $\delta^{13}C$, total PLFA-C (ng PLFA C g^{-1} soil) and microbial group, and APE_{rel} for microbial groups using SAS v. 9.3 (SAS Institute, Cary, North Carolina, USA). Statistics for the aboveground biomass and plant biomass APE were run as a repeated measured analysis with ecotype and sampling time as main effects and plot*ecotype and sampling*plot*ecotype as random effects. Where necessary, data were log transformed to meet assumptions of normality and equal variance. *P*-values are noted in the text after Bonferroni adjustment and significant differences considered less $p < 0.05$.

The PLFA relative abundance (mol%) and APE_{rel} biomarker data were analyzed using distance-based redundancy analysis (dbRDA) to examine microbial compositional differences between cultivar soils. We chose to use Bray's distance for the dbRDA (Legendre and Anderson, 1999). A principal coordinate analysis (PCoA) was performed on the distance matrix, from which the eigenvalues (obtained in the PCoA) were used within a redundancy analysis. Ellipsoids represent standard errors around the multivariate-group centroids. Permutation-based analysis of variance (ANOVA) was performed on all dbRDA models to determine significance among group differences.

3. Results

3.1. Climate variables

The growing season of 2012 was the driest, warmest year on record for Nebraska (High Plains Regional Climate Center) with precipitation 40% less (440 mm) than the 30 year average of 740 mm (Fig. 1). Average MAT was 12.2 °C (30 year average 9.6 °C) and growing season (May–September) averaged 22 °C. This resulted in a Palmer drought index rating of extreme drought March–August 2012 (NOAA, https://www.ncdc.noaa.gov/sotc/drought/201213#west-sect).

3.2. Comparison of switchgrass ecotype biomass & root traits

The two ecotypes had differing production, with the lowland ecotype, Kanlow, having more than two times the aboveground and root biomass compared to Summer (8337 vs. 2946 g m^{-2} and 4115 vs. 1881 g m^{-2} respectively, $p < 0.007$) averaged over all depths and sampling times (Fig. 2). Aboveground biomass increased from the initial sampling to anthesis and post-frost ($p < 0.002$) averaged over both ecotypes, with Kanlow having roughly double the biomass of Summer ($p < 0.005$) averaged over sampling time. Across the three sampling time points, Kanlow root biomass stayed roughly three times greater than Summer ($p < 0.0001$) but decreased over the season ($p < 0.008$), with no interaction between ecotype and sampling time. Kanlow lost 78% of initial root biomass and Summer lost 60% from initial sampling to post-frost. Shoot:root ratio increased from 0.79 initially to 2.14 at anthesis and 5.94 after frost ($p = 0.0004$), but did not differ between Kanlow (3.51) and Summer (2.14).

The two ecotypes had different root morphology and production, with the lowland ecotype, Kanlow, having more, coarser roots and the upland ecotype, Summer, having fewer, thinner roots (Table 1). Kanlow had double the root mass density (RMD) (4.40 mg cm^{-3}), compared to Summer (1.99 mg cm^{-3}) averaged over all depths and sampling points, while Summer had double the specific root length (SRL) (46.6 vs. 22.4 m g^{-1} root, respectively). There was no difference between ecotypes in root length density (RLD). Root distribution and morphology changed over depth, with RMD decreasing with soil depth and SRL increasing ($p > 0.0001$).

RMD decreased in the 0–10 and 10–30 cm depths over the season (ecotype*depth interaction, $p = 0.001$), but not in the lower soil profile. The decrease in RMD was greater under Kanlow than Summer, which showed no change over the season in RMD (sampling*ecotype *depth interaction, $p = 0.003$).

3.3. Switchgrass ^{13}C enrichment

Both Summer and Kanlow were significantly ^{13}C enriched in both aboveground and root biomass after ^{13}C pulse-labeling (Table 2), with Summer enrichment in leaves and tillers 1.3–1.9 times greater than of Kanlow after 48 h ($p < 0.035$) and remained throughout the study. Summer roots were 11.0 and 7.9 times more enriched in the 0–10 and 10–30 cm depths, respectively, compared to Kanlow at the initial sampling ($p = 0.011$). Enrichment significantly decreased with depth ($p < 0.0001$) and over the season ($p = 0.036$) across both ecotypes. Enrichment decreased from initial sampling to anthesis in the leaves and tillers ($p < 0.0001$) and increased in roots in the 30–60, 60–90 and 120–150 cm depths ($p < 0.020$, Table 2).

3.4. PLFA & microbial community composition

Microbial biomass was greater under Kanlow (4.59 μg PLFA-C g^{-1} soil) compared to Summer (3.59 μg PLFA-C g^{-1} soil, $p < 0.003$) and decreased over the season ($p < 0.0001$), corresponding to decreases in root biomass and RMD (Fig. 3). Microbial biomass decreased through soil depth from 9.4 to 1.1 ng PLFA-C g^{-1} soil ($p < 0.0001$). Microbial biomass under Kanlow in the 90–120 cm depth at the initial sampling and 60–90 cm depth at anthesis was greater than Summer, but not post-frost, causing a significant sampling*depth interaction ($p = 0.034$).

Microbial community composition did not differ between ecotypes, but was substantially different through depths ($p = 0.001$) and over time ($p = 0.001$, Figs. 4 and 5). The soil microbial community was dominantly bacterial, and bacterial relative abundance increased over time from 54.4% to 61.5% due to an increase in gram-positive bacteria (27.3–43.0% from initial to post-frost) (Fig. 5). Both AMF (4.4–2.8%) and saprotrophic fungi (8.5–7.2%) decreased over time, leading to a significant decrease in fungal:bacteria ratio.

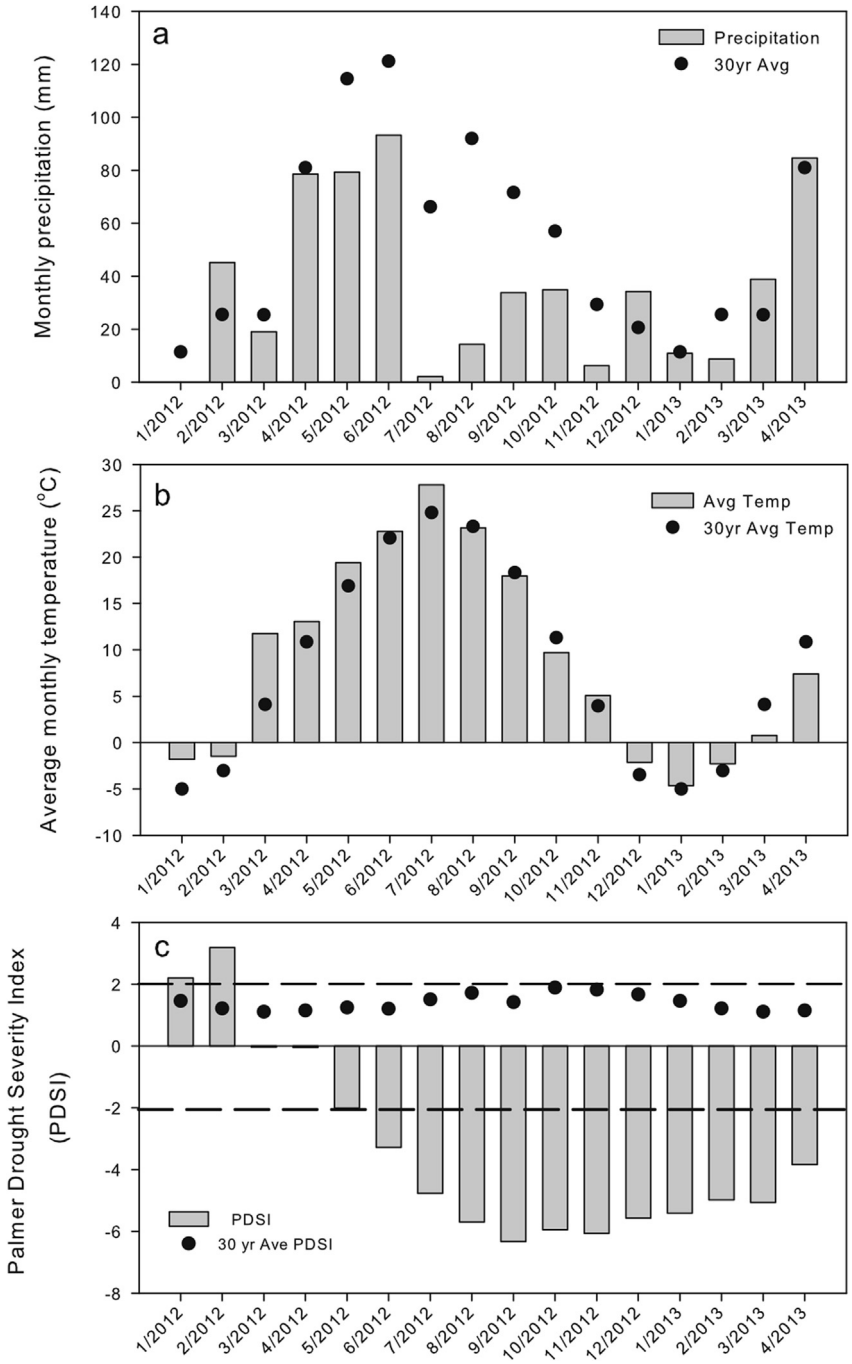

Fig. 1. Monthly precipitation (a), average temperature (b) and Palmer Drought Severity Index (PDSI) (c) for Nebraska during the study period. PDSI values of −1.0 to −2.0 = mild drought; −2.0 to −3.0 = moderate drought; −3.0 to −4.0 = severe drought; and greater than −4.0 = extreme drought.

Actinomycetes decreased slightly (22.6–17.5%).

Microbial community composition changed through depth with actinomycetes and gram-positive bacteria more abundant in the 30–90 cm depths and gram-negative and saprotrophic fungi more abundant in the surface 0–30 cm depths (Fig. 5).

3.5. PLFA ^{13}C enrichment

Microbial community uptake of rhizodeposit C differed between Kanlow and Summer ($p = 0.001$) over time ($p = 0.001$) and through the soil profile ($p = 0.001$, Fig. 6). When expressed as a relative enrichment within group, initial rhizodeposit uptake was greater in the gram-negative community under Kanlow (44.1 ± 2.3% APE_{rel}, 16:1ω7, 17:0cy, 18:1ω7) and the saprotrophic fungi under Summer (48.5 ± 2.2% APE_{rel}, c18:1ω9, 18:2ω9,12, Fig. 7). This difference decreased over time as the microbial community turned over and more C was assimilated by the gram-positive bacterial community at anthesis and by the actinomycetes at post-frost (Fig. 7). By post-frost, there were still overall microbial community differences between Kanlow and Summer in APE_{rel}.

3.6. Soil C & N

Although there was no difference in soil properties beneath the

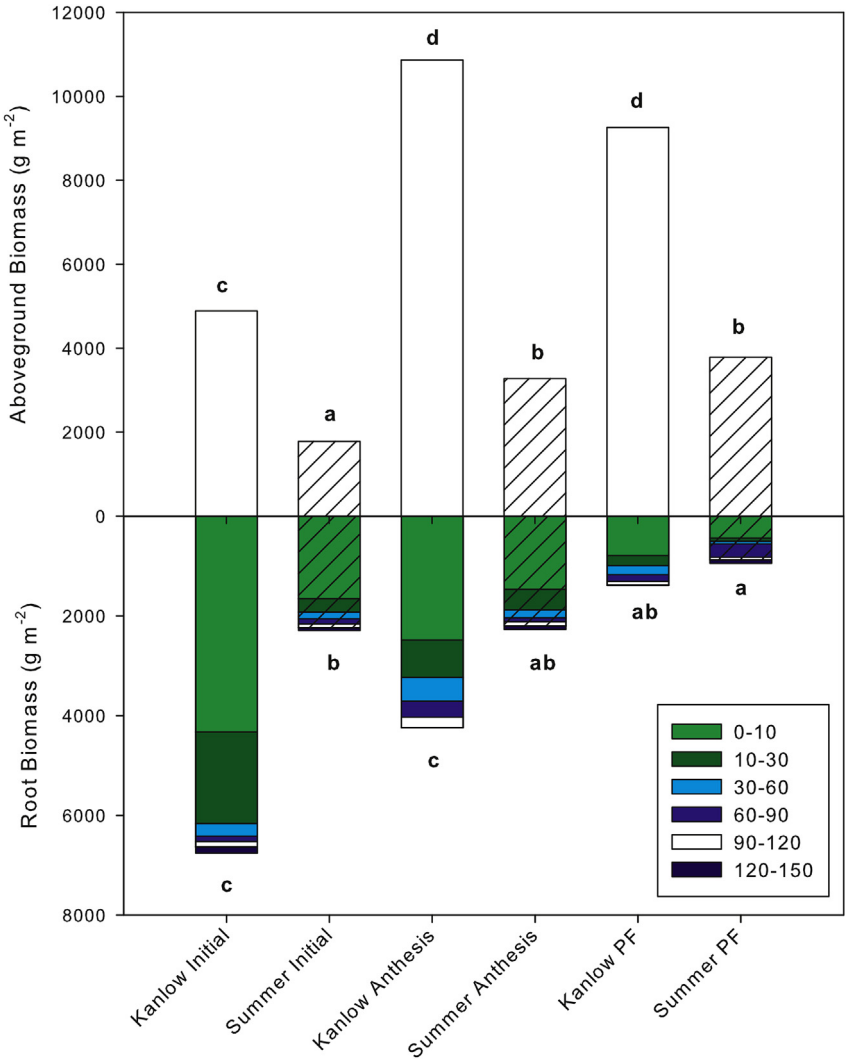

Fig. 2. Aboveground and root biomass (g m^{-2}) for Kanlow and Summer over the season (initial, anthesis, and post-frost (PF) sampling). * Initial sampling data redrawn from Roosendaal et al. (2016). **Anthesis sampling was 11 July 2012 for Summer and 17 September 2012 for Kanlow. n = 3.

two ecotypes at the beginning of the experiment, rhizosphere soil C and N concentration increased in the 0–10 cm depth under Kanlow and the 30–60 cm depth under Summer over the experiment (Table 3). Decreases in SOC were evident in the 10–30 cm and 120–150 cm depths of both cultivars. Rhizosphere N was greater post-frost under Summer in the 30–60 and 90–120 cm compared to initial sampling.

There was a slight, but significant increase in $\delta^{13}C$ in the rhizosphere soil over the experiment, from −17.0 to −15.8, averaged over species and depths (Table 3). This effect was driven by significant enrichment in the 30–60 cm depth of both species and the 0–10 cm and 120–150 cm depths under Kanlow. There was no significant difference in rhizosphere $\delta^{13}C$ between ecotypes.

4. Discussion

Variation in root allocation patterns across broad vegetation types determines soil profile SOC (De Deyn et al., 2008; Jobbágy and Jackson, 2000). We show here that differences in root allocation, morphology and architecture among switchgrass ecotypes likely promoted rhizodeposit-C uptake into different soil microbial communities and can impact rhizosphere SOC through those plant-microbial associations. Kanlow had three times the root biomass with coarser root morphology compared to Summer, which had finer root structure. Rhizodeposit-C uptake was associated with a more saprotrophic fungal community with the upland ecotype, Summer, and a more gram-negative bacterial community under the lowland ecotype, Kanlow. Rhizosphere soil ^{13}C was slightly enriched at the end of the experiment, with no difference between the ecotypes, suggesting that despite much smaller root biomass, root derived-C may have been more efficiently transferred to the soil beneath the fine-rooted ecotype, Summer. The two ecotypes also had different rhizosphere C and N content post-frost, indicating differences between them in root turnover. These two ecotypes illustrate contrasting mechanisms of C accumulation and stabilization through microbial processing of rhizodeposit C and through root death and particulate organic matter accumulation. The relative importance of these mechanisms in SOC sequestration could be mediated by extreme climate events.

4.1. Ecotype root biomass and trait response to drought

Upland switchgrass ecotypes are often more drought-tolerant compared to lowland ecotypes (Barney et al., 2009; Stroup et al.,

Table 1
Root mass density (mg cm^{-3}) root length density (cm cm^{-3} soil), and specific root length (cm g^{-1} root) with standard deviation for the two switchgrass ecotypes Kanlow and Summer. Asterisks indicate significant differences between ecotypes Kanlow and Summer within each depth. nd = not determined. †**Initial data from** Roosendaal et al. (2016). **Anthesis sampling was 11 July 2012 for Summer and 17 September 2012 for Kanlow.

Sampling Time	Depth cm	Root Mass Density mg cm^{-3}		Root Length Density cm cm^{-3}		Specific Root Length m g^{-1} root	
		Kanlow	Summer	Kanlow	Summer	Kanlow	Summer
Initial†	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)***
Anthesis**	0–10	15.18 (5.11)	7.40 (1.78)***	11.5 (5.53)	13.58 (3.30)	7.58 (2.40)	18.40 (1.57)***
	10–30	2.09 (0.69)	0.93 (0.47)***	3.10 (0.49)	2.91 (0.95)	15.97 (5.78)	32.81 (5.07)**
	30–60	0.76 (0.60)	0.26 (0.05)**	1.23 (0.40)	1.17 (0.61)	21.37 (12.82)	44.36 (19.50)***
	60–90	0.76 (0.35)	0.12 (0.04)***	1.22 (0.69)	0.70 (0.16)	15.49 (2.39)	62.47 (8.45)***
	90–120	nd	0.12 (0.02)	nd	1.04 (0.19)	nd	89.77 (18.35)
	120–150	nd	0.13 (0.07)	nd	1.44 (0.53)	nd	120.94 (48.11)
	0–150	**4.33 (1.9)**	**1.49 (0.38)***	**4.01 (1.71)**	**3.47 (0.92)**	**15.5 (3.33)**	**61.46 (11.83)***
Post-Frost	0–10	14.75 (5.37)	9.47 (5.39)	16.30 (7.92)	11.20 (3.95)	11.48 (5.32)	13.49 (6.33)
	10–30	1.14 (0.29)	0.64 (0.27)**	3.12 (0.73)	2.16 (0.88)	27.44 (0.60)	34.67 (5.68)
	30–60	1.33 (1.04)	0.40 (0.15)***	2.24 (1.00)	1.26 (0.56)	21.08 (10.97)	31.03 (6.94)*
	60–90	0.83 (0.58)	0.61 (0.45)	1.58 (0.83)	1.71 (0.78)	27.82 (20.33)	35.37 (22.58)
	90–120	0.49 (0.29)	0.34 (0.08)	1.44 (0.35)	1.92 (0.77)	37.03 (21.45)	60.48 (33.15)
	120–150	0.55 (0.29)	0.21 (0.12)	1.96 (0.68)	1.82 (0.81)	42.22 (26.15)	90.57 (12.44)
	0–150	**3.18 (0.68)**	**2.00 (0.87)**	**4.44 (1.26)**	**3.44 (0.76)**	**27.84 (12.9)**	**43.11 (15.35)**

*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 2
Main effects of ecotype, sampling and ecotype*sampling interactions on ^{13}C enrichment (APE ng C g^{-1} DW) of leaves, stems, crown and roots for the two switchgrass ecotypes Kanlow and Summer over the sampling times (initial, anthesis, and post-frost) and by depth. Lowercase letters indicate significant differences between sampling times averaged over ecotype. Uppercase letters indicate main ecotype effects averaged over sampling. DW = dry weight; nd = not determined. †Initial data from Roosendaal et al. (2016). **Anthesis sampling was 11 July 2012 for Summer and 17 September 2012 for Kanlow.

		^{13}C enrichment APE (ng C g^{-1} DW)										
		Initial†			Anthesis**			AfterFrost				
		Kanlow	Summer	Avg.	Kanlow	Summer	Avg.	Kanlow	Summer	Avg.	Avg. Kanlow	Avg. Summer
Leaves		474.4	630.5	552.4 a	260.3	526.3	393.3 b	173.5	347.0	260.2 c	302.7 A	501.3 B
Tillers		756.4	1469.9	1113.1 a	146.2	378.6	262.4 b	112.1	337.9	225.0 b	338.2 A	728.8 B
Crown		4.7	70.8	37.7 a	10.9	44.9	27.9 a	14.2	48.7	31.4 a	9.9 A	54.8 B
	Depth											
Roots	0–10	10.0	119.9	64.9 a	27.4	96.0	61.7 ab	41.1	163.7	102.4 b	26.1 A	126.5 B
	10–30	11.0	76.6	43.8 a	17.2	48.6	32.9 a	20.7	52.4	36.6 a	16.3 A	59.2 B
	30–60	16.2	36.8	26.5 a	46.5	80.9	63.7 b	29.8	36.7	33.3 a	30.8 A	51.5 A
	60–90	18.2	29.1	23.7 a	68.8	94.9	81.8 b	20.5	31.2	25.8 a	35.8 A	51.7 A
	90–120	8.7	33.9	21.3 a	18.7	48.2	36.4 a	13.2	23.7	18.5 a	12.9 A	35.3 A
	120–150	8.7	26.2	15.7 a	nd	34.7	34.7 b	14.1	21.5	17.1 ab	11.4 A	28.5 A
	Wt. Avg. 0-150	18.7	62.5	40.6 a	48.1	96.9	72.5a	31.5	58.7	45.1a	32.8 A	72.7 B

2003), although physiological measures such as water-use-efficiency appear to be quite plastic and do not always clearly separate across ecotypes (Liu et al., 2015; Zegada-Lizarazu et al., 2012). In response to a very dry year where switchgrass received 40% less precipitation compared to the 30-year average, both ecotypes lost a large proportion of root biomass. Root system responses to water availability and dry soil are often specific to the degree and can be opposing depending on the level of dryness (Comas et al., 2005). For example, small shortfalls in water availability can increase root production in plants, while extremely dry soil will limit root production; likewise, some species, even with succulent fine roots, retain these roots under moderate drought but may not under extreme conditions (Comas et al., 2005). Garten et al. (2010) showed an increase in root production during the latter part of the growing season in rainfed switchgrass in the southeast US. The upland ecotype, Summer, appeared to be more drought tolerant and retained more of the initial root biomass (60%) compared to the lowland ecotype, Kanlow (78%, Fig. 1, Table 1). Other studies document a reduction in shoot:root ratio with drought, but inconsistent results with drought on root biomass (Barney et al., 2009). Greater root length per biomass investment in root structures (e.g. greater SRL, m g^{-1}) and greater association with saprotrophic fungi may enhance soil water uptake by Summer if these fungi help roots maintain better connections with the soil as it dries (Rapparini and Peñuelas, 2014). In addition, there may be physiological differences in plant membrane composition, such as phenolic content that confer drought resistance to switchgrass ecotypes (De Micco and Aronne, 2012).

Despite losing root biomass over the year, there was little change in root architecture, but consistent differences between

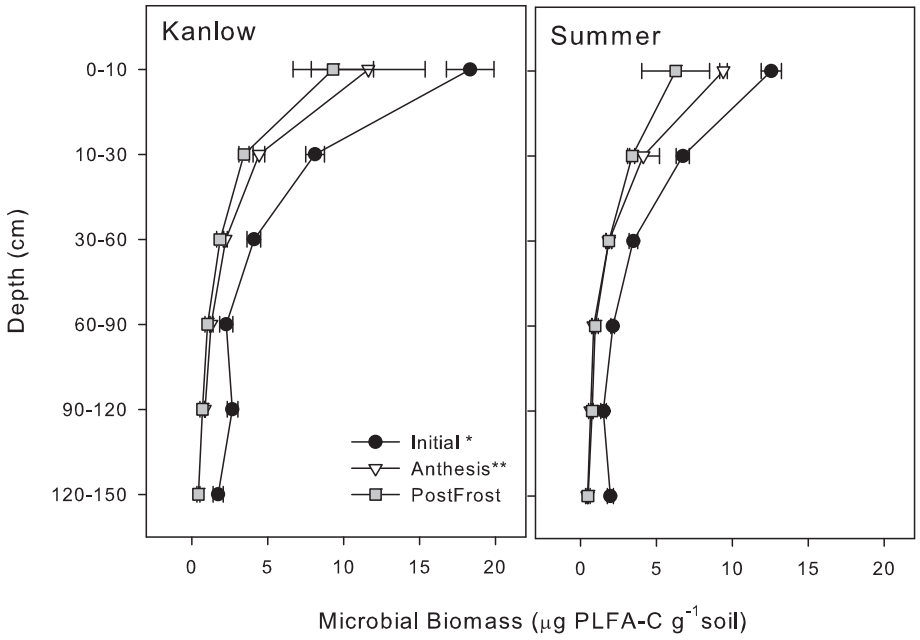

Fig. 3. Microbial biomass PLFA-C (μg PLFA-C g^{-1} soil) for Kanlow and Summer over the season (initial, anthesis, and post-frost (PF) sampling) and through the soil profile. * Initial sampling data redrawn from Roosendaal et al. (2016). **Anthesis sampling was 11 July 2012 for Summer and 17 September 2012 for Kanlow. n = 3.

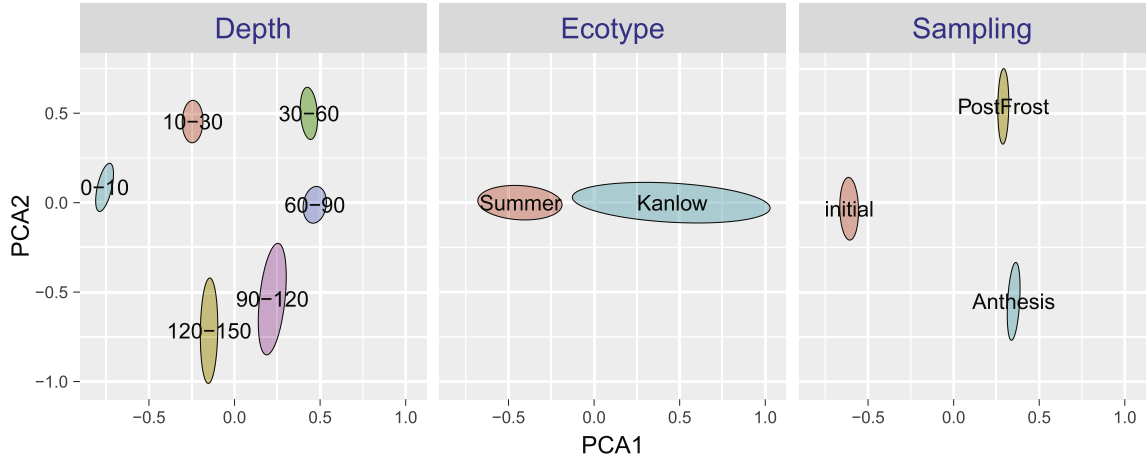

Fig. 4. Distance-based redundancy analysis of PLFA biomarkers (mol%) for microbial community composition for main effects ecotype, depth, and sampling. Ellipsoids represent standard errors around the multivariate-group centroids.

ecotypes (Table 1). Kanlow averaged 2.2 times more root mass density compared to Summer, while Summer had two times more specific root length. Upland switchgrass ecotypes have greater SRL compared to lowland cultivars when grown in the US Midwest (de Graaff et al., 2013). Consistent differences between switchgrass ecotypes throughout drought stress confirm that root morphology and architecture appear to be genetically determined (Comas and Eissenstat, 2004; Fischer et al., 2006).

4.2. Microbial community amount and abundance

Microbial biomass (PLFA-C) was positively related to root length across ecotypes, sampling times, and depths (R^2 = 0.70, p = 0.0001), suggesting that root length was more important in supporting microbial communities than root biomass. Fine roots have more surface area for root exudation (Guo et al., 2004), have a lower C:N ratio (Comas and Eissenstat, 2009; de Graaff et al., 2013; Garten et al., 2010), and faster turnover (Xia et al., 2010), all providing substrate for the microbial community.

The slight difference in soil microbial community composition between ecotypes at the initial sampling (Roosendaal et al., 2016) was not maintained over the study. This suggests that the overall rhizosphere microbial communities, including root and soil organic matter decomposers, is similar across ecotypes and, perhaps not unexpectedly, given the dominant composition of bacteria due to the agricultural history of this site.

Microbial biomass decreased over the season and through the soil profile in response to drought conditions (Fig. 3). The relative abundance of gram-positive bacteria increased over the study (Fig. 5) and these bacteria are more tolerant of water stress compared to gram-negative bacteria due to their thicker cell wall (Fenchel et al., 2012; Schimel et al., 2007). Drought commonly induces a shift in microbial community towards gram-positive bacteria (Fuchslueger et al., 2014), but soil fungi have broad hyphal

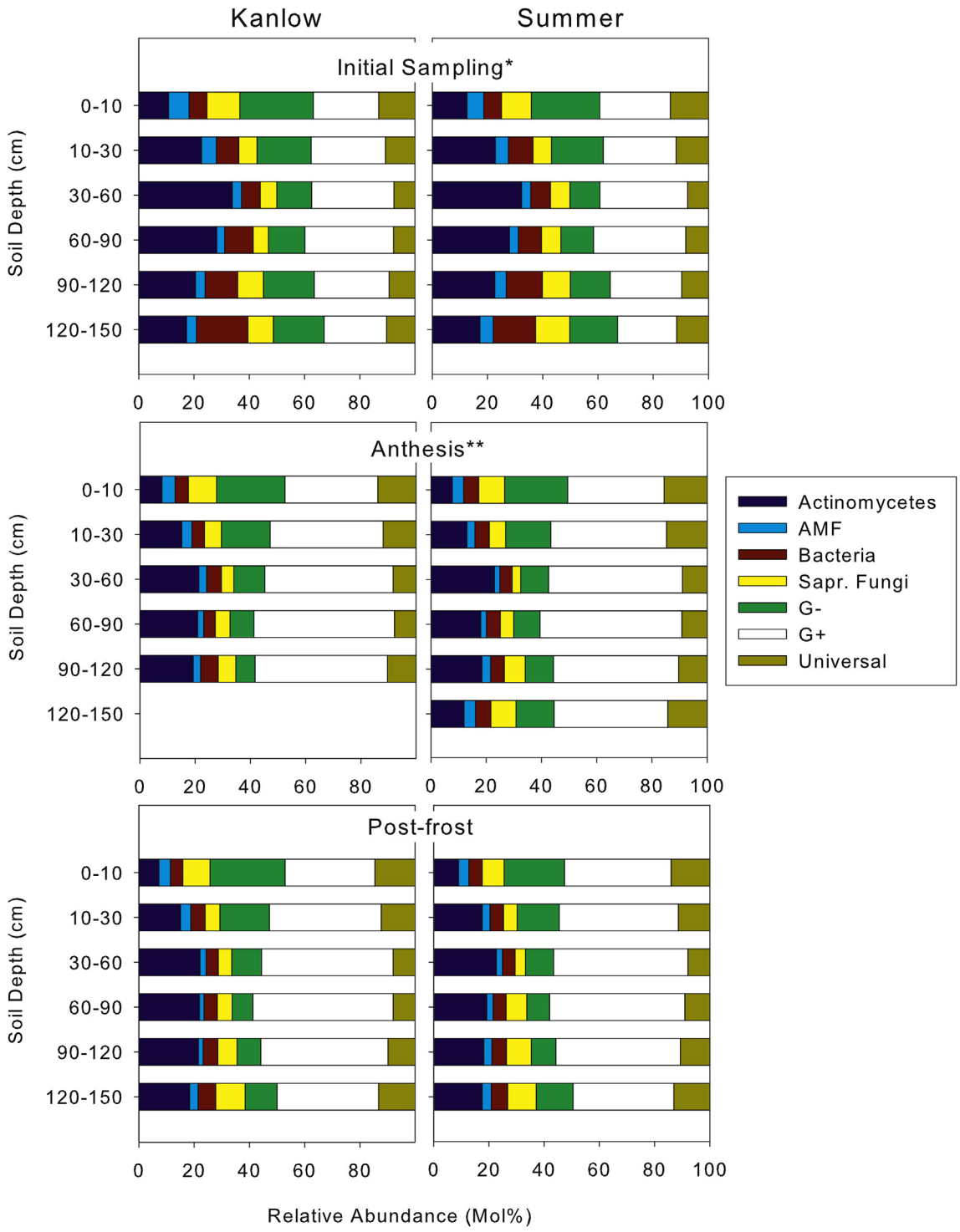

Fig. 5. Relative abundance (mol%) of microbial groups for Kanlow and Summer over the season (initial, anthesis, post-frost) through the soil profile. * Initial sampling data redrawn from Roosendaal et al. (2016). **Anthesis sampling was 11 July 2012 for Summer and 17 September 2012 for Kanlow. n = 3.

networks that can transport water (Allen, 2007; Schimel et al., 2007) and have been reported to be drought resistant in some studies (de Vries et al., 2012).

4.3. Rhizodeposit uptake differed between ecotypes

Switchgrass ecotypes had different soil microbial communities take up rhizodeposit C and the community composition converged over the year (Fig. 7). The fine-rooted Summer ecotype had rhizodeposit uptake at the initial sampling primarily in saprotrophic fungi (18:2ω 9,12, & c18:1ω9, Supplementary Table 1), while the coarser-rooted Kanlow ecotype had more rhizodeposit uptake in gram-negative bacteria (18:1ω7, Supplementary Table 2) (Roosendaal et al., 2016). These results suggest broadly different plant-microbial relationships that could reflect differing micro-evolutionary strategies in nutrient acquisition and drought

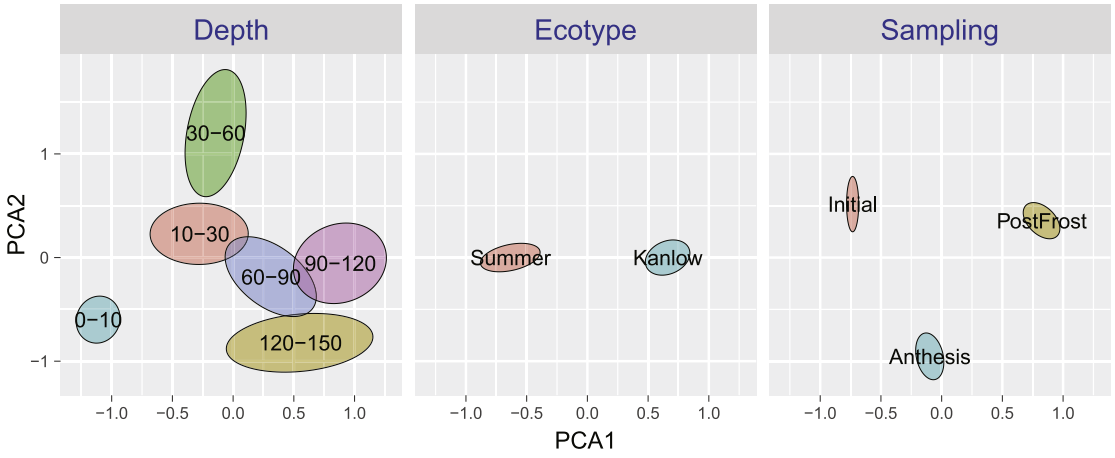

Fig. 6. Distance-based redundancy analysis of microbial community composition relative APE ^{13}C enrichment (APE_{rel} ^{13}C enrichment) for main effects ecotype, depth, and sampling. Ellipsoids represent standard errors around the multivariate-group centroids.

tolerance adapted by the two ecotypes.

The fine-rooted, upland ecotype may have developed microbial associations with fungi that assist in nutrient and water acquisition to survive stress. The finer roots of Summer had greater rhizodeposit-C uptake by saprotrophic fungi, which has been noted in other pulse-chase ^{13}C experiments (Denef et al., 2007; Jin and Evans, 2010). Finer roots turn over rapidly (Xia et al., 2010) and may promote a more saprotrophic fungal community for N acquisition and recycling. Saprotrophic fungal communities can be stimulated by AMF to decompose litter for available N (Herman et al., 2012). Soil fungi colonizing roots may also promote drought tolerance to plants through a wide hyphal network that can access water in small soil pores (Allen, 2007). Summer lost a smaller proportion of root biomass through the drought and could be a function of smaller root size, and/or greater microbial associations with the fungal community.

The rhizodeposit C uptake primarily in gram-negative bacteria under the coarser-rooted ecotype Kanlow may reflect a growth, not survival strategy. Some bacterial endophytes associated with switchgrass and have been shown to increase plant growth (Xia et al., 2013). Plant root exudation patterns can select different microbial communities even between plant cultivars, resulting in strikingly different microbial communities in lab settings (Broeckling et al., 2008; Gschwendtner et al., 2010). To our knowledge, this is the first field demonstration of plant-specific microbial interactions between switchgrass ecotypes.

Over time, rhizodeposit C moved into the gram-positive community under Summer and the gram-positive and actinomycete communities under Kanlow. Drought can reduce plant-microbial C transfer and cause a shift in microbial community to slow-growing bacterial species (Fuchslueger et al., 2014, 2016; Schimel et al., 2007). Fuchslueger et al. (2014) found that drought reduced plant-bacterial, but not plant-fungal C transfer in a mountain meadow exposed to drought treatments. These results suggest a shift in microbial community toward drought-tolerant bacterial species, which do not rely on plant-C, and could disrupt plant-microbial C flow.

4.4. Impacts on C cycling

Variation in root architecture between ecotypes illustrates to two contrasting mechanisms of C stabilization; one through microbial processing of rhizodeposit C and the other through root death and particulate organic matter accumulation. The importance of microbial processing in SOC stabilization is well documented, with impacts of microbial C use efficiency in SOC stabilization recently recognized (Cotrufo et al., 2013, 2015; Grandy and Neff, 2008). Stable SOC is largely microbially-derived (Knicker, 2011; Schmidt et al., 2011) and may be influenced by soil microbial composition. Fungal cells are more recalcitrant and remain in the soil longer than bacteria (Jin and Evans, 2010; Six et al., 2006) and can also promote other soil C protection mechanisms, like soil aggregation (De Deyn et al., 2008). Despite a much greater root biomass under Kanlow, rhizosphere $\delta^{13}C$ was not significantly different between the two ecotypes, suggesting greater microbial C input under the finer rooted ecotype, Summer, whose microbial associations were predominately saprotrophic fungi. Fine-root C also may contribute more to soil C as they are more difficult to separate for the soil.

Switchgrass root death and turnover contribute to SOC sequestration, but these effects can take years to decades to observe (Garten et al., 2010; Stewart et al., 2016). Surprisingly, switchgrass ecotype differences in root death contributed to changes in rhizosphere SOC concentration over the course of one season, with significant increases in Kanlow in the 0–30 cm depths and in Summer in the 30–60 cm depths, with decreases under Summer in other depths. The SOC content is highly related to C inputs to the soil and in all cases where ecotypes were different, Kanlow had greater SOC content, corresponding to greater root biomass. Although the fine-rooted ecotype, Summer contributed similar amounts of labeled C belowground, root death from Kanlow contributed more to increased surface soil rhizosphere SOC contents.

5. Conclusions

The rhizosphere is known to be a dynamic environment with changes in nutrient availability, water content and plant chemical signals influencing the associated microbial community. In contrast, the soil below 30 cm is often assumed to be nutrient limited and rather static, aside from rhizosphere ‘hotspots’. We show that despite containing a relatively small proportion of the overall root biomass and soil C, it nevertheless can be influenced directly by plant-specific rooting traits and indirectly through microbial community changes. Plant species with greater root length and associated fungal communities could incorporate rhizodeposit

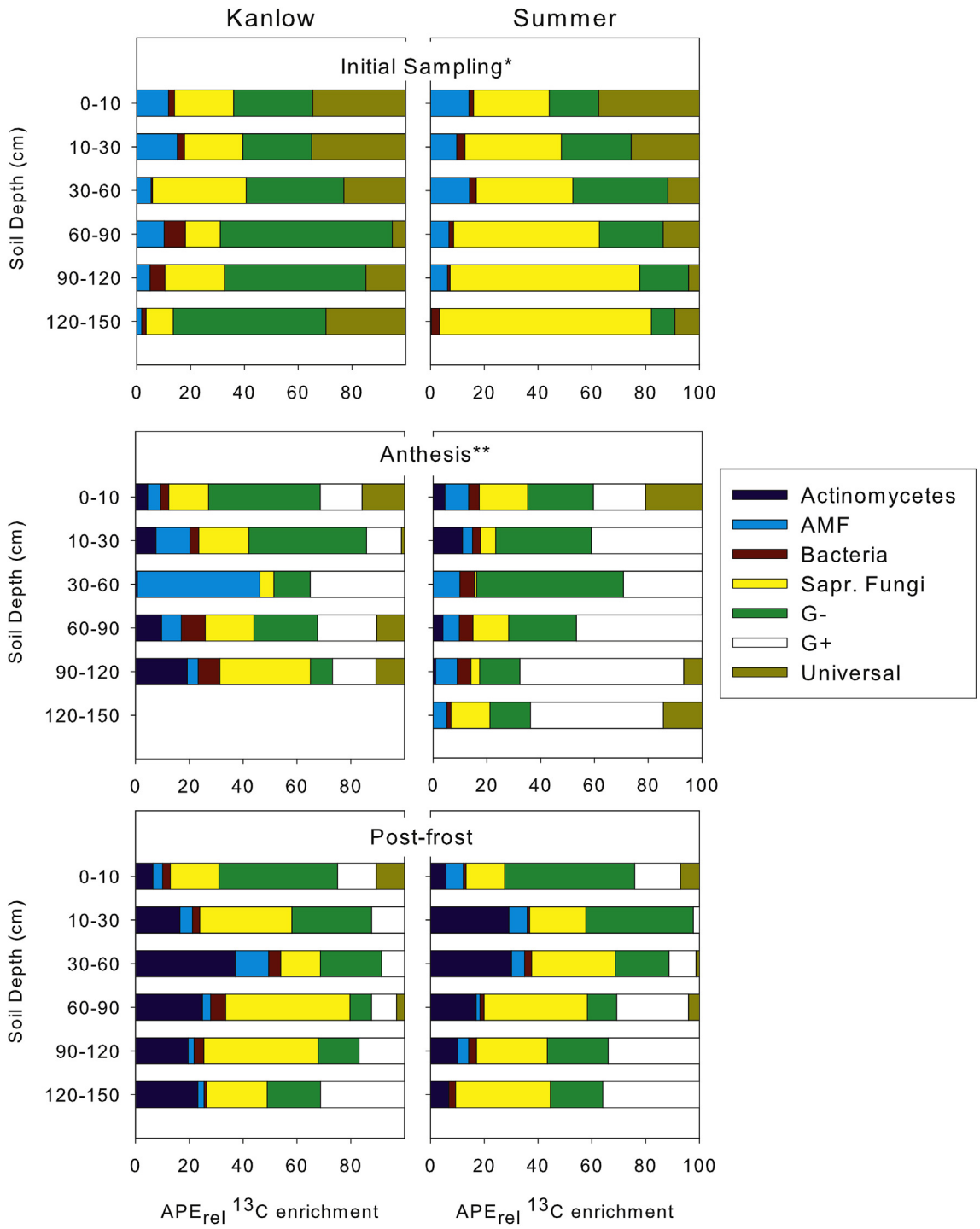

Fig. 7. Relative APE ^{13}C enrichment (APE$_{rel}$ ^{13}C enrichment) of microbial groups for Kanlow and Summer over the season (initial, anthesis, post-frost) through the soil profile. * Initial sampling data redrawn from Roosendaal et al. (2016). **Anthesis sampling was 11 July 2012 for Summer and 17 September 2012 for Kanlow. n = 3.

C throughout the soil profile and stabilize soil C via associations with clay minerals.

Extreme drought events have the potential to add significant root C stocks to prairie soils though root death. Surprisingly, between 60 and 78% of standing root biomass was added over this single drought event. Although root biomass was removed before C analyses, the C input was great enough to increase rhizosphere C content in the 0–30 cm soils under Kanlow and the 30–60 cm depth under Summer. Decomposition of both coarse and fine roots into soil C will depend on root chemistry, nutrient availability, and microbial accessibility. The dynamic interplay between individual plants and their associated microbial community has been clearly illustrated in laboratory studies, but these data suggest that ecotype interactions with specific microbial communities can direct rhizodeposit C flow and C accrual deep in the soil profile. The fine-rooted, upland ecotype may have developed fungal associations that assist in nutrient and water acquisition to survive stress. In contrast, the gram-negative bacterial communities associated with

Table 3
Soil C, N (g kg^{-1} soil), C:N ratio and $\delta^{13}C$ for the two switchgrass ecotypes Kanlow and Summer over the sampling times (initial, anthesis, and post-frost). Asterisks indicate significant differences between ecotypes Kanlow and Summer within each depth. Lowercase letters indicate significant differences between sampling times within ecotype and depth.

Depth	Sampling	Soil Organic C				Soil Total N				Soil C:N ratio				SOC $\delta^{13}C$			
		g C kg^{-1} soil				g N kg^{-1} soil								‰			
		Kanlow		Summer		Kanlow		Summer		Kanlow		Summer		Kanlow		Summer	
0–10	Initial	20.0	a	19.5	a	1.76	a	1.76	a	11.34	a	11.05	a	−16.5	b	−16.2	a
	Anthesis	25.5	ab	22.6	a	2.03	ab	1.93	a**	12.53	b	11.66	a*	−18.1	c	−16.3	a**
	Post-Frost	28.1	b	20.5	a*	2.27	b	1.78	a	12.32	b	11.52	a	−14.9	a	−16.0	a
10–30	Initial	16.2	a	15.7	a	1.38	a	1.36	a	11.73	a	11.61	a	−15.2	a	−14.8	a
	Anthesis	16.4	ab	15.9	a	1.41	a	1.38	a	11.63	a	11.55	a	−14.4	a	−15.0	a
	Post-Frost	15.9	b	15.4	b	1.39	a	1.38	a	11.42	a	11.15	a	−14.2	a	−14.7	a
30–60	Initial	12.0	a	9.7	a	1.05	a	0.87	a	11.37	a	11.05	a	−14.0	a	−14.6	a
	Anthesis	14.0	a	10.3	ab*	1.24	a	0.95	ab	11.36	a	10.87	a	−14.0	ab	−14.1	ab
	Post-Frost	13.7	a	11.9	b	1.25	a	1.14	b	10.95	a	10.46	a	−13.2	b	−13.6	b
60–90	Initial	5.6	a	5.9	a	0.59	a	0.60	a	9.47	b	9.81	a	−15.9	a	−16.3	a
	Anthesis	7.5	b	4.5	b**	0.74	b	0.52	a**	10.22	c	8.62	b	−15.3	a	−16.8	a**
	Post-Frost	5.2	a	4.6	b	0.63	ab	0.60	a	8.22	a	7.66	c	−15.9	a	−16.5	a
90–120	Initial	3.4	ab	3.5	a	0.45	a	0.43	a	7.52	b	8.18	c**	−18.9	a	−18.8	a
	Anthesis	4.7	b	3.3	ab***	0.57	a	0.44	ab	8.27	c	7.46	b*	−17.8	a	−19.2	a
	Post-Frost	3.0	a	3.0	b	0.47	a	0.47	b	6.37	a	6.33	a	−17.0	a	−15.8	a
120–150	Initial	2.4	a	2.5	a	0.37	a	0.37	a	6.25	a	6.77	a	−21.9	a	−21.2	a
	Anthesis	–		2.4	ab	–		0.37	a	–		6.41	a	–		−21.9	a
	Post-Frost	2.0	b	2.0	b	0.39	a	0.41	a	5.02	b	5.01	a	−17.6	b	−20.1	a

the coarser-rooted ecotype, Kanlow, may reflect a growth strategy as some gram-negative *Protobacteria* have been shown to act as plant growth promoters. These broadly different plant-microbial relationships could reflect differing micro-evolutionary strategies in nutrient acquisition and drought tolerance and illustrate the importance of the microbial community in plant survival strategies.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.04.021.

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