GENETIC VARIABILITY OF CULTIVARS SHAPE BIOCHEMICAL PROFILES IN A BIOENERGY CROPPING SYSTEM

by

Megan Kelly-Slatten

A thesis

submitted in partial fulfillment

of the requirements for the degree of

Master of Science in Biology

Boise State University

August 2019

BOISE STATE UNIVERSITY GRADUATE COLLEGE

DEFENSE COMMITTEE AND FINAL READING APPROVALS

of the thesis submitted by

Megan Kelly-Slatten

Thesis Title: Genetic Variability of Cultivars Shape Biochemical Profiles in a Bioenergy Cropping System

Date of Final Oral Examination: 20 June 2019

The following individuals read and discussed the thesis submitted by student Megan Kelly-Slatten, and they evaluated her presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

Marie-Anne de Graaff, Ph.D.	Chair, Supervisory Committee
Kevin Feris, Ph.D.	Member, Supervisory Committee
Julie D. Jastrow, Ph.D.	Member, Supervisory Committee

The final reading approval of the thesis was granted by Marie-Anne de Graaff, Ph.D., Chair of the Supervisory Committee. The thesis was approved by the Graduate College.

DEDICATION

I dedicate this to my family who has been there to support me through my many years of school.

ACKNOWLEDGEMENTS

I would like to thank Argonne National Laboratory and Environmental Molecular National Sciences Laboratory for the resources to complete this project. I personally want to thank Malak Tfaily for helping me develop a working protocol for my samples, and Julie Jastrow for helping me harvest samples.

Additionally, I am so thankful for all the advice and guidance given to me by Marie-Anne de Graaff. You have crafted my passion for ecosystem ecology and my yearning to learn more and become a better researcher. I look forward to working alongside you for my PhD. I would also like to thank my committee members Julie Jastrow and Kevin Feris for their knowledge and their encouragement to pursue my masters. I am grateful for all of the help and support of each and every member in the de Graaff Laboratory as well as the Biology graduate student community.

Finally, I would like to thank my family who has been nothing but supportive as I have experienced my ups and downs of graduate school. My passion to pursue what I love and what makes me happy is something my parents have always encouraged, and I would not have ended up a researcher without their love and guidance.

ABSTRACT

The largest terrestrial carbon (C) pool on Earth is the soil, surpassing both biotic and atmospheric C pools combined. The majority of C stabilized in soil is root-derived, and root derived C is the preferred food source for the soil microbial community. Recent studies have indicated that the perennial bioenergy crops *Panicum Virgatum* (hereafter: switchgrass) and *Andropogon Gerardii* (hereafter: big bluestem) accumulate significant amounts of soil C owing to their extensive root systems, and that soil C accumulation rates are driven by inter- and intra-specific variability in plant traits. While soil C accumulation in the short term (i.e. after two growing seasons) was linked to root morphology and associated root derived C input rates, this relationship did not hold up in the long term (i.e. after four growing seasons). Given the importance of soil metabolic profiles for microbial assimilation of C and subsequent C stabilization of microbial residues, this study aimed to evaluate how six cultivars of candidate bioenergy grasses (three cultivars of switchgrass and three cultivars of big bluestem) affect the soil biochemical profile across a 30 cm depth profile in the soil. To assess the soil's biochemical profile, we performed a water-methanol-chloroform sequential extraction, which allowed us to assess the composition of the water dissolved C pool, and also the sorbed C pool. Our study yielded two main results: 1) soil depth significantly impacted the soil biochemical profiles, with recently deposited water dissolved C dominating at shallower depths and older, more stable C dominating at greater depths, and 2) soil biochemical profiles differed among plant cultivars, but not species, indicating

the importance of genetic variability in driving the soil C cycle. Our data suggest that cultivar variations in molecular abundance across soil biochemical profiles may explain variance in plant-derived C. Models currently use root biomass as the sole parameter to predict soil C influx and stabilization, but our data indicate that the chemical composition of root-derived C influx, driven by genetic variability of the cultivar, may be another important predictor of how roots might affect soil C cycling. By further understanding how variables like cultivar type impact the formation of soil C, we can better adapt planting strategies for biofuel and agricultural industries to promote soil C formation, improve its persistence, and help mitigate the effects of climate change. Additional research into evaluating differences among cultivar exudate composition, microbial community composition, and soil respiration would help to disentangle the complicated process by which plant-derived C contributes to soil C formation and stabilization.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

C	Carbon
BB	Big Bluestem
SG	Switchgrass
BO	Bonanza
BSL	Big Bluestem Southlow
CR	Cave-in-Rock
KA	Kanlow
SSL	Switchgrass Southlow
ST	Suther

CHAPTER ONE: INTRODUCTION

The largest terrestrial carbon (C) pool on Earth is the soil, surpassing both biotic and atmospheric C pools combined (Lal, 2004). Owing to the large size of the C pool, small changes in the quantity of C stored in the soil can influence atmospheric CO_2 levels and affect climate change (Lal, 2004). The transition from unmanaged to managed ecosystems typically leads to a loss of soil C due to soil disturbance that increases soil microbial activity and decomposition of soil C (Lal, 2004). For instance, disturbances associated with the establishment of bioenergy production can release substantial amounts of previously sequestered soil C back into the atmosphere (Gelfand et al., 2011). This loss may be mitigated by management strategies that promote soil C accumulation, such as planting species or cultivars that increase soil C sequestration (Adkins et al. 2016; Morris et al., 2016). Currently, two species of perennial, warm-season grasses, switchgrass (*Panicum virgatum*) and big bluestem (*Andropogon gerardii*), are being evaluated as possible bioenergy crops. Integral to this evaluation is development of a mechanistic understanding of their impacts on soil C formation and stabilization. We know that these species and their cultivars affect soil C storage differently (Adkins et al., 2016; Adkins et al., in review), but the mechanisms that regulate these differences are largely unknown.

It has been well established that the majority of C stabilized in soil is root-derived (Rasse et al., 2005; Kong & Six, 2010). Root-C input is driven by the processes of root turnover and exudation by living roots (Fig. 1). Root exudates are composed of organic

(carbohydrates, amino acids, phenolic compounds, fatty acids, sterols, vitamins, enzymes, nucleosides) and inorganic compounds (bicarbonate, hydroxide, H+) (Carvalhais et al., 2011). The sum of root litter and exudate input into the soil is commonly referred to as rhizodeposition (Lynch & Whipps, 1990). Root derived C is the preferred food source for the soil microbial community (Bolan et al, 2011), which metabolizes rhizodeposits as an energy substrate, leading to biosynthesis of stable C, and to C loss owing to metabolic CO_2 production (Frey et al., 2013; Liang et al., 2017) (Fig. 1). The ratio of C turned into microbial biomass *versus* the amount of C released from soil as CO_2 plays a large role in determining soil C stabilization (Kallenbach et al., 2015; Wallenstein et al., 2007). This ratio is dependent on rhizosphere chemistry, which regulates the soil microbial structure and its physiology (Badri & Vivanco, 2009; Huang et al., 2014; Mao et al., 2014), thus affecting microbial C use efficiency (*i.e.* the relative ratio of C turned into microbial biomass *versus* the amount of C released from soil as CO_2) (Baudoin et al., 2003; Kallenbach et al., 2016; Wallenstein et al., 2007). Given that the chemical composition of rhizodeposition has been shown to vary by plant species and plant genotype (Huang et al., 2014), this genetic variation in rhizosphere chemistry may lie at the root of differential impacts of plant species and cultivars on soil C stabilization.

Until recently it was assumed that more complex molecules (i.e. including lignin, lipids, and tannins), usually derived from plant litter are more persistent in the soil in the long-term. This is because they are more difficult for soil microbes to break down and digest (Bolan et al., 2011; Rovira & Vallejo, 2002), thus suppressing CO_2 respiration. However, more recently, several studies have challenged this notion, because they have found that the majority of C stabilized in soil is derived from microbial residues that were

originally formed by microbial assimilation of root exudates (Chenu et al., 2019; Kallenbach et al., 2015; Wallenstein et al., 2007). Particularly, labile plant derived C compounds, derived from root exudates (i.e. composed of proteins, carbohydrates, condensed and uncondensed hydrocarbons) and suspended in the water dissolved C pool have proven to be an important mediator of long-term soil C stabilization (Rasmussen et al., 2018). This is because microbes can easily access this labile C and assimilate it into microbial residues that are stable and critical for long term C storage (Gleixner, 2013; Miltner et al., 2012; Zhu et al., 2017; Kallenbach et al., 2015). These studies indicate that variability in rhizodeposits from different plants are likely to impact soil C stabilization through their impacts on soil microbial community functioning.

Rhizosphere chemistry also affects soil C stabilization, because it affects sorption of C to soil minerals (Mikutta, 2009; Sanderman et al., 2014; Scow et al., 1995). Sorbed soil C or C that has been chemically adhered to a mineral or clay soil particle is less vulnerable to microbial attack, and is thus more persistent in soil (Scow et al., 1995; Sollins et al. 1996; Baldock & Skjemstad, 2000). This is because the physical protection offered by the mineral or clay particle lowers the amount of interaction the C has with the microbial community, helping to stabilize the C and reduce its chance of being respired (Sanderman et al., 2014; Scow et al., 1995). Additionally, sorption of C lowers its ability to disperse through the soil, making it is less mobile and prone to leaching than water dissolved C (Scow et al., 1995). Thus, a larger sorbed C pool increases soil C stabilization (Sanderman et al., 2014). The molecular structure of C, availability of exchangeable polyvalent cations, and the mineral composition of the soil are the main drivers in determining C composition of the sorbed C pool (Bollag, 2017; Bolan et al.,

2011; Mikutta, 2009; Rovira & Vallejo, 2002; Sanderman et al., 2014; Scow et al., 1995). Due to their molecular structure, lipids have a higher binding affinity to minerals and make up a large portion of the sorbed C pool (Bollag, 2017; Bolan et al., 2011; Rovira & Vallejo, 2002). Additionally, microbial residues adhere to minerals and make up a large portion of the sorbed C pool (Poirier et al., 2005; Schrumpf et al., 2013). Since labile C is important for microbial residue formation, labile C can indirectly influence the size of the sorbed C pool and soil C stability (Gleixner, 2013; Miltner et al., 2012; Zhu et al., 2017). Thus, differences in rhizosphere chemistry are likely to impact soil C stabilization by affecting the amount of C entering into the sorbed C pool (Mikutta, 2009; Sanderman et al., 2014; Scow et al., 1995).

With this study we set out to evaluate how six cultivars of candidate bioenergy grasses (three cultivars of switchgrass and three cultivars of big bluestem) affect the soil biochemical profile across a 30 cm depth profile in the soil. By examining the soil biochemical profiles of different species and cultivars we can begin to understand how genetic variability in plants may impact the chemical composition of root-derived soil C inputs, and implications for soil C stabilization (Bailey et al., 2017; Keiluweit et al., 2017). Given the extensive root systems of switchgrass and big bluestem (Stewart et al., 2017), and their depth-dependent impacts on soil C and microbial communities (Stewart et al., 2017; Syswerda et al. 2011), we quantified root impacts on soil biochemical profiles in 10 cm depth increments to a depth of 30 cm. To assess the soil's biochemical profile, we performed a water-methanol-chloroform sequential extraction (Tfaily et al., 2017). The sequential extraction allows for quantification of both the composition of the water dissolved C pool, and the sorbed C pool (Tfaily et al., 2017). Analysis of both

pools is an essential first step in understanding how genetic variability in plants may affect the soil C cycle differently.

CHAPTER TWO: METHODS

Experimental Field Site and Field Sampling

The study site was established in June 2008 at the U.S. Department of Energy National Environmental Research Park at Fermilab in Batavia, IL (88°13′47″W, 41°50′29″N). Since 1971 the landscape had been dominated by perennial, cool-season C3 grasses. In 2007, all vegetation was removed by a combination of herbicide application (glyphosate) and prescribed burning, and then in June 2008 the switchgrass *(Panicum virgatum*) and big bluestem (*Andropogon gerardii*) plots were planted and established. Plots were arranged into randomized complete blocks. Switchgrass plots (36m x 20m) consisted of three monoculture treatments (Cave-in-Rock, Kanlow, and Southlow; n=3), and in 2008 were drill-seeded in 20-cm rows with 6.7kg of pure live seed (PLS) ha^{-1}. Big bluestem plots (2m x 3m) consisted of three monoculture treatments (Bonanza, Southlow, and Suther; n=4), and in 2008 were hand sown in 20-cm rows.

In June 2018, 10 years after the establishment of the plots, we collected soil cores using a 4.8-cm diameter soil corer. For the three switchgrass cultivars, three soil cores were collected on randomly selected crowns to a depth of 30 cm and composited within each replicate plot (n=3). For the three big bluestem cultivars, two soil cores were collected on randomly selected crowns to a depth of 30 cm and composited within each replicate plot (n=4). All cores were divided into 10 cm depth increments (0-10, 10-20, 20-30 cm). The samples were frozen and shipped to the de Graaff Laboratory at Boise State University where they were kept frozen (-20℃) until processed. All soil samples

were weighed, after which two representative composite sub-samples (10 g) were immediately removed from the root zone soil and transferred back to the freezer (-20℃).

Sequential Extraction – Biochemical Profile

To assess how plant species, cultivars and soil depth affect soil biochemical profiles, we performed a water-methanol-chloroform sequential extraction. The water extraction quantifies the relative abundance of a wide variety of organic molecules dissolved in the soil solution. The methanol extraction also measures organic molecules in the water dissolved C pool, broadening the scope of organic molecules extracted. The chloroform extraction quantifies the relative abundances of organic molecules sorbed to soil minerals. Due to the overlap of organic matter captured by the water and methanol extractions (Tfaily et al., 2017), only water and chloroform extractions were analyzed to map the soil biochemical profile.

One of the frozen sub-samples was lyophilized for 48 hours, and then a water-methanol-chloroform sequential extraction adapted from Tfaily et al. (2017) was performed in order to determine the biochemical profile of each sample. From each lyophilized sample, 1 g of soil was transferred to a glass vial, and 2 mL of DI water was added, after which the vial was shaken for exactly 2 hours on Eberbach Variable Speed Reciprocal Shaker. The vial was then centrifuged at 1500xg for 10 minutes, and then using a glass Pasteur pipette the water was removed and stored (-20C). Next 2 mL of MeOH was added to the sample vial and vortexed for 30 seconds before shaking for 2 hours. The vial was centrifuged at 1500xg for 10 minutes, and then using a glass Pasteur pipette the MeOH was removed and stored (-20C). Finally, 2 mL of $CHCL_3$ was added to the sample vial and vortexed for 30 seconds before shaking for 2 hours. The $CHCL_3$ was

removed and stored at (-20℃). Each extraction layer (water and $CHCL_3$) was shipped on ice to the Environmental Molecular Sciences Laboratory (EMSL), a DOE-BER national user facility located in Richland, WA.

A 12 Tesla Bruker SolariX FTICR spectrometer located at EMSL was used to collect high resolution mass spectra of the organic material in the extracts. A standard Bruker ESI source was used to generate negatively charged molecular ions. Samples were then introduced directly to the ESI source. The instrument was externally calibrated weekly to a mass accuracy of <0.1 ppm using a tuning solution from Agilent, which contains the following compounds: $C_2F_3O_2$, $C_6HF_9N_3O$, $C_{12}HF_{21}N_3O$, $C_{20}H_{18}F_{27}N_3O_8P_3$, and $C_{26}H_{18}F_{39}N_3O_8P_3$ with an *m*/*z* ranging between 112 and 1333. The instrument settings were optimized by tuning on a Suwannee River Fulvic Acid (SRFA) standard. Blanks (HPLC grade MeOH) were also run at the beginning and the end of the day to monitor potential carry over from one sample to another. The instrument was flushed between samples using a mixture of water and methanol. The ion accumulation time (IAT) was varied (0.1 and 0.3 s) to account for differences in C concentration between samples. Ninety-six individual scans were averaged for each sample and internally calibrated using organic matter homologous series separated by 14 Da (CH_2 groups). The mass measurement accuracy was <1 ppm for singly charged ions across a broad m/z range (i.e. $200 < m/z < 1200$). To further reduce cumulative errors, all sample peak lists for the entire dataset were aligned to each other prior to formula assignment to eliminate possible mass shifts that would impact formula assignment. Putative chemical formulas were assigned using Formularity software (Tolić et al., 2017). Chemical formulas were assigned based on the following criteria: S/N > 7, and mass measurement error < 1 ppm, taking into

consideration the presence of C, H, O, N, S and P and excluding other elements. Peaks with large mass ratios (*m*/*z* values > 500 Da) often have multiple possible candidate formulas. These peaks were assigned formulas through propagation of CH_2, O, and H_2 homologous series. Additionally, to ensure consistent choice of molecular formula when multiple formula candidates are found the following rules were implemented: we consistently pick the formula with the lowest error with the lowest number of heteroatoms, and the assignment of one phosphorus atom requires the presence of at least four oxygen atoms. Peaks that were present in the blanks were subtracted from the sample data sets. Additionally, all single peaks i.e. peaks that are present in only one sample were removed and are not included in the downstream analysis. Overall, the organic compounds with assigned peaks from each extraction were classified into one of the following molecular groups: lipids, unsaturated-hydrocarbons, condensed-hydrocarbons, proteins, amino sugars, carbohydrates, lignin, or tannins. To investigate the compositional changes in soil organic matter with the cultivar treatment and depth, we calculated a composite composition by combining the common compounds that were observed in spectra produced by the two solvents (i.e., H_2O, and $CHCl_3$) and the compounds that were uniquely extracted by each of the two solvents (Tfaily et al., 2017; Tfaily et al., 2015). Within each sample, the percent abundance of each molecular group was calculated from the total compounds extracted.

Extraction efficiency of organic matter varies among soil types (Tfaily et al., 2017). Due to this, we use the relative abundance of each molecular group as an estimation for the biochemical profile of the entire soil. However, some relative

abundances may be over or underestimated due to C that we were unable to extract from the soil samples.

Statistics

Generalized linear models were created for each molecular group (lipids, unsaturated-hydrocarbons, condensed-hydrocarbons, proteins, amino sugars, carbohydrates, lignin, tannins) in base R version 3.6.0. Variables were tested for normality and then analysis of variance (ANOVA) was used to assess if molecular abundance varied between species, cultivar, depth, and interactions between those same factors with R package car. For each ANOVA that showed significance a Tukey HSD, run with R package agricolae, was used to determine which groups varied.

Principal components analysis (PCA) was used to assess how species, cultivar, and depth impacted the entire set of molecular abundances for the water and chloroform extracts with the princomp function in R.

CHAPTER THREE: RESULTS

Sequential Extraction – Biochemical Profile

For all organic molecular groups, the water extractions yielded higher percent abundances compared to the chloroform extractions. The one exception was the lipid percent abundance, which was higher in the chloroform extractions (Tab. 1, Tab. 2).

Water Extraction – Water Dissolved C

For the water extractions, 52.8-64.4% of the mass spectrometer peaks were assigned to organic molecular class groups, while 35.6-47.2% of the peaks were unclassified.

Overall, species did not affect the chemistry of the soil solution, as indicated by similar abundances of each one of the dissolved organic molecules across species (Tab. 1). However, when comparing all six cultivars across all depths there was a difference in the relative abundance of unsaturated hydrocarbons (Tab. 1). Soil derived from the switchgrass cultivars Kanlow and Southlow had 0.3-0.6% lower mean abundances of unsaturated-hydrocarbons relative to soil associated with the switchgrass cultivar Cave-in-Rock, and relative to soil from all big bluestem cultivars ($p=0.0485$, Tab. 1).

Depth significantly affected the relative abundance of different water dissolved chemicals (Fig. 2, Fig. 3). Across both species and cultivars, the top soil (0-10cm) had significantly less lipids ($p<0.0001$), lignin ($p=0.0001$), and carbohydrates ($p=0.0002$) compared to the 10-20 cm and 20-30 cm depth increments (Tab. 1). Specifically, the top soil had 0.5-0.7% lower mean abundance of lipids, 0.7% lower mean abundance of

lignin, and 0.5-0.6% lower mean abundance of carbohydrates compared to the 10-20 cm and 20-30 cm depth increments. However, the top soil had the highest levels of tannins with a 0.5-0.6% higher mean abundance of tannins compared to the 10-20 cm and 20-30 cm depth increments ($p<0.0001$, Tab. 1). The 20-30 cm depth increment had 0.3% higher mean abundance of condensed-hydrocarbons ($p<0.0001$, Tab. 1), and had 0.2-0.5% lower mean abundance of amino-sugars ($p=0.0002$), 0.7-1% lower mean abundance of proteins ($p<0.0001$), and 0.4-0.5% lower mean abundance of unsaturated-hydrocarbons ($p<0.0001$) relative to the other depth increments (Tab. 1).

Species did not affect soil chemistry in each one of the depth increments, however, the effect of cultivar on the relative abundance of organic molecules varied by depth increment (Tab. 1). In the 0-10 cm depth increment, soil chemistry was similar across cultivars, but in the 10-20 cm depth increment, the relative abundance of carbohydrates and lignin varied between cultivars. Specifically, the mean percent abundance of carbohydrates was 0.5-1.25% higher in the soil solution derived from the switchgrass cultivar Kanlow and big bluestem cultivar Suther compared to all other cultivars ($p=0.0476$, Tab. 1). Furthermore, the mean percent abundance of lignin was 0.75-1.25% lower in the soil solution derived from big bluestem cultivars Southlow and Suther than the cultivar Bonanza and all switchgrass cultivars ($p=0.0069$, Tab. 1). Finally, in the 20-30 cm depth increment, the relative abundances of proteins and amino-sugars differed among cultivars. Specifically, soil from the switchgrass cultivar Kanlow and big bluestem cultivar Bonanza had 0.4-1% lower mean abundances of dissolved proteins compared to all other cultivars ($p=0.0395$, Tab. 1). In addition, soil derived from

the switchgrass cultivar Kanlow had the 0.5-1.25% lower mean abundance of amino-sugars ($p=0.0383$, Tab. 1).

Chloroform Extraction – Sorbed C

For the chloroform extractions, 12.9-46.9% of the mass spectrometer peaks were assigned to organic molecular class groups, while 53.1-87.1% of the peaks were unclassified. Across all chloroform samples, lipids made up more than half of the classified organic peaks.

Overall, species type did not impact the sorbed chemical profile, as supported by similar observed molecular abundances across the big bluestem and switchgrass chloroform extractions (Tab. 2). However, across all depths, there were significant differences among the six cultivars within the sorbed chemical profile (Fig. 4, Fig. 5). The switchgrass cultivar Kanlow had 5.5-8.5% higher mean abundance of lipids compared to Cave-in-Rock and Southlow cultivars as well as all big bluestem cultivars ($p=0.0194$, Tab. 2). Additionally, the switchgrass cultivar Southlow had 1-2% higher mean abundance of lignin ($p<0.0001$) and 0.7-1.7% higher mean abundance of proteins ($p<0.0001$) compared to Cave-in-Rock, Kanlow and all big bluestem cultivars (Tab. 2). Lastly, the big bluestem cultivar Southlow had 0.06% higher mean abundance of unsaturated-hydrocarbons compared to switchgrass cultivar Southlow ($p<0.0001$), and 0.05% higher mean abundance of tannins compared to all switchgrass and big bluestem cultivars ($p=0.0001$, Tab. 2).

Across both species and cultivars combined, soil depth had an impact on the sorbed chemicals for lignin, proteins, and lipids. The top soil (0-10 cm) had 0.5-1% higher mean abundance of lignin ($p=0.0021$), and 0.9-1% higher mean abundance of

proteins ($p=0.0014$) compared to the 10-20 cm and 20-30 cm layers (Tab. 2). While, the 20-30 cm soil layer had 3.5-4.5% higher mean abundance of lipids compared to the upper layers ($p=0.0059$, Tab. 2).

Species type did not affect the sorbed-chemical profile within each of the soil depth increments; however, cultivar type did impact the relative abundances of molecules across depth increments (Tab. 2). Within the 0-10 cm depth increment, the percent abundance of unsaturated-hydrocarbons and lignin varied between cultivars (Tab. 2). Specifically, soil derived from the big bluestem cultivar Southlow had 0.2% higher mean abundance of unsaturated-hydrocarbons compared only to the switchgrass cultivar Cave-in-Rock ($p<0.0001$, Tab. 2). Additionally, the mean percent abundance of lignin was 1.6-2% higher in soil derived from the switchgrass cultivar Southlow in the 0-10 cm depth increment compared to soil derived from the big bluestem cultivars Southlow and Bonanza ($p=0.0008$). Within the 10-20 cm soil depth increment, lipids, lignin, and proteins differed between cultivars. Cultivar Kanlow had an 8-17% higher mean percent abundance of lipids in the 10-20 cm layer than all other cultivars ($p=0.02449$, Tab. 2). Switchgrass cultivar Southlow had a 1.7-2.3% higher mean percent abundance of lignin in the 10-20 cm layer compared to big bluestem cultivars Bonanza and Southlow ($p<0.0001$, Tab. 2). The switchgrass cultivar Southlow also had 1.7% higher mean abundance of proteins in the 10-20 cm soil increment compared to the big bluestem cultivar Southlow ($p=0.0022$, Tab. 2). Finally, within the 20-30 cm soil increment, lipids, condensed-hydrocarbons, and lignin varied among cultivars (Tab. 2). The switchgrass cultivar Cave-in-Rock had 3-16% lower mean percent abundance of lipids ($p=0.0025$), and 0.6-0.9% higher mean abundance of condensed-hydrocarbons ($p<0.0001$) in the 20-

30 cm soil increment compared to all other cultivars (Tab. 2). Additionally, Cave-in-Rock had 1.6% higher mean abundances of lignin in the 20-30 cm depth increment compared only to big bluestem cultivar Southlow ($p=0.0011$, Tab. 2).

CHAPTER FOUR: DISCUSSION AND CONCLUSION

Our study yielded two main results: 1) soil depth significantly impacted soil biochemical profiles, but not every compound consistently increased or decreased in abundance with depth, and 2) soil biochemical profiles differed among plant cultivars, but not species, indicating the importance of genetic variability in driving the soil C cycle.

Soil depth significantly impacted the soil biochemical profiles, but not every compound consistently increased or decreased in abundance with depth (Fig. 6, Fig. 7). The relative abundance of unsaturated-hydrocarbons, amino sugars, proteins and tannins in the water dissolved pool was higher in the 0-10 cm depth increment than at greater depths. Furthermore, the abundance of lignin was higher in the sorbed C pool at 0-10 cm depth relative to greater depths. A greater relative abundance of these compounds at shallower compared to greater depths may be explained by greater inputs of root exudates, or plant litter to the topsoil, where the majority of plant roots are located (Bolan et al., 2011; Jobbagy & Jackson, 2001; Mikutta, 2009). The higher abundance of lignin in the sorbed C pool indicates that fresh plant inputs of lignin are quickly binding to mineral surfaces (Bolan et al., 2011). Greater input of these compounds at shallower depths is also supported by the high abundance of proteins in the sorbed C pool at 0-10 cm depth. These proteins may have been derived either directly from roots, or they may originate from microbial biomass (Kögel-Knabner 2017; Taylor & Williams, 2010), which is generally higher at shallower depths owing to greater inputs of root derived C.

In contrast to these results, the relative abundance of carbohydrates, lignin and lipids in the water dissolved pool increased with soil depth, as did the relative abundance of lipids in the sorbed pool. The increase in percent lipids and carbohydrates in the water dissolved C pool with depth likely reflects the decline in fresh root-derived inputs, resulting in a higher proportion of more persistent C. Both lipids and carbohydrates form an important structural component of soil microbes (Kögel-Knabner, 2002), and their residues are highly persistent in soil (Schmidt et al., 2011). Furthermore, a greater relative abundance of lignin at depth may be due to lower microbial activity, which may especially affect decomposition of substrates that are characterized by a complex chemical structure (Bolan et al., 2011; Rovira & Vallejo, 2002). Several studies have found that inputs of labile substrates synergistically increases the decomposition of more complex substrates (de Graaff et al., 2011). Thus, lower inputs of labile substrates at greater depths may lead to reduced decomposition rates of more persistent substrates. Finally, differential sorption affinities of the substrates and impacts of soil depth on sorption may explain differences in biochemical profiles among depths. The higher abundance of sorbed lipids in the 20-30 cm layer relative to the 0-10 cm layer may be explained by a higher water dissolved C absorption rate with depth (Jardine et al., 1989). The affinity of both lignin and lipids to bind to soil minerals may explain the depletion of these compounds in the water dissolved pool in the topsoil. In both cases, an increase in the compound in the water dissolved C pool was accompanied by an increase in the sorbed C pool. This indicates that lignin and lipids have a high binding affinity to minerals (Bolan et al., 2011). In our soils, lipids made up more than half of the assigned peaks for all the sorbed C. Our data confirm that shallower depths of soil tend to have

higher amounts of recently deposited water dissolved C, while at greater depths the C pool is dominated by older, more stable C (Bernoux et al., 1998; Jobbagy & Jackson, 2001).

The switchgrass cultivars Kanlow and Southlow were the most distinct in their biochemical profiles relative to each other and other cultivars. Kanlow had different molecular abundances of both labile C and persistent C in comparison to all the other cultivars. Within the water dissolved C pool, Kanlow had higher abundances of carbohydrates in the 10-20 cm depth increment, and lower abundances of proteins and amino sugars in the 20-30 cm depth increment compared to other cultivars. Amino sugars are a unique product of microbial activity (Kögel-Knabner, 2002), thus the lower amino sugars for Kanlow relative to other cultivars may indicate a decrease in microbial residue formation. This could lead to a reduction in the formation and maintenance of stable C at depth (Rovira & Vallejo, 1997; Rovira & Vallejo, 2002). The differences of carbohydrate and protein abundance in the water dissolved C pool for Kanlow suggests that Kanlow may be releasing exudates that are different than those exuded by other cultivars. Namely, the water dissolved C pool is composed of fresh C inputs from plants (ie. exudates) (Fuß, 2009). Additionally, Kanlow had a 5.5-8.5% higher mean relative abundance of lipids in the sorbed C pool compared to all other cultivars across all depths. The amount of C sorption relies heavily on the source of C and the types of minerals present in the soil (Mikutta, 2009; Sanderman et al., 2014; Scow et al., 1995). Given that mineral composition is similar across our soils, and that aboveground biomass is removed on an annual basis, the differences between sorbed chemical profiles among cultivars further supports that cultivars vary in root-derived inputs of C. The high levels of lipids in

the Kanlow sorbed C pool suggests that Kanlow has a higher lipid plant structure, releases exudates with higher concentrations of lipids, or has a microbial community that is composed of or exudes lipids (Burns et al., 2013; Kögel-Knabner, 2017). Previous work has also identified that switchgrass cultivars vary in rhizosphere C chemistry (Stewart et al., 2017). Thus, there appears to be genetic variability in the chemical composition of root exudates or root litter, and this variability may affect soil C stabilization.

Soil C data collected in 2012, four years after plant establishment, show that Kanlow has a lower concentration of plant-derived C compared to the other cultivars. (Adkins et al., in review). Given that Kanlow has a similar root structure to switchgrass cultivars Cave-in-Rock and Southlow (de Graaff et al., 2013), the lower plant-derived C input may be contributed to the differences in C inputs and their interactions with the microbial community. Recent studies have found that lipid abundance is negatively correlated with total soil C (Bailey et al., 2017), supporting the theory that persistent C is not the main predictor of soil C stabilization (Chenu et al., 2019; Kallenbach et al., 2015; Schmidt et al., 2011; Wallenstein et al., 2007). Instead labile C inputs interacting with microbes and forming microbial residues is a stronger predictor of soil C formation (Chenu et al., 2019; Kallenbach et al., 2015; Schmidt et al., 2011; Wallenstein et al., 2007). Thus, the lower abundances of amino sugars in the Kanlow biochemical profile, which may indicate less microbial residue formation, may explain lower plant-derived C in Kanlow relative to other switchgrass cultivars.

The switchgrass cultivar Southlow also had contrasting molecular abundances compared to the other cultivars, but only in the sorbed C pool. Across all soil depths the

switchgrass cultivar Southlow had higher abundances of lignin and proteins compared to all other cultivars in the sorbed C pool. Proteins that adhere to minerals are more likely to be derived from microbial inputs, and may represent stable microbial residues (Kögel-Knabner 2017; Taylor & Williams, 2010). Therefore, a greater relative abundance in proteins in the sorbed C pool may indicate stable C formation. Furthermore, lignin is a more persistent plant derived substrate that may aid in soil C retention, at least in the shorter term. Lignin is persistent in soil because it can only be fully decomposed by certain fungi, and due to its oxidative decomposition it can only be broken down in anaerobic conditions (Kirk & Farrell, 1987; Kögel-Knabner, 2002). Thus, higher abundances of lignin can contribute to soil C stabilization in the short-term while environmental conditions limit the decomposition of lignin (Schmidt et al., 2011). In 2012, the switchgrass cultivar Southlow had elevated levels of plant-derived C compared to other switchgrass cultivars. Our data suggest that this may be linked directly to increased protein formation and relatively high quantities of lignin in root tissue material.

Our data, along with previous studies on soil C storage in these ecosystems, suggest that both root-C input rates and chemistry of root-derived C input may drive root derived soil C stabilization. Although notable differences were not observed between species, genetic variation of cultivars led to variability in cultivar biochemical profiles. This is interesting, given that soil C accumulation differs significantly between big bluestem and switchgrass, with a much greater rate of C accumulation in big bluestem soils since the start of the experiment (Adkins et al., in review). Lower root derived C in switchgrass relative to big bluestem, may be related to lower root derived C inputs. Indeed, our preliminary data indicate that big bluestem cultivars have more root biomass

than switchgrass cultivars, and root C input is a function of root biomass (Kong & Six, 2010; Rasse et al., 2005). Models currently use root biomass as the sole parameter to predict soil C influx and stabilization (Woolf & Lehmann, 2019), but our data indicate that the chemical composition of root derived C influx, driven by genetic variability of the cultivar, may be another important predictor of how roots might affect soil C cycling, particularly when root biomass is similar across cultivars. Therefore, adapting planting strategies for biofuel and agricultural industries like cultivar selection that promote soil C formation and stability may help mitigate the effects of climate change. Further research into evaluating differences among cultivar exudate composition, microbial community composition, and soil respiration would help to disentangle this complicated process of how plant-derived C contributes to soil C formation and stabilization.

REFERENCES

Adkins, J., Jastrow, J. D., Morris, G. P., Six, J., & de Graaff, M. A. (2016). Effects of switchgrass cultivars and intraspecific differences in root structure on soil carbon inputs and accumulation. Geoderma, 262, 147-154.

Adkins, J., Jastrow, J. D., Morris, & de Graaff, M. A. (2019). Unpublished: Effects of fertilization, plant species, and intra-specific diversity on soil carbon in biofuel cropping systems.

Badri, D. V., & Vivanco, J. M. (2009). Regulation and function of root exudates. Plant, Cell & Environment, 32(6), 666-681.

Bailey, V. L., Smith, A. P., Tfaily, M., Fansler, S. J., & Bond-Lamberty, B. (2017). Differences in soluble organic carbon chemistry in pore waters sampled from different pore size domains. *Soil Biology and Biochemistry*, *107*, 133-143.

Baldock, J. A., & Skjemstad, J. O. (2000). Role of the soil matrix and minerals in protecting natural organic materials against biological attack. *Organic geochemistry*, *31*(7-8), 697-710.

Baudoin, E., Benizri, E., & Guckert, A. (2003). Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. *Soil Biology and Biochemistry*, *35*(9), 1183-1192.

Bernoux, M., Cerri, C. C., Neill, C., & de Moraes, J. F. (1998). The use of stable carbon isotopes for estimating soil organic matter turnover rates. *Geoderma*, *82*(1-3), 43-58.

Bolan, N. S., Adriano, D. C., Kunhikrishnan, A., James, T., McDowell, R., & Senesi, N. (2011). Dissolved organic matter: biogeochemistry, dynamics, and environmental significance in soils. In *Advances in agronomy* (Vol. 110, pp. 1-75). Academic Press.

Bollag, J. M. (2017). Soil Biochemistry: Volume 6: Volume 6.

Burns, R. G., DeForest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E., Wallenstein, M. D., ... & Zoppini, A. (2013). Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biology and Biochemistry*, *58*, 216-234.

Carvalhais, L. C., Dennis, P. G., Fedoseyenko, D., Hajirezaei, M. R., Borriss, R., & von Wirén, N. (2011). Root exudation of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. Journal of Plant Nutrition and Soil Science, 174(1), 3-11.

Chenu, C., Angers, D. A., Barré, P., Derrien, D., Arrouays, D., & Balesdent, J. (2019). Increasing organic stocks in agricultural soils: Knowledge gaps and potential innovations. *Soil and Tillage Research*, *188*, 41-52.

de Graaff, M. A., Six, J., Jastrow, J. D., Schadt, C. W., & Wullschleger, S. D. (2013). Variation in root architecture among switchgrass cultivars impacts root decomposition rates. Soil Biology and Biochemistry, 58, 198-206.

de Graaff, M. A., Schadt, C. W., Rula, K., Six, J., Schweitzer, J. A., & Classen, A. T. (2011). Elevated CO2 and plant species diversity interact to slow root decomposition. *Soil Biology and Biochemistry*, *43*(11), 2347-2354.

Frey, S. D., Lee, J., Melillo, J. M., & Six, J. (2013). The temperature response of soil microbial efficiency and its feedback to climate. Nature Climate Change, 3(4), 395.

Fuß, R. (2009). Diffusion processes of soluble organic substances in soil and their effect on ecological processes (Doctoral dissertation, Technische Universität München).

Gelfand, I., Zenone, T., Jasrotia, P., Chen, J., Hamilton, S. K., & Robertson, G. P. (2011). Carbon debt of Conservation Reserve Program (CRP) grasslands converted to bioenergy production. Proceedings of the National Academy of Sciences, 108(33), 13864-13869.

Gleixner, G. (2013). Soil organic matter dynamics: a biological perspective derived from the use of compound-specific isotopes studies. Ecological research, 28(5), 683-695.

Huang, X. F., Chaparro, J. M., Reardon, K. F., Zhang, R., Shen, Q., & Vivanco, J. M. (2014). Rhizosphere interactions: root exudates, microbes, and microbial communities. Botany, 92(4), 267-275.

Jardine, P. M., McCarthy, J. F., & Weber, N. L. (1989). Mechanisms of dissolved organic carbon adsorption on soil. *Soil Science Society of America Journal*, *53*(5), 1378-1385.

Jobbágy, E. G., & Jackson, R. B. (2001). The distribution of soil nutrients with depth: global patterns and the imprint of plants. *Biogeochemistry*, *53*(1), 51-77.

Kallenbach, C. M., Frey, S. D., & Grandy, A. S. (2016). Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature communications*, *7*, 13630.

Kallenbach, C. M., Grandy, A. S., Frey, S. D., & Diefendorf, A. F. (2015). Microbial physiology and necromass regulate agricultural soil carbon accumulation. Soil Biology and Biochemistry, 91, 279-290.

Keiluweit, M., Wanzek, T., Kleber, M., Nico, P., & Fendorf, S. (2017). Anaerobic microsites have an unaccounted role in soil carbon stabilization. *Nature communications*, *8*(1), 1771.

Kirk, T. K., & Farrell, R. L. (1987). Enzymatic" combustion": the microbial degradation of lignin. *Annual Reviews in Microbiology*, *41*(1), 465-501.

Kögel-Knabner, I. (2017). The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter: fourteen years on. *Soil Biology and Biochemistry*, *105*, A3-A8.

Kögel-Knabner, I. (2002). The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry, 34(2), 139-162.

Kong, A. Y., & Six, J. (2010). Tracing root vs. residue carbon into soils from conventional and alternative cropping systems. Soil Science Society of America Journal, 74(4), 1201-1210.

Lal R (2004) Soil carbon sequestration to mitigate climate change. Geoderma 123, 1-22.

Liang, C., Schimel, J. P., & Jastrow, J. D. (2017). The importance of anabolism in microbial control over soil carbon storage. *Nature Microbiology*, *2*(8), 17105.

Lynch, J. M., & Whipps, J. M. (1990). Substrate flow in the rhizosphere. *Plant and soil*, *129*(1), 1-10.

Mao, Y., Li, X., Smyth, E. M., Yannarell, A. C., & Mackie, R. I. (2014). Enrichment of specific bacterial and eukaryotic microbes in the rhizosphere of switchgrass (P anicum virgatum L.) through root exudates. Environmental microbiology reports, 6(3), 293-306.

Mikutta, Robert (2009). "Biogeochemistry of mineral–organic associations across a long-term mineralogical soil gradient (0.3–4100kyr), Hawaiian Islands". *Geochimica et cosmochimica acta* (0016-7037), 73 (7), p. 2034.

Miltner, A., Bombach, P., Schmidt-Brücken, B., & Kästner, M. (2012). SOM genesis: microbial biomass as a significant source. Biogeochemistry, 111(1-3), 41-55.

Morris, G. P., Hu, Z., Grabowski, P. P., Borevitz, J. O., Graaff, M. A., Miller, R. M., & Jastrow, J. D. (2016). Genotypic diversity effects on biomass production in native perennial bioenergy cropping systems. GCB Bioenergy, 8(5), 1000-1014.

Poirier, N., Sohi, S. P., Gaunt, J. L., Mahieu, N., Randall, E. W., Powlson, D. S., & Evershed, R. P. (2005). The chemical composition of measurable soil organic matter pools. *Organic Geochemistry*, *36*(8), 1174-1189.

Qiu, Q., Wu, L., Ouyang, Z., Li, B., Xu, Y., Wu, S., & Gregorich, E. G. (2015). Effects of plant-derived dissolved organic matter (DOM) on soil CO2 and N2O emissions and soil carbon and nitrogen sequestrations. *Applied Soil Ecology*, *96*, 122-130.

Rasmussen, C., Heckman, K., Wieder, W. R., Keiluweit, M., Lawrence, C. R., Berhe, A. A., ... & Marin-Spiotta, E. (2018). Beyond clay: towards an improved set of

variables for predicting soil organic matter content. *Biogeochemistry*, *137*(3), 297-306.

Rasse, D. P., Rumpel, C., & Dignac, M. F. (2005). Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and soil*, *269*(1-2), 341-356.

Rovira, P., & Vallejo, V. R. (2002). Labile and recalcitrant pools of carbon and nitrogen in organic matter decomposing at different depths in soil: an acid hydrolysis approach. *Geoderma*, *107*(1-2), 109-141.

Rovira, P., & Vallejo, V. R. (1997). Organic carbon and nitrogen mineralization under Mediterranean climatic conditions: the effects of incubation depth. *Soil Biology and Biochemistry*, *29*(9-10), 1509-1520.

Sanderman, J., Maddern, T., & Baldock, J. (2014). Similar composition but differential stability of mineral retained organic matter across four classes of clay minerals. *Biogeochemistry*, *121*(2), 409-424.

Schmidt, M. W., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., ... & Nannipieri, P. (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, *478*(7367), 49.

Schrumpf, M., Kaiser, K., Guggenberger, G., Persson, T., Kögel-Knabner, I., & Schulze, E. D. (2013). Storage and stability of organic carbon in soils as related to depth, occlusion within aggregates, and attachment to minerals. *Biogeosciences*, *10*, 1675-1691.

Scow, K. M., Fan, S., Johnson, C., & Ma, G. M. (1995). Biodegradation of sorbed chemicals in soil. *Environmental health perspectives*, *103*(suppl 5), 93-95.

Sollins, P., Homann, P., & Caldwell, B. A. (1996). Stabilization and destabilization of soil organic matter: mechanisms and controls. *Geoderma*, *74*(1-2), 65-105.

Stewart, C. E., Roosendaal, D., Denef, K., Pruessner, E., Comas, L. H., Sarath, G., ... & Soundararajan, M. (2017). Seasonal switchgrass ecotype contributions to soil organic carbon, deep soil microbial community composition and rhizodeposit uptake during an extreme drought. *Soil Biology and Biochemistry*, *112*, 191-203.

Syswerda, S. P., Corbin, A. T., Mokma, D. L., Kravchenko, A. N., & Robertson, G. P. (2011). Agricultural management and soil carbon storage in surface vs. deep layers. *Soil Science Society of America Journal*, *75*(1), 92-101.

Taylor, E. B., & Williams, M. A. (2010). Microbial protein in soil: influence of extraction method and C amendment on extraction and recovery. *Microbial ecology*, *59*(2), 390-399.

Tfaily, M. M., Chu, R. K., Toyoda, J., Tolić, N., Robinson, E. W., Paša-Tolić, L., & Hess, N. J. (2017). Sequential extraction protocol for organic matter from soils and sediments using high resolution mass spectrometry. Analytica chimica acta, 972, 54-61.

Tfaily, M. M., Chu, R. K., Tolić, N., Roscioli, K. M., Anderton, C. R., Paša-Tolić, L., ... & Hess, N. J. (2015). Advanced solvent based methods for molecular characterization of soil organic matter by high-resolution mass spectrometry. *Analytical chemistry*, *87*(10), 5206-5215.

Tolić, N., Liu, Y., Liyu, A., Shen, Y., Tfaily, M. M., Kujawinski, E. B., ... & Hess, N. J. (2017). Formularity: software for automated formula assignment of natural and other organic matter from ultrahigh-resolution mass spectra. *Analytical chemistry*, *89*(23), 12659-12665.

Wallenstein, M. D., Denef, K., Boot, C. M., Cotrufo, M. F., & Paul, E. A. (2007). Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global Change Biology (2013) 19, 988–995.

Woolf, D., & Lehmann, J. (2019). Microbial models with minimal mineral protection can explain long-term soil organic carbon persistence. *Scientific reports*, *9*(1), 6522.

Zhu, Z., Ge, T., Hu, Y., Zhou, P., Wang, T., Shibistova, O., ... & Wu, J. (2017). Fate of rice shoot and root residues, rhizodeposits, and microbial assimilated carbon in paddy soil-part 2: turnover and microbial utilization. Plant and Soil, 416(1-2), 243-257.

Table 1. Totals from water extractions

Table 1. Total % lipids, unsaturated-hydrocarbons, condensed-hydrocarbons, proteins, amino sugars, carbohydrates, lignin, and tannins in water extractions from samples of *Andropogon gerardii* and *Panicum virgatum* cultivars at varying depths collected at the U.S. Department of Energy National Environmental Research Park at Fermilab in Batavia, IL in June 2018.

Species	Cultivar	Depth (cm)	Lipids (%)	Unsaturated-Hydrocarbons(%)	Condensed-Hydrocarbons (%)	Proteins (%)	Amino Sugars (%)	Carbohydrates (%)	Lignin (%)	Tannins (%)
Andropogon gerardii	Bonanza	0-10	6.91 ± 0.07	1.73 ± 0.09	1.54 ± 0.08	12.68 ± 0.21	6.74 ± 0.27	13.30 ± 0.68	14.60 ± 0.57	2.39 ± 0.07
		10--20	7.49 ± 0.20	1.57 ± 0.08	1.40 ± 0.04	12.36 ±0.26	6.48 ± 0.06	14.42 ± 0.22	15.83 ± 0.24	1.88 ± 0.07
		20-30	7.11 ± 0.33	1.13 ± 0.14	1.97 ± 0.12	11.27 ±0.17	6.33 ± 0.29	14.63 ± 0.52	18.69 ± 0.35	1.85 ± 0.12
	Southlow	0-10	6.90 ± 0.25	1.73 ± 0.08	1.56 ± 0.09	12.89 ±0.55	6.79 ± 0.25	14.39 ± 0.29	15.02 ± 0.14	2.36 ± 0.10
		10--20	7.40 ± 0.10	1.68 ± 0.07	1.54 ± 0.11	12.67 ±0.35	6.82 ± 0.25	14.73 ± 0.15	14.68 ±0.12	1.85 ± 0.02
		20-30	7.13 ± 0.16	1.22 ± 0.06	1.67 ± 0.05	12.11 ±0.27	6.51 ± 0.13	14.50 ± 0.24	14.91 ±0.22	1.85 ± 0.03
	Suther	0-10	6.45 ± 0.20	1.77 ± 0.03	1.66 ± 0.14	12.91 ±0.37	7.36 ± 0.14	14.73 ± 0.05	14.84 ±0.15	2.91 ± 0.18
		10--20	7.01 ± 0.23	1.81 ± 0.11	1.55 ± 0.04	12.41 ± 0.23	7.03 ± 0.12	15.37 ± 0.45	14.81 ±0.19	1.95 ± 0.06
		20-30	7.19 ± 0.28	1.04 ± 0.10	1.95 ± 0.08	11.76 ± 0.12	6.36 ± 0.35	15.10 ± 0.46	16.20 ±0.40	2.03 ± 0.06
Panicum virgatum	Cave-in-Rock	0-10	6.93 ± 0.19	1.48 ± 0.05	1.57 ± 0.10	12.93 ± 0.33	6.95 ± 0.19	13.95 ± 0.48	15.24 ± 0.21	2.58 ± 0.29
		10--20	7.02 ± 0.12	1.28 ± 0.15	1.79 ± 0.31	12.11 ± 0.34	6.37 ± 0.34	14.10 ± 0.35	15.83 ±0.42	2.26 ± 0.10
		20-30	7.19 ± 0.11	0.94 ± 0.06	1.75 ± 0.16	11.98 ± 0.38	6.05 ± 0.11	14.50 ± 0.23	16.21 ±0.44	2.11 ± 0.14
	Kanlow	0-10	6.86 ± 0.25	1.38 ± 0.12	1.48 ± 0.08	12.74 ± 0.43	6.60 ± 0.36	11.89 ± 1.35	14.28 ± 0.67	2.51 ± 0.08
		10--20	7.6 ± 0.27	1.21 ± 0.06	1.61 ± 0.07	12.79 ± 0.26	7.10 ± 0.15	15.40 ± 0.11	15.52 ± 0.05	2.35 ± 0.01
		20-30	7.77 ± 0.03	0.98 ± 0.13	1.75 ± 0.09	11.29 ± 0.27	5.51 ± 0.37	14.04 ± 0.39	15.64 ± 0.28	1.64 ± 0.09
	Southlow	0-10	6.85 ± 0.11	1.61 ± 0.14	1.47 ± 0.07	13.08 ± 0.35	6.74 ± 0.37	12.77 ± 1.12	15.04 ± 0.27	2.64 ± 0.12
		10--20	7.64 ± 0.17	1.00 ± 0.08	1.50 ± 0.08	12.72 ± 0.12	6.92 ± 0.38	14.79 ± 0.55	15.66 ± 0.63	2.36 ± 0.22
		20-30	7.35 ± 0.10	0.92 ± 0.10	1.82 ± 0.11	11.98 ± 0.25	6.77 ± 0.10	15.47 ± 0.21	15.88 ± 0.33	2.14 ± 0.28
Sources of Variation										
	Species		ns	ns	ns	ns	ns	ns	ns	ns
	Cultivar		ns	*	ns	ns	ns	ns	ns	ns
	Depth		*	*	*	*	*	*	*	*
	Cultivar*Depth		ns	ns	ns	*	*	*	*	ns

Mean ± SE

*significant at the 0.05 level

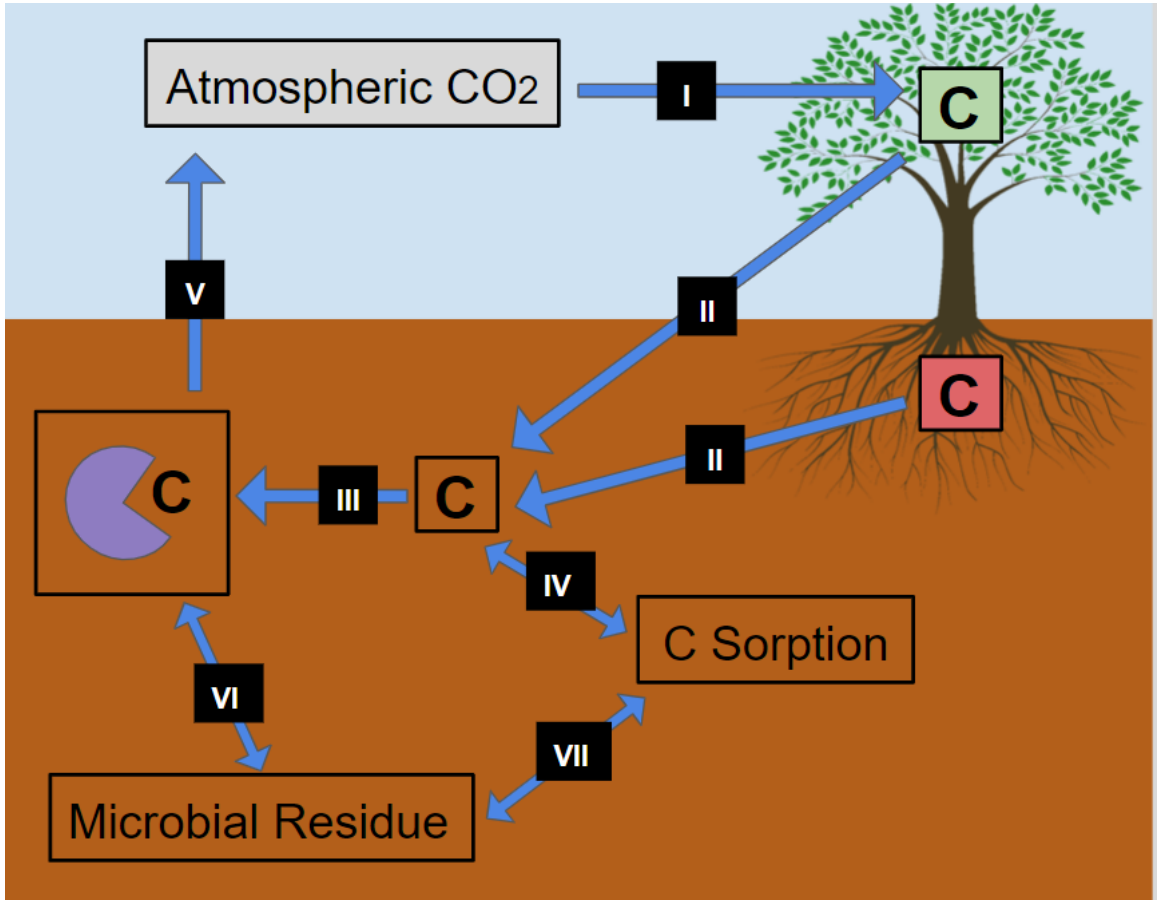
Table 2 Totals from chloroform extractions

Table 2. Total % lipids, unsaturated-hydrocarbons, condensed-hydrocarbons, proteins, amino sugars, carbohydrates, lignin, and tannins in chloroform extractions from samples of *Andropogon gerardii* and *Panicum virgatum* cultivars at varying depths collected at the U.S. Department of Energy National Environmental Research Park at Fermilab in Batavia, IL in June 2018.

Species	Cultivar	Depth (cm)	Lipids (%)	Unsaturated-Hydrocarbons(%)	Condensed-Hydrocarbons (%)	Proteins (%)	Amino Sugars (%)	Carbohydrates (%)	Lignin (%)	Tannins (%)
Andropogon gerardii	Bonanza	0-10	29.00 ± 1.12	0.40 ± 0.03	0.36 ± 0.10	2.54 ± 0.28	0.08 ± 0.02	0.03 ± 0.01	1.43 ± 0.27	0.01 ± 0.01
		10--20	24.33 ± 3.59	0.36 ± 0.04	0.51 ± 0.12	2.19 ± 0.25	0.06 ± 0.02	0.04 ± 0.04	1.01 ± 0.22	0.01 ± 0.01
		20-30	30.45 ± 0.77	0.43 ± 0.05	0.52 ± 0.04	2.25 ± 0.25	0.11 ± 0.02	0.03 ± 0.03	1.01 ± 0.06	0.01 ± 0.01
	Southlow	0-10	31.27 ± 1.52	0.51 ± 0.02	0.51 ± 0.11	2.80 ± 0.24	0.09 ± 0.01	0.00 ± 0	1.70 ± 0.11	0.03 ± 0.02
		10--20	21.24 ± 4.78	0.36 ± 0.08	0.27 ± 0.08	1.57 ± 0.35	0.07 ± 0.03	0.01 ± 0.01	0.44 ± 0.09	0.04 ± 0.02
		20-30	33.13 ± 2.33	0.53 ± 0.05	0.50 ± 0.11	1.73 ± 0.13	0.08 ± 0.02	0.02 ± 0.01	0.81 ± 0.03	0.02 ± 0.01
	Suther	0-10	27.27 ± 0.88	0.44 ± 0.02	0.72 ± 0.15	3.29 ± 0.26	0.10 ± 0.02	0.02 ± 0.01	2.14 ± 0.26	0.02 ± 0.01
		10--20	28.24 ± 0.36	0.44 ± 0.02	0.68 ± 0.13	2.69 ± 0.26	0.11 ± 0.02	0.01 ± 0.01	1.48 ± 0.25	0.00 ± 0
		20-30	40.12 ± 2.92	0.42 ± 0.04	0.39 ±0.09	2.17 ± 0.44	0.12 ± 0.03	0.03 ± 0.01	1.03 ± 0.21	0.01 ± 0.01
Panicum virgatum	Cave-in-Rock	0-10	28.90 ± 3.70	0.30 ± 0.05	0.35 ± 0.14	3.70 ± 0.96	0.09 ± 0.03	0.02 ± 0.01	2.06 ± 0.53	0.00 ± 0
		10--20	27.48 ± 2.38	0.34 ± 0.03	0.58 ± 0.18	2.11 ± 0.41	0.07 ± 0.04	0.03 ± 0.01	1.41 ± 0.50	0.02 ± 0.02
		20-30	28.80 ± 1.77	0.35 ± 0.05	1.09 ± 0.15	3.23 ± 0.32	0.10 ± 0.01	0.03 ± 0.02	2.32 ± 0.32	0.00 ± 0
	Kanlow	0-10	33.79 ± 1.51	0.38 ± 0.01	0.41 ± 0.12	3.04 ± 0.63	0.11 ± 0.03	0.00 ± 0	1.94 ± 0.32	0.00 ± 0
		10--20	37.11 ± 2.10	0.37 ± 0.08	0.42 ± 0.12	2.36 ± 0.07	0.08 ± 0.02	0.04 ± 0.04	1.53 ± 0.14	0.00 ± 0
		20-30	36.85 ± 3.50	0.36 ± 0.09	0.50 ± 0.06	2.20 ± 0.31	0.16 ± 0.05	0.01 ± 0.02	0.96 ± 0.12	0.00 ± 0
	Southlow	0-10	29.10 ± 1.00	0.31 ± 0.04	0.72 ± 0.09	4.24 ± 0.38	0.09 ± 0.01	0.02 ± 0.02	3.31 ± 0.38	0.01 ± 0.01
		10--20	32.04 ± 4.69	0.30 ± 0.05	0.84 ± 0.27	3.49 ± 0.52	0.09 ± 0.02	0.00 ± 0	2.71 ± 0.56	0.00 ± 0
		20-30	30.57 ± 1.39	0.30 ± 0.05	0.39 ± 0.15	2.29 ± 0.57	0.09 ± 0.04	0.01 ± 0.01	1.83 ± 0.71	0.00 ± 0
Sources of Variation										
	Species		ns	ns	ns	ns	ns	ns	ns	ns
	Cultivar		*	*	ns	*	ns	ns	*	*
	Depth		*	ns	ns	*	ns	ns	*	ns
	Cultivar*Depth		*	*	*	*	ns	ns	*	ns

Mean ± SE

*significant at the 0.05 level



I. Atmospheric CO_2 is taken up by plants and converted to C building blocks. This plant C is both aboveground in the leaves and branches, and belowground in the roots and root exudates.

II. Above and belowground plant C contributes to soil C formation through leaf litter turnover, root turnover, and root exudate release.

III. Soil C in the water dissolved C pool can interact with the soil microbial community. The outcome of this interaction is driven by chemistry of C inputs and physiology of the microbe.

IV. Soil C in the water dissolved C pool can also become sorbed or chemically bonded to a mineral or clay soil particle. Sorption is driven by soil mineralogy and C chemistry. Sorbed C can remain in the soil or it can re-enter into the water dissolved C pool.

V. C that interacts with the soil microbial community can be ingested and respired into the atmosphere as CO_2.

VI. C can also be ingested by the microbial community and incorporated into the microbial biomass. When microbes die this C becomes microbial residue.

VII. Microbial residue can either stay in the active C pool and interact with the microbial community possibly being respired or stored as microbial biomass, or it can sorb and be physically protected from microbial interaction.

Figure 1. Conceptual model depicting the relationship between atmopsheric C, plant derived C, and soil C. See text in grey box.

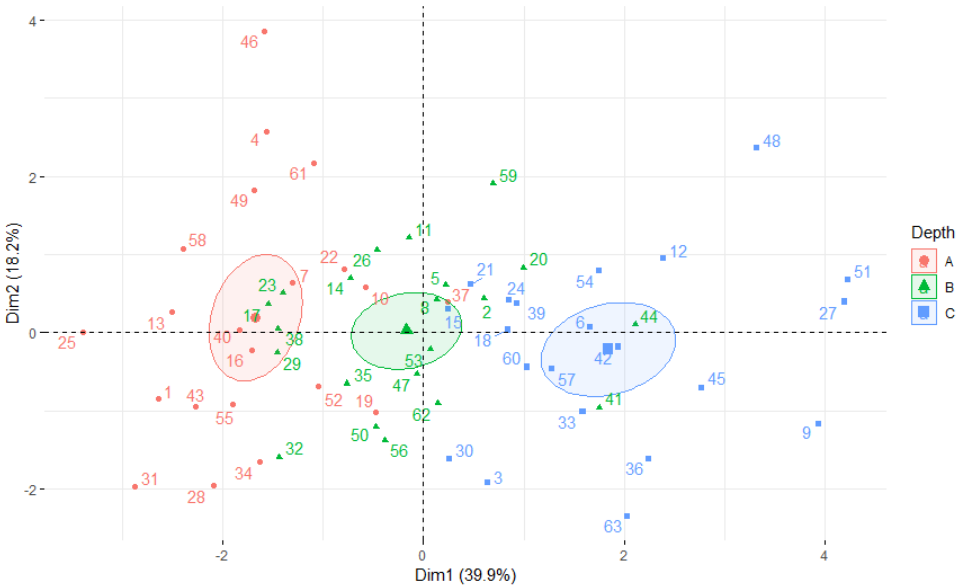


Figure 2. Principal Component Analysis of water-soluble chemicals from samples of *Andropogon gerardii* and *Panicum virgatum* collected at the U.S. Department of Energy National Environmental Research Park at Fermilab in Batavia, IL in June 2018. Ellipses depicted 95% confidence intervals for the different depths (A: 0-10, B: 10-20, and C: 20-30 cm).

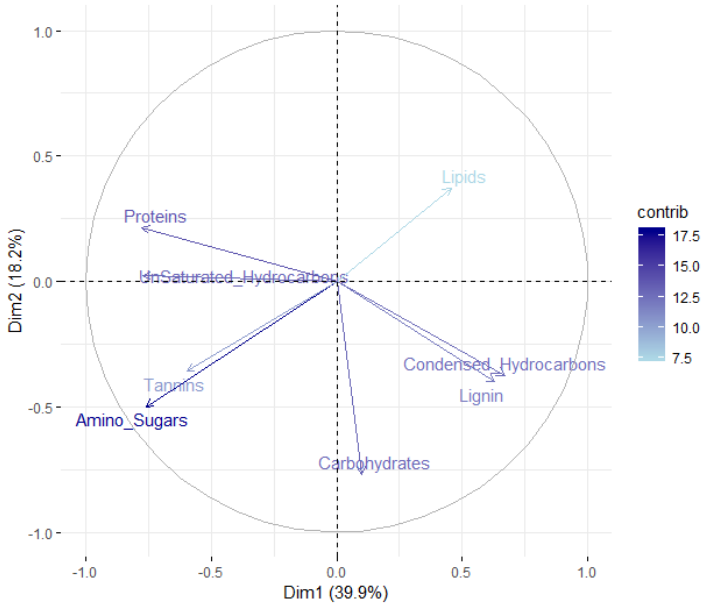


Figure 3. Principal Component Analysis of water-soluble chemicals from samples of *Andropogon gerardii* and *Panicum virgatum* collected at the U.S. Department of Energy National Environmental Research Park at Fermilab in Batavia, IL in June 2018. Arrows indicate the amount of variance each molecular group contributed to the overall variance.

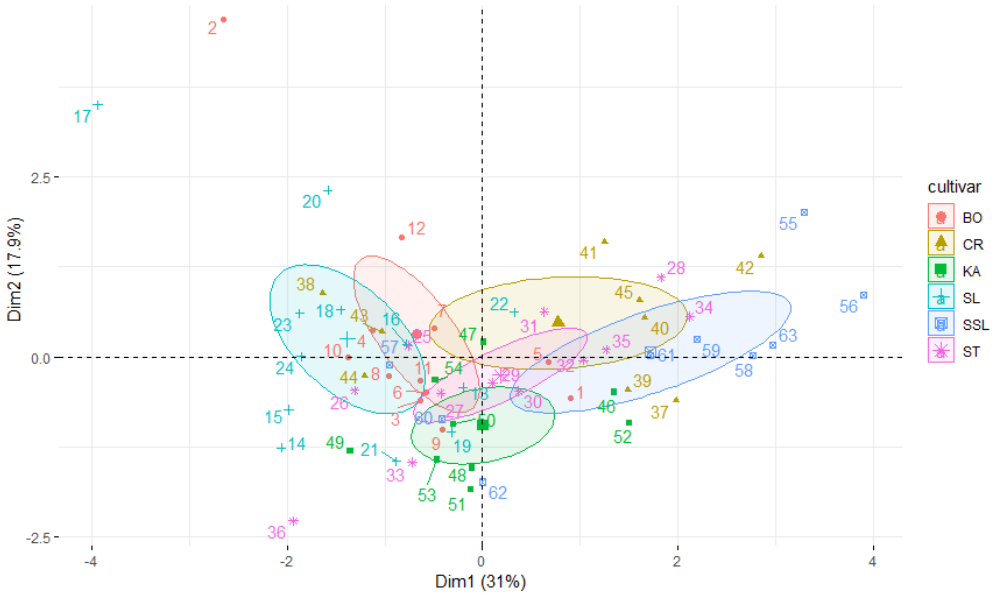


Figure 4. Principal Component Analysis of chloroform extracted chemicals from samples of *Andropogon gerardii* and *Panicum virgatum* collected at the U.S. Department of Energy National Environmental Research Park at Fermilab in Batavia, IL in June 2018. Ellipses depicted 95% confidence intervals for the different cultivars.

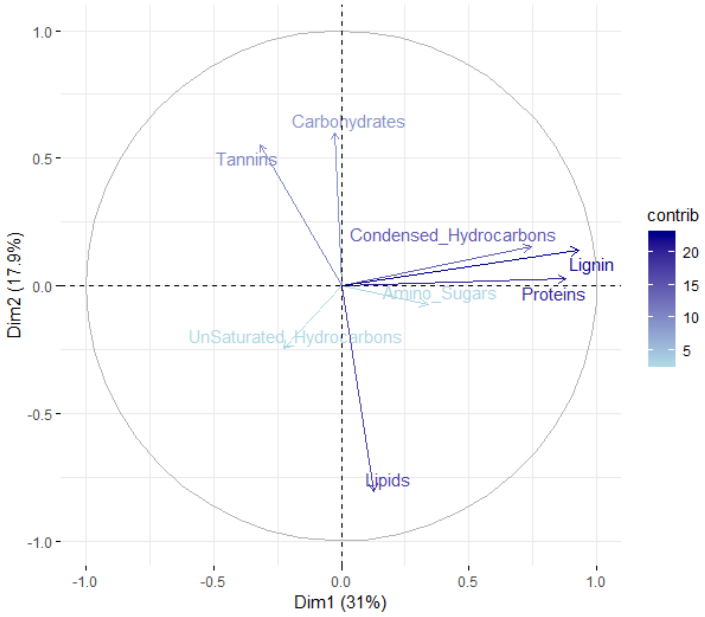


Figure 5. Principal Component Analysis of chloroform extracted chemicals from samples of *Andropogon gerardii* and *Panicum virgatum* collected at the U.S. Department of Energy National Environmental Research Park at Fermilab in Batavia, IL in June 2018. Arrows indicate the amount of variance each molecular group contributed to the overall variance.

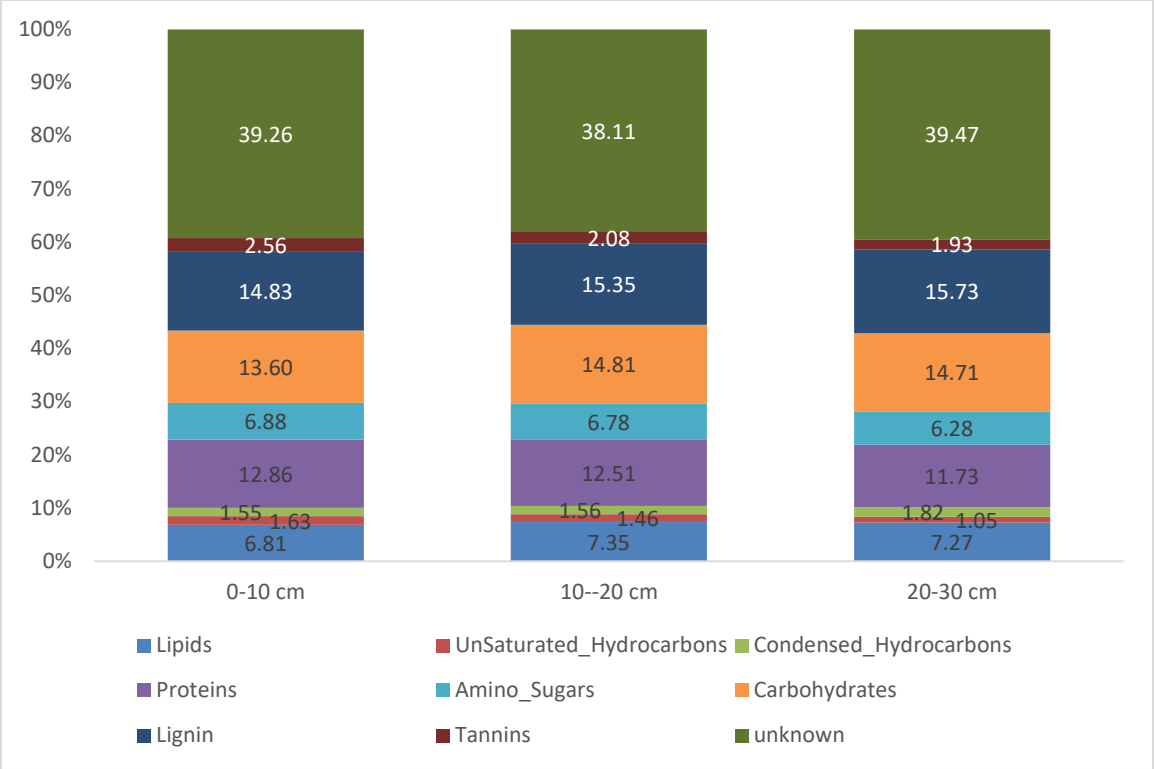


Figure 6. Average relative abundance of each molecule at the different soil depth increments in the water dissolved C pool.

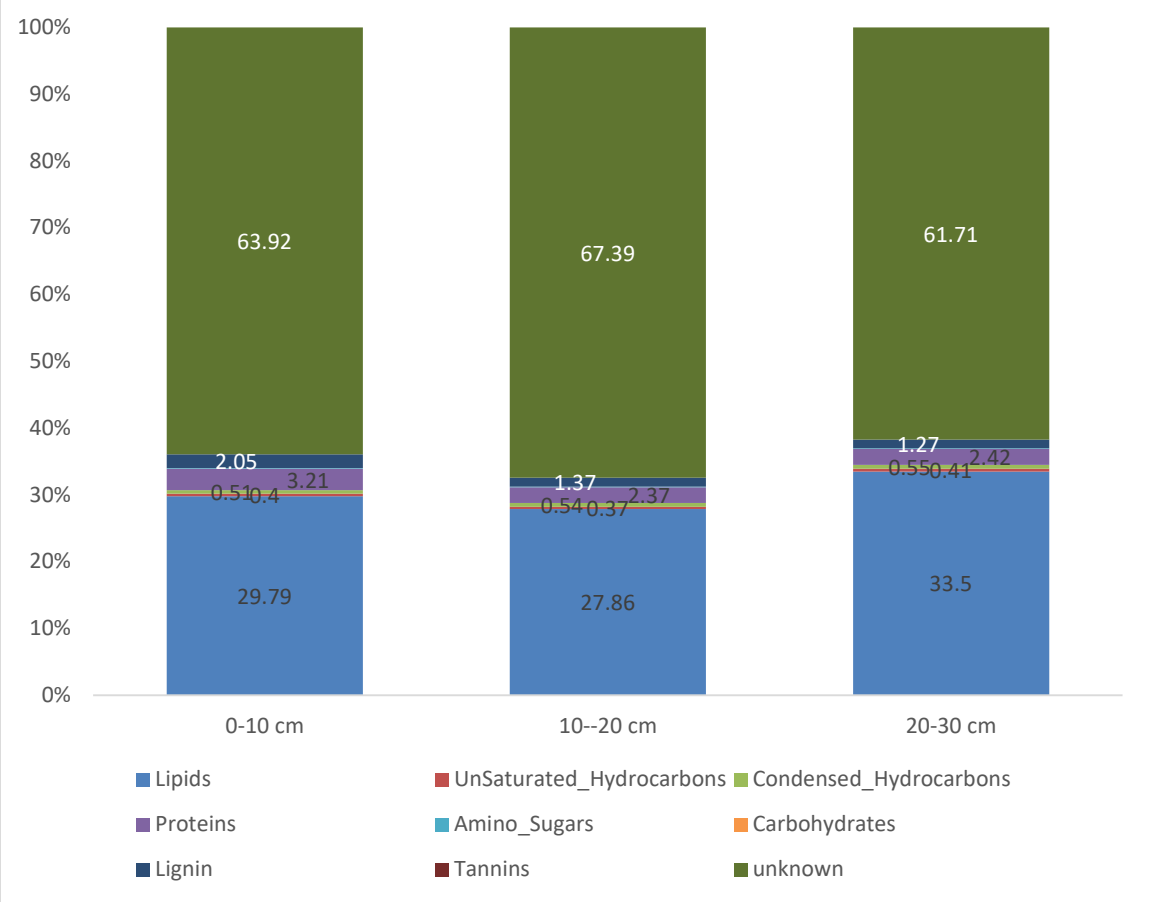


Figure 7. Average relative abundance of each molecule at the different soil depth increments in the sorbed C pool.