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Investigating the climate mitigation performance of a green roof system in the Flint Hills Ecoregion, USA

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ABSTRACT

Rooftop green infrastructure enhances sustainable urban development by reducing atmospheric CO_2 as carbon is sequestered in plants and substrates. However, it is uncertain what substrate types, depths, and plant combinations sequester the greatest amounts of carbon in green roofs across different ecoregions, including the U.S. Great Plains. This research sought to evaluate carbon sequestration potential of two experimental green roof beds of 10 cm (4 in) and 20 cm (8 in) and two substrate types in Manhattan, Kansas, USA. Microbial and root biomass and their interactions were measured as early indicators of changes in soil organic carbon (SOC). Soil and root biomass samples were taken from beds of two depths with two substrates (K and R) and three plant communities (all *sedum*, *sedum* and grass, and native grasses and forbs) for a total of 48 plots. Microbial biomass was measured by phospholipid fatty acids (PLFA) analysis in 2019 and root biomass in 2020. Root biomass and microbial biomass was greater in native grass in shallower beds. Shallower beds can partially offset the need for deeper beds and should perform well in mitigating climate change if beds are irrigated during very dry periods.

Key words: *experimental green roof; climate change mitigation; carbon sequestration; substrate types and depths; microbial biomass; root density*

INTRODUCTION

Global warming is caused by greenhouse gases trapping the sun's energy in the form of long wavelength infrared light. Increases in anthropogenic greenhouse gas emissions, specifically carbon dioxide, increases atmospheric temperatures. Dramatical reduction in 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).

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 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 carbon dioxide levels in the air (Sohn 2009). Plants through photosynthesis absorb CO_2 and store carbon in their leaves, roots, and other tissues. Studies by Gaumont-Gauy and Halsall (2013) consider photosynthesis the primary production of carbon input to green roofs. Gaumont-Guay and Halsall (2013) and Starry (2016) calculated the Net Primary Production (NPP) from a green roof (*Sedum* green roof with 8 cm of substrate depth in Vancouver) was 440 g C m^{-2} yr^{-1}. However, the authors stated that *Sedum* performed better in the winter climate of the Pacific Northwest (Gaumont-Guay and Halsall 2013; Starry 2016).

On the other hand, estimation of belowground carbon accumulation is more challenging because of its complex nature and strong connections with the local climate. Substrate characteristics, plant communities, substrate depth, and roof age all play a role in regulating belowground accumulation of carbon (Buffam and Mitchell 2015). Additionally, Buffam and Mitchell argued that little is known about the nutrient dynamics within green roof ecosystems. And that more studies, including full roof-scale experiments, computer modelling, and long-term monitoring, are needed for improved understanding of these ecosystems (Buffam and Mitchell 2015).

Research conducted in Michigan indicated that green roofs sequester carbon in plants and soils (Getter et al., 2009; Whittinghill et al., 2014). Carbon is transferred to the substrate via plant litter and exudates (Getter et al., 2009). Net ecosystem production is beneficial since this created ecosystem will be a net carbon sink, at least in the short term (Getter 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).

Green roofs as climate change mitigation strategy

Several opportunities exist, starting from the planning and design of plant- and water-based spaces in urban landscapes (Demuzere et al., 2014) to develop climate-resilient urban areas and reduce emissions. This research explored the potential contribution of green roofs to climate change mitigation and was conducted with two contextual aspects in mind: 1) The regional environment (Flint Hills Ecoregion); and 2) The local setting and design context (Manhattan, Kansas, USA) and the Kansas State University (KSU) Architecture, Planning and Design Experimental Green Roof (APD-EGR) design and implementation, which includes its unique 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 capacity (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 result from climate change (Laukkonen et al., 2009). According to the United Nations Development Program (United Nations Development 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 (Berardi et al., 2014) can help reduce global warming impacts at regional and global scales (Laukkonen et al., 2009).

The use of regionally adapted vegetation is critical. Akther et al. (2018) synthesized the effects of 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 is meaningfully different (Akther et al., 2018). Therefore, we need more ecoregion-focused 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. The United States Environmental Protection Agency (USEPA) has designated the Flint Hills as an ecoregion, distinct from other grasslands of the Great Plains (Chaplin et al., 2007).

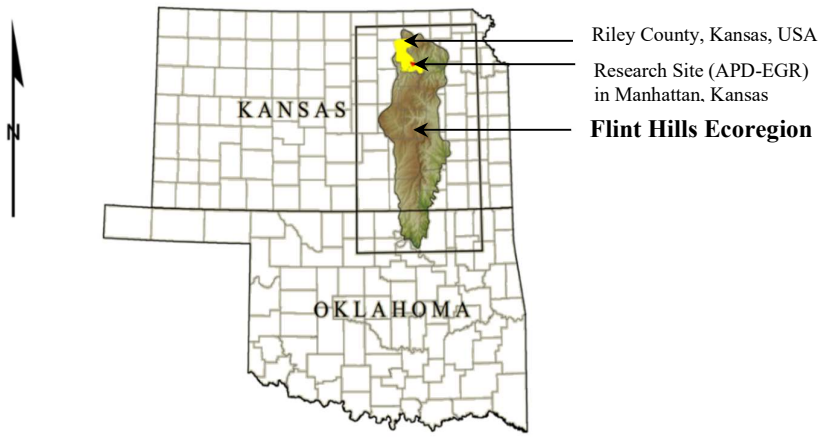

Figure 1. The Flint Hills Ecoregion in Kansas. By M. M. Lekhon Alam, adapted from Chapman et al. (2001).

The research site is in a location having 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 from April to September (Tollerud et al., 2018). Nevertheless, very dry periods can occur throughout the year, including during the hottest parts of the growing season.

Scope, goal, and research question of the study

Broadly, this study examined how green roofs may help reduce carbon dioxide (CO_2) emissions in the Flint-Hills Ecoregion directly and indirectly. The study examined the impact of the APD-EGR green roof beds and plots in terms of carbon sequestration and evaluated the climate change mitigation potential of the APD-EGR for two different substrate depths and two different types of substrates (or engineered growing media). The research focused on 10 cm (4 in) and 20 cm (8 in) substrate depths.

This study investigates different APD-EGR variables and their contribution to carbon sequestration considering Kansas BuildEx® and rooflite® substrates at two depths, with three plant mix types. This paper focuses on belowground biomass samples for plant mix types A, B, and C (Table 1). Carbon sequestration contributions were examined by measuring microbial biomass and root biomass to understand indicators of changes in SOC. The primary research question was: How do Kansas BuildEx® (K) and rooflite® (R) substrates, microbial communities, and substrate depths (20 cm versus 10 cm) impact carbon sequestration for the APD-EGR in the Flint Hills Ecoregion?

Research hypothesis and approach

Hypothesis for this research: Green roofs reduce CO_2 directly from the atmosphere to a greater degree when there is: 1) greater substrate depth (as with the 20 cm APD-EGR bed), 2) a substrate having greater water holding capacity (as assumed to be for Kansas BuildEx® given that this substrate was understood to be less porous than the rooflite® substrate), 3) a

greater abundance of soil microbes in a substrate, and 4) higher organic matter and more root biomass (which should change with the age of the green roof, but may be higher at the outset for rooflite® given its physical, material composition). This research used a quantitative assessment of belowground plant biomass and microbial biomass in the two substrate types to ascertain the estimated carbon sequestration contributions of different green roof conditions.

RESEARCH CONTEXT AND METHODOLOGY

Between July 2017 and June 2018, the KSU 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 10 cm (4 in) and 20 cm (8 in) deep APD-EGR beds (Figures 2 and 3). The focus of this paper was the 24 shallowest and 24 deepest plots.

Research setting and climatic context

A cross-section of the APD-EGR shows the components of the green roof system (Figure 4). A total of 48 roughly 1.2 x 1.2 m (4 x 4 ft) experimental green roof plots were established at the two examined substrate depths, with 24 plots in each bed of approximately 10 cm and 20 cm deep substrates (Figure 5). Manhattan, Kansas has an average annual precipitation of 904.75 mm (35.62 inches), 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 was 33.4°C (92.1°F; July), and the lowest monthly mean minimum temperature was -7.5°C (18.6°F; January). Per monitoring data collected on the roof, air, surface, and sub-surface temperatures on the APD-EGR frequently exceed 32.2°C (90°F) from June to August.

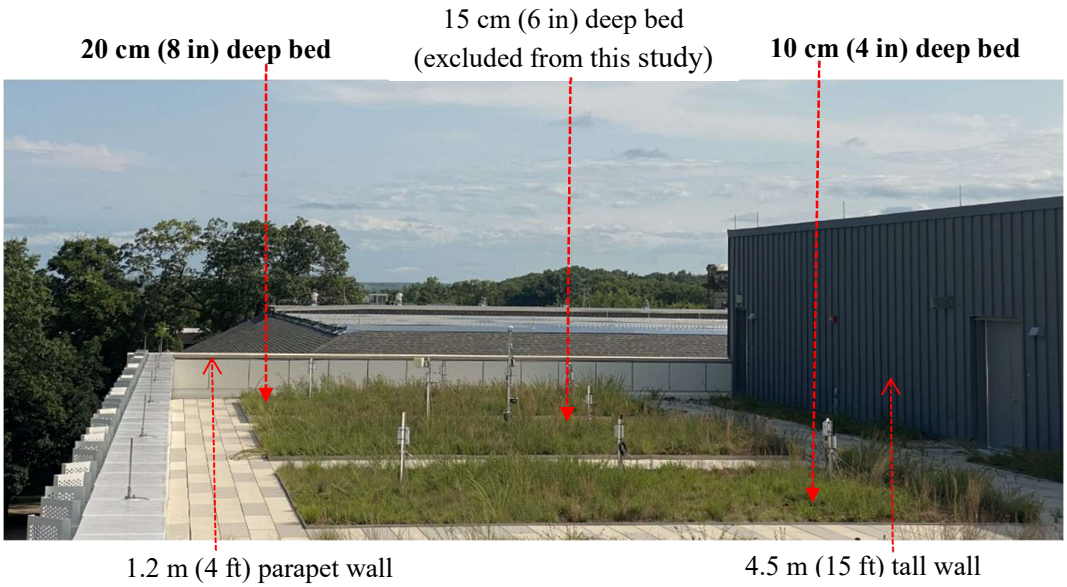

Figure 2. APD-EGR site surroundings. The 10 cm bed is in the foreground on the north side of the 3-bed experimental green roof. Photo by M. M. Lekhon Alam, 15 July 2021.

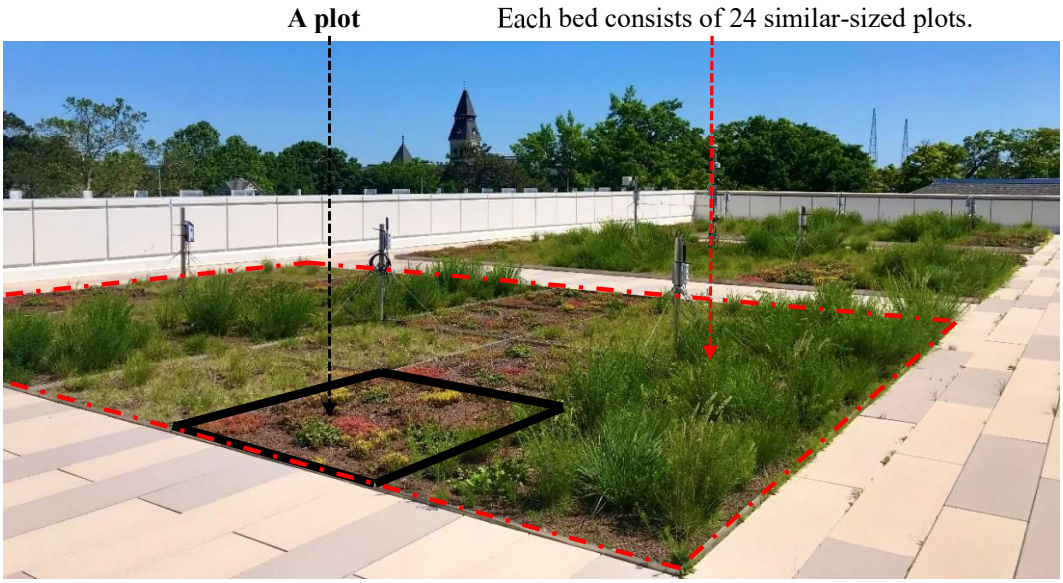

Figure 3. Basic layout of research site. Photo by M. M. Lekhon Alam, May 2020.

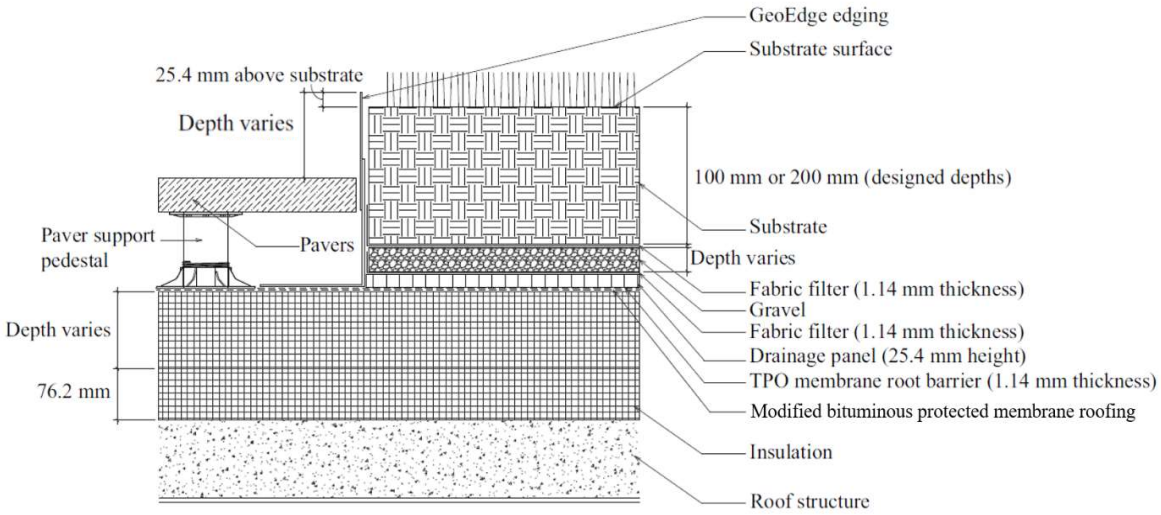

Figure 4. Section of APD-EGR with green roof component shown. Drawn by M. M. Lekhon Alam (Alam 2022; Liu et al., 2019). Adapted from APD-EGR construction drawings prepared by Confluence designers between 2015-2017.

Plots have one of two types of substrates: a sandy, dense Kansas BuildEx® or more porous rooflite® extensive green roof substrate. Vegetation was planted on the APD-EGR in three mixes of 18 plants, with 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 a 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 APD-EGR.

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 the approximately 10 cm and 20 cm deep substrate plots (four plots for each unique substrate type and vegetative mix) considering the ease of making comparisons (one depth is the shallowest and the other is the deepest of the three established APD-EGR depths). Comparisons between the two distinct depth conditions on the APD-EGR were expected to show the most significant differences in regard to total microbial biomass and carbon-storage performance for the two substrate types used on the APD-EGR.

Table 2. Carbon sequestration research setting at APD-EGR considering two substrate types, three vegetative plant mixes, and two substrate depths, 10 cm and 20 cm deep, with four plots sampled for each unique plot type (the combination of substrate type, plant mix type, and substrate depth), taken from (Alam 2022).

Initial APD-EGR Carbon Sequestration Research at Manhattan, Kansas, USA													
Composites**	10 cm deep bed						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).**

APD-EGR substrates, K and R affect soil moisture

Lab analyses discussed by Decker (2021) established that for two APD-EGR depths (10 cm and 20 cm) Kansas BuildEx® (K) held more water (by volume) within the substrate profile than rooflite® (R). The physical properties (per 2018 lab analyses of APD-EGR substrate samples) for substrate types K and R, are meaningfully different, with R being more porous (Table 3).

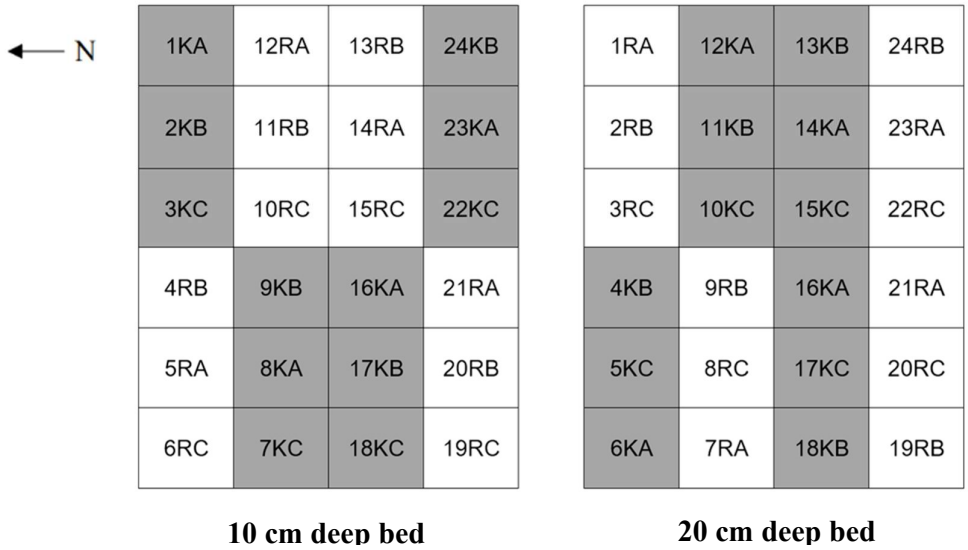

Figure 5. Plant mixes A, B, and C in the Kansas BuildEx®, K *(marked with gray color),* and the rooflite® extensive mc, R, substrates in the 10 cm and 20 cm deep beds, with plots in each bed numbered from 1 to 24; taken from (Alam 2022).

Table 3. Reporting substrate properties of K and R. Substrates were tested at Turf and Soil Diagnostics lab in Linwood, Kansas in 2018. Both substrates are able to retain water despite having different properties.

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 this research was to examine cause-and-effect relationships (Thomas et al., 2015). Independent variables for the APD-EGR 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 estimated the soil/substrate carbon (C) sequestration potential of the APD-EGR by measuring microbial biomass using Phospholipid Fatty Acid Analysis (PFLA) and root biomass within the two different substrates (Kansas BuildEx® and rooflite®) from two growing seasons (2019 for PLFA, and 2020 for root biomass). Data from two depths (10 cm and 20 cm) were analyzed and compared.

PLFA analyses of the two substrate types and substrate depths were conducted at the KSU Department of Agronomy Soil Microbial Agroecology Lab (SMAL). Additionally, root biomass analyses for the volume of the core were performed to complement the PLFA analyses. These tasks allowed the research team to estimate and better understand carbon

dynamics within the two substrate types and depths. This research suggests additional experimental approaches are needed for estimating the aboveground plant biomass of an ecosystem since belowground biomass studies can be too destructive for *in-situ* green roof systems. It is evident from many studies that a significant amount of carbon is stored in living plant tissues located above the earth's surface (Shen et al., 2021). Ideally, a long-term analysis of both belowground and aboveground biomass samples would be undertaken to gather more evidence of carbon sequestration on the APD-EGR. Completing such studies in non-destructive ways is deemed important, and so PLFA analysis may remain the best approach given the situation. It is uncertain how future PLFA studies will be done on the APD-EGR given budgetary and personnel limitations.

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 were the independent variables for this study. 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). The substrate sampling protocols, and laboratory procedures used to conduct the PLFA analyses at the SMAL are described in more detail.

Statistical Analyses:
Statistical evaluation of data was performed using IBM SPSS Statistics 28. 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 (indoor procedures were conducted at SMAL.

Soil Sampling for PLFA Analyses (substrate samples taken from 10 cm and 20 cm beds):
Composite soil samples (25 g) were collected and stored in labelled (Figure 5) plastic bags from each treatment/plot from the two beds for laboratory analyses after identifying uniform areas near plants in the APD-EGR plots. The sampling probe (18 mm) was cleaned with acetone between samples to avoid contamination. Barren areas in each plot were avoided during sampling. A total of 48 substrate samples were collected from the APD-EGR (24 in the 10 cm bed and 24 in the 20 cm bed) on October 3, 2019, and stored at -4°C until the samples were analyzed.

Soil Sampling for Root Biomass Analysis (substrate samples from 10 cm and 20 cm beds):
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. K-State

researchers collected 48 APD-EGR soil/substrate samples at the end of the third growing season for root biomass analysis on November 6, 2020.

Procedure for Root Biomass Analysis:
Root biomass was 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 10 cm and 20 cm plots (24 x 2 for 48 total samples).

- Volumetric cores were taken from the 10 cm bed and 20 cm deep bed, thus producing four replications (reps) for each unique plot type (plant mix and soil type). The samples were collected at a consistent distance (~3–6 cm) from a plant selected near the southeastern corner of each plot. A 5 cm (2-inch) diameter corer was used to collect one core per plot. Although the APD-EGR plots had four replicates (or reps), these reps were not combined, so statistical analyses could be used to compare and contrast the findings among all 48 plots in the approximately 10 cm and 20 cm deep beds.
- Some substrate cores did not come out as complete and consistent core lengths given the sandy and gravelly nature of the substrate. Because the volume of a cylinder is $\boldsymbol{V = \pi r^2 h}$, researchers kept track of each core depth and height (h). The most effective approach was to measure the core depths (h) manually during the sampling process, allowing the collection of "substrates with roots" from the 5 cm (2-inch) volumetric core using a soil probe and/or trowel, as needed to remove the core. (Note that these holes were later filled in with extra/stored K or R substrate.)
- In the laboratory, each core required visual observation to first remove coarse material (Wilsey and Polley 2006). Large roots were hand-picked and removed from the soil samples, and then the substrate samples were passed through 4-mm, 2-mm, and 1-mm sieves, respectively, with the roots being collected with tweezers from each sieve. All roots were gathered in metal tins and washed over a 0.25-mm screen/sieve. The metal tins were labeled with plot numbers and weighed before the roots were placed in them. Because only the K-type substrate had ~2% of clay a root washer was not needed to separate the clay from the roots. Samples were refrigerated until the roots were washed.
- After washing, the root samples in the 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 the root biomass density (p = m/V) for the volume of the core, using the formula $\boldsymbol{V = \pi r^2 h}$.
- Roots were gathered in a separate metal tin for each sample/plot.

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 (approximately 10 cm and 20 cm deep beds) on total microbial biomass (total MB) as well as their interactions using IBM SPSS Statistics 28.

Figure 6. Collecting and analyzing root biomass samples from 10 cm and 20 cm deep APD-EGR beds. Photographs taken by M. M. Lekhon Alam, November 2020. (a) measuring consistent distance (~3–6 cm) from a plant; (b) trying to reach the bottom of the plot with a soil probe while also taking the height to determine ***h*** value for the analysis; (c) a volumetric core of soil was taken from the plot; (d) Large roots were hand-picked from the soil; (e) a portion of root biomass from a soil core; (f) Roots were gathered in a separate metal tin for each sample/plot.

From the three-way ANOVA, total microbial biomass was significantly different between the two depths, with higher biomass in the 10 cm deep bed (Mean=45.1) compared to the 20 cm 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), ($F(2, 35) = 3.56$, $p = 0.039$) (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).

Discussion and 2019 PLFA result interpretations

Analysis 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 10 cm bed compared to the 20 cm 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.

Table 4. Descriptive statistics of total microbial biomass.

Variables		Mean (nmol PLFA/g soil)	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Depth	10 cm	45.1	2.44	40.1	50.0
	20 cm	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 10 cm and 20 cm deep bed (SPSS outputs).

Variables	Sum of squares	df	Mean square	F	p-value
Depth	1406.941	1	1406.941	9.845	0.003***
Substrate	144.236	1	144.236	1.009	0.322
Plant	665.376	2	332.688	2.328	0.112
Depth × Substrate	559.830	1	559.830	3.917	0.056*
Depth × Plant	1017.427	2	508.714	3.560	0.039**
Substrate × Plant	724.506	2	362.253	2.535	0.094
Depth × Substrate × Plant	92.277	2	46.139	0.323	0.726

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

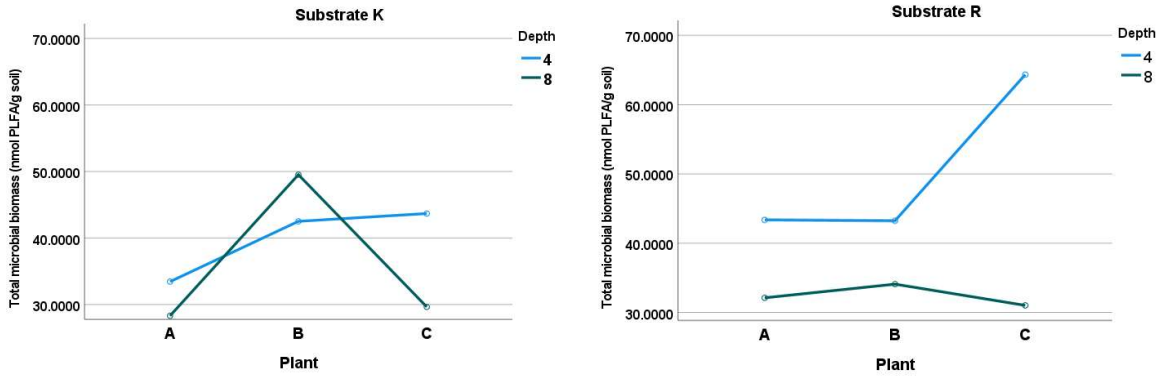

Figure 7. The average amount of total microbial biomass in conditions at two depths (10 cm and 20 cm), belowground biomass samples of plant mixes A, B and C, and two substrates (K and R).

The three-way ANOVA statistical results (Tables 4 and 5) indicate that belowground biomass containing grasses (plant mixes B and C) had higher microbial biomass. The 10 cm bed had greater microbial biomass than the 20 cm bed. The rooflite® extensive mc (R) contained more microbial biomass than the Kansas BuildEx® (K) in the shallower 10 cm bed (Table 5 and Figure 7). The higher plant root density caused by limited substrate depth resulted in higher microbial biomass in the two substrates on the APD-EGR. Future studies should seek more conclusive evidence to support the above claims and determine the mechanism of the carbon storage capacity of substrates.

COMPLEMENTING THE 2019 PLFA (MICROBIAL BIOMASS) RESULTS WITH AN ANALYSIS OF ROOT BIOMASS FROM THE 2020 GROWING SEASON

Root biomass analysis

To explore the extent of soil microbes and their effects on carbon sequestration potential, data for root biomass, microbial biomass, and total carbon in the soil is needed. In general, aboveground biomass assesses productivity (Barrachina et al., 2015; Lauenroth et al., 1986) correlate to carbon belowground. Thus, data on root density supports both microbial biomass and carbon sequestration.

Data analyses and results

A three-way ANOVA was used to evaluate the effect of belowground biomass of three plant mixes (A, B, and C), substrates K and R, and soil depths (10 cm and 20 cm) on root density and their interactions. Tukey's HSD post-hoc test determined a pairwise comparison between two sets of groups using IBM SPSS Statistics 28. Root density was significantly different among plant mixes A, B, and C ($F(2, 36) = 18.92$, $p = 0.000$) (Table 6). The root density in plant mix C (Mean=0.253) and plant mix B (Mean=0.233) were higher than in plant mix A sample (Mean=0.075) at $p = 0.000$.

Table 6. Descriptive statistics of root density data.

Variables		Mean (g/cm^3)	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Depth	10 cm	0.206	0.018	0.169	0.243
	20 cm	0.168	0.018	0.131	0.205
Substrate	K	0.193	0.018	0.156	0.230
	R	0.181	0.018	0.144	0.218
Plant Mix	A	0.075	0.022	0.029	0.120
	B	0.233	0.022	0.188	0.279
	C	0.253	0.022	0.207	0.298

Root density interpretations and discussion

From the three-way ANOVA, the belowground biomass containing grasses (B and C) had significantly (Table 7) higher root density overall than *Sedum* in both 10 cm and 20 cm beds. In the case of K and R substrates (Fig. 8), the belowground root density of three different APD-EGR plant mixes (more evident to B and C) was higher in the 10 cm bed as compared to the 20 cm bed.

Table 7. Three-way ANOVA results of root density data sets for the 10 cm and 20 cm beds (SPSS outputs).

Variables	Sum of squares	df	Mean square	F	p-value
Depth	0.018	1	0.018	2.210	0.146
Substrate	0.002	1	0.002	0.222	0.641
Plant	0.305	2	0.153	18.920	0.000***
Depth * Substrate	0.002	1	0.002	0.237	0.630
Depth * Plant	0.011	2	0.006	0.701	0.503
Substrate * Plant	0.010	2	0.005	0.644	0.531
Depth * Substrate * Plant	0.017	2	0.008	1.043	0.363

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

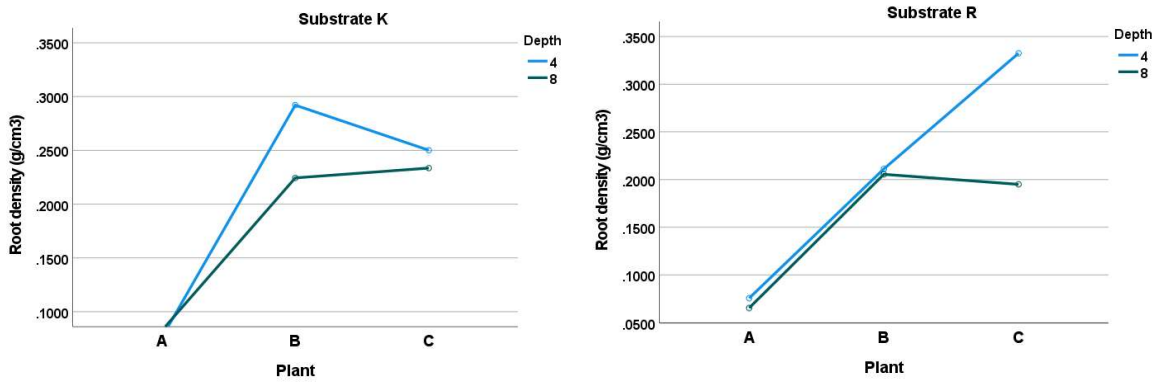

Figure 8. The average amount of root density in conditions at two depths (10 cm and 20 cm), belowground biomass samples of plant mixes A, B, and C, and two substrates (K and R).

It is important to note that native short grass roots have significantly more belowground biomass than *Sedum* spp. (Sutton 2013) and that the higher root biomass of these perennial grasses contributes more carbon to the soil (Sainju et al., 2017).

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 the 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 to answer and link the depths of APD-EGR substrates to carbon sequestration potential and possible longer term performance. The analysis discussed in this paper identified potential causes of root biomass on the two APD-EGR beds and hypothesized the effect of depth from the empirical studies.

In the shallower 10 cm deep (4 in) bed, roots proliferated within the entire bed more than the 20 cm deep (8 in) bed because the roots have less space. In the 10 cm bed, belowground biomass for A, B, and C plant mixes may become root-bound since their roots were observed from the soil sample columns to reach the bottom of the bed and thus over time may occupy all available substrate space; therefore, the root density of each shallow plot will tend to increase. Statistical analysis of PLFA data from 2019 has provided significant evidence of interactions at varying depths. Depth was the most important factor in microbial biomass and root density.

Visual observations between July and October 2022, after supplemental water was eliminated between January 2022 and mid-July 2022, clearly reveal severe vegetative stress and widespread plant dieback, indicating that deeper substrates provide greater support for plant survival than the shallower substrates under severe drought stress (Figure 9). Irrigation during extended dry periods is deemed essential in the Flint Hills Ecoregion to provide full or nearly full coverage (Skabelund et al., 2014), particularly on green roofs receiving approximately eight hours or more of full sunlight each day (Skabelund et al., 2022).

Figure 9. Visible plant stress during an extended dry period was greater in the 10 cm bed (left) than the 20 cm bed (right) following cessation of irrigation between January 2022 and July 20, 2022, on the APD-EGR. These two 5 September 2022 photos were taken by Lee R. Skabelund.

RESEARCH FINDINGS IN CONTEXT

Fundamentals of SOC

Soil organic matter (SOM) is composed of soil microbes, including bacteria and fungi, decaying material from once-living organisms, such as plant and animal tissues, fecal material, and products formed from their decomposition (Alam 2022; Ontl and Schulte 2012). Soil C includes both SOM and the inorganic C in carbonate minerals (Jobbágy and Jackson 2000). Soil C is a carbon sink, within the global C cycle, playing a role in biogeochemistry, climate-change mitigation, and the construction of global climate models (Amelung et al. 2020). SOC levels are directly related to the amount of OM contained in soil (Ontl and Schulte 2012), and result from the interactions of ecosystem processes, photosynthesis, respiration, and decomposition (Ontl and Schulte 2012). The decomposition of plant biomass by soil microbes results in C loss from the soil in the form of CO_2 due to microbial respiration (Alam 2022; Ontl and Schulte 2012). Soil respiration is a measure of the CO_2 released by the microbial decomposition of SOM and the respiration of plant roots and soil fauna (Alam 2022; USDA n.d.).

This study focused on estimating microbial biomass as an early indicator of changes in SOC. Microbes decompose SOM releasing CO_2 and plant-available nutrients. Soils with more organic (labile) C tend to have 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 supporting 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 an enhanced microbial population (McNear Jr 2013). Thus, living root-soil interfaces are nutrient-rich, and act 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 is positively correlated with microbial biomass. K-State researchers suggest that the amount of microbial biomass is likely due to the higher density of roots in the 10 cm bed than in the 20 cm bed. We also suggest that "soil depth constraints" may create higher microbial populations in substrate R than in substrate K, which helps to retain more carbon.

Although there are some limitations (noted above and below), the study suggests that shallower beds with rooflite® (R) substrate, having lower bulk density, higher pore space, and lower water holding capacity than the Kansas BuildEx® (K) substrate, 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. However, to retain plant health and carbon sequestration, supplemental irrigation during dry periods is essential. Based on 2018-2022 observations by the research team, supplemental irrigation should only be needed once a week on the APD-EGR, even during the driest and hottest periods of the growing season.

CONCLUSIONS, RESEARCH LIMITATIONS, AND FUTURE OPPORTUNITIES

This study has the following limitations. Substrate depths are known to vary in both the 10 cm and 20 cm beds (with some beds having depths as shallow as 6.35 cm in the 10 cm bed and others as great as 24-25 cm in the 20 cm bed), but these variations were not examined for this initial carbon sequestration study. Additionally, this research did not measure nor interpret plant residue (aboveground vegetative biomass) data at the end of the 2019 and 2020 growing seasons.

To assess the total amount of carbon in each substrate type, the study suggests the need for analysis of total carbon and nitrogen, and soil respiration. APD-EGR researchers hope that microbial biomass and root biomass research can continue during future growing seasons on this and other *in-situ* green roofs to provide multi-year baselines and important references for longer-term studies of carbon sequestration on the APD-EGR and at green roofs studied in other parts of the world.

LITERATURE CITED

Akther, M., J. He, A. Chu, J. Huang, and B. Van Duin. 2018. "A review of green roof applications for managing urban stormwater in different climatic zones." *Sustainability,* 10 (8): 2864.

Alam, M.M.L. 2022. "Climate change mitigation potential of green roofs: Exploring urban heat island indicators and a green roof's capacity to sequester carbon in the Flint Hills Ecoregion." PhD dissertation, Kansas State University (https://krex.k-state.edu/handle/2097/42822).

Amelung, W., D. Bossio, W. de Vries, I. Kögel-Knabner, J. Lehmann, R. Amundson, R. Bol, C. Collins, R. Lal and J. Leifeld. 2020. "Towards a global-scale soil climate mitigation strategy." *Nature communications,* 11 (1): 1-10.

Anderson, K.L., and C.I. Fly. 1955. "Vegetation-soil relationships in Flint Hills bluestem pastures." *Rangeland Ecology and Management/Journal of Range Management Archives,* 8 (4): 163-169.

Barrachina, M., J. Cristóbal, and A. Francesc Tulla. 2015. "Estimating above-ground biomass on mountain meadows and pastures through remote sensing." *International Journal of Applied Earth Observation and Geoinformation,* 38: 184-192.

Berardi, U., GhaffarianHoseini, A., and GhaffarianHoseini, A. 2014. "State-of-the-art analysis of the environmental benefits of green roofs." *Applied energy*, *115*, 411-428.

Best, B.B., R.K. Swadek, and T.L. Burgess. 2015. "Soil-based green roofs." In *Green Roof Ecosystems*, 139-174. Springer.

Bligh, E.G., and W.J. Dyer. 1959. "A rapid method of total lipid extraction and purification." *Canadian Journal of Biochemistry and Physiology,* 37 (8): 911-917.

Buffam, I. and M.E. Mitchell. 2015. "Nutrient cycling in green roof ecosystems." *Green roof ecosystems,* 107-137.

Chaplin, S., P. Simms, T. Cook, and E. Dinerstein. 2007. "Flint Hills tall grasslands." https://www.worldwildlife.org/ecoregions/na0807.

Chapman, S.S., J.M. Omernik, J.A. Freeouf, D.G. Huggins, J.R. McCauley, C.C. Freeman, G. Steinauer, R.T. Angelo and R.L. Schlepp. 2001. "Ecoregions of Nebraska and Kansas (color poster with map, descriptive text, summary tables, and photographs)." *US Geological Survey, Reston, Virginia,* 1 (1,950,000).

Decker, A.B. 2021. *An experimental design to assess soil-plant-water relations on a Kansas green roof*. PhD dissertation, Kansas State University (https://krex.k-state.edu/dspace/handle/2097/41683).

Demuzere, M., K. Orru, O. Heidrich, E. Olazabal, D. Geneletti, H. Orru, A. G. Bhave, N. Mittal, E. Feliu, and M. Faehnle. 2014. "Mitigating and adapting to climate change: Multi-functional and multi-scale assessment of green urban infrastructure." *Journal of Environmental Management,* 146: 107-115. https://doi.org/https://doi.org/10.1016/j.jenvman.2014.07.025.

Fioretti, R., A. Palla, L. G. Lanza, and P. Principi. 2010. "Green roof energy and water related performance in the Mediterranean climate." *Building and Environment* 45 (8): 1890-1904. https://doi.org/https://doi.org/10.1016/j.buildenv.2010.03.001.

Frasier, I., E. Noellemeyer, R. Fernández and A. Quiroga. 2016. "Direct field method for root biomass quantification in agroecosystems." *MethodsX,* 3: 513-519.

Gaumont-Guay, D., and R. Halsall. 2013. "The carbon balance of a Pacific west coast green roof." Proceedings of Cities Alive: 11th Annual Green Roof and Wall Conference, San Francisco, California.

Getter, K.L., D.B. Rowe and B.M. Cregg. 2009. "Solar radiation intensity influences extensive green roof plant communities." *Urban Forestry and Urban Greening,* 8 (4): 269-281.

Getter, K.L., D.B. Rowe, G.P. Robertson, B.M. Cregg and J.A. Andresen. 2009. "Carbon Sequestration Potential of Extensive Green Roofs." *Environmental Science and Technology,* 43 (19): 7564-7570. https://doi.org/10.1021/es901539x.

Hoyle, F.C., D.V. Murphy and I.R.P. Fillery. 2006. "Temperature and stubble management influence microbial CO2–C evolution and gross N transformation rates." *Soil Biology and Biochemistry,* 38 (1): 71-80.

Ismail, W. and M. Abdullah. 2016. "Mitigating climate change through green roof implementation: a review of significant literature." *Journal of Architecture, Planning and Construction Management,* 6 (2).

Jaffal, I., S. Ouldboukhitine and R. Belarbi. 2012. "A comprehensive study of the impact of green roofs on building energy performance." *Renewable Energy,* 43: 157-164. https://doi.org/https://doi.org/10.1016/j.renene.2011.12.004.

Jobbágy, E.G. and R.B. Jackson. 2000. "The vertical distribution of soil organic carbon and its relation to climate and vegetation." *Ecological Applications,* 10 (2): 423-436.

Jones, D.L., A. Hodge and Y. Kuzyakov. 2004. "Plant and mycorrhizal regulation of rhizodeposition." *New Phytologist* ,163 (3): 459-480.

Knapp, M. 2017. "Weather Reports for Research Field Locations." *Kansas Agricultural Experiment Station Research Reports,* 3 (6): 3.
Korenblum, E., Y. Dong, J. Szymanski, S. Panda, A. Jozwiak, H. Massalha, S. Meir, I. Rogachev and A. Aharoni. 2020. "Rhizosphere microbiome mediates systemic root metabolite exudation by root-to-root signaling." *Proceedings of National Academy of Sciences,* 117 (7): 3874-3883.
KSU. 2012. "Konza Prairie Site Description." Konza Prairie Biological Station (KPBS). http://www.konza.ksu.edu/general/knzsite.aspx?currMenu=9anddepth=1.
Kuronuma, T., H. Watanabe, T. Ishihara, D. Kou, K. Toushima, M. Ando and S. Shindo. 2018. "CO_2 payoff of extensive green roofs with different vegetation species." *Sustainability,* 10 (7): 2256.
Lauenroth, W.K., H.W. Hunt, D.M. Swift and J.S. Singh. 1986. "Estimating aboveground net primary production in grasslands: a simulation approach." *Ecological Modelling,* 33 (2-4): 297-314.
Lin, B., C. Yu, A. Su and Y. Lin. 2013. "Impact of climatic conditions on the thermal effectiveness of an extensive green roof." *Building and Environment,* 67: 26-33. https://doi.org/https://doi.org/10.1016/j.buildenv.2013.04.026.
Liu, J., P. Shrestha, L.R. Skabelund, T. Todd, A. Decker and M.B. Kirkham. 2019. "Growth of prairie plants and sedums in different substrates on an experimental green roof in Mid-Continental USA." *Science of the Total Environment,* 697: 134089.
McNear, D.H. 2013. "The rhizosphere-roots, soil and everything in between." *Nature Education Knowledge,* 4 (3): 1.
Ontl, T.A. and L.A. Schulte. 2012. "Soil carbon storage." *Nature Education Knowledge,* 3 (10).
Quideau, S.A., A.C.S. McIntosh, C.E. Norris, E. Lloret, M.J.B. Swallow and K. Hannam. 2016. "Extraction and analysis of microbial phospholipid fatty acids in soils." *Journal of Visualized Experiments,* (114): e54360.
Raaijmakers, J.M., T.C. Paulitz, C. Steinberg, C. Alabouvette and Y. Moënne-Loccoz. 2009. "The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms." *Plant and Soil,* 321 (1-2): 341-361.
Refahi, A.H. and H. Talkhabi. 2015. "Investigating the effective factors on the reduction of energy consumption in residential buildings with green roofs." *Renewable Energy,* 80: 595-603. https://doi.org/https://doi.org/10.1016/j.renene.2015.02.030.
Sailor, D. 2009. Cooling Our Cities: An Interview with Dr. David Sailor. edited by Brian Phelps. Green Infrastructure Digest: sustainable site solutions for a sustainable world. https://hpigreen.com/2009/12/23/cooling-our-cities-an-interview-with-dr-david-sailor/.
Sainju, U.M., B.L. Allen, A.W. Lenssen and R.P. Ghimire. 2017. "Root biomass, root/shoot ratio, and soil water content under perennial grasses with different nitrogen rates." *Field Crops Research,* 210: 183-191.
Shen, X., M. Jiang, X. Lu, X. Liu, B. Liu, J. Zhang, X. Wang, S. Tong, G. Lei and S. Wang. 2021. "Aboveground biomass and its spatial distribution pattern of herbaceous marsh vegetation in China." *Science China Earth Sciences,* 64 (7): 1115-1125.
Skabelund, L.R., C. Blocksome, M. Hamehkasi, H.J. Kim, M. Knapp and D. Brokesh. 2014. "Semi-arid green roof research 2009–2014: resilience of native species." Proceedings of Cities Alive: 12th Annual Green Roof and Wall Conference, Nashville, Tennessee.
Skabelund, L.R., Y. Gao, J.H. Taylor, N. Laney, E. Musoke-Flores, M.M.L. Alam and A. Decker. 2022. "Dominant vegetation types on two steep slope prairie-like green roofs (Kansas/USA)." Cities Alive: 19th Annual Green Roof and Wall Conference: 8.1 Research Track, 2022 Conference Proceedings. Philadelphia, Pennsylvania.
Sohn, E. 2009. "Green Roofs Offset Global Warming, Study Finds." *NBC News*.
Starry, O.S. 2016. "Ecosystem ecology as a framework for organizing and advancing greenroof research." *Israel Journal of Ecology and Evolution,* 62 (1-2): 97-102.
Sutton, R.K. 2013. "Rethinking extensive green roofs to lessen emphasis on above-ground biomass."
Thomas, J.R., J.K. Nelson and S.J. Silverman. 2015. *Research methods in physical activity*. Human kinetics.
Tollerud, H., J. Brown, T. Loveland, R. Mahmood and N. Bliss. 2018. "Drought and land-cover conditions in the great plains." *Earth Interactions,* 22 (17): 1-25.

United Nations Development, Programme. 2007. *Fighting climate change: Human solidarity in a divided world*. Springer.

USDA. n.d. *Soil Respiration.* United States Department of Agriculture (https://www.nrcs.usda.gov/).

White, P.M. and C.W. Rice. 2009. "Tillage effects on microbial and carbon dynamics during plant residue decomposition." *Soil Science Society of America Journal,* 73 (1): 138-145.

Whittinghill, L.J., D.B. Rowe, R. Schutzki and B.M. Cregg. 2014. "Quantifying carbon sequestration of various green roof and ornamental landscape systems." *Landscape and Urban Planning,* 123: 41-48. https://doi.org/https://doi.org/10.1016/j.landurbplan.2013.11.015.

Wilsey, B.J. and H.W. Polley. 2006. "Aboveground productivity and root–shoot allocation differ between native and introduced grass species." *Oecologia,* 150 (2): 300-309.