SOIL CHARACTERISTICS AND ECOSYSTEM-LEVEL EFFECTS OF WOODY SPECIES ENCROACHMENT IN TALLGRASS PRAIRIE

By

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Abstract: Grasslands across the globe currently experience numerous threats resulting in lost ecosystem services and global biodiversity. In the North American Great Plains, it is estimated that as little as 1% of the historical range of the tallgrass prairie ecosystem remains intact, and these tracts are threatened by a variety of global change phenomena. One of the greatest current threats to grasslands worldwide is the expansion of woody species. Many studies document aboveground consequences of woody species encroachment and have noted changes in multiple trophic levels. However, little research exists on belowground ecosystem-level effects following woody plant establishment, such as shifts in soil microbial community composition, including arbuscular mycorrhizal (AM) fungal abundance, and soil nutrient dynamics. Mycorrhizal interactions have previously been shown to play a critical role in native plant species dominance and contribute to healthy soil function of tallgrass prairie ecosystems. My study assesses abiotic and biotic soil characteristics of native tallgrass prairie and adjacent areas with established stands of the following woody species: *Juniperus virginiana*, *Cornus drummondii*, *Gleditsia triacanthos*, and *Rhus aromatica*. My results indicate woody encroachment and establishment result in species-specific alterations in biotic and abiotic soil characteristics. For example, encroachment by *J. virginiana* resulted in increased plant-available P and more alkaline soils, while soils associated with *R. aromatica* exhibited greater soil organic matter. While *C. drummondii* or *G. triacanthos* did not demonstrate significant shifts in nutrient availability, these species altered other soil abiotic characteristics including gravimetric moisture content and soil aggregate stability. *Juniperus virginiana* and *G. triacanthos* were associated with greater abundance of AM fungi, both within the roots and in surrounding soil, compared to native soils or soils associated with *C. drummondii* or *R. aromatica.* Alteration in soil characteristics may be one mechanism facilitating the rapid conversion of grasslands to woodland species, and may provide challenges to ecosystem restoration following removal of the woody species.

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CHAPTER I

INTRODUCTION

Among the many global change phenomena is the expansion of species to areas beyond their historic native range. Invasion of non-native species and the encroachment of native species can have a variety of negative effects on communities and ecosystems, including loss of ecosystem services and productivity through displacement of wildlife and native flora and loss of grazing lands (Samson *et al.* 1994 and 2004). Globally, grasslands may be the most widely, and perilously, threatened ecosystem (Ricketts 1999, Samson *et al.* 2004).

The tallgrass prairie of the North American Great Plains once occupied vast tracts of land from what is now southern Canada to northern Texas, but this vital ecosystem now occupies less than 10% of its historic range, with some experts estimating that as little as 1% of historic prairie remains (Samson *et al.* 1994). In addition to ecosystem losses caused by conversion to row-crop agriculture, the anthropogenic removal of fire has contributed to the loss of tallgrass prairie via woody species encroachment. While expansion of native woody species such as *Juniperus virginiana, Cornus drummondii, Gleditsia triacanthos,* and *Rhus aromatica* have become problematic in grasslands throughout the Great Plains, little is known about the resultant effects of encroachment on soil properties or ecosystem-level processes.

Many belowground effects (e.g. changes in soil dynamics) are coupled with or influence aboveground effects (community and ecosystem-level factors) and biotic properties are inextricably linked with abiotic properties (Bardgett et al. 2014). Native woody expansion may alter soil biotic properties such as arbuscular mycorrhizal (AM) associations, resulting in changes

in soil legacy dynamics. For example, greater root biomass and increased heterogeneity of soil nutrient resources have been linked to woody species encroachment (Pärtel et al. 2007), which may influence resource acquisition strategies with subsequent alterations in AM fungal abundance and composition.

The importance of arbuscular mycorrhizal (AM) associations in facilitating the growth and success of native prairie grasses has been well demonstrated (Johnson et al. 2010, Hartnett et al. 1999, Wilson et al. 1998). Furthermore, AM associations produce feedbacks in the soil that have been shown to alter plant community structure (Hartnett et al. 1999, Bever 2003, Miller et al. 2012).

AM fungal biomass surrounding roots (extra-radical hyphae) strongly influence soil aggregate stability. The extra-radical hyphae, together with the fibrous roots, form "sticky-string bags" that contribute to the consolidation of soil particles into macroaggregates, basic building blocks of soil structure, through physical entanglement of soil particles and binding of particles via production of extracellular polysaccharides and stable glycoproteins that act as a long-term soil binding agents (Tisdall and Oades 1982; Miller and Jastrow 2000, Wilson et al. 2009, Sikes et al. 2010).

The encroachment of woody plant species can have cascading effects through the terrestrial ecosystem. Many ecosystem-level functions are determined by biotic root associations involving interactions with microbial communities, especially AM fungi (Bardgett et al. 2014). AM symbioses can influence carbon fluxes between the biosphere and the atmosphere, resulting in ecosystem-level effects. A key AM fungal-mediated process involved in the storage of carbon in soils is the transfer of photosynthate from host plants to hyphae. Mycorrhizal biomass may operate as a carbon sink, thus relocating and increasing soil organic matter (Miller and Jastrow 2000; Six et al. 1998).

Despite the well-documented importance of plant-soil-microbial interactions on community and ecosystem-level processes, little is understood of the potential cascading effects

of soil microbial community alterations following encroachment of woody species into native grasslands. Therefore, the major objectives of my project are to examine soil biotic and abiotic parameters in grassland sites with native tree encroachment and adjacent prairie sites dominated by native warm-season grasses.

This study examines belowground biotic and abiotic characteristics in soils associated with encroaching woody species and C4- dominated prairie by testing various abiotic and biotic soil factors. Abiotic characteristics include: soil water-stable aggregation, soil pH, soil moisture, plant-available nitrogen and phosphorous, soil organic matter (SOM) and soil organic carbon (SOC). Soil biotic characteristics assessed include: arbuscular mycorrhizal (AM) fungal root colonization, annual production of AM hyphae, standing root biomass, and relative abundance of selected soil microbial groups determined through phospholipid fatty acid analyses (PLFA) and neutral lipid fatty acid analyses (NLFA).

Hypothesis H_1: Overall, encroaching woody species will be associated with greater inter-radical (assessed through percent root colonization) and extra-radical (assessed through hyphal in-growth bags) AM hyphal production, as compared to native prairie grasses. This hypothesis is based on a previous study in which J. virginiana was characteristically higher in both inter- and extra-radical hyphal production (Coppick 2009). However, I predict AM associations will be highly species-specific and may range widely between woody species.

Hypothesis H_2: AM fungal abundance will be positively correlated with stable soil structure and SOM. This hypothesis is based on a long-term ecological field study by Wilson et al. (2009) that found that AM fungi abundance was strongly associated with soil aggregation, possibly due to the presence of AM hyphae.

Hypothesis H_3: Shifts in abiotic soil characteristics, such as plant-available phosphorus, soil pH, and gravimetric soil moisture, will be dependent upon the identity of the encroaching woody species and result in species-specific alterations.

CHAPTER II

METHODOLOGY

STUDY SITE

Field work was conducted at Konza Prairie Biological Station (KPBS), near Manhattan, Kansas, USA. This 3,487 hectare preserve is divided into watershed-level experimental plots examining effects of varying fire intervals and presence of native and non-native grazers. Research was conducted in upland areas of three watersheds: 20B, a heavily encroached watershed experiencing fire suppression with its last burn occurring in April 1991; FA, a C_4-dominated grassland that is burned annually in the fall; and 1D, another C_4-dominated grassland that is annually burned in the spring. Watersheds FA and 1D were burned during the course of this research project: FA in November of 2014, 2015, and 2016, and 1D in April 2014, April 2015, and May 2016. In all sampling locations across all watersheds, the soil is a silty clay loam that is notably shallow and phosphorus limited.

This study focused on four encroaching woody species present in upland areas of KPBS watershed 20B: *Juniperus virginiana* (Eastern redcedar), *Cornus drummondii* (roughleaf dogwood), *Gleditsia triacanthos* (honey locust), and *Rhus aromatica* (fragrant sumac). Within each species, six replicate individuals of similar physical characteristics were selected for sampling. Data were collected for one growing season for *G. triacanthos* and *R. aromatica*. Because *J. virginiana* and *C. drummondii* are abundant encroachers, data were collected for two growing seasons to allow for temporal comparison of these problematic species. Two of the selected species represent single-stemmed tree species (*J. virginiana, G. triacanthos*), and two

represent clonal, multi-stemmed shrub species (C. drummondii, R. aromatica). C4 grass-dominated interstitial areas between individual trees (watershed 20B) and in nearby annually burned grasslands (watersheds FA and 1D) were included as controls.

Plant size of *J. virginiana* and *G. triacanthos* individuals was determined by assessing trunk diameter at breast height and tree height. Size of *C. drummondii* and *R. aromatica* was determined by assessing area of expansion (diameter) and number of stems per m^2. Individuals were nonrandomly selected for consistent size classes among members of the same species; additionally, only trees separated from other trees by a minimum distance of 2.0 m were sampled to minimize possible root overlap or misidentification. Average size and growth characteristics of selected individuals are given in Table 1. Plant species composition of each control area was conducted by using a 0.25 m^2 frame to assess percent cover using modified Daubenmire cover class (Daubenmire 1959). Soil and root samples were collected from each individual single-stemmed tree directly adjacent to the trunk and at the dripline (within C_4 grass-dominated plant communities). Shrub species with multi-stem morphologies were sampled at the center of the shrub island and at the leading edge (areas with C_4 grass vegetation). Soil and root samples were also collected from within each 0.25 m^2 control (C_4 grass-dominated) area (see Figure 1 for illustration of sampling locations).

ABIOTIC CHARACTERISTICS

Soil Aggregation

Aggregate stability is a useful indicator in water availability, root growth, and resistance to erosion, with soils that have greater proportion of macroaggregates generally considered more stable than those favoring microaggregates. Soils were field collected at 0-10 cm depth and wet sieved via the use of an automated wet sieving device.

The wet sieving apparatus, a modified Yoder machine, utilizes five stacked sieves: 4 mm and 2 mm (representing macroaggregation); 1 mm, 0.5 mm, and 0.25 mm (representing

microaggregation). For each soil sample, 50.0 g of soil was placed into the uppermost sieve. Sieves were attached to the Yoder machine and slaked in distilled water for 10 minutes. Samples were wet sieved for 10 minutes at 30 rotations per minute (Mikha and Rice 2004). After sieving, soil was removed from each sieve, dried, and weighed to calculate relative abundance of macro- and microaggregates.

Soil Moisture

As soil moisture is a driving factor in soil microbial community composition, soil was collected to obtain gravimetric moisture content. For each soil sampling location, 5.0g of wet soil was oven heated and dried at 70° C for at least 48 hours, then weighed to obtain dry soil weight. Gravimetric soil moisture content was obtained by calculating the difference of wet weight to dry weight of the soil sample.

Soil Nutrient Analyses

Subsamples of sieved soil from each sampling location were tested by the OSU Soil, Water, and Forage Analytical Laboratory for nutrient analyses. Before testing, soil samples were dried at 65° C overnight and ground to homogeneity to pass a 2.0 mm sieve. Each sample was tested for content of: soil organic matter (SOM), plant-available phosphorus (P), potassium (K), and nitrates (NO_3). Soil organic carbon was determined using a LECO Truspec dry combustion carbon analyzer (Nelson 1996). Potassium and plant-available phosphorus were determined using Mehlich-3 extraction solution (Mehlich 1984) and quantified by a Spectro Blue ICP spectrometer (Soltanpour 1996). Soil NO_3-N was extracted using 1M KCl solution and analyzed using the Lachat Quickchem 8000 Flow Injection Autoanalyzer (LACHAT 1994). Soil pH was measured by glass electrode in a 1:1 soil:water suspension (Sims 1996).

BIOTIC CHARACTERISTICS

Extra-radical Hyphal (ERH) Biomass

ERH production was quantified by using mesh in-growth bags and a procedure adapted from Schweiger and Jakobsen (2000) and Wallander *et al.* (2001). We used hyphal in-growth bags constructed from nylon mesh (40-µm mesh, 10 × 5 × 2 cm) that allows mycelia to grow into the bag but excludes roots. Bags were filled with 80 cm^3 of sterilized, acid-washed quartz sand (0.36–2.0 mm) and sealed. At the beginning of the growing seasons 2014 and 2015, six replicate hyphal in-growth bags were inserted into the soil (0-10 cm depth) at the base of each tree trunk or center of shrub clone, at the dripline or leading edge of each woody individual, and in the C_4 grass-dominated control areas. Bags were collected at the end of the growing season and combined for further analyses. ERH were isolated from the soil and standing crop quantified following the protocol of Miller *et al.* (1995). The sand from all six replicate bags was combined and ERH extracted by using 53-µm- and 38-µm-diameter nested sieves in sequence to collect the mycelia. The collected mycelia were freeze-dried and weighed; dry weight is used as a measure of relative annual production of hyphae.

Root Colonization

Fine, fibrous roots from each individual tree and from C_4 grasses were carefully washed to remove soil. To prevent damage to AM fungal structures, roots were not subjected to drying by heat (McGonigle *et al.* 1990). Proper samples included only secondary and tertiary roots, as primary roots typically have diameters too large for accurate analysis; the target diameter for roots was 0.25 mm or less. Samples were divided into individual tissue cartridges (Fisherbrand™ SURE-TEK Tissue Processing/Embedding Cassettes with lids) for staining and microscopic assessments.

Roots were bleached by soaking in 10% KOH solution for 24 hours. After bleaching, a dilute 1% HCl solution was added to acidify the staining solution. Roots were stained with 0.05%

trypan blue in lacto-glycerol for 10 minutes at 110°C. After staining, roots were washed and stored in 30% lactic acid.

For exceptionally thick roots (such as those of *J. virginiana*), samples were bleached for an hour in 10% KOH solution at 100°C, followed by 45 minutes in alkaline H_2O_2 (10:1 H_2O_2 : NH_4OH). Cleared roots were stained with a 5% ink-vinegar solution (5% acetic acid) using Shaffer black ink then heated at 110°C for 10 minutes. Roots were rinsed with water before acidified using a 2-3 drops of acetic acid. Roots were stored in 30% lactic acid.

Following staining, roots were examined microscopically at 200x magnification using a digital microscope (Hirox KH 7700). Three 4 cm samples of fresh live roots from each tree and from grasses within each control area were examined. Root colonization is based on visual observations on a 1 cm^2 grid (McGonigle *et al.* 1990) and combines percentage of root length colonized by hyphae, vesicles, and arbuscules to determine total percent colonization.

Microbial Community/ Quantification of the Mycorrhizal Fungus

A major advancement in quantifying the AM fungi association is the use of marker phospholipids (PLFA) and their neutral lipid (NLFA) counterparts (Allison & Miller 2005). PLFAs are constituents of biological membranes that can be used to estimate the biomass of fungi, because biovolume and cell surface area are well correlated (Tunlid 1992). The NLFAs, on the other hand, are the basic storage product of many fungi and serve as the primary energy reserve in fungi (Olsson 1999; Larsen & Bodker 2001). PLFA and NLFA 16:1ω5c can be used to quantify AM fungal biomass and energy reserve status, respectively (Olsson 1999). 18:2ω6,9 can be used quantitatively for saprophytic fungi. Phospholipid fatty acids (PLFA) and neutral lipid fatty acids (NLFA) were extracted from soil samples using a modification of the Bligh and Dyer (1959) extraction method (White *et al.* 1997). Total lipid extract was separated into PLFA and NLFA using silicic acid chromatography; the fatty acids were then cleaved from the glycerol backbone

using KOH saponification; and the harvested fatty acids were methylated to form fatty acid methyl esters (FAMEs) (White *et al.* 1997; Allison & Miller 2005). Gas chromatography and mass selection detection (Hewlett Packard) was used to analyze the FAMEs. In addition to measuring AM fungal biomass, the PLFA and NLFA allow for a simple measure of microbial diversity by determining the differences in evenness between treatments and provide valuable data related to C processing and storage (Billings & Ziegler 2005; Olsson & Johnson 2005).

Biomarkers used to select for the functional group of gram-positive bacteria consisted of i-15:0, a-15:0, i-17:0, and i-16:0. For gram-negative bacteria, selected biomarkers were 16:1ω7, cy19:0, cy17:0ω9, 2-OH 14:0, 2-OH 16:0, 3-OH 14:0, and 18:1ω9 trans. For inter-radical AM fungal biomass, biomarkers consisted of 16:1ω5c, 20:1ω9, and 22:1ω13. Biomarkers selected for the functional group of saprophytic fungi were 18:2ω9,12 and 18:1ω9c. The abundances associated with these biomarkers were used to calculate a total nmol per gram of soil for each functional group as well as total microbial biomass by adding all functional groups with non-specific markers (14:0, 15:0, 16:0, 17:0, 18:0, and 20:0).

Root Biomass and Morphology

Soil cores (10 cm diameter x 10 cm deep) containing roots were collected at the end of each growing season. Roots were separated from soil through gently agitating each individual sample in water and collecting the roots via repeated flotation and sieving over 2 mm sieve. Collected root samples from each plot were oven dried at 60°C for at least 48 hours and weighed to measure dry root biomass.

To allow for finer description of carbon recalcitrance and potential carbon storage from belowground biomass, I conducted root morphology analyses on fresh whole root samples collected directly from the field. Cubes of soil with dimensions of 10 cm x 10 cm x 10 cm were taken from near the trunk of *J. virginiana* trees, the center of *C. drummondii* clones, and in C_4-dominated areas located on watershed 1D. Samples were carefully harvested and transported to

minimize breakage of fine secondary and tertiary roots, and soil cubes were kept intact to preserve root structure integrity during transport. To remove soil, samples were gently rinsed with lukewarm water, allowed to air dry for 24 hours, and weighed to obtain total dry root biomass. Because the growth pattern of grass roots and rhizomes present in 1D sampling areas was extremely dense, these grass-dominated root samples were soaked in a dilute sodium hexametaphosphate $(NaPO_3)_6$ solution for one hour to deflocculate attached clay particles.

After washing and drying, the roots were analyzed using WinRHIZO image analysis system (V4.1c, Regent Instruments). Root architecture was analyzed for total root length, root volume, root surface area, average root diameter, and total length of roots of certain diameters. The diameter cover classes were divided increments of 0.2 mm up through 3.0 mm for a total of 13 cover classes. For example, cover class 1 included all roots of a diameter between 0.0 mm to 0.2 mm, cover class 2 included all roots of diameter between 0.2 mm to 0.4 mm, and so on. Roots with diameter at or exceeding 3.0 mm were combined into a single cover class. These data allow for a comparison of fine root biomass to coarse root biomass, which can be used as a measure of carbon lability.

STATISTICAL ANALYSES

Soil water-stable aggregation, gravimetric moisture, nutrient content, as well as root biomass, extra-radical hyphal biomass, mycorrhizal root colonization, and soil PLFA/NLFA profiles were analyzed using generalized linear mixed models (GLIMMEX) methods. Results are reported as least square means. All tests of significance are performed at the nominal 0.05 level unless otherwise stated. The data analysis for this paper was generated using SAS® version 9.4.

CHAPTER III

RESULTS

ABIOTIC CHARACTERISTICS

Soil Aggregation

Soils of annually burned grasslands (watersheds 1D and FA) were characterized by significantly higher proportions of microaggregates to macroaggregates ($p = 0.0001$). This trend was reversed in grass-dominated sites in the encroached watershed, which had a higher ratio of macroaggregates to microaggregates (i.e. soils were more highly aggregated) ($p = 0.0045$). In soils associated with *G. triacanthos*, macroaggregates were more abundant than microaggregates at both the base of trunk ($p = 0.0092$) and the dripline ($p = 0.0029$). The difference of macroaggregates to microaggregates was not statistically significant in within-species comparisons (i.e. comparing microaggregates from the dripline of *C. drummondii* to macroaggregates from the dripline of *C. drummondii*) (Figure 2).

Soil Moisture

Gravimetric soil moisture content did not differ significantly for any species by location comparisons, with the exception of *C. drummondii*. Compared to other species, values for gravimetric soil moisture were significantly lower in soils associated with *C. drummondii*, compared to all other soils sampled ($p = 0.0049$). However there was no statistical difference between soil moisture from the dripline of *C. drummondii* to the center of the clone (data not shown).

Soil Nutrient Analyses

P: Plant-available phosphorus (P) ranged from 22.5 ppm (base of *J. virginiana*) to 10.16 ppm (C_4-dominated areas on watershed 20B), consistent with previous observations that KPBS soils are characteristically P-limited systems (Fahnestock & Knapp 1994; Myster 2011). Soils at the base of *J. virginiana* exhibited approximately double the amount of plant-available P compared to other sample sites; otherwise, no significant difference was found (Table 2).

K: Soil potassium concentrations ranged from 316.56 ppm (C_4-dominated areas on encroached watershed 20B) to 430.11 ppm (annually burned grassland site 1D). Interstitial areas on watershed 20B exhibited significantly lower soil potassium levels (ppm) than either of the annually burned grassland sites. However, presence of woody species did not significantly alter soil potassium levels from that of annually burned grasslands or interstitial areas (data not shown).

SOM: Soil organic matter (SOM) content of sampled soils ranged from 15.63% at the base of *J. virginiana* trunks to 6.99% in annually burned grasslands. Grassland controls were not significantly different from one another regardless of fire interval. Compared to grassland controls, *J. virginiana* exhibited greater SOM content at the base of the trunk (15.63% versus 7.55%, $p \leq 0.05$), but not at the dripline (Table 3). *Rhus aromatica* exhibited elevated levels of SOM (an average increase of 3%) compared to C_4-dominated interstitial sites (20B).

NO_3: Surface nitrogen (NO_3) was significantly lower in annually burned grasslands (17.43 ppm for grasslands burned in the fall and 14.62 ppm for grasslands burned in the spring) compared to infrequently burned grasslands and soils associated with most woody encroachers. Soils near the base of *C. drummondii* trunks and at the dripline of *R. aromatica* were not statistically different from either annually burned grasslands, encroached grasslands, or other woody species (Table 4).

pH: Soils at most sampling locations demonstrated a pH value near 6.5, consistent with previous research done on the upland soils of KPBS (Myster 2011). However, soils sampled near the base of *J. virginiana* trunks showed increased pH to approximately 7.5, representing an increase in soil alkalinity. This trend was not shared by soils at the dripline of *J. virginiana* individuals (data not shown).

BIOTIC CHARACTERISTICS

Extra-Radical Hyphal (ERH) Biomass

C_4-dominated interstitial sites on the encroached grassland (20B), grasslands annually burned in the spring (1D), and grasslands annually burned in the fall (FA) did not differ significantly from one another. Both annually burned grasslands and interstitial areas on encroached watershed 20B exhibited significantly greater levels of extra-radical hyphal biomass compared to soils sampled at the dripline, base of trunk, or center of clone of woody species (Figure 3). No significant differences existed in hyphal abundance between soils sampled at the base of woody species' trunks or center of woody clones compared to soils sampled at the driplines.

Root Colonization

Mycorrhizal colonization of fine roots ranged from 56.5% to 28.96% (Table 5). Grasslands burned annually in the spring exhibited the highest percentage of root colonization with arbuscules, vesicles, and/or hyphae present in 56.5% of fine roots. No significant difference was noted between woody species, infrequently burned grasslands, or grasslands annually burned in the fall, with the exception of roots sampled at the dripline of *R. aromatica* which showed slightly lower levels of colonization.

Microbial Community/ Quantification of the Mycorrhizal Fungus

Grassland control sites did not significantly differ from soils associated with woody encroachers in total microbial biomass, levels of gram-positive bacteria, or in levels of arbuscular mycorrhizal fungi or saprophytic fungi. Annually burned grasslands and C_4-dominated sites in encroached grasslands were statistically similar to one another in gram-positive bacteria, gram-negative bacteria, AM fungi, and saprophytic fungi (these sample locations are averaged together and reported as "Grassland controls" in Figures 4 through 8). However, woody species differed from one another in most microbial functional groups.

Total microbial biomass was not significantly different between grassland controls and woody encroachers. The driplines of *J. virginiana* and *R. aromatica* exhibited significantly lower levels of microbial biomass compared to soil associated with the respective trunks or compared to other woody species. Total microbial biomass was significantly lower at the driplines of both *J. virginiana* and *R. aromatica* compared to all other woody species. Soils associated with both these species also exhibited greater total microbial biomass at the base of the trunk, compared to the driplines (Figure 4). Biomass of gram-positive bacteria at the dripline of *J. virginiana* (8.19 nmol per gram of soil) was significantly lower than soil from all other woody species locations with the exception of the dripline of *R. aromatica* (9.71 nmol per gram of soil). *Gleditsia triacanthos* exhibited greater abundances of both AM and saprophytic fungi compared to soil associated with all other woody encroachers, but were not statistically different from grassland controls.

Soils at the dripline of *J. virginiana* individuals exhibited lower levels of gram-positive bacteria compared to all other sampling locations, except the grassland controls and the dripline of *R. aromatica* shrubs. No other significance was noted in gram-positive bacterial communities (Figure 5). The base of *R. aromatica* trunks exhibited greater levels of gram-negative bacteria than the grassland controls and the driplines of *C. drummondii*, *J. virginiana*, and *R. aromatica*. Grassland controls did not differ from one another, but had lower gram-negative bacterial

biomass levels compared to all base of trunk locations, and compared to the driplines for both *J. virginiana* and *R. aromatica* (Figure 6).

Standing crop abundance of arbuscular mycorrhizal fungi was lower near the dripline of *R. aromatica* compared to grassland controls and soils dominated by other woody species. *Gleditsia triacanthos* exhibited greater abundances of AM fungi than other woody encroachers at both the dripline and at the base of the trunk (Figure 7). Abundance of saprophytic fungi was lowest in the dripline locations for *J. virginiana* (6.68 nmol/gram) and *R. aromatica* (4.84 nmol/gram). These two locations were significantly lower in saprophytic fungal biomass than the grassland controls and from all other sampled locations (Figure 8).

Root Biomass and Morphology

For analysis, roots were separated into 13 distinct classes based on diameter. Grassland control samples were primarily composed of fine roots with a diameter smaller than 0.2 mm, with an average length of 65.5 cm in roots of this diameter class. Each subsequent diameter class had shorter average lengths, with the exception of diameter class 13. Root length means decreased as diameter class increased. This trend was not demonstrated by woody species *C. drummondii* or *J. virginiana*, in which the longest roots had diameters between 0.2 mm and 0.4 mm (Figure 9).

CHAPTER IV

DISCUSSION

Woody species encroachment did not result in uniform shifts across all species, but rather exhibited effects on belowground ecosystem dynamics dependent upon the identity of the woody encroacher. My data support hypothesis H_3 that alterations in soil dynamics are species-specific: the shifts observed in soils associated with *J. virginiana* were not similar to soils associated with *G. triacanthos*, or vice versa. For example, gravimetric soil moisture was not significantly altered by the presence or dominance of woody species, compared to grassland controls, with the exception of soils associated with clonal species *C. drummondii*, which exhibited drier soils. The 16 stem / m^2 density of these clonal shrubs outshaded competing plant species while exposing soil to the air, potentially resulting in increased atmospheric drying of the topsoil (personal observation).

While virtually all perennial plants in natural grasslands form AM symbioses, the degree to which individual plant species depend on the relationship varies among plant taxa, plant life history stage, plant evolutionary background, resource availability, and season. For example, Wilson and Hartnett (1998) measured the mycorrhizal responsiveness of 95 species by growing plants with and without AM fungi. C_4 (warm-season) grasses benefitted more from mycorrhizas and had higher levels of root colonization than C_3 (cool-season) grasses; and perennial plants benefitted more from mycorrhizas than annual plants. The generality of these patterns has been further supported in a meta-analysis (Hoeksema 2010). However, Wilson and Hartnett (1998)

focused solely on grasses and forbs, not examining variation among woody species. My results suggest that this variation in response among herbaceous species occurs in woody species as well.

The degree to which dominant species rely on the symbiosis may play an important role in the composition of plant communities (Hartnett & Wilson 2002). When the dominant plant species are highly dependent on mycorrhizal fungi for acquiring essential nutrients, and subordinates are not, mycorrhizal fungi increase the competitive ability of the dominants and decrease plant species diversity, as observed in C_4-dominated grasslands (Hartnett & Wilson 1999; O'Connor *et al.* 2002). However, if dominant plant taxa are weakly mycorrhizal and subordinate species strongly mycorrhizal, as in many C_3 grasslands, AM increase plant species diversity (Urcelay & Diaz 2003; Bingham & Biondini 2009). Encroaching woody species may be poor hosts for AM fungi and may, therefore, reduce the density of AM fungi, inhibiting the growth of the warm-season grasses which are highly dependent on AM fungi. As plants can allocate preferentially to the most beneficial fungal partner (Vogelsang & Bever 2009; Kothamasi *et al.* 2011), it is possible that encroaching plants may alter AM fungal communities to promote their own success and inhibit highly dependent native grassland species. Therefore, benefits conferred by AM fungi may extend beyond improving growth of dependent species, to inhibition of growth and survivorship of less mycorrhizal dependent plant species. If encroaching woody species are associated with greater abundance of AM fungi than native plant species, as was observed with *G. triacanthos* in the current study (based on AM fungal biomass), they may disrupt the tight association with native grasses and forbs, with potentially negative influences on native plant communities.

My data support hypothesis H_3: alterations in soil characteristics are species-specific. Notably, soils associated with *J. virginiana* exhibited numerous significant divergences from native C_4-dominated grassland controls, including increased plant-available phosphorus, elevated soil pH, decreased total microbial biomass at the dripline and increased microbial biomass at the base of the trunk, elevated soil organic matter, thicker average root diameter, and increased soil

nitrate content. These effects may be size class dependent; this project focused on mature, well-established trees with average crown heights of 5.9 m, average crown base diameters of 2.5 m, and average base area under the crown of 4.9 m^2. The growth characteristics of these trees has been shown to result in significant reduction of herbage under the crown area which may reduce or eliminate any regulatory feedback effects from differing plant species (Engle *et al.* 1987; Engle & Kulbeth 1992).

Aggregated soils protect carbon-rich detritus from microbial degradation; therefore an increase in aggregation is important for soil carbon sequestration (Six *et al.* 1998; Jastrow *et al.* 2000). Aggregation physically structures the soil and influences virtually all nutrient cycling processes and soil biota (Diaz-Zorita & Grove 2002). Miller and Jastrow (1990) found a strong direct correlation linking the length of extraradical hyphae and fine roots to the formation of soil macroaggregates; therefore, a loss in density of AM fungal or fine root density, as compared to native grass communities, may have profound consequences on soil aggregate stability. Roots sampled from *C. drummondii* and *J. virginiana* exhibited thicker average root diameter (an increase of approximately 0.2 mm) compared to roots sampled from C_4-dominated grasslands burned annually in the spring (Figure 9), while grassland sites showed greater levels of extra-radical hyphae (Figure 3) and greater mycorrhizal colonization of roots in grasslands burned annually in the spring (Table 5). Annually burned grasslands had significantly higher proportions of microaggregates to macroaggregates, compared to the C_4-dominated sites in the encroached grassland or soils associated with woody species (i.e. were less well-aggregated), suggesting that presence of woody species may improve soil stability by increasing water-stable aggregation.

My data do not support hypothesis H_1 that soils associated with woody species would exhibit higher levels of hyphal biomass. Woody species had significantly lower levels of extra-radical hyphae compared to grassland controls (Figure 3), which could represent a shift in the recalcitrance and storage of carbon as tallgrass ecosystems become more dominated by woody encroachers. Hyphal cell walls, cytoplasm, and extracellular polysaccharides represent a

relatively labile organic pool in soils, and AM fungi in grassland soils represent a significant contribution of carbon, with as much as 20-30% of the microbial biomass carbon being composed of AM fungal biomass (Leake 2004). The combination of both recalcitrant and labile compounds from roots and AM fungi can stimulate the heterotrophic members of the microbial community, leading to a release of nutrients stored in soil organic matter – the so-called "priming effect" (Cheng *et al.* 2014). While it has been shown that roots from some species can affect SOM decomposition twice as much as roots from other species (Cheng *et al.* 2003), the root traits underlying these species-specific effects are largely unknown. However, Fontaine *et al.* (2011) suggests that fungi play a dominant role in producing priming effects on decomposition of SOM. Indeed, in my study, soil associated with *J. virginiana* and *R. aromatica* exhibited greater levels of SOM and a lower relative abundance of both arbuscular mycorrhizal and saprophytic fungal biomass. As suggested by Fontaine *et al.* (2011), the decreased abundance of microbial decomposers in soils is presumably associated with a reduction in decomposition of SOM.

Root traits and root biomass also play important roles in soil carbon cycling indirectly via the composition of soil microbial communities. For example, root traits that promote the growth of fungi over that of bacteria, such as high lignin and low root nitrogen content, promote soil carbon storage (De Deyn *et al.* 2008; Bardgett *et al.* 2013). This is due to inherent differences in fungal and bacterial metabolism; fungi respire less carbon per unit biomass carbon gained and fungal metabolites reside in soil longer than bacterial metabolites (Plante *et al.* 2006). Fungal mycelia are composed of complex, nutrient poor membranes such as melanin and chitin, whereas bacterial membranes are composed largely of phospholipids that are quickly broken down and mineralized (White *et al.* 1979). Therefore, alterations in root biomass and subsequent changes in soil microbial communities associated with encroachment of woody species may have substantial consequences in ecosystem-level carbon dynamics and biogeochemical cycles. For example, the combined effects of *J. virginiana* increasing plant-available phosphorus in soils near the base of

the trunk with the decrease in relative abundance of gram-positive bacteria may indicate a shift in microbial community composition.

My results support the need for individualized plans in both management of existing tallgrass prairie lands and restoration of encroached sites to tallgrass reference sites. Challenges facing areas converted to juniper woodlands dominated by *J. virginiana* will not be identical to shifts experienced by *Rhus*-dominated lands, for example. Restoration efforts attempting to revert encroached lands back to native C_4 tallgrass prairie may need to be tailored to match the unique woody encroacher dominance in order for restoration to be successful.

Molecular analyses qualifying the identity and species composition of microbial communities could refine our understanding of soil alterations following woody encroachment. I intend to continue this research at Kansas State University; examining the effects of fire return interval and woody species encroachment on microbial communities in the tallgrass prairie.

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TABLES

Table 1: Growth characteristics by tree species identity (n = 6). Individuals were nonrandomly selected for similar characteristics to eliminate variation due to age. Clonal morphology species *C. drummondii* and *R. aromatica* were measure for stem density while single-stemmed species *J. virginiana* and *G. triacanthos* were measured for diameter at breast height (DBH). Width represents the average diameter of zone of influence (crown base area or clonal island diameter).

Species Identity	Height (m)	Width (m)	DBH (cm)	Stem Density (stem / m^2)
Cornus drummondii	1.3	8.8		16
Gleditsia triacanthos	2.7	1.2	26.5	
Juniperus virginiana	5.9	2.5	51.1	
Rhus aromatica	1.0	4.2		20

Table 2: Plant-available phosphorus (P) measured in parts per million, arranged by level of significance. Grassland control sites are located on the following Konza Prairie Biological Station watersheds: 1D (annually burned in the spring), FA (annually burned in the fall), and 20B (sampled at C_4-dominated interstitial zones in an infrequently burned watershed). Soil samples were collected at the base of the trunk or at the dripline for *Juniperus virginiana* and *Gleditsia triacanthos*, and at the center or edge of the clone for *Cornus drummondii*, and *Rhus aromatica*. Values with different letters differ significantly from one another ($p \leq 0.05$).

Species	Location	Soil P (ppm)
J. virginiana	Base of Trunk	22.50 [a]
R. aromatica	Base of Trunk	13.46 [b]
J. virginiana	Dripline	13.19 [b]
C. drummondii	Base of Trunk	13.12 [b]
G. triacanthos	Base of Trunk	12.49 [b]
R. aromatica	Dripline	12.34 [b]
C. drummondii	Dripline	12.34 [b]
Grassland controls	1D	12.33 [b]
G. triacanthos	Dripline	11.83 [b]
Grassland controls	FA	10.77 [b]
Grassland controls	20B	10.16 [b]

Table 3: Soil organic matter (SOM) content of sampled soils arranged from highest percentage to lowest. Grassland control sites are located on the following Konza Prairie Biological Station watersheds: 1D (annually burned in the spring), FA (annually burned in the fall), and 20B (sampled at C_4-dominated interstitial zones in an infrequently burned watershed). Soil samples were collected at the base of the trunk or at the dripline for *Juniperus virginiana* and *Gleditsia triacanthos*, and at the center or edge of the clone for *Cornus drummondii*, and *Rhus aromatica*. Values with different letters differ significantly from one another ($n = 6$, $p \leq 0.05$).

Species	Location	SOM (%)
J. virginiana	Base of Trunk	15.63[a]
R. aromatica	Base of Trunk	10.61[b]
R. aromatica	Dripline	10.17[b]
C. drummondii	Dripline	9.23[b]
C. drummondii	Base of Trunk	9.12[bc]
G. triacanthos	Base of Trunk	8.91[bc]
G. triacanthos	Dripline	8.66[bc]
J. virginiana	Dripline	8.66[bc]
Grassland controls	1D	8.60[bc]
Grassland controls	FA	7.5[bc]
Grassland controls	20B	6.99[c]

Table 4: Surface nitrogen in nitrates (NO_3) arranged from highest concentration in ppm to lowest concentration. Grassland control sites are located on the following Konza Prairie Biological Station watersheds: 1D (annually burned in the spring), FA (annually burned in the fall), and 20B (sampled at C_4-dominated interstitial zones in an infrequently burned watershed). Soil samples were collected at the base of the trunk or at the dripline for *Juniperus virginiana* and *Gleditsia triacanthos*, and at the center or edge of the clone for *Cornus drummondii*, and *Rhus aromatica*. Values with different letters differ significantly from one another ($n = 6$, $p \leq 0.05$).

Species	Location	NO_3 (ppm)
G. triacanthos	Base of Trunk	54.29 [a]
G. triacanthos	Dripline	45.63 [a]
J. virginiana	Dripline	38.05 [a]
Grassland controls	20B	37.96 [a]
R. aromatica	Base of Trunk	37.62 [a]
J. virginiana	Base of Trunk	37.34 [a]
C. drummondii	Dripline	32.83 [a]
C. drummondii	Base of Trunk	31.83 [ab]
R. aromatica	Dripline	30.12 [ab]
Grassland controls	FA	17.34 [b]
Grassland controls	1D	14.62 [b]

Table 5: Colonization of fine roots by arbuscular mycorrhizal fungi, arranged from highest percentage colonized to lowest. Results are percentage of observed roots that had arbuscules, vesicles, or mycorrhizal hyphae present. For each sample, 6 representative fine roots were stained and examined microscopically; each root was subsampled for 5 observations at 200x magnification (n = 30). Grassland control sites are located on the following Konza Prairie Biological Station watersheds: 1D (annually burned in the spring), FA (annually burned in the fall), and 20B (sampled at C_4-dominated interstitial zones in an infrequently burned watershed). Soil samples were collected at the base of the trunk or at the dripline for *Juniperus virginiana* and *Gleditsia triacanthos*, and at the center or edge of the clone for *Cornus drummondii*, and *Rhus aromatica*. Values with different letters differ significantly from one another ($p \leq 0.05$).

Species	Location	Mycorrhizal Colonization (%)
Grassland controls	1D	56.50 [a]
J. virginiana	Base of Trunk	43.19 [bc]
C. drummondii	Leading Edge	38.51 [bc]
C. drummondii	Center of Clone	38.47 [bc]
R. aromatica	Center of Clone	36.92 [bc]
J. virginiana	Dripline	36.51 [bc]
G. triacanthos	Base of Trunk	36.48 [bc]
G. triacanthos	Dripline	35.75 [bc]
Grassland controls	FA	35.70 [bc]
Grassland controls	20B	35.30 [bc]
R. aromatica	Leading Edge	28.96 [c]

FIGURES

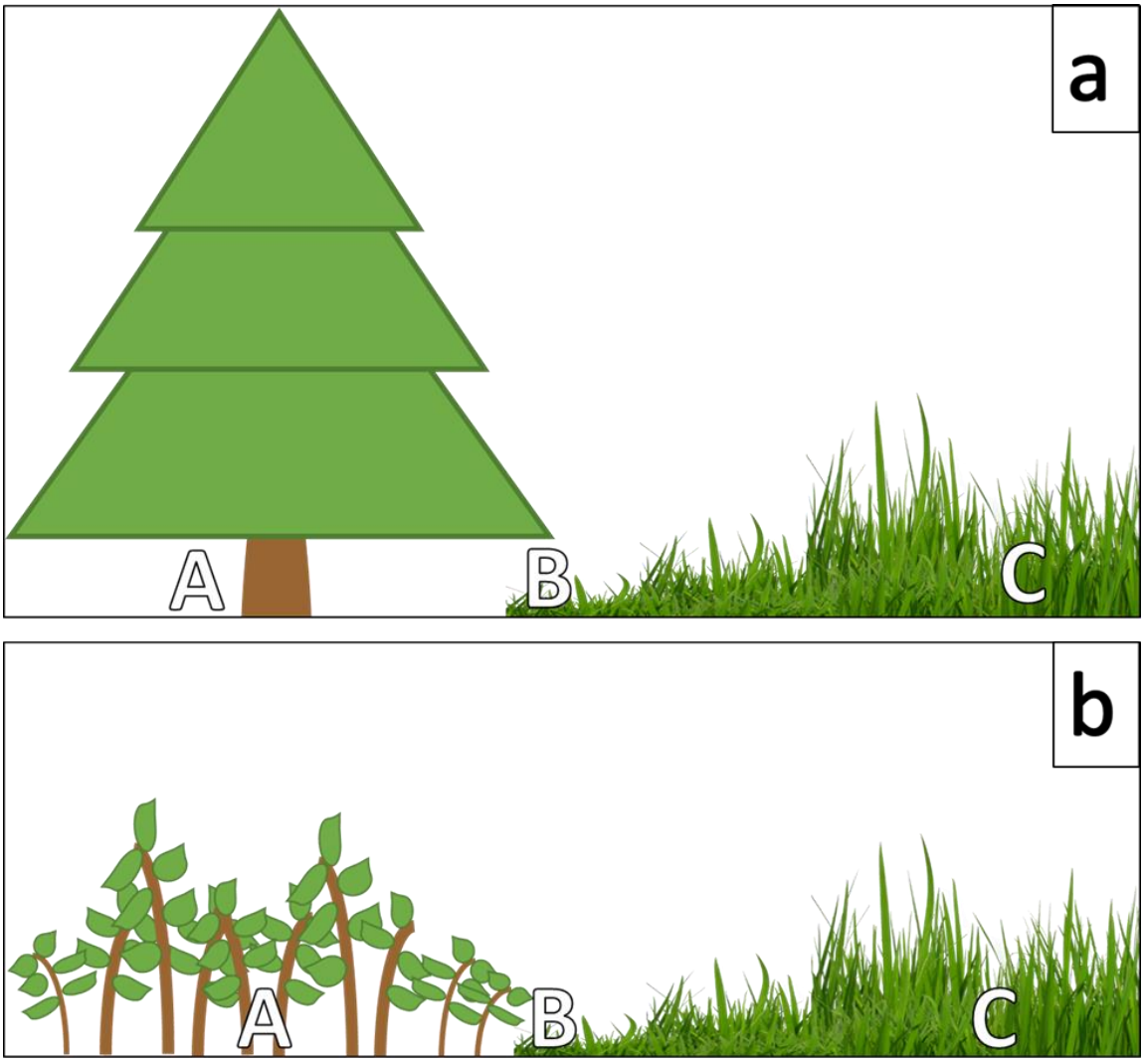

Figure 1: Sampling locations on watershed 20B relative to individuals of woody species; 1a illustrates locations for single-stemmed tree species *J. virginiana* and *G. triacanthos* while 1b illustrates multi-stemmed clonal shrub species *C. drummondii* and *R. aromatica*. The sampling site denoted by "A" is referred to as base of trunk; "B" is referred to as dripline; and "C" denotes grass control samples taken on 20B. Control samples from annually burned watersheds are not represented in this schematic.

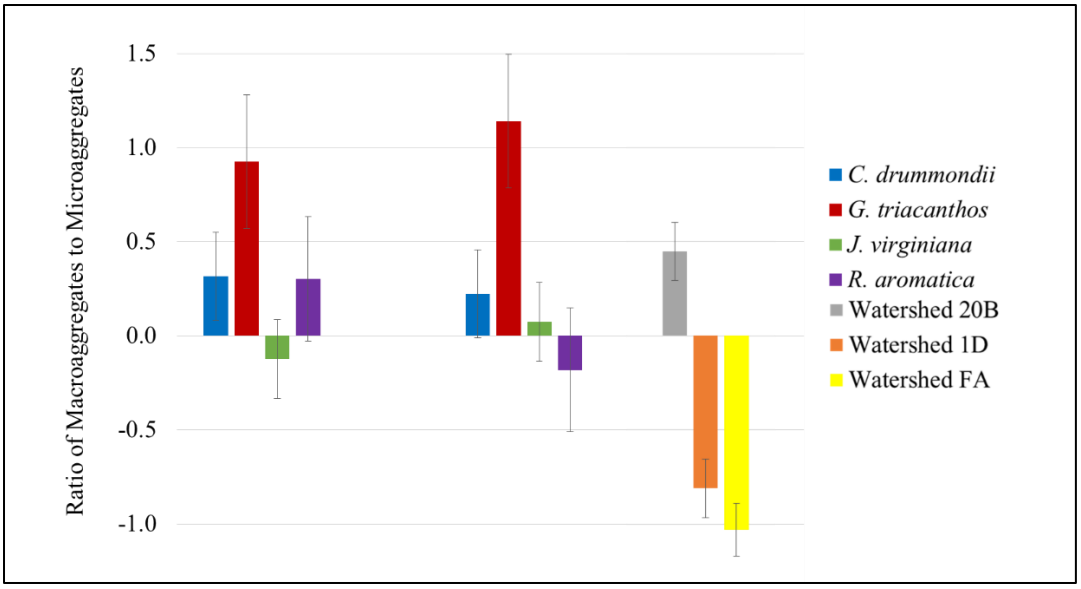

Figure 2: Soil stability measured as the ratio of macroaggregates (larger than 1.0 mm in diameter) to microaggregates (smaller than 1.0 mm in diameter). Positive ratios indicate a greater proportion of macro- to microaggregates, or more well-aggregated soils. Negative ratios indicate a greater proportion of micro- to macro-aggregates, or less well-aggregated soils. C_4-dominated grassland controls differed significantly from one another and are represented individually by watershed: 1D is the watershed burned annually in the spring, FA is annually burned in the fall, and 20B is the interstitial control sites on the heavily encroached watershed.

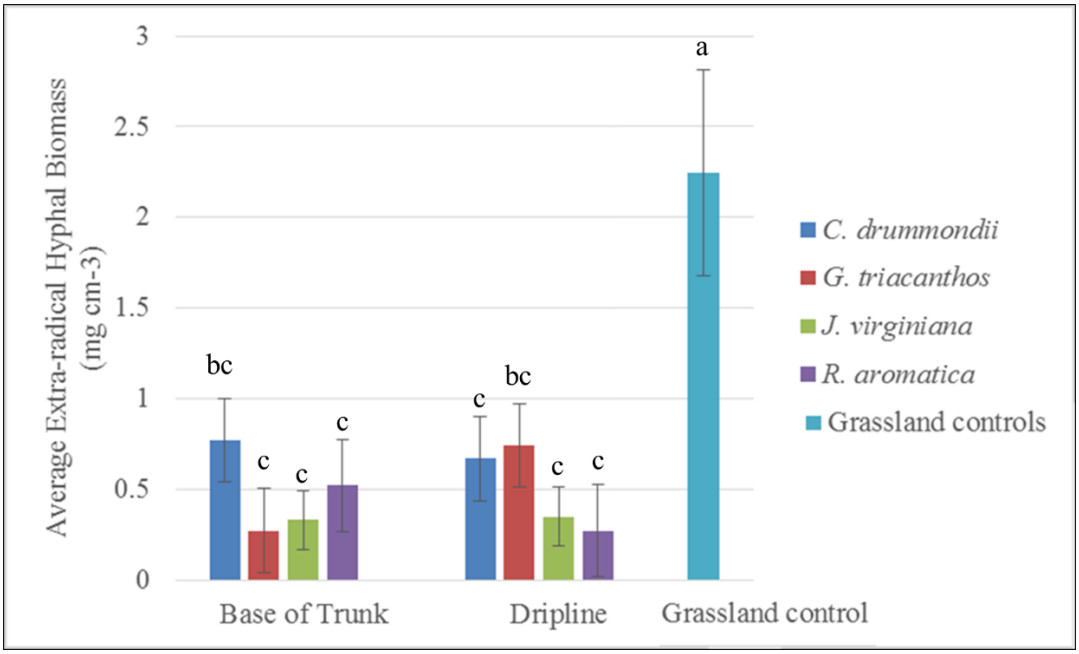

Figure 3. Extra-radical hyphal biomass produced during the season at each sampling location. Grassland control sites are located on the following Konza Prairie Biological Station watersheds: 1D (annually burned in the spring), FA (annually burned in the fall), and 20B (sampled at C_4-dominated interstitial zones in an infrequently burned watershed). Soil samples were collected at the base of the trunk or at the dripline for *Juniperus virginiana* and *Gleditsia triacanthos*, and at the center or edge of the clone for *Cornus drummondii*, and *Rhus aromatica*.

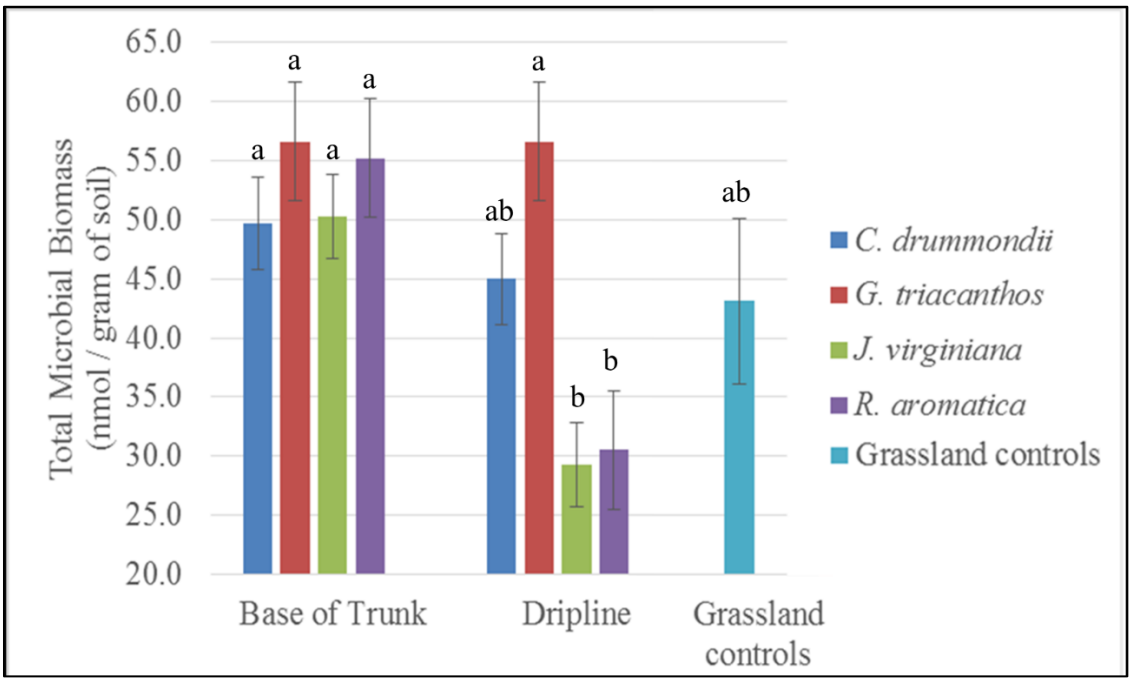

Figure 4: Total microbial biomass as determined by phospholipid fatty acid (PLFA) analysis in nmol per gram of soil. Data is separated by base of trunk/center of clone, dripline/leading edge, and grassland control sampling locations. Annually burned grasslands (1D and FA) and C_4-dominated areas in encroached grassland (20B) are combined, averaged, and presented as Grassland controls on the rightmost portion of the graph.

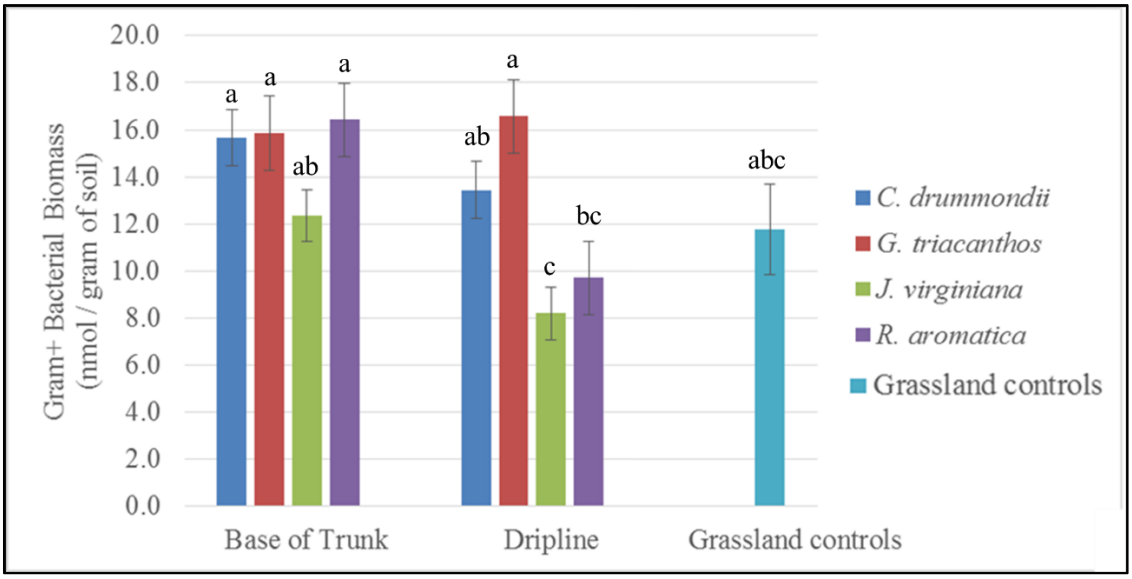

Figure 5: Phospholipid fatty acid analysis of relative abundance of gram-positive bacteria in nmol per gram of soil. Data is separated by base of trunk/center of clone, dripline/leading edge, and grassland control sampling locations. Annually burned grasslands (1D and FA) and C_4-dominated areas in encroached grassland (20B) are combined, averaged, and presented as Grassland controls.

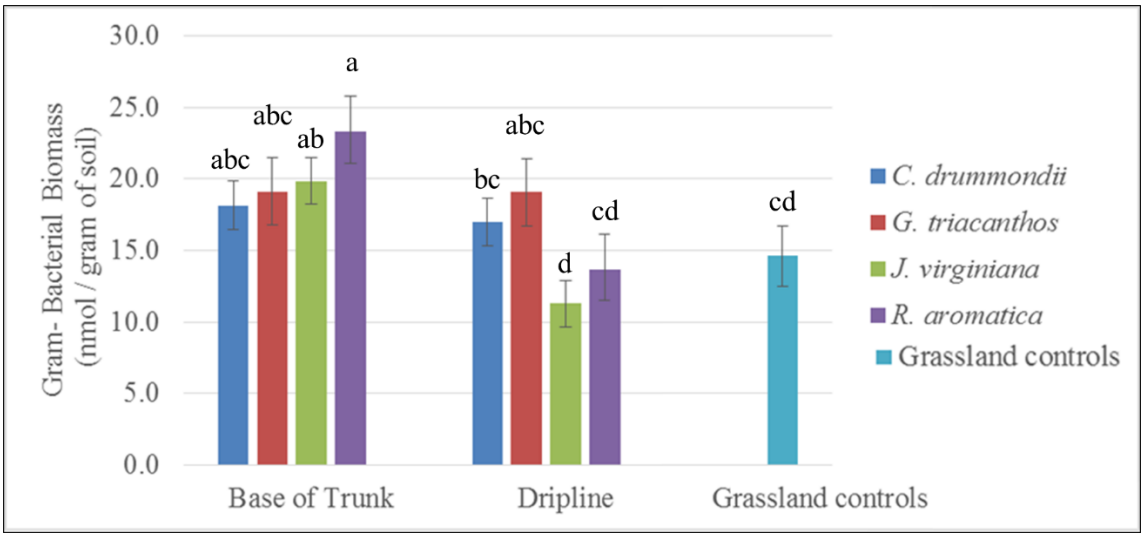

Figure 6: Phospholipid fatty acid analysis of relative abundance of gram-negative bacteria in nmol per gram of soil. Data is separated by base of trunk/center of clone, dripline/leading edge, and grassland control sampling locations. Annually burned grasslands (1D and FA) and C_4-dominated areas in encroached grassland (20B) are combined, averaged, and presented as Grassland controls.

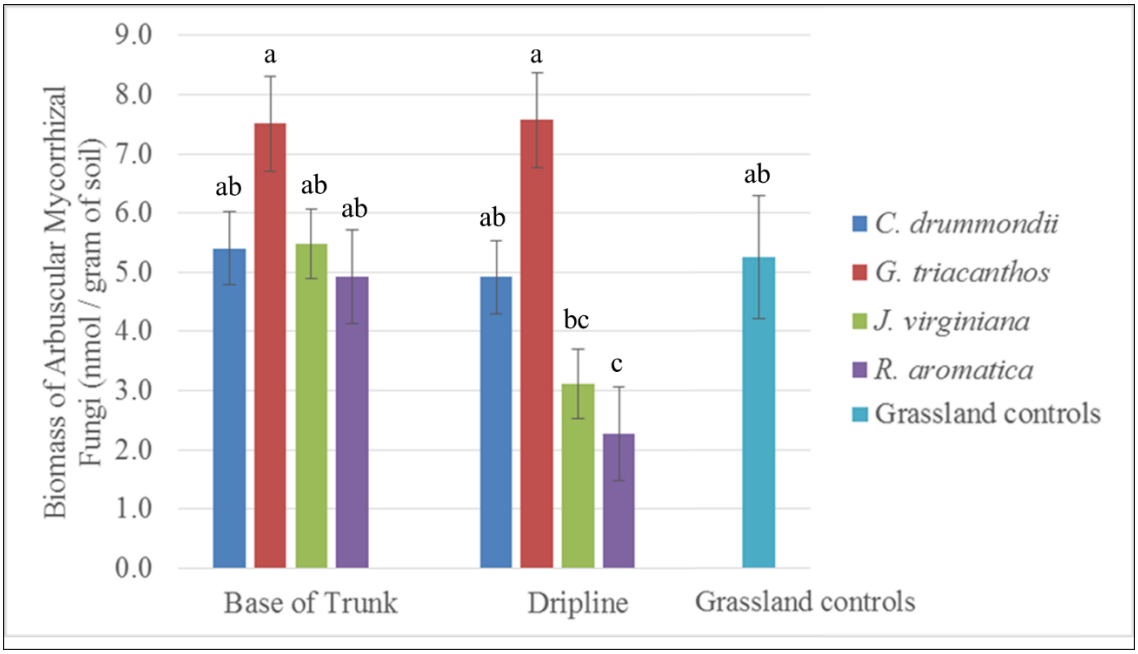

Figure 7: Relative abundance of arbuscular mycorrhizal fungal biomass (nmol per gram of soil) as determined by phospholipid fatty acid analysis. Data is separated by base of trunk/center of clone, dripline/leading edge, and grassland control sampling locations. Annually burned grasslands (1D and FA) and C_4-dominated areas in encroached grassland (20B) are combined, averaged, and presented as Grassland controls.

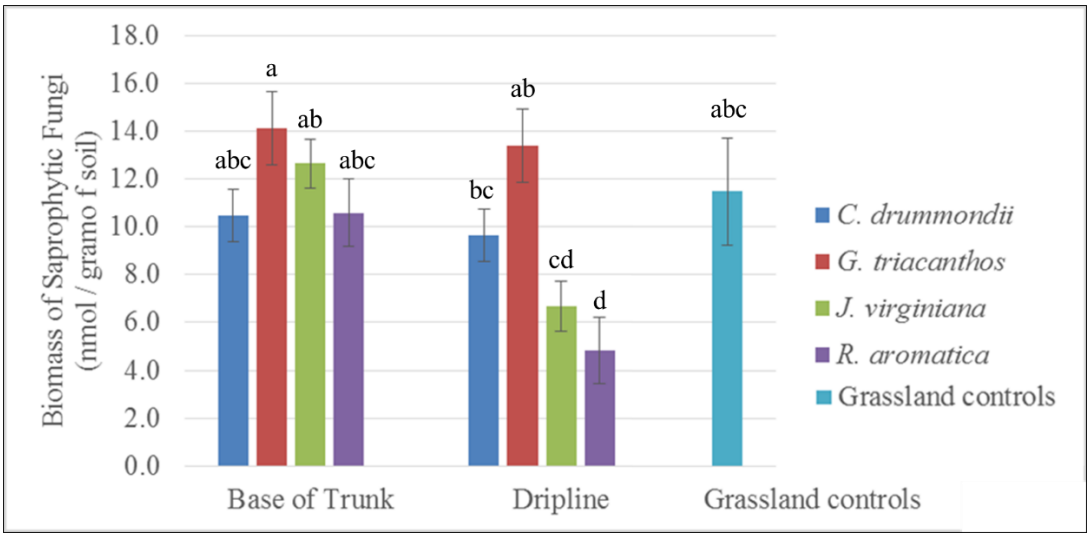

Figure 7: Relative abundance of saprophytic fungal biomass (nmol per gram of soil) as determined by phospholipid fatty acid analysis. Data is separated by base of trunk/center of clone, dripline/leading edge, and grassland control sampling locations. Annually burned grasslands (1D and FA) and C_4-dominated areas in encroached grassland (20B) are combined, averaged, and presented as Grassland controls.

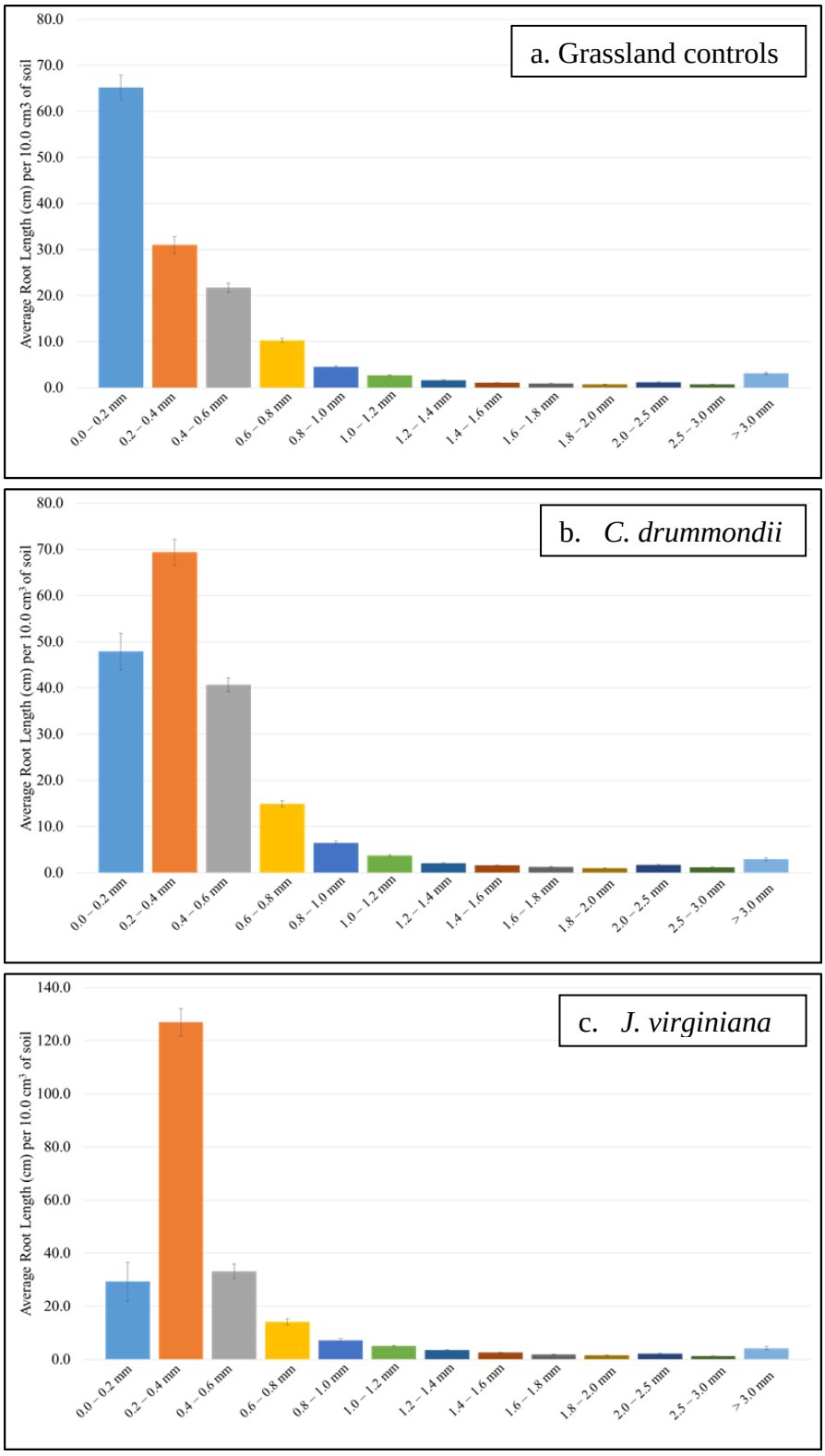

Figure 9: Root morphology as determined by average root length as a function of diameter, separated by diameter classes. Diameters increase in size on the x-axis from < 0.2 mm to ≥ 3.0 mm. The y-axis presents average root length in cm. Roots from grassland sites burned annually in the spring are presented in Figure 9a; Figure 9b presents values for roots sampled from the center of *Cornus drummondii* clones; and Figure 9c presents values for roots sampled near the base of *Juniperus virginiana* trunks. Root architecture was analyzed using WinRHIZO image analysis system (V4.1c, Regent Instruments).

VITA

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