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2.2: Research Track

CLIMATE CHANGE MITIGATION POTENTIAL OF GREEN ROOFS: EXPLORING A GREEN ROOF'S CAPACITY TO SEQUESTER CARBON IN THE FLINT HILLS ECOREGION

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Abstract

Green roofs can help mitigate climate change. Rooftop green infrastructure enhances sustainable urban development by reducing atmospheric CO_2 as carbon is sequestered in plants and substrates. However, researchers are uncertain as to what substrate types, depths, and plant combinations sequester the greatest amounts of carbon in green roofs across different ecoregions, and research is needed to understand the benefits and limitations of green roofs in specific locations such as the U.S. Great Plains. This research evaluates the carbon sequestration performance of two experimental green roof beds with different depths (~8-inch or ~20 cm and ~4-inch or ~10 cm) and two substrate types in Manhattan, Kansas. The study was focused on estimating microbial biomass and exploring its interactions with root biomass, believing that it is an early indicator of changes in total soil organic carbon (C). Soil and root biomass samples from 48 plots (24 samples per the two depth examined) containing three different green roof plant mixes were collected in 2019 for soil PLFA (phospholipid fatty acids) analysis, and in 2020 for root biomass. Considering the real-life situation and possible research obstacles, methods have been proposed in this study to obtain root density data for each substrate type and depth. A three-way analysis of variance (ANOVA) was conducted to better understand the consequences of shallow and deep green roof growing media and what factors affect its ability to sequester carbon in the Flint Hills Ecoregion. Depth appears to be the key factor related to this research. The paper concludes that greater concentration of root material and microbial biomass in grass plots in shallower beds can (partially) offset the need for deeper beds and should perform better in mitigating climate change.

Key Words: Experimental Green Roof, Climate Change Mitigation, Carbon Sequestration, Substrate Types and Depths, Microbial Biomass, Root Density

Introduction

The installation of a green roof on any building potentially allows for sequestering the primary greenhouse gas carbon dioxide from the atmosphere (Getter et al., 2009; Kuronuma et al., 2018). Green roofs are seen as a practical way to reduce some types of pollution, reduce energy costs, retain stormwater during weather events, and sequester carbon (Fioretti et al., 2010; Refahi and Talkhabi, 2015; Whittinghill et al., 2014).

Like any vegetated area, a patch of rooftop vegetation should lower levels of carbon dioxide in the air (Sohn, 2009). Plants "breathe in" greenhouse gases and store carbon in their leaves, roots, and other tissues. Significant research was conducted in Michigan and Maryland, indicating that green roofs sequester carbon in plants and soils (Getter, Rowe, Robertson, et al., 2009; Whittinghill et al., 2014). Carbon is transferred to the substrate via plant litter and exudates (Getter et al., 2009). However, the length of time that carbon remains in the soil before total ecosystem respiration has yet to be quantified for green roofs. Net ecosystem production is beneficial since this created ecosystem will be a net carbon sink, at least in the short term (Getter, Rowe, Robertson, et al., 2009). A green roof that offsets the carbon debt of green roof materials creates a positive impact on climate change and sustainability (Getter et al., 2009; Kuronuma et al., 2018; Sailor, 2009; Sohn, 2009).

Global warming is driven in large part by the increase in atmospheric temperatures caused by burning fossil fuels and releasing greenhouse gases (including CO_2). Dramatically reducing greenhouse gas emissions is essential to mitigate negative climate change impacts (Fioretti et al., 2010; Jaffal et al., 2012). Green roofs can contribute to this mitigation effort. With different climates, plant materials, and construction conditions, regional research is needed to demonstrate the benefits of green roofs in specific locations (Lin et al., 2013).

Green roofs as climate change mitigation strategy

Several opportunities exist, starting from planning and design of green (and blue) spaces in urban landscapes (Demuzere et al., 2014) to develop climate-resilient urban areas and reduce emissions. This research explored the contribution of green roofs to climate change mitigation and was conducted with two contextual aspects in mind: A. The Regional Environment (Flint Hills Ecoregion, Manhattan, Kansas, USA); and B. The Local Setting and Experimental Green Roof (APD-EGR) design and implementation (construction and ongoing management).

The importance of green roof substrates and living vegetation

In combination, green roof substrates and living vegetation have the potential to sequester carbon from the environment (Getter et al., 2009; Whittinghill et al., 2014), thus helping to reduce global warming impacts (Jaffal et al., 2012). The substrate's water-holding capability (Best et al., 2015) is dependent on substrate type and depth. In combination with living vegetation (well-adapted to the regional and local climate and microclimate), a green roof's depth and composition can be designed to optimize potential benefits and reduce problems related to climate change (Ismail and Abdullah, 2016).

The Flint Hills Ecoregion and regional-scale green roof studies

Understandably, the globally increasing vulnerabilities to natural and human-made disasters are a consequence of climate change (Laukkonen et al., 2009). According to the United Nations Development Program (UNDP, 2007), it is necessary to ensure future human survival by

inventing new strategies to be implemented worldwide that align with regional architecture, planning/design, and development considering climate change mitigation. From these discussions, implementing green roofs in substantial numbers worldwide to mitigate climate change (Knight, 2011) can help reduce global warming impacts at regional and global scales (Laukkonen et al., 2009).

The use of regionally adapted vegetation is seen as critical. Akther et al. (2018) synthesized the effects of the influential factors statistically, including design and hydrologic variables on green roof performance, and explored their impact in different climatic zones. These authors concluded that the performance of green roofs in different climatic zones are meaningfully different (Akther et al., 2018). Therefore, we need more regional-scale research.

The Flint Hills Ecoregion (Figure 1) is defined by gently sloping, prairie-dominated hills of limestone and shale (Anderson and Fly, 1955). Hot continental summer temperatures and cool winters (accentuated by cold arctic blasts) are prevalent in this region. Tallgrass prairie is the dominant vegetation (Anderson and Fly, 1955). Soils along ridgelines are typically thin, and may be comparable to green roof substrates, especially in terms of the harsh growing conditions they induce on vegetation.

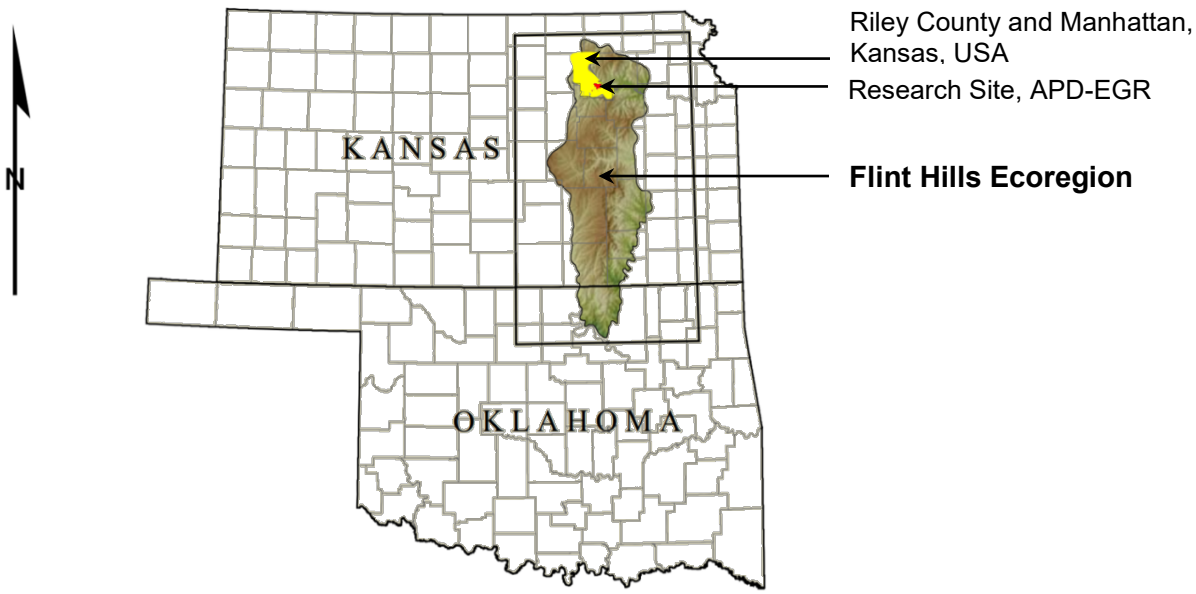

Figure 1. The Flint Hills Ecoregion in Kansas. By M. M. Lekhon Alam, adapted from Chapman et al. (2001).

The United States Environmental Protection Agency has designated the Flint Hills as an ecoregion, distinct from other grasslands of the Great Plains (Chaplin et al., 2007). The research site has a continental climate characterized by warm, wet summers and dry, cold winters (KSU, 2012). The continental climate accounts for substantial daily and seasonal temperature fluctuations; the ecoregion typically receives 30-38 inches (760-965 mm) of annual precipitation, with most falling during the growing season, especially April to September (Tollerud et al., 2018). Nevertheless, very dry periods can occur throughout the year, including during the growing season.

Scope, goal, and research question of the study

The intent of the study examines the impact of the selected APD-EGR green roof beds and plots in terms of carbon (C) sequestration and evaluate the climate change mitigation potential of the APD-EGR for two different substrate depths and types of engineered growing media. The research focuses on the 4-inch (10.16 cm) and 8-inch (20.32 cm) substrate depths.

This study seeks to provide scientific evidence for APD-EGR's contribution to C sequestration considering Kansas BuildEx® and rooflite® substrates at two depths. Also, the study seeks to propose a treatment (substrate + plant mix type + depth) that will potentially sequester the largest amount of carbon from green roofs in the Flint Hills ecoregion. The study focuses only on the three different belowground biomass samples for plant mix types A, B, and C (Table 1). Performance was examined by measuring microbial biomass and root biomass to better understand possible indicators of changes in total soil organic carbon (SOC). How green roofs can help reduce carbon dioxide (CO_2) emissions in the Flint-Hills Ecoregion, directly and indirectly, is the focus of this study. A primary research question is: How do Kansas BuildEx® **(K)** and rooflite® **(R)** substrates, microbial communities, and substrate depths (approximately 8-inch versus 4-inch) impact carbon sequestration for the APD-EGR in the Flint Hills Ecoregion?

Research approach and hypothesis

This research is being conducted using quantitative assessment of belowground plant biomass and microbial biomass as methods to ascertain carbon sequestration. The research hypothesis is stated as follows: Green roofs reduce CO_2 directly from the atmosphere to a greater degree when there is: 1) greater substrate depth (as in the 8-inch APD-EGR bed), 2) substrate having greater water holding capacity (as should be for Kansas BuildEx® given that this substrate is less porous), 3) a greater abundance of soil (substrate) microbes, and 4) higher organic matter and more root biomass (which should change with the age of green roof, but which may be higher at the outset for rooflite® given its physical, material composition).

Research Context and Methodology

Between July 2017 and June 2018, the Kansas State University (KSU) Architecture, Planning, and Design's Experimental Green Roof (APD-EGR) was constructed above the Seaton-Regnier Hall studios in the Flint Hills Ecoregion at Manhattan, Kansas (39.1897° N, 96.5831° W). The approximately 4-inch (~10 cm), 6-inch (~15 cm), and 8-inch (~20 cm) deep APD-EGR beds (with the 4-inch bed closest to the camera on the north side of this three-bed experimental green roof; figures 2 and 3). Our focus for this paper are the 48 shallowest and deepest plots.

Research setting

A cross section of the APD-EGR and shows the components of the green roof system (Figure 4). A total of 48 roughly 4 x 4 feet (1.2 x 1.2 meter) experimental green roof plots were established at the two examined substrate depths, with 24 plots in each bed of approximately 4-inch (~10 cm) and 8-inch (~20 cm) deep substrates (Figure 5). Manhattan, Kansas has an average annual precipitation of 35.62 inches (904.75 mm), based on 30-year averages (Knapp, 2017). Based on 20-year weather data from the National Oceanic and Atmospheric Administration (NOAA, 2000-2019); the highest monthly mean maximum temperature between 2000-2019 was 92.1°F or 33.4°C (July), while the lowest monthly mean minimum temperature was 18.6°F or -7.5°C (January). Air, surface, and sub-surface temperatures on the APD-EGR frequently exceed 90°F (32.2°C) from June to August.

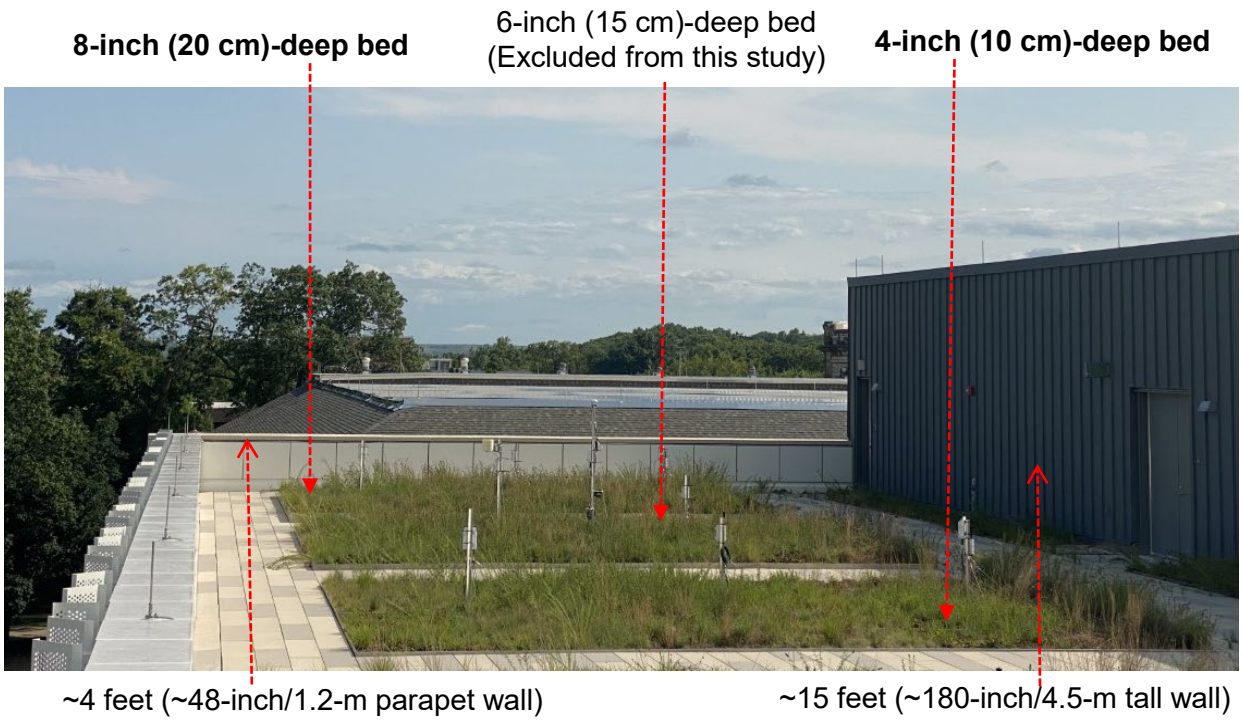

Figure 2. APD-EGR site surroundings (photo by M. M. Lekhon Alam, July 15, 2021).

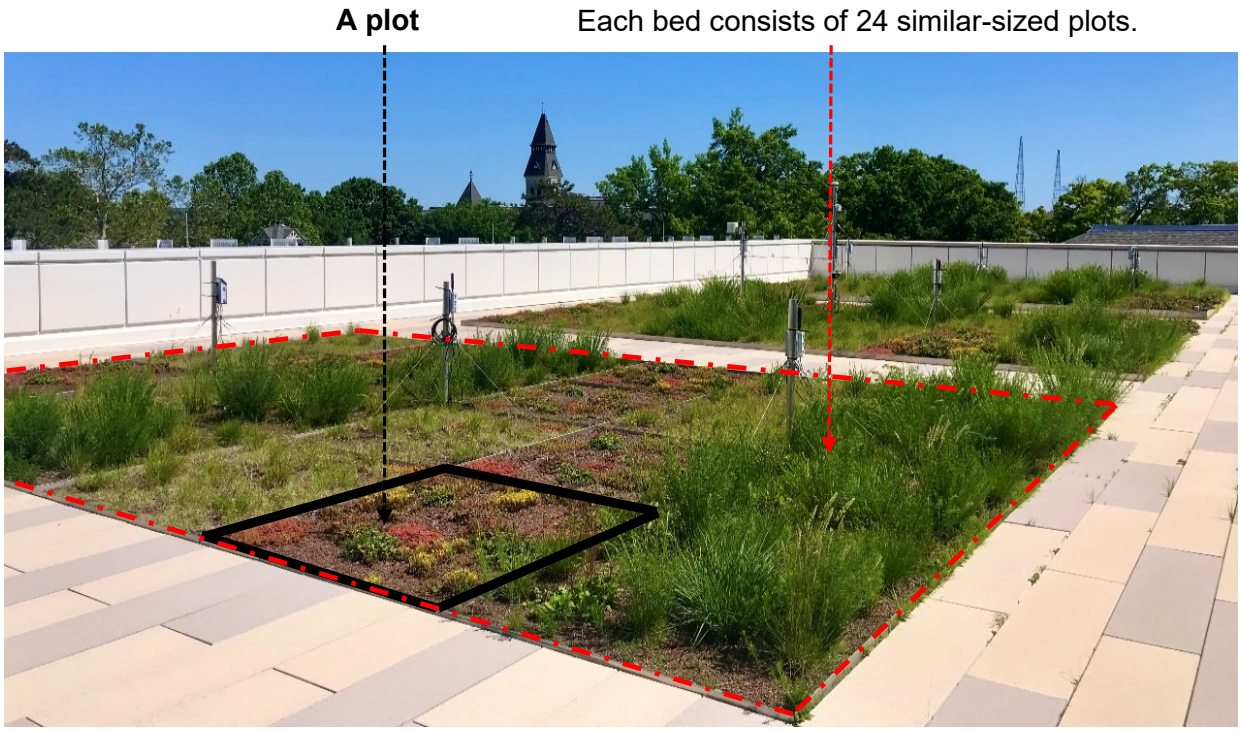

Figure 3. Basic layout of research site (photo by M. M. Lekhon Alam, May 2020).

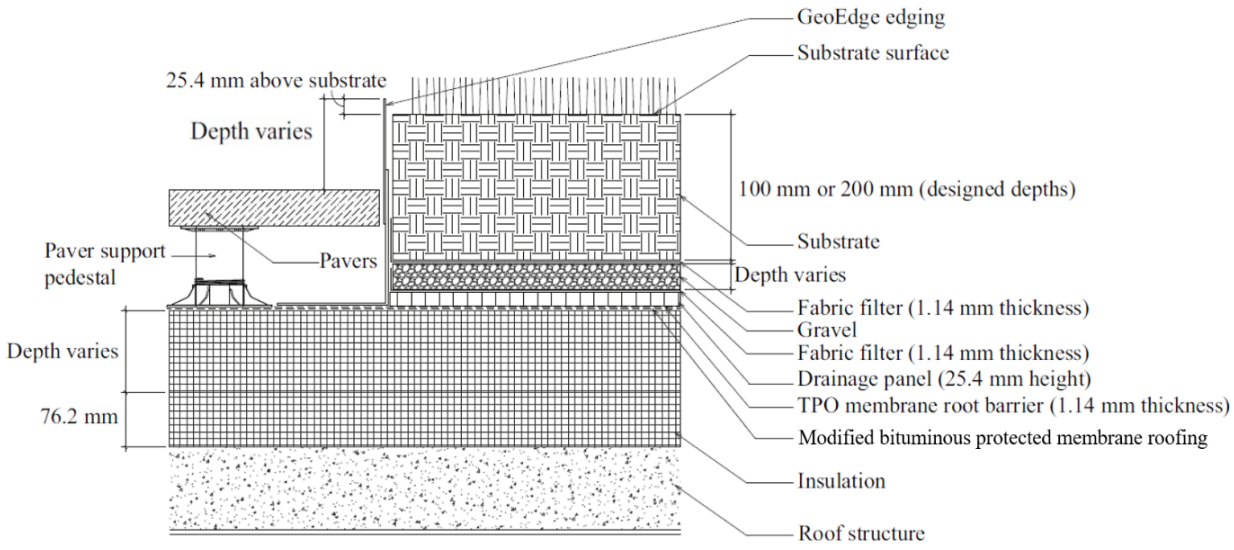

Figure 4. Section of APD-EGR with green roof component shown. Drawn by M. M. Lekhon Alam; adapted from the APD-EGR construction drawings.

Plots have one of two types of substrates: a sandy, dense Kansas BuildEx® or more porous rooflite® extensive mc green roof media. Vegetation was planted on the APD-EGR in three mixes of 18 plants (three plants of each species for each mix type: (A) six *Sedums*, (B) two *Sedums* and four native grasses, and (C) four native grass-like plants and two native forbs planted in the repeating order (1-6) (Table 1). The grasses and forbs are native to or are now commonly found within the Flint Hills Ecoregion.

Table 1. Plant mixes on the architecture planning and design experimental green roof.

All *Sedum* species (Mix A)	***Sedum* and grass species (Mix B)**	**Native grasses and forbs (Mix C)**
Sedum album f. *murale (1)*	*Bouteloua curtipendula (1)*	*Carex brevoir (1)*
Sedum ellacombeanum (2)	*Bouteloua dactyloides (2)*	*Dalea purpurea (2)*
Sedum hybridum 'Immergrüchen' *(3)*	*Bouteloua gracilis (3)*	*Koeleria pyrammidata (3)*
Sedum kamschaticum var. *floriforum* 'Weihenstephaner Gold' *(4)*	*Schizachyrium scoparium (4)*	*Packera obovata (4)*
Sedum sexangulare (5)	*Sedum reflexum (5)*	*Schizachyrium scoparium (5)*
Sedum spurium (6)	*Sedum rupestre (6)*	*Sporobolus heterolepis (6)*

This study focused on 4-inch (10 cm) and 8-inch (20 cm) deep substrate plots (four plots for each unique substrate type and vegetative mix) considering: (1) ease of making comparisons, (2) one depth is the shallowest and the other is the deepest of the three established APD-EGR depths, hence we could compare two distinct depth conditions on the APD-EGR, and (3) total microbial biomass and carbon-storage performance of the two substrate types were expected to show the most significant differences for the two substrate depths.

Table 2. Research settings at APD-EGR considering two substrate types and three vegetative plant mixes (4-inch [10 cm] and 8-inch [20 cm] deep beds), with four plots sampled for each unique plot type (the combination of depth, substrate type, and plant mix).

Initial APD-EGR Carbon Sequestration Research at Manhattan, Kansas, USA													
Composites**	**4-inch (10 cm) deep bed**						**8-inch (20 cm) deep bed**						**Total Replicates**
	24 Plots						24 Plots						
	A		B		C		A		B		C		
	Sedum only		*Sedum* and native grass mix		native grasses and forbs		*Sedum* only		*Sedum* and native grass mix		native grasses and forbs		
KA	4						4						**8 KA**
KB			4						4				**8 KB**
KC					4						4		**8 KC**
RA		4						4					**8 RA**
RB				4						4			**8 RB**
RC						4						4	**8 RC**

** ‘KA,’ ‘KB,’ and ‘KC’ indicate a Kansas BuildEx® **(K)** substrate plot—planted with *Sedum* only **(A)**, *Sedum* and native grass mix **(B)**, and native grasses and forbs **(C)**. ‘RA,’ ‘RB,’ and ‘RC’ indicate a rooflite® extensive mc **(R)** substrate plot—planted with *Sedum* only **(A)**, *Sedum* and native grass mix (**B**), and native grasses and forbs **(C).**

Figure 5. Plant mixes **A**, **B**, and **C** in the Kansas BuildEx®, **K** *(marked with gray color),* and the rooflite® extensive mc, **R**, substrates in the 4-inch and 8-inch-deep beds, with plots in each bed numbered from 1 to 24.

APD-EGR substrates, K and R affect soil moisture

Laboratory analyses reported and discussed by Decker (2021) established that for two APD-EGR depths (4 and 8 inches) Kansas BuildEx® (K) held more water (by volume) in the substrate profile than rooflite®. The physical properties per the 2018 lab analyses of APD-EGR substrate samples for the two substrate types, K and R, are as noted (Table 3).

Table 3. Reporting substrate properties of K and R. Substrates were tested at Turf and Soil Diagnostics lab in Linwood, Kansas in 2018.

Properties	Substrate, **K**	Substrate, **R**
Clay (<0.002 mm)	2.9%	1.3%
Silt (0.002-0.063 mm)	4.5%	5.8%
Sand (0.063-2.0 mm)	67.6%	52.4%
Larger particles (>2 mm)	25%	40.5%
Dry Bulk density (g/cm3)	1.47	0.98
Saturated density (g/cm3)	1.77	1.33
Maximum water retention	29.50	35.00
Total pore space (%)	42.50%	58.00%

Method and rationale for estimating soil carbon sequestration:
The objective of experimental research was to examine cause-and-effect relationships (Thomas, Nelson, and Silverman, 2015). Independent variables were manipulated at the outset by varying green roof substrate types and depths and vegetative mix types to observe the effects on different dependent variables.

This study estimates the soil (substrate) carbon (C) sequestration potential of the APD-EGR by measuring the microbial biomass (Phospholipid Fatty Acid Analysis) and root biomass of the two different substrates (Kansas BuildEx® and rooflite®) from two growing seasons (2019 for PLFA, and 2020 for root biomass) to compare these variables across two depths for the 4-inch and 8-inch green roof plots.

Phospholipid Fatty Acid (PLFA) analyses (Indoor Portion) of the two substrate types and substrate depths were conducted in the KSU Department of Agronomy Soil Microbial Agroecology Lab (SMAL). Additionally, the study performed root biomass analyses for the volume of the core to complement the PLFA analyses, helping the research team better understand the carbon dynamics within the two substrate types and depths. Finally, the study suggests different experimental approaches and studies that can be undertaken to gather more evidence related to carbon sequestration on the APD-EGR. It is important to note that aboveground plant biomass was collected at the end of each growing season in 2018, 2019, and 2020, but this data is not discussed in this paper.

Phospholipid Fatty Acid (PLFA) analyses:
In 2019, PLFA analyses were conducted to determine microbial biomass and proportions of microbial communities, including arbuscular mycorrhizal fungi (AMF), Gram-positive bacteria, Gram-negative bacteria, actinomycetes, and saprophytic fungi as dependent variables (Quideau et al., 2016). Plant (vegetation) mix type, soil (substrate) type, and substrate depth are the independent variables for this study. The total lipids were extracted from freeze-dried soil using a modification of the Bligh and Dyer lipid extraction method (Bligh and Dyer, 1959; White and Rice, 2009). Substrate sampling protocols and the laboratory procedures used to conduct the PLFA analyses at the SMAL lab are described in more detail below.

Statistical Analyses:
Statistical evaluation of data was performed using IBM SPSS Statistics 27. Significant differences among different dependent and independent variables were tested using the three-way analysis of variance (ANOVA) with Tukey's HSD post-hoc analysis.

The PLFA analysis has two primary protocols:

- **Outdoor Portion:** Protocols for collecting soil samples from the APD-EGR.
- **Indoor Portion:** Protocols for analyzing soil samples in the lab.

Soil Sampling Protocols (Outdoor Portion):
Protocols for collecting soil samples from the APD-EGR are given below.

Soil Sampling Protocols for PLFA Analyses
(Substrate samples were taken from 4 [10 cm] and 8 [20 cm]-inch beds):

- **Step 1:** Label all plastic bags before collecting samples from each APD-EGR bed.
- **Step 2:** Identify uniform areas near plants in the plots to be tested. Avoid sampling areas that might give misleading results, such as barren areas in a plot.
- **Step 3:** From each area, take enough samples from the two beds (4-inch and 8-inch) to properly represent the area— referring to Figure 5 for each plot location and sample number. Make sure that the probe reaches the bottom of the plot (which is the upper surface of the filter fabric at the base of the substrate in each plot). Keep the samples for each plot separate in bags to organize each sample. Clean the probe with acetone after use in each plot.
- **Step 4:** Repeat this substrate sampling procedure for each bed and plot. Phospholipid fatty acid samples were stored at -4°C until analysis.

We collected 48 substrate samples from the APD-EGR in the 4-inch and 8-inch beds to use for PLFA analysis. Samples were collected October 3, 2019 (7:30 am to 11:30 am) and stored in a refrigerator until the samples were analyzed.

Procedure for Root Biomass Analysis (substrate samples taken from 4-inch and 8-inch beds):
Root biomass were estimated by extracting roots from soil cores (Wilsey and Polley, 2006). Note that root biomass is typically carried over from year 1 to year 2, so it is appropriate to call root biomass “peak biomass” rather than productivity (Wilsey and Polley, 2006). For the APD-EGR, researchers collected soil samples from each of the 4-inch and 8-inch plots (48 total samples).

- Volumetric cores were taken within 4-inch and 8-inch beds, thus giving four replications (Reps) for each unique plot type (plant mix and soil type). Samples were collected at a consistent distance (~3-6 cm) from a plant selected near the southeast corner of each plot. A 2-inch (5.08 cm) diameter core was used to collect one core per plot (employing a hand trowel as needed). Although the APD-EGR plots have four Reps, the Reps were not combined so that statistical analyses could be used to compare and contrast the findings among all 48 plots within the 4-inch and 8-inch beds.
- A number of substrate cores did not come out as a complete and consistent core length given the sandy and gravelly nature of the substrate. Because the volume of a cylinder is: $\boldsymbol{V = \pi r^2 h}$, the study needed to keep track of each core depth and height (h). The most effective approach was to measure core depths (h) manually during the sampling process, allowing researchers to collect “substrates with roots” from the 2-inch (5.08 cm) diameter volumetric core using a soil probe and then using a trowel as needed.
- In the lab, every core requires visual observation to remove the coarse material first (Wilsey and Polley, 2006). Large roots were hand-picked from soil samples, and then substrate samples were passed through 4 mm, 2 mm, and 1 mm sieves, respectively, and roots were collected with tweezers from each sieve. All roots were gathered in metal tins and washed over a 0.25 mm screen/sieve. Metal tins were labeled with plot numbers

and weighed before collecting roots. Notes: Samples were refrigerated until the roots were washed. Because only Kansas BuildEx® had ~2% of clay, researchers did not use a root washer to separate the clay from the roots.

- After washing, the root samples in metal tins were oven-dried at 55-60°C for 48 hours (Frasier et al., 2016) then weighed (metal tin + dry roots) using a precision scale. The final step was to calculate root biomass density (p=m/V) for the volume of the core using the formula $\boldsymbol{V = \pi r^2 h}$.

Since 2020 was the third growing season, the APD-EGR was expected to have relatively stable root systems and fairly stable root biomass within the two substrate types. We collected 48 soil samples at the end of the third growing season for root biomass analysis November 6, 2020 (8:30 am to 2:30 pm) at the APD-EGR.

Figure 6. Collecting and analyzing root biomass samples from 4 (10 cm) and 8 (20 cm)-inch-deep beds. Photographs by M. M. Lekhon Alam, November 2020.

Observation and Interpretation of PLFA Data from the Year 2019

Data analyses and results

A three-way ANOVA was performed to evaluate the effect of plant mixes (A, B and C), substrates (K and R), and soil depths (4 [10 cm] and 8 [20 cm]-inch-deep beds) on total microbial biomass (total MB) as well as their interactions using IBM SPSS Statistics 27.

From the three-way ANOVA, total microbial biomass was significantly different between two depths, with higher biomass in 4-inch-deep bed (Mean=45.1) compared to 8-inch-deep bed (Mean=34.1) at APD-EGR (Table 4), ($F(1, 35) = 9.845$, $p = 0.003$). There was significant two-way interaction between two depths, and plant mixes (A, B and C), ($F(2, 35) = 3.56$, $p = 0.039$) as shown in Table 5. Also, the study found a marginally significant two-way interaction between depths and substrates, ($F(1, 35) = 3.917$, $p = 0.056$) (Table 5).

Table 4. Descriptive statistics of total microbial biomass.

Variables		Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Depth	4-inch	45.1	2.44	40.1	50.0
	8-inch	34.1	2.50	29.0	39.2
Substrate	K	37.8	2.51	32.8	42.9
	R	41.4	2.44	36.4	46.3
Plant Mix	A	34.3	2.98	28.2	40.4
	B	42.3	2.98	36.3	48.4
	C	42.2	3.11	35.9	48.5

Table 5. Three-way ANOVA results of 2019 PLFA data sets for the 4 and 8-inch-deep bed (SPSS outputs).

Variables	Sum of squares	df	Mean square	F	p-value
Depth	1406.941	1	1406.941	9.845	.003***
Substrate	144.236	1	144.236	1.009	.322
Plant	665.376	2	332.688	2.328	.112
Depth × Substrate	559.830	1	559.830	3.917	.056*
Depth × Plant	1017.427	2	508.714	3.560	.039**
Substrate × Plant	724.506	2	362.253	2.535	.094
Depth × Substrate × Plant	92.277	2	46.139	.323	.726

*** Significant at 1% level. ** Significant at 5% level. * Marginally significant at 10% level.

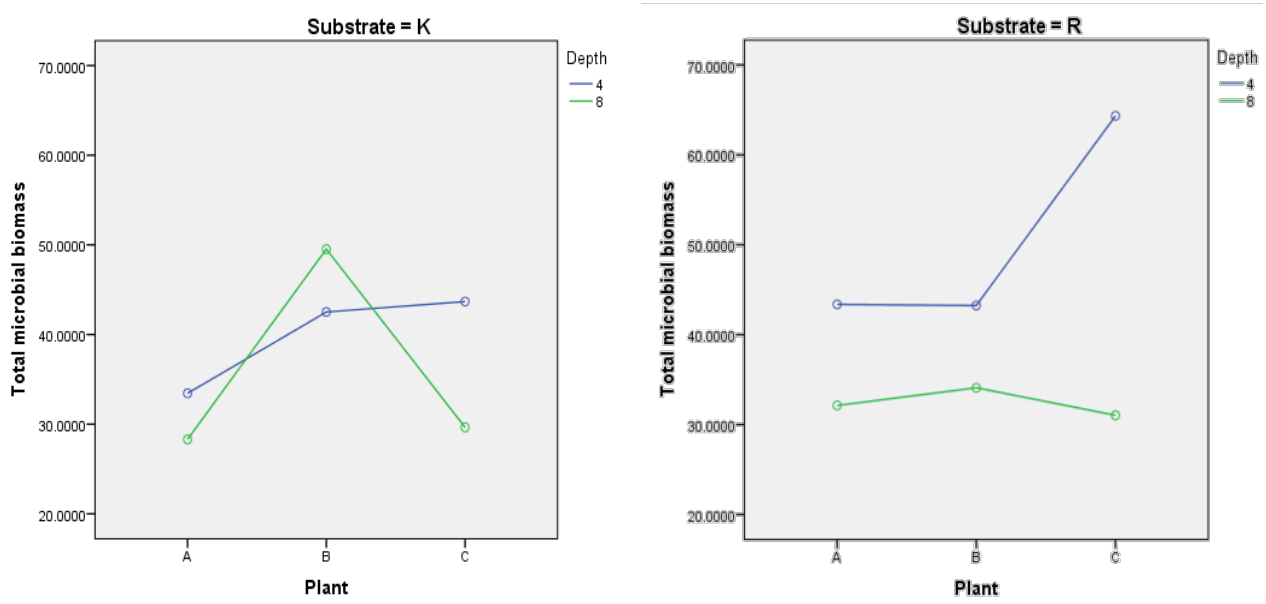

Figure 7. The average amount of total microbial biomass in conditions at two depths (4 and 8-inch), belowground biomass samples of plant mixes A, B and C, and two substrates (K and R).

Discussion and 2019 PLFA result interpretations

One of the primary independent variables of this study – "different plant mixes" – focused only on the three different belowground biomass samples for plant mixes A, B, and C. A few significant findings related to two substrate depths were observed for the 4-inch bed compared to the 8-inch-deep bed at the APD-EGR. There were substantial differences in the concentration of microbial biomass between the belowground biomass samples for plant mixes A, B, and C and the two depths (4-inch and 8-inch).

The three-way ANOVA statistical results (Table 4 and Table 5) indicated that belowground biomass containing grasses (plant mixes B and C) appeared to have higher microbial biomass. The 4-inch bed had higher microbial biomass than the 8-inch- bed. The rooflite® extensive mc (R) contained more microbial biomass than the Kansas BuildEx® (K) in the shallower 4-inch bed at APD-EGR (Table 5 and Figure 7). It is possible that the growing density of plant roots caused by substrate depth limitations is one of the main reasons for the accumulation of high carbon in the two substrates on the APD-EGR. Future studies should seek more conclusive evidence to support the above claims and determine exactly what causes substrate carbon storage capacity to vary.

Complementing the 2019 PLFA (Microbial Biomass) Results with Analysis of Root Biomass from the 2020 Growing Season

Root biomass analysis

To explore the extent of soil microbes and its effects on carbon sequestration potential, data regarding root biomass, microbial biomass, and total carbon in the soil is necessary. Aboveground biomass assess productivity (Lauenroth et al., 1986), which co-relates or translates to how much carbon is occurring belowground in general. Thus, it is possible to explain the main points of this study (also complements the PLFA results) by direct measurement or relative density of root biomass.

Data analyses and results

A three-way ANOVA was used to evaluate the effect of three belowground biomass samples of plant mixes (A, B, and C), substrates K and R, and soil depths (4-inch and 8-inch-deep) on root density and their interactions. In addition to this, Tukey's HSD post-hoc test determined a pairwise comparison between two sets of groups using IBM SPSS Statistics 27.

Root density was found to be significantly different among belowground biomass samples of plant mixes A, B and C, ($F(2, 36) = 18.92$, $p = 0.000$) (Table 6). The Tukey's HSD post-hoc test indicated that the belowground root density in plant mix C (Mean=0.253) and plant mix B (Mean=0.233) were higher than plant mix A sample (Mean=0.075) at $p = 0.000$.

Table 6. Descriptive statistics of root density data.

Variables		Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Depth	4-inch	.206	.018	.169	.243
	8-inch	.168	.018	.131	.205
Substrate	K	.193	.018	.156	.230
	R	.181	.018	.144	.218
Plant Mix	A	.075	.022	.029	.120
	B	.233	.022	.188	.279
	C	.253	.022	.207	.298

Root density interpretations and discussion

Root biomass from 4-inch and 8-inch beds were examined to understand better the soil C sequestration potential. From three-way ANOVA, the belowground biomass samples containing grasses (B and C) appeared to have overall significantly (Table 7) higher root density than *Sedum* plots in both 4-inch and 8-inch beds. In the case of K and R substrates (Figure 8), the belowground root density of three different plant mixes (more evident to B and C) was relatively higher in the 4-inch- bed as compared to the 8-inch bed.

Table 7. Three-way ANOVA results of root density data sets for the 4 and 8-inch beds (SPSS).

Variables	Sum of squares	df	Mean square	F	p-value
Depth	.018	1	.018	2.210	.146
Substrate	.002	1	.002	.222	.641
Plant	.305	2	.153	18.920	.000***
Depth * Substrate	.002	1	.002	.237	.630
Depth * Plant	.011	2	.006	.701	.503
Substrate * Plant	.010	2	.005	.644	.531
Depth * Substrate * Plant	.017	2	.008	1.043	.363

*** Significant at the 1% level. ** Significant at the 5% level.

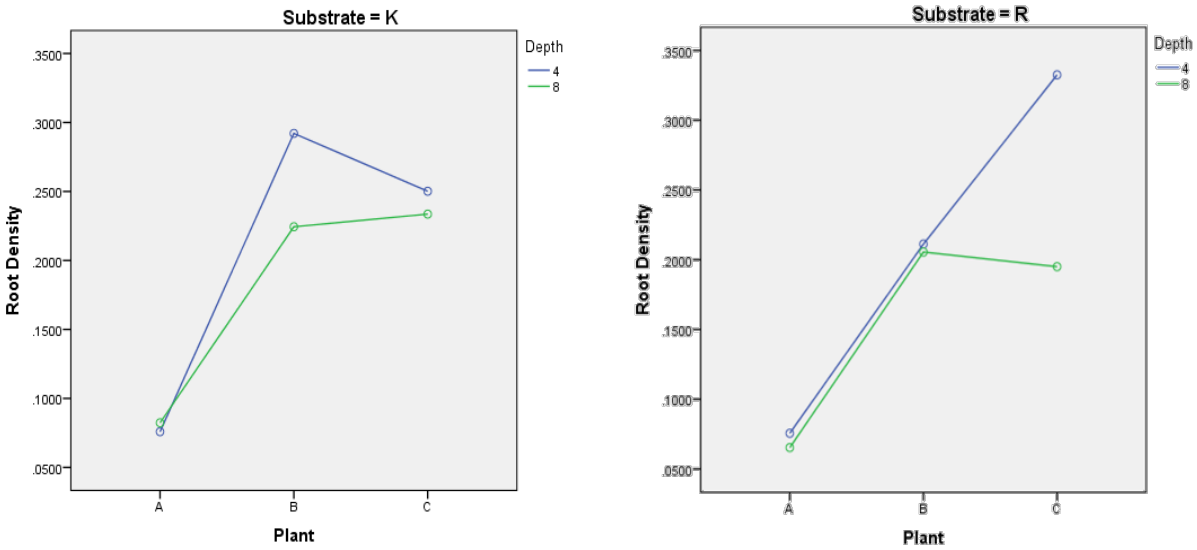

Figure 8. The average amount of root density in conditions at two depths (4-inch and 8-inch), belowground biomass samples of plant mixes A, B and C, and two substrates (K and R).

Native short grass roots had significantly more belowground biomass than *Sedum* spp. (findings which are supported by Sutton, 2013), and the higher root biomass of these perennial grasses contributes more C to the soil (Sainju et al., 2017). Insofar as we know, root biomass in this study has never been done before in the context of the Great Plains, USA.

This study was not done to recommend any depth, but to understand the consequences of shallow and deep green roof growing media (substrates) and what factors affect its ability to sequester carbon in this region. Also, this study was not concerned with different root systems of various plants but focused on the overall root density of substrates. The study investigated

the potential influences of plant roots in two different substrates that may help answer and link all statistical analyses performed for the APD-EGR related to carbon sequestration. The analysis discussed in this paper identifies some of the potential causes of root biomass concentration in APD-EGR and hypothesizes the effect of depth from the empirical studies.

In the shallower rooting depth of the 4-inch bed, roots proliferate within the entire profile more than in the 8-inch-deep bed because they have the least amount of space. In the 4-inch bed, belowground biomass for A, B, and C plant mixes should become root-bound, and their roots should likely hit the bottom of the plot and over time occupy all available substrate space; therefore, the root density of each shallow plot tends to increase. Statistical analysis of PLFA data from 2019 has provided significant evidence of interactions of varying depth. Depth was found to be the most important factor.

Research findings in context and suggestions for future study

From the very beginning, the study was focused on estimating microbial biomass, believing that microbes are an early indicator of changes in total soil organic carbon (C). Microbes decompose soil organic matter releasing carbon dioxide and plant-available nutrients. Soils with more organic (labile) C tend to have a higher microbial biomass (Hoyle et al., 2006). Based on other studies, exudates released by plant roots are the main food source for microorganisms and a driving force of their population density and activities (Raaijmakers et al., 2009). Processes in the rhizosphere are complex, and the plant-root interface is a hotspot of microbial interactions (Korenblum et al., 2020; Raaijmakers et al., 2009). The rhizosphere is the area around a plant root inhabited by a unique population of microorganisms influenced (McNear, 2013). Thus, living root-soil interfaces are nutrient-rich, which acts as a source of energy for microbes (Jones et al., 2004). APD-EGR research, based on data collected and analyzed to date, hypothesizes that the greater root density on the APD-EGR may be positively correlated with microbial biomass obtained directly from the substrate in some way.

We suggest that the amount of microbial biomass is likely due to the higher density of roots in the 4-inch bed than in the 8-inch bed. We also suggest that “soil depth constraints” may be one of the main reasons for creating higher microbial conditions in substrate R than in substrate K, which helps to retain more carbon. Although there are some limitations, the study suggests that shallower beds with R substrate (having lower bulk density, higher pore space, and lower water holding capacity than K) should have a greater amount of sequestered carbon per substrate volume, which can (at least partially) offset the need for deeper beds and may effectively contribute to climate change mitigation in similar ways as deeper substrate profiles.

Conclusions: Discussion of Research Limitations and Opportunities

The study has limitations. Substrate depths are known to vary in some plots, but these variations were not examined for this initial carbon sequestration study. This research did not interpret plant residue (aboveground vegetative biomass) data. Lastly, to assess the total amount of carbon in each substrate type, the study suggests the need for analysis of soil nematode communities, total carbon and nitrogen, and soil respiration. APD-EGR researchers hope that the microbial biomass and root biomass research can continue during future growing seasons to provide a multi-year baseline and important reference for longer-term studies of carbon sequestration that may be completed on the APD-EGR and also at green roofs being studied in other parts of the world.

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