Project Title:	Using root and soil traits to forecast woody encroachment dynamics in mesic grassland
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Executive Summary

Grasslands are a widespread and globally important biome providing key ecosystem services including C storage and regulation of the water cycle. Grasslands face multiple threats, including changes in drought intensity and woody encroachment - a process that results in increased woody plant abundance corresponding with decreased herbaceous plant abundance. The combination of reduced soil moisture and shifts in plant dominance from herbaceous to woody are likely to alter C pools in the soil profile. We currently do not have the capacity to predict either the magnitude or rates of change in these C pools. In order to predict changes in grassland vegetation structure and the associated impacts on C cycling requires greater understanding of changes in soil C pools at multiple soil depths, and the responses of these pools to changes in precipitation. To support and perform this parameterization of Land Surface Models, we performed detailed investigations of root (anatomical and physiological) and soil traits (microbial function) at varying soil depths, to capture the belowground impacts of changing dominant plant growth forms (grasses to shrubs) and the impacts of frequent drought.

We found that five consecutive years of moderate drought, in combination with fire, was not sufficient to reduce shrub biomass production or stem density of the predominant encroaching shrub *Cornus drummondii*. In addition, we found evidence that *C. drummondii* is highly resilient to changes in water availability. This resilience is likely a reflection of a unique leaf physiological strategy to maximize C gain throughout the canopy, and higher fine root abundance than typical woody plants, improving competitive ability for resource acquisition. To our knowledge, we collected the deepest (1 m) belowground net primary productivity (BNPP) measurements ever conducted in grasslands, to compare plant C pools in grassy v shrubby areas. We found that grass fine root production was higher in surface soils than shrub fine root production, and that shrub BNPP appears to be more heavily influenced by annual precipitation (lower BNPP during drier years). This was coupled with detailed root excavations of entire shrub assemblages to assess the rooting depth distributions and magnitude of coarse root biomass. The assessment of belowground C stocks between grassy and shrubby areas revealed that root stocks, soil microbial respiration, 30-day mineralizable C, C-cycling enzyme activities, and total soil C concentrations were lower under dogwoods than grasses, despite a higher proportion of woody-plant-derived C under dogwoods. Cumulative C stocks show that woody encroachment should not be expected to increase soil C, and that there is a significant pool of particulate C in native tallgrass prairies that is vulnerable to loss as a consequence of woody plant encroachment. Cumulatively, this information is being used to parameterize a vegetation demographic model, BiomeE. A sensitivity analysis revealed that there were two types of encroachment that were linked with different characteristics of shrubs in the model. First, steady monotonic increases reflect the shrubs ability to produce small cheap clonal growth and low investment in shallow roots. Second, rapid expansion during years of particularly favorable conditions – namely dry years followed by fire – were linked with slow growth rates and lower water transportation capacity. This allowed shrubs to persist through drought conditions and then take advantage of more favorable conditions the following year.

The experiment-modeling framework described here will improve our understanding of interactions and feedbacks between aboveground and belowground processes, by linking plant-soil-microbial traits. The details of these coupled interactions (plant-soil-microbial) will improve the representation of subsurface processes in LSMs and will improve forecasts of dynamic changes in ecosystem structure in grassland ecosystems.

Overview of Project:

As noted in the Executive summary, our goals were to improve our ability to project ecosystem consequences of shrub encroachment in the US Great Plains region using a set of objectives that combined observational, experimental and modeling approaches to. We focused on 3 main objectives: (1) Quantify differences in aboveground (stem and leaf biomass) and belowground C pools (root C, microbial C, bulk soil C) using detailed excavations of entire mixed shrub-grass assemblages. We subsampled portions of the rhizosphere for detailed root physiological and microbial activity measurements; (2) Using rainout shelters built over mature shrub-grass communities, we experimentally reduced the amount of precipitation to compare responses among shrubs and grasses (water use, growth, stress, and soil and microbial C and microbial C-cycling activity); (3) Using a demographic LSM (BiomeE), we created scenario projections to assess the impacts of available water on shrub-grass cover in the central Great Plains region, and the resulting effects of these dynamics on ecosystem services (aboveground production, above- and belowground C budgets).

Remarkably (given a global pandemic and our ambitious goals), we did not experience any measurable departures from our original goals and didn't encounter any problems that couldn't be remedied by our team. Given that we had 4 complementary, yet independent research teams to accomplish the tasks in our original proposal, we formatted the final report to illustrate the accomplishments of each group. Organizing the report in this fashion provides transparency and accountability into which group accomplished which tasks. Each section provides a short overview, a synthesized section regarding the information learned, the students and technicians that form the backbone of the scientific accomplishments, and then the measurable outcomes of our work.

<u>Nippert Lab (Kansas State University)</u>

Description:

The Nippert Lab led the Shrub Rainout Manipulation Plots (ShRaMPs) experiment, in addition to projects focused on shrub canopy physiology and root structural changes with shrub encroachment.

1) <u>ShRaMPs</u> – Rainout shelters (control vs. 50% rainfall exclusion) were constructed in neighboring watersheds with different burn frequencies (1-year vs. 4-year burn) at Konza Prairie Biological Station (KPBS) to assess how dominant C_4 grasses and encroaching shrubs are impacted by multi-year drought, and how this is mediated by fire frequency. We found that five consecutive years of moderate drought, in combination with fire, was not sufficient to reduce shrub biomass production or stem density in *Cornus drummondii*. Frequent fire reduced shrub biomass, but not stem density or shrub cover. In addition, we found evidence that *C. drummondii* is highly resilient to changes in water availability, with the ability to shift to deeper soil water sources when surface soils dry and osmotically adjust within individual growing seasons. *C. drummondii* also maintains high rates of water-use even when there are no additional increases in carbon capture, which is expected to further exacerbate the negative ecohydrological impacts of shrub encroachment in tallgrass prairie.

Associated publications:

Keen, RM, 2023 Ecohydrological implications of clonal shrub encroachment in tallgrass prairie. Doctoral Dissertation, Kansas State University.

Keen RM, Bachle S, Bartmess M, Nippert JB (*<u>In press</u>, Oecologia*) Combined effects of fire and drought are not sufficient to slow shrub encroachment in tallgrass prairie.

Keen RM, Helliker BR, McCulloh KA, Nippert JB (*In revision, Journal of Ecology*) Save or spend? Diverging water-use strategies of grasses and encroaching clonal shrubs.

2) <u>Shrub canopy physiology</u> – We also showed that the remarkably high LAI (leaf area index) values maintained by *C. drummondii* are facilitated by substantial vertical leaf trait plasticity across their canopies. Leaves at the top of canopies had high leaf mass per area and high photosynthetic capacity, while leaves in the lower canopy had low LMA values and the unique ability to maintain light compensation points below ambient light levels. Understanding these mechanisms is valuable, as high LAI values allow *C. drummondii* to shade out understory grasses and reduce fuel loads for future fires, establishing new feedbacks that promote the transition from grassland to shrubland.

Associated publications:

Tooley, EG, 2022 The unique canopy structure, leaf morphology, and physiology of *Cornus drummondii*. Master's thesis, Kansas State University.

Tooley EG, Nippert JB, Bachle S, Keen RM (2022) Intra-canopy leaf trait variation facilitates high leaf area index and compensatory growth in a clonal woody encroaching shrub. Tree Physiology. https://doi.org/10.1093/treephys/tpac078

Tooley EG, Nippert JB, Ratajczak Z (*In review, Agricultural and Forest Meteorology*) Evaluating methods for measuring the leaf area index of encroaching shrubs in grasslands: from leaves to optical methods, 3-D scanning, and airborne observations.

3) <u>Root architecture and physiology</u> –The Nippert lab also led efforts to understand belowground changes in rooting structure and physiology as shrub cover increases in tallgrass prairie. Deep (1-m) root cores were collected at KPBS in watersheds with varying fire frequencies (1-year, 4-year, and 20-year burns) and under both grass and shrub cover. We found that fine root biomass in surface soils was lower under shrub cover, but not to the degree we expected, and that the 1-year burn treatment had the highest fine root biomass overall. Specific root length was substantially lower under grass cover compared to shrub cover, particularly at deeper soil depths – higher specific root length indicates that these roots are more absorptive and have faster turnover rates compared to roots with lower specific root length, which has implications for carbon cycling at depth. Belowground net primary productivity (BNPP) was also measured in separate 1-m deep ingrowth cores. We found that grass fine root production was higher in surface soils than shrub fine root production, and that shrub BNPP appears to be more heavily influenced by annual precipitation (lower BNPP during drier years). In addition, we excavated 1 m^3 sections of root systems of *C. drummondii* to assess the rooting depth distributions and magnitude of coarse root biomass associated with encroaching clonal shrubs. Increasing *C. drummondii* cover introduces a substantial amount of belowground C stored in coarse woody roots that is not present in un-encroached grasslands. These rooting systems have been scanned by a 3D scanner and are being analyzed to better understand the complex rooting architecture of clonal shrubs.

Associated publications:

Two manuscripts from this project are currently *in prep*. The first is focused on differences in root biomass, physiology, and belowground net primary productivity under grass vs. shrub cover in tallgrass prairie. The second is focused on changes in rooting architecture in *C. drummondii* using 3D root scans.

Other publications with DOE support acknowledged:

Keen, R.M., Nippert, J.B., Sullivan, P.L., Ratajczak, Z., Ritchey, B., O'Keefe, K. and Dodds, W.K., 2023. Impacts of Riparian and Non-riparian Woody Encroachment on Tallgrass Prairie Ecohydrology. *Ecosystems*, *26*(2), pp.290-301.

Wedel ER, O'Keefe K, Nippert JB, Hoch B, O'Connor RC (2021) Spatio-temporal differences in leaf physiology are associated with fire, not drought, in a clonally integrated shrub. AoB PLANTS. https://doi.org/10.1093/aobpla/plab037

Rastetter EB, Kwiatkowski BL, Kicklighter DW, Barker Plotkin A, Genet H, Nippert JB, O'Keefe K, Perakis SS, Porder S, Roley SS, Ruess RW, Thompson JR, Wieder WR, Wilcox K, Yanai RD (2022) N and P constrain C in ecosystems under climate change: Role of nutrient redistribution, accumulation, and stoichiometry. Ecological Applications. e2684. doi:10.1002/eap.2684

Students and postdoctoral researchers (represents students directly supported by DOE funding):***
*Rachel Keen – PhD student (2019-2023)
*Greg Tooley – MS student (2020-2022) [summer salary support only]
Anna Shats – MS student (2020-2021)
*Lauren Gill – Undergraduate student (2020-2022)
Emily Wedel – PhD student (2019-2023)
Michael Bartmess – MS student (2017-2020)
Ryan Donnelly – Undergraduate student (2020)
Ella Elmendorf – Undergraduate student (2023)

McCulloh Lab (University of Wisconsin, Madison)

Description:
The McCulloh lab led two main projects – the investigation of root anatomical traits in grasses and shrubs and the investigation of hydraulic responses to drought in grasses and shrubs.

1) Root anatomical traits – Root anatomical traits were compared across different soil depths and fire frequencies. We found three main findings: (1) shallow woody roots had a high capacity to move water while deep woody roots had greater mechanical strength; (2) shallow grass roots had unique trait combinations that likely allow these plants to rapidly use water but tolerate dry soils under multiple fire regimes; and (3) hydraulic safety versus efficiency tradeoffs translate from anatomical to integrative root traits. Overall, these results provide anatomical evidence to explain water-use dynamics in tallgrass prairie and also provide novel insight regarding functional strategies that may facilitate the conversion from grassland to shrubland in less frequently burned tallgrass prairie.

Associated publications:
O'Keefe K, Bachle S, Keen R, Tooley EG, Nippert JB (2021) Root traits reveal safety and efficiency differences in grasses and shrubs exposed to different fire regimes. Functional Ecology. https://doi.org/10.1111/1365-2435.13972

Associated data products:
O'Keefe K, Bachle S, Keen R, Tooley EG, Nippert JB (2021). Data from: Root traits reveal safety and efficiency differences in grasses and shrubs exposed to different fire regimes. Dryad Digital Repository, https://doi.org/10.5061/dryad.dr7sqvb0g

2) Hydraulic responses to drought – We measured above- and belowground hydraulic responses to drought in shrubs and grasses burned at different frequencies. We showed that: (1) *A. gerardii* roots had a substantially greater capacity to move water than *C. drummondii* roots; (2) belowground hydraulic functioning was linked with aboveground processes; (3) above- and belowground *C. drummondii* hydraulics were not negatively impacted by the rainfall reductions imposed. These results suggest that a multi-year drought will not ameliorate rates of woody expansion and highlight key differences in aboveground and belowground hydraulics for dominant species within the same ecosystem.

Associated publications:
O'Keefe K, Nippert JB, Keen RM, McCulloh KA (*In revision, Oecologia*) Contrasting shrub and grass hydraulic responses to experimental drought.

Other publications with DOE support acknowledged:
O'Keefe K, Smith DD, McCulloh KA (2023) Linking stem rehydration kinetics to hydraulic traits using a novel method and mechanistic model. Annals of Botany. https://doi.org/10.1093/aob/mcad066
McCulloh KA, Augustine SP, Goke A, Jordan R, Krieg CP, O'Keefe K, Smith DD (2022) At least it is a dry cold: the global distribution of freeze–thaw and drought stress and the traits that may impart poly-tolerance in conifers. Tree Physiology. https://doi.org/10.1093/treephys/tpac102

Students and postdoctoral researchers (represents students directly supported by DOE funding)*:**
*Kim O'Keefe – Postdoctoral researcher (2017-2021)

Wilcox Lab (University of North Carolina, Greensboro)

Description: The Wilcox lab led two main efforts. First, the lab led an effort to synthesize current capabilities of process-based models to represent grassland processes and provide directions forward for empirical and modeling scientific communities. Second, we incorporated empirical information from Nippert, McCulloh, and Zeglin labs and used this to test mechanisms of shrub encroachment in tallgrass prairie.

1) Improving representation of grasslands in process-based models – We gathered empirical scientists and process-based modelers together in a synthesis effort that (1) conducted a model simulation exercise to assess current model capabilities to capture vegetation responses to global change drivers in grassland ecosystems, and (2) detailed several empirical and modeling research directions to improve representation of herbaceous vegetation in models. This research was a necessary first step to improve grassland representation in models and will lead to future improvements of herbaceous vegetation in earth system models.

Associated publications

Wilcox, K.R., Chen, A., Avolio, M.L., Butler, E.E., Collins, S., Fisher, R., Keenan, T., Kiang, N.Y., Knapp, A.K., Koerner, S.E., Kueppers, L., Liang, G., Lieungh, E., Loik, M., Luo, Y., Poulter, B., Reich, P., Renwick, K., Smith, M.D., Walker, A., Weng, E., Komatsu, K.J. 2023. Accounting for herbaceous communities in process-based models will advance our understanding of grassy ecosystems. Global Change Biology, 29(23), pp.6453-6477.

Wilcox, K.R., 2023. Catching up with the trees: empirical advancements to improve herbaceous representation in models. New Phytologist, 239(2), pp.452-455.

2) Mechanisms of shrub encroachment in tallgrass prairie – We first modified the vegetation demographic model BiomeE (Weng et al. 2022) to reflect physiological, morphological, and demographic characteristics measured in this project. After model calibration, we ran a sensitivity analysis to assess characteristics of shrubs that led to encroachment. This analysis showed us that there were two types of encroachment that were linked with different characteristics of shrubs in the model. First, steady monotonic increase in shrub area were aided by shrub ability to produce small cheap clonal growth and low investment in shallow roots. Second, rapid expansion during years of particularly favorable conditions – namely dry years followed by fire – were linked with slow growth rates and lower water transportation capacity. This allowed shrubs to persist through drought conditions and then take advantage of more favorable conditions the following year. Indeed, these 'waves of change' are documented in the long-term record at Konza Prairie, where there are abrupt increases in shrub cover during and after dry years. We also conducted model experiments designed to simulate increasing atmospheric CO2, high frequency extreme droughts, and presence or absence of fire. These simulations were run singly and in combination with one another. When absence of fire, increasing CO2, and increased frequency of drought were all stacked upon one another, encroachment rates became substantially faster highlighting the need to account for co-occurring global change drivers when predicting future states of ecosystems.

Associated publications

We have two publications in preparation associated with this research effort. The first one is being led by Anping Chen, who is using satellite data to derive the spatiotemporal patterns of shrub encroachment over the tallgrass prairie. This is being done with 30-m Landsat data stack and submeter- and meter-resolution imagery from GEE and Planet lab for labeling training pixels. The second publication is being led by Kevin Wilcox and is focused on the BiomeE sensitivity analysis and global change simulations.

Other publications with DOE acknowledged:

Wilcox, K.R., Collins, S.L., Knapp, A.K., Pockman, W., Shi, Z., Smith, M.D. and Luo, Y., 2023. Assessing carbon storage capacity and saturation across six central US grasslands using data–model integration. Biogeosciences, 20(13), pp.2707-2725.

Students and postdoctoral researchers (represents students directly supported by DOE funding)*:**

*Kim O'Keefe – Postdoctoral researcher (2021)
*Anping Chen – Research scientist (2022)
Sheila Cloud (2018-2022)
Ashley Dupuis (2019-2021)
Joshua Ajowele (2020-2023)

<u>Zeglin Lab (Kansas State University)</u>

Description:

The Zeglin Lab led work on controls on soil C stocks and microbial activity, and data collection on belowground C efflux in shrub-encroached areas and drought (ShRaMPs) experiments described above.

<u>1) Soil carbon stocks in woody-encroached grasslands.</u> Woody encroachment affects carbon (C) dynamics, but C-cycling responses in soil horizons below 20-cm depth are unknown. Understanding subsoil C response is critical, because most plant biomass in grasslands is belowground, and most soil C is at depth. We asked how shrub encroachment affects soil C cycling to 1-m depth at replicated dogwood-invaded or native grass-dominated locations. Because woody invaders have deeper roots, we expected higher C inputs and C-cycling activity deeper in the soil profile, under dogwoods than grasses. We collected replicate 1-m soil cores under dogwood islands and open grass areas in both heavily and weakly invaded areas, measured plant and soil C pools at 10-cm increments, and used isotopic C signatures to differentiate C3-woody-plant-derived from C4-grass-derived pools and fluxes. We also conducted laboratory priming experiments in soils collected from non-invaded and heavily invaded areas, to learn whether increased dissolved C inputs would stimulate or repress decomposition of standing soil C stocks.

Results partially support our predictions, in that the $\delta^{13}C$ of soil microbial respiration was lower under woody plants than grasses (-19.9 (1.0) < -17.1 (1.1), mean (SE), $p=10^{-16}$) over the whole soil profile, and this difference was greater at both 0-10 cm and 50-100 cm depths (p=0.0002). This shows that soil microbes use more C_3-plant-derived dissolved C, particularly at depth, under encroaching dogwood plants. Surprisingly, however, root stocks, soil microbial respiration, 30-day mineralizable C, C-cycling enzyme activities, and total soil C concentrations were lower under dogwoods than grasses, despite a higher proportion of woody-plant-derived C under dogwoods. There was also a strong positive priming response to sugar addition in soils collected from intact grasslands (40-110%), but no priming under dogwoods. After soil C stocks were summed through the 1-m soil profile (g C ha-1), woody encroachment had no significant effect: Grassland soils hold large amounts of C, so changes in stocks may not be significant until many decades pass. However, our results clearly show that woody encroachment should not be expected to increase soil C, and that there is a significant pool of particulate C in native tallgrass prairies that is vulnerable to loss as a consequence of woody plant encroachment.

<u>2) Belowground CO_2 efflux in response to shrub encroachment and drought.</u> We also measured belowground CO_2 efflux under grasses in in 1-y, grasses and shrubs in 4-y, and shrubs in 20-y fire-return interval watersheds, as well as under shrubs in a long-term drought experiment in1-y and 4-y fire-return interval watersheds. In both datasets, CO_2 efflux was higher in the annually burned treatment than the 4-y or 20-y fire return interval treatments ($p=10^{-16}$), and this effect overshadowed grass/shrub ($P=0.56$) or direct drought effects ($P=0.17$). Drought decreased belowground CO_2 efflux only in 4-y fire return interval treatments (Burn*Drought, $P = 0.0001$), while CO_2 efflux was higher and unaffected by drought in the annually burned treatments. Fire management had a stronger effect on belowground C loss to the atmosphere than woody encroachment or drought.

Associated publications:
One manuscript on soil carbon stocks and their vulnerability under grass cover, not shrub cover, is *in prep*.

Students and postdoctoral researchers (**represents personnel directly supported by DOE funding)***:**
Hannah Dea* – MS student (2019)
Kyle Cochran* – technician (2019-2020)
Harley-Payj Leatherman – undergraduate student (2021)
Domnique Byrd – undergraduate student (2021)