CONSEQUENCES OF *JUNIPERUS VIRGINIANA* ENCROACMENT IN A TALLGRASS PRAIRIE: SOIL PHYSICAL AND BIOLOGICAL ALTERATIONS

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CHAPTER I

CONSEQUENCES OF *JUNIPERUS VIRGINIANA* ENCROACMENT IN A TALLGRASS PRAIRIE: SOIL PHYSICAL AND BIOLOGICAL ALTERATIONS

ABSTRACT

The invasion of *J. virginiana* into grasslands has multi-scale effects including loss of biodiversity, loss of habitat for wildlife species, and alterations in community-level and biogeochemical functions. Most previous studies focus on aboveground changes, therefore, in this study I assess belowground changes in soil characteristics including soil microbial communities, nutrient availability, moisture, and aggregate stability following establishment of *J. virginiana* into tallgrass prairie. To assess the influence of tree density, I included forested areas with moderate levels of encroachment, as well as sites that are highly encroached, and compare these sites to an adjacent non-encroached native prairie. To assess the impact of *J. virginiana* at the individual tree level, a single *J. virginiana* tree, paired with an uncut control, was cut and removed from each site. My results show significant differences in microbial communities, nutrient availability, moisture, and aggregate stability in forested sites compared to the native prairie sites. The removal of an individual *J. virginiana* tree did not significantly affect soil characteristics, compared to a corresponding uncut control

tree. Possible explanations for this lack of response could be due to the relatively short (6 month) duration of this study or that there is a continued influence from surrounding *J.virginiana* trees. Understanding how microbial communities and these plant-fungal associations are influenced by *J. virginiana* invasions may be a critical aspect of the ecology and management of this invading species, as well as the conservation and restoration of native ecosystems.

INTRODUCTION

The tallgrass prairie ecosystem once occupied great expanses throughout central North America. However, primarily due to extensive conversion to row-crop agriculture since the 1830s, 82-99% of this highly productive, floristically diverse and formerly extensive tallgrass prairie ecosystem has been lost (Samson and Knopf 1994). Today, the last remaining tracts of tallgrass prairie in Kansas, Oklahoma, and Texas are threatened by a variety of global change phenomena (Vitousek et al. 1988). The invasion and encroachment of woody plant species into the remaining grasslands, due primarily to the anthropogenic removal of fire, is possibly the greatest current threat to the remainder of this endangered ecosystem (Briggs et al. 2002, 2005). In the south central Great Plains of North America, the primary species leading to woody encroachment is *Juniperus virginiana* (eastern redcedar) (Ferguson et al. 1968, Schmidt and Leatherberry 1995; Batek et al. 1999). *Juniperus virginiana* is native to the tallgrass prairie ecosystem. However, this species was historically restricted to areas protected from intense grassland fires such as rocky outcrops or shallow soil. In the absence of fire, *J. virginiana* increases in density and size at rates that allow canopy closure in less than 40 years (Briggs et al. 2005). Great Plains grasslands provide the primary resources for livestock production in North America and provide habitat for a wide variety of wildlife species. Therefore,

conservation of the remaining tracts and remnants of the tallgrass prairie is considered essential and efforts have intensified to conserve this ecosystem and the species contained therein (Samson and Knopf 1994).

Recently, the terminology of invasive species has become a complex issue, distinguishing between native and non-native plant species invasion (Falk-Petersen et al. 2006; Pierce and Reich 2010). However, encroachment (referring to native plant species) and invasion (referring to exotic or non-native plant species) are functionally similar processes. Increasing the dominance of one species, either native or non-native, associated with the loss of ecosystem diversity and function leads to similar corresponding effects on native community composition (Meiners et al. 2001). Further, the mechanisms and impacts of invasion following replacement of native species with *J. virginiana*, and the pathways that lead to restoration are similar to many outlined for many non-native biological invasions (Didham et al. 2005; Pierce and Reich 2010). Therefore, I focused on the effects of invasion by *J. virginiana* on ecosystem structure and function, from the perspective of the conservation of a declining ecosystem. In this study, alterations in ecosystems structure and function will be examined by assessing soil biotic and physical properties following establishment of *J. virginiana*.

Previous research has reported *J. virginiana* invasion into grasslands has multi-scale effects including loss of biodiversity, loss of habitat for wildlife species (Gehring and Bragg 1992; Briggs et al. 2002a; 2002b.), and alterations in community-level and biogeochemical functions (Norris et al. 2001a; Smith and

Johnson 2003; Norris et al. 2007). For example, aboveground net primary productivity of *J. virginiana* forests have been reported to be substantially greater than similar locations of grasslands, leading to an increase in aboveground C storage, the potential for increases in belowground C storage, and alterations in nutrient cycling (Norris et al. 2001b; Smith and Johnson 2003; Norris et al. 2007). Most previous studies describing impacts of invasion by native or non-native species focus on aboveground features, with little attention given to the belowground microbial communities, although soil organisms play important roles in regulating ecosystem-level processes (Levine et al. 2004). The association of arbuscular mycorrhizal (AM) fungi with a plant species can be the central force shaping the species' ecology (Herre et al. 1999), and invading plant species have the potential to alter the density and/or composition of the mycorrhizal fungal community (van der Heijden et al. 1998). These plant-fungal associations have been reported to both constrain (Vogelsang et al. 2004; Vogelsang and Bever 2009) and facilitate (Shah et al. 2009) the ability of a non-native species to successfully invade, and have been shown to influence the trajectory of the invasion process (Vogelsang and Bever 2009). However, to my knowledge, no previous research has been conducted to examine the effects of invasion by native species on soil microbial communities or AM fungal abundance. Soil organisms play important roles in regulating ecosystem-level processes and the association of arbuscular mycorrhizal (AM) fungi with a plant species can be a central force shaping plant species' ecology. Understanding how microbial communities and these plant-fungal associations are influenced by *J. virginiana*

invasions may be a critical aspect of the ecology and management of this invading species, as well as the conservation and restoration of native ecosystems. Therefore, in this study, I assess soil characteristics including soil microbial communities, nutrient availability, moisture, and aggregate stability following establishment of *J. virginiana* into tallgrass prairie. To assess the influence of tree density, I included forested areas with moderate levels of encroachment, as well as sites that are highly encroached, and compare these sites to an adjacent non-encroached native prairie. To assess the impact of *J. virginiana* at the individual tree level, a single *J. virginiana* tree, paired with an uncut control, was cut and removed from each site. Specifically, I tested the following hypotheses:

H_1: The encroachment of *J. virginiana* into a tallgrass prairie will have significant effects on the soil microbial communities, as compared to the tallgrass prairie control site.

H_2: Soil abiotic characteristics, such as nutrient availability, soil moisture, and soil aggregate stability, of the forested areas will be altered by establishment of eastern redcedar trees.

H_3: Tree removal will reverse the effects of redcedar encroachment on both biotic (microbial community composition) and abiotic (nutrient availability, moisture, and aggregate stability) soil characteristics. Therefore, tree removal sites will be more similar to the non-

encroached prairie site than soil collected beneath uncut (control) trees.

METHODS

Experimental design

My experiment was located at the Oklahoma State University Research Station about 18 km southwest of Stillwater, Oklahoma USA (36°06'N; 97°23'W). The region is dominated by a continental climate with an average of 204 frost-free days and 846 mm annual precipitation, 65% of which falls from May to October (Meyers 1982). Typical climax vegetation in the study area is characterized by little bluestem (*Schizachryium scoparium* [Michx.] Nash), indiangrass (*Sorghastrum nutans* (L.) Nash), switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon gerardii Vitman*), Carex spp. and perennial forbs. Fire has been removed from a portion of my study site, with consequential establishment of eastern redcedar. These forested areas were characterized by moderately encroached areas with approximately 45% canopy cover and highly encroached areas with a canopy cover of approximately 80%, as determined by aerial photographs (Limb et al. 2010). Twelve eastern redcedar (*Juniperus virginiana* L.) trees were randomly selected from moderately encroached areas, hereafter referred to as open canopy forests. Open canopy areas were characterized by redcedar trees occupying areas of approximately 45% cover with open grass interspaces (native warm-season grass dominated spaces

between redcedar trees). Twelve additional trees were randomly selected in highly encroached areas, hereafter referred to as closed canopy forests. Closed canopy forests were characterized by redcedar trees producing canopy closure of approximately 80%, with little to no vegetation in the interspaces between trees. Six randomly selected trees from each forest type (open and closed canopies) were cut to ground level and removed from the area. For each removed tree, an adjacent paired redcedar tree of similar diameter (dbh) was selected and tagged as an uncut control. Six randomly selected sites were established throughout an adjacent tallgrass prairie. The prairie sites were frequently burned and were not encroached by redcedar.

Plot establishment and soil collection

Permanent 1 m^2 plots were established at the base of the removed trees, beneath the control trees, and in the interspace area between removed and control trees (Fig. 1). Permanent plots were also established in the adjacent native prairie. Soil was collected December 2010, March 2011, and June 2011. Within the meter square area, five soil samples (0- 5 cm in depth) were collected and composited for assessment of microbial communities and nutrient analyses (described below). The samples were placed on ice in the field and stored at 5^0 C after transporting to the lab until soil nutrient, microbial communities and moisture analyses were completed.

Assessment of AM fungal root colonization

Soil samples were homogenized by hand and approximately 0.5 g (dry wt) live roots of uniform maturity and appearance (approximately 0.25 – 0.50 mm in diameter) were selected from each soil sample. Root samples were washed free of soil, oven dried at 60 C for 24 hours. Quantification of the percentage root length colonized by arbuscular mycorrhizal fungi followed a modified protocol based on procedures outlined by Johnson et al. (1999) and Vierheilig et al. (1998). Roots were cleared with 10% (w/v) KOH, heated at 100º C for 60 min. and then rinsed several times with tap water. Roots were then soaked for 45 min in alkaline H_2O_2 (10:1 H_2O_2 : NH_4OH) to further remove pigmentation, then rinsed several times with tap water. Cleared roots were boiled for 3 min in a 5% ink-vinegar solution (5% acetic acid) using Shaffer black ink. Roots were de-stained by rinsing in tap water, and acidified with a few drops of 5% acetic acid. Percent AM fungal colonization of the stained roots was measured by the grid-line intersect method at 200x magnification using a compound microscope (McGonigle et al. 1990).

Soil microbial community analysis

Assessing alterations in microbial communities is extremely difficult due to the vast microbial diversity of most soils and the inability to culture and identify most microbes. One method currently used to overcome this problem is phospholipid fatty acid (PLFA) analysis. Fatty acids are components of cell membranes and generally constitute a relatively constant proportion of the

biomass of an organism. Therefore, alterations in PLFA abundance reflect alterations in soil microbial communities and can act as indicators of microbial community composition. Some fatty acids are considered 'key signatures' (Zelles 1999) and are used to differentiate different taxa or estimate bacterial or fungal biomass. Other fatty acids are ubiquitous and therefore cannot be used to distinguish between taxonomic groups, but can be useful in estimating total microbial biomass.

Soil was freeze-dried and finely ground with a mortar and pestle. Five grams of each sample were mixed with a phosphate buffer, methanol, and chloroform for lipid extraction. (0.8:2.0:1.0 v/v). The soil-solvent mixture was separated by centrifugation and the supernatant, and was decanted. A 1:2 (v/v) chloroform-methanol solution was added to the soil, repeating the centrifugation and collection of the supernatant each time. Phosphate buffer was then added and the mixture separated overnight. After phase separation, the chloroform layer containing the lipids was recovered and reduced by nitrogen flow at 60˚C. The lipids were separated into neutral lipids, glycolipids, and phospholipids by solid phase extraction (SPE) by eluting with chloroform, acetone, and methanol, respectively. Phospholipids and neutral lipids were hydrolyzed and the fatty acids methylated. The methylated fatty acids were extracted with hexane and then evaporated under nitrogen at 37˚C. Phospholipid fatty acid (PLFA) analysis was performed using an Agilent 7890A gas chromatograph with an Agilent 5975C series mass selective detector.

The nomenclature used to describe the identified fatty acid is as follows (Bossio and Scow, 1998): total number of C atoms:number of double bonds, cis or trans isomers identified by c or t. Prefixes of a or i indicate anteiso branching or iso branching, respectively. We selected the following biomarkers: 16:1ω5c for AM fungi (Olsson et al., 1995); 3-OH 14:0, 16:1ω7, cy17:0, 2-OH 16:0, 18:1ω9c, cy19:0 for gram negative bacteria; i15:0, a15:0, i16:0, i17:0 for gram positive bacteria; 14:0, 15:0, 16:0, 17:0, 18:0, 20:0 for common (non-specific) microbes ; 18:2ω9,12c and 18:1ω9c for saprophytic fungi (McKinley et al. 2005). Common non-specific fatty acid biomarkers were included in our analysis to express alterations in overall microbial biomass, although these cannot allow assessment of shifts of specific microbial community groups. PLFA data is reported in percent of the total mole fraction which can be interpreted as a relative abundance.

Root biomass and aboveground herbaceous plant biomass and plant species richness

To assess belowground plant biomass, roots were harvested from a 5 cm (depth) x 10 cm (length) soil core (using a square auger) collected directly adjacent to the trunk of the control trees, removed trees, in the interspace and in the native prairie. Roots were washed free of soil, dried at 60 ^{0}C for three days, and weighed. Within each permanent 1 m^2 plot (described above), all herbaceous cover was clipped to ground level, identified and separated to species. The plants were dried at 60 ^{0}C for five days and weighed by species to obtain aboveground biomass. Richness (number of species per m^2) was

assessed. Belowground and aboveground plant biomass as well as species richness were collected in June 2011 only.

Aggregate stability

Soil samples were collected (0 - 5 cm depth) for wet-aggregate stability analysis (Yoder, 1936; Low, 1954). Field moist soils were sieved through an 8 mm sieve to remove large roots, and small stones. The soil sieved through the 8 mm was then sieved through a 4 mm sieve, with aggregates that remain on the 4 mm sieve used for analysis. Fifty grams of air-dried soil was placed in the top sieve of the wet-sieving apparatus that contains 5 sieves (4mm, 2mm, 1mm, 0.5mm, and 0.25mm). These 5 sieves combine into one column with the 4mm being the top layer. Two columns of sieves were attached by spring to the sieving apparatus. Samples were lowered into water and allowed to soak for 10 minutes, then a 10 minute action cycle was initiated by lifting the sieves into and out of the water at a rate of 30 rotations per minute. When complete the soil was removed from each sieve, dried, and weighted. Aggregate stability/size distribution (dry wt of soil remaining in each sieve / total dry wt of initial soil sample) was calculated. The geometric mean diameter (GMD) was calculated as $GMD = \exp\left[\sum_i^n = 1\ wi \log\ \bar{x}I\ / \sum_i^n = 1\ wi\right]$. Where $\bar{x}i$= *mean diameter,* w_i = *aggregate weight for size class and* $\sum_i^n = 1\ wi$ = *total weight of the sample.* Soils for aggregate analysis were collected in December 2010 and June 2011.

Soil bulk density

To assess soil bulk density, soil cores were taken directly adjacent to the root biomass cores with a 5 cm cylindrical auger to a depth of 5 cm. Soil wet-weight was assessed, soil was then dried at 90 ^{0}C for three days, at which time soil dry weight was assessed, and bulk density was calculated as dry mass / soil volume. Bulk density was assessed in December 2010.

Soil nutrient analyses

Soil was sieved through a 2 mm sieve to remove root fragments and rocks. Samples were analyzed by the Soil, Water and Forage Analytical Laboratory at Oklahoma State University for pH, total nitrogen, total soil organic carbon (SOC), extractable inorganic N (NO_3-N) and plant-available phosphorous. Phosphorous was analyzed using a Spectro ICP (H_2PO_4-P; Mehlich 3 test)(Gavlak et al., 2003). Extractable inorganic N was analyzed using a flow-injection analyzer (Gavlak et al. 2003), SOC and total nitrogen were tested with a Leco TruSpec combustion analyzer (Bremner 1996; Nelson and Sommers 1996)

Soil temperature and moisture

Soil temperature data were collected from mesonet.org. Soil moisture content was assessed in the lab. Five g of field-collected soil was oven-dried for 72 hours at 110 ^{0}C, weighed, and percent moisture content calculated.

Photosynthetically Active Radiation (PAR)

PAR light readings were taken in June 2011 using a Decagon AccuPAR ceptometer.

Statistical analysis

For each response variable, the effects of canopy (open, closed, or prairie), vegetative treatment (control tree, removed tree, or interspace) and date (December, March, or June) were analyzed with less significant difference via a three-way ANOVA using SAS version 9.2 software SAS Institute Inc, Cary, NC, USA), and the significant levels of differences are reported for $p \leq 0.05$. Our analysis revealed no significant date x site interactions for soil organic carbon, total N, pH, or PAR. Thus, data from the three sampling dates were combined and reanalyzed as a two-way analysis of variance to compare canopy x vegetative treatment. The Pearson correlation analyses were used to examine the relationships between soil microbial biomass, soil moisture, and soil aggregate stability.

RESULTS

Soil microbial communities (PLFA analysis)

Soil microbial biomass can be assessed by distinct functional groups using specific biomarkers determined by phospholipid fatty acid. In this study, I selected several distinct microbial groups important to soil function: gram positive bacteria, gram negative bacteria, AM fungi, and saprophytic fungi. All of these functional groups decreased noticeably over time in both the open and closed canopy sites (December 2010-March 2011-June 2011) and for all treatments (removed, interspace, control). However, each of these functional groups from soils of the native prairie sites increased significantly from March 2011 to June 2011 (Fig 2 A-D; Fig 3 A-D; Fig 4 A-B). Both open and closed canopy forests had consistently greater bacterial and fungal biomass than the native prairie in December 2010 and March 2011. In fact, the lowest bacterial or fungal biomass in the open or closed canopy sites was still greater than the largest bacterial or fungal biomass in the native prairie (Fig 4 A-B). These results differed in June 2011, with the microbial biomass in the native prairie nearly equaling or surpassing either the open or closed canopy sites (Fig 2 A-D; Fig 3 A-D; Fig 4 A-B). Soil moisture of the forested sites was strongly correlated with soil microbial biomass (Fig 5 A). However, this strong relationship was not

observed between soil moisture and microbial biomass in the prairie sites (Fig 5 B).

Intra-radical AM fungal root colonization

Percent AM fungal root colonization was not significantly different between the open or closed canopy sites. However, the forested sites were significantly greater in percent colonization than the native prairie sites (Table 1).

Root biomass and aboveground herbaceous plant biomass and plant species richness

Root biomass was significantly greater in the closed and open canopy sites, as compared to the native prairie. However, herbaceous aboveground plant biomass was profoundly greater in the prairie sites, as compared to either closed or open forested sites (Table 1). Plant species richness was also significantly greater in the native prairie, as compared to that of either the open or closed canopy sites (Table 1).

Soil nutrients

Extractable nitrogen (NO_3) was consistently greater in the native prairie sites, compared to any of the forested areas, at all sampling dates (Fig 6 A-B). In the closed canopy sites, plant-available phosphorus was consistently lower in June than that of the native prairie sites, although these differences were not apparent in December or March (Fig. 7A). Available phosphorus was not consistently different between the soils of the open canopy sites or the native prairie sites

(Fig. 7B). Soil organic carbon was significantly greater in the open and closed canopy sites compared to the native prairie sites. The percent total organic nitrogen and soil pH were not significantly different across the open or closed canopy sites or the native prairie sites (Table 2).

Aggregate stability and bulk density

The geometric mean diameter (GMD) of aggregates of the closed and open canopy soils averaged ~ 4 in December then decreased significantly to ~ 2.5 in June. The GMD of native prairie aggregates were not significantly different between December and June. The closed and open canopy sites were significantly greater in GMD in December, as compared to the native prairie sites, but were not significantly different in June (Table 3). Across the forest sites, aggregate stability was strongly correlated with total microbial biomass (Fig 8 A). However, no relationship was observed between aggregate stability and microbial biomass in the native prairie sites (Fig 8 B). Soil bulk densities were extremely consistent in all of my sites (Table 3).

Soil moisture

In December, soil moistures were significantly lower in all vegetative areas of the closed canopy sites, compared to the prairie sites (Fig 9 A). However, there was not a significant difference between any of the open canopy sites and the native prairie sites (Fig 9 B). All forested sites were higher in soil moisture in December 2010, compared to June 2011, with the exception of the interspace areas in the closed canopy sites (Fig 9 A-B). The soil moisture of the interspace

areas of the closed canopy sites was characteristically low at each of my sampling dates (Fig 9 A). For each of my sampling dates, the native prairie sites consistently had greater soil moisture, compared to any of the soils within the closed forest sites (Fig 9 A). Soil moisture was not significantly different between the native soils and the removed areas of the open canopy sites at any sampling date (Fig 9 B), but was greater in the March and June sampling of the control (uncut) and interspace areas of these forested sites (Fig 9 B).

Photosynthetically Active Radiation (PAR)

As expected, PAR was significantly greater in the prairie, as compared to either the open or closed canopies (Table 4). However, the open canopy sites were not significantly different from the closed canopy sites (Table 4).

Soil Temperature

Soil temperature data were gathered using mesonet.org for soil temperature with and without vegetative cover. The average soil temperature in December was 7^0C beneath vegetative cover and 6^0C in areas absent of vegetation. In March, the average was 11^0C beneath vegetative cover and 9^0C in areas absent of vegetation, and in June the average soil temperature was 27^0C beneath vegetative cover and 29^0C in areas absent of vegetation (Table 5).

DISCUSSION

Replacement of native herbaceous species with encroaching eastern redcedar have previously been reported to have multi-scale effects; such as the loss of biodiversity, loss of wildlife habitats, and alterations in biogeochemical functions (Gehring and Bragg 1992; Briggs et al. 2002a; 2002b; Pierce and Reich 2010). Most previous studies have focused on aboveground parameters with little attention given to belowground microbial communities. The results of my study suggest potentially important alterations in soil microbial communities following the establishment of eastern redcedar. These data support my first hypothesis that the encroachment of *J. virginiana* into a tallgrass prairie will have significant effects on soil microbial communities. My results indicate that the open and closed canopy forest sites, regardless of the vegetative treatment, had significantly ($p \leq 0.05$) greater gram positive, gram negative, AM fungal, saprophytic fungal and total microbial biomass (which includes non-specific, microorganisms), compared to the native prairie at both the December and March sampling dates. In fact, the difference in microbial biomass in the open and closed canopy sites was 4 to 8 times greater than the microbial biomass of the native prairie sites. Interestingly, this trend did not hold true for June when all microbial functional groups and total microbial biomass significantly increased in the native prairie sites while microbial biomass significantly decreased in both

open and closed canopy sites. In fact, these opposing shifts in microbial biomass resulted in equivalent microbial biomass between the native prairie sites and the open or closed canopy sites in the June sampling. My data suggest the most probable driving factors resulting in the significant decrease in microbial biomass in forested sites, with an increase in biomass in the prairie sites, is the combined effects of moisture availability and dominant host plant phenology (coniferous redcedar with year-round photosynthetic activity vs perennial grasses that are senescent throughout the winter months).

My data indicate the major mechanism driving microbial biomass production in the forested areas is soil moisture availability. There was a reduction in soil moisture in both forested sites from the winter to the summer sampling dates, and these reductions corresponds to the reduction in microbial biomass of these soils. Furthermore, microbial biomass was tightly correlated with soil moisture across these forested sites. The native prairie maintained relatively high soil moisture throughout the testing months. However, several forested sites were characterized by extremely low soil moisture levels. For example, the interspace area in the open canopy sites was low in December, with approximately 14% moisture, and was less than 5% in June. These low levels of moisture in the forest interspaces compared to the native prairie could be explained by the profoundly lower vegetative cover in the forested sites (Table 1). Low biomass cover in agricultural soils has been shown to increase soil evaporation rates (Smika and Unger 1986). That soil moisture in the open and closed canopy sites generally decreased even with increases in rainfall (Table 5)

is in agreement with Smith and Stubbendieck (1990). In their study, increases in eastern redcedar canopy cover resulted in increased diversion of rain with concomitant low levels of soil moisture, even following rainfall events. That the closed canopy sites were lower in soil moisture in our December sampling, as compared to the open canopy sites, may be a reflection of higher diversion of rainfall with greater redcedar cover (80% in the closed canopy sites vs 45% in the open canopy sites). Additionally, greater loss in soil moisture of the open canopy sites may be a reflection of greater evaporation from the soil in the spring and summer months, due to lower shade and litterfall cover (Pierce and Reich 2010). While soil moisture of the native prairie sites decreased between December and March, these losses in moisture were less substantial and, thus, moisture was generally greater than that of either the open or closed canopy sites in March and June. For example, in June, the percent soil moisture of the native prairie sites were 53 and 54% higher than that of the average June moisture levels of open and closed canopy sites, respectively. Under low soil moisture, microbial activity has been shown to decrease significantly, as microbes reduce their activity until moisture availability increases (Csonka 1989).

The increase in the microbial biomass in June 2011 of the native prairie sites cannot be explained by alterations in soil moisture, and my correlation analysis did not indicate a relationship between moisture and microbial biomass. Although soil moisture of the prairie remained relatively constant, as compared to the forested sites, percent moisture of the prairie sites was also reduced in the spring and summer sampling, as compared to winter. However, although the

moisture was reduced, moisture levels of the prairie sites were 33 to 50% greater than the closed or open canopy forest sites in both spring and summer sampling dates. Therefore, microbial biomass of the prairie sites may be driven by the phenology of the dominant host plants, rather than by soil moisture availability. The forested sites are dominated by eastern redcedar, a coniferous tree species that photosynthesizes year-round; the prairie sites are dominated by warm-season grasses that senesce throughout the winter months. Most soil microorganisms are generally C limited (Hogberg et al. 2001), with the possible exception of mycorrhizal fungi. These symbionts are not generally C limited unless the plant host restricts belowground C allocation (Smith and Read 1997). It has been reported that mycorrhizal activity and mycorrhizal root colonization decreases in winter months when host plants senesce, thereby limiting soil C translocation (Bentivenga and Hetrick 1992). In my study, PLFA analysis indicates extremely low levels of AM fungi in prairie soils during the December sampling. These levels increased 4 fold by June, when plant activity is heightened, with increased C allocation to the belowground microbial communities. Other studies have also shown that increasing C in the soil by increasing plant production resulted in increased microbial biomass (Broughton and Gross 2000; Yao et al. 2000).

Overall, microbial biomass production was substantially greater in the forest sites than the prairie sites. The soils of the open and closed canopy sites were 36% and 50% greater in SOC, respectively, as compared with the native prairie sites. The ability of eastern redcedar to maintain a greater C store in the

soil compared to the native prairie is a possible mechanism for increased AM fungal biomass production, contributing to the greater microbial biomass in the forested sites, as compared to the native prairie sites, throughout winter and spring seasons. Root biomass in the open and closed canopies was also significantly greater than that of the native prairie, presumably substantially contributing to the overall increases in SOC of these forested sites. Greater root biomass of redcedar forests, compared to the grasslands they have replaced, has also been reported for encroached areas of Kansas (Norris et al. 2001a). Several previous studies have found SOC accumulation in soils encroached by *Juniperus* spp. and other coniferous trees (Klemmedson and Tiedemann 2000; Bates et al. 2002; Smith and Johnson 2003; Grunzweig et al. 2007 and McKinley and Blair 2008). McKinley et al. (2006) reported that soils from areas that were encroached by eastern redcedar were 24% greater in SOC than adjacent prairie soils, values that are similar to those in my study. It has been suggested that substantially more carbon is held in woodlands because of greater aboveground productivity (Norris et al. 2001b) and lower soil respiration rates (Smith and Johnson 2004).

It is important to note that the shifts in the microbial communities, whether decreasing or increasing, remained approximately the same across all functional groups. In other words, alterations in microbial biomass production were not due to rapid increases in any of the functional groups examined in this study, but rather were due to an overall increase across all functional groups. This is in

agreement with Hogberg et al. (2007), who showed that microbial community structure is generally stable under varying natural conditions.

Based on the PLFA data, the forest sites were profoundly greater in AM fungal biomass in the winter and summer sampling, compared to the prairie sites. and percent inter-radical AM fungal colonization of forest roots were significantly greater when observed microscopically, as well (Table 1). Higher levels of colonization may be a reflection of host plant association. Eom et al. (2000) reported that AM fungal species abundance was significantly affected by the host species. Therefore, higher levels of colonization may be in response to shifts in host plant species as redcedar establishes. As previously described, eastern redcedar trees and supply carbon to the symbiotic fungi year-long; whereas the native prairie grasses senesce and belowground carbon supply is limited. Therefore, there may be a shift in AM fungal species due to changes in the dominant host (native grasses to coniferous tree), resulting in higher colonization. However, it is also possible the AM fungal species remain consistent in both forest and grass communities, but the coniferous host can supply greater belowground C allocations, and therefore, root colonization is greater in the conifer dominated communities.

Soil abiotic conditions are also important in microbial community composition and productivity. Indeed, my second hypothesis states that abiotic factors will be strongly affected by the encroachment of eastern redcedar. For example, soil temperature is frequently correlated with soil microbial biomass productivity (Zogg et al. 1997). Soil temperature increased slightly from

December to March, and increased substantially in June, based on data collected from mesonet.org station near my study site in Stillwater, OK.

Soil nutrients were also expected to be altered by the encroachment of eastern redcedar (Hypothesis 2). Alterations in nutrient dynamics in native soils may have important consequences. For example, plant available N in forest and grassland soils may influence plant productivity, as well as soil microbial productivity, and ultimately potential rates of C accretion and storage. In my study, NO_3 concentrations were typically greater in prairie soils, compare to forest sites. Greater primary productivity of redcedar forests, relative to the prairie they replace, may increase N demand and elicit immobilization of substantial quantities of N in plant biomass, litter, and soil organic matter (McKinley and Blair 2008; Norris et al. 2001b). Mean aboveground plant productivity of redcedar forests has been reported as 2.5 times greater than adjacent prairie (Norris et al. 2007). Immobilization of N in plant tissue and soil pools, coupled with alterations in litter N quality that reduce decomposition rates may reduce labile N pools and subsequently N availability.

Soil phosphorus remained fairly consistent in forest and grassland sites. Phosphorus data indicated forest and prairie sites contained relatively low plant-available P, typical of tallgrass prairie soils within the Great Plains (Johnson et al. 2010; Wilson et al. 2009). Soil bulk density did not vary significantly between sites, with all values close to 1 g/cm^3, typical of grassland soils with relatively high soil organic matter (Brady and Weil 2008). Soil pH also did not vary significantly between sites.

In the December sampling, soils of both closed and open canopy sites contained greater percentages of water-stable macroaggregates, as compared to the adjacent prairie sites. As with the microbial biomass, the percent water-stable macroaggregates decreased significantly from December to June in the open and closed canopy sites, but did not decrease significantly in the native prairie sites.

Soil aggregation is a process by which aggregates of varying sizes are joined together by organic and inorganic material. These processes include the formation then stabilization of these soil aggregates (Amezketa 1999). There are three main organic groups that help form and stabilize these aggregates based on age and degradation of organic matter: transient, temporary and persistent binding agents (Tisdall and Oades 1982). Transient binding agents decompose rapidly by soil microorganisms. Temporary binding agents are roots, hyphae, particularly AM fungal hyphae as well as saprophytic fungi. Persistent binding agents are associated by polyvalent metal and strongly sorbed polymers (Tisdall and Oades 1982). My study suggests that the temporary binding agents are the major player in the high percentage of soil macroaggregates in the open and closed canopy sites in December. In my study, soil microbial biomass decreased in June in all forested sites, reflecting the loss of soil macroaggregates and concomitant increase in microaggregates. Indeed, microbial biomass of these forested sites was significantly correlated with abundance of water-stable macroaggregates. Previous research has shown that aggregate formation and stabilization is promoted by AM fungi hyphae and fibrous root growth (Jastrow,

1987; Miller and Jastrow, 1990; Jastrow et al, 1998) and Wilson et al. (2009) found soil aggregation was tightly correlated with the abundance of AM fungal hyphae. In my study, AM fungi and other microbial functional groups are greater in the open and closed canopy sites than that of the native prairie sites in both winter and spring sampling. However, in June, microbial biomass, including AM fungal abundance, was generally reduced in all forest sites, corresponding to decreases in soil moisture. The macroaggregate stability in the open and closed canopy sites significantly decreased from December to June. I did not observe any change in macroaggregate stability in the native prairie sites over the same time period. However, the abundance of macroaggregates in the native prairie was significantly lower than either open or closed canopy sites in December, and the reduction in forest soil aggregation resulted in values similar to that of the native prairie soils in the June sampling. That microbial biomass in the native prairie soils increased in the growing season, while the forest soils exhibited a substantial decrease in microbial activity, may be why the macroaggregates in the native prairie soils did not significantly decrease from December to June, as was observed in the forest sites. In the forested sites, the abundance of all microbial biomass, and especially that of the AM and saprophytic fungi, was high in the winter and spring sampling, with AM fungal values often exceeding 8 nmol/ g soil and saprophytic fungal values exceeding 35 nmol/g soil. Corresponding values from the adjacent prairie soils were less than 1 nmol/ g soil and less than 3 nmol/ g soil for AM and saprophytic fungi, respectively. These data add support for transient macroaggregation stability in these forest sites formed and stabilized

by soil microbial communities, including AM and saprophytic fungi and key to their formation and stability.

Summarizing my data for the abiotic parameters measured, I am able to accept my second hypothesis, but only in regard to N availability and soil aggregation. Extractable NO_3 was typically greater in prairie sites, compared to the encroached sites. In the December sampling, soil macroaggregate stability was greater in the forest soils, compared to soils of the prairie sites. However, plant-available P, soil temperature, soil bulk density or soil pH were not altered by establishment of eastern redcedar trees.

Herbaceous plant biomass and plant species richness were profoundly reduced in the open and closed canopy sites compared to the native prairie sites (Table 1) which agrees with many studies that have reported encroachment by eastern redcedar results in a significant decrease in vegetation, compared to the grasslands they replace (Bard 1952; Gehring and Bragg 1992; Engle et al. 1987; Smith and Stubbendieck 1990; van Els et al. 2010). This reduction in herbaceous biomass and plant species richness corresponds with a significant reduction in PAR in the forest sites, compared to the adjacent prairie sites (Table 1).

In my third hypothesis, I projected that the removal of individual *J.virginiana* trees would result in microbial communities diverging back to that of the native prairie; microbial communities of soil collected beneath cut and removed trees would become more similar to the non-encroached prairie site than soil collected beneath uncut (control) trees. However, my data does not

support this hypothesis. Microbial communities from the removed sites were not significantly different from that of the corresponding control (uncut) sites in any of my forested areas. One possible reason that my data do not support this hypothesis is that the duration of this study (6 months) was not adequate to observe long-term changes. Ansley and Rasmussen (2005) reported that herbaceous vegetation recovery took at least 3 years following eastern redcedar removal. Another explanation is that the surrounding eastern redcedar influence soil biotic and abiotic properties well beyond their own canopies, as described by Linneman and Palmer (2006), thereby slowing or preventing significant changes in soil properties.

However, while the removed tree sites were typically similar to the control sites in microbial community abundance, my data indicates the interspace areas had characteristically lower microbial biomass production, compared to that of the soil collected directly beneath either the removed tree trunk, or the uncut control. The interspace areas were typically intermediate between the forested and the prairie soils, in microbial biomass, but also in PAR and herbaceous vegetation.

CONCLUSION

The encroachment of *J.virginiana* into the tallgrass prairie does have significant effects on several soil abiotic and biotic properties. Most notable are the increase in soil organic carbon, soil microbial communities and aggregate stability in forested sites compared to the tallgrass prairie sites. Soil microbial communities decrease in biomass in response to available soil moisture within the forested sites, but not in the tallgrass prairie. Alternatively, soil microbial biomass of the grassland sites appears to be directed by the phenology of the dominant host plant. Aboveground biomass of herbaceous plant species and plant species richness under and near *J. virginiana* is severely decreased, further altering the tallgrass prairie landscape. The removal of an individual *J. virginiana* tree did not result in changes compared to the uncut control. Therefore, my research suggests that management be aimed at whole stand eradication for the most rapid recovery of encroached grasslands. It is possible that with more time the soil biotic and abiotic characteristics would diverge to that of a non-invaded prairie, even with the removal of individual trees. However, it is also likely that re-invasion would occur since the surrounding area was moderately (open canopy) and highly encroached (closed canopy). The interspace areas were generally intermediate in both biotic and abiotic characteristics, with values between the forested and prairie soils. This suggests *J.virginiana* not only changes the soil characteristics directly beneath its canopy, but also extends into soils beyond its

canopy. The recovery of the tallgrass prairie ecosystem requires immediate and thorough removal of *J.virginiana* in encroached areas. *J.virginiana* does have a place in our native rangelands, but without management, it can quickly become a spreading monoculture, displacing the natural diversity of native tallgrass prairies.

LITERATURE CITED

Amézketa, E. 1999. Soil Aggregate Stability: A Review. Journal of Sustainable Agriculture. 14: 2, 83 – 151

Ansley, J.R. and Rasmussen, G.A. 2005. Managing native invasive juniper species using fire. Weed Technology. 19:517–522

Bard, G.E. 1952. Secondary succession on the piedmont of New Jersey. Ecology Monographs. 22(3):195–215

Batek, M.J., Rebertus, A.J., Schroeder, W.A., Haithcoat, T.L., Compas, E. and Guyette, R.P.1999. Reconstruction of early nineteenth-century vegetation and fire regimes in the Missouri Ozarks. Journal of Biogeography. 26:397–412

Bates, J., Svejcar, T.S. and Miller, R.F., 2002. Effects of juniper cutting on nitrogen mineralization. Journal of Aridland Environments. 51:221–234

Bentivenga, S. P. and Hetrick B. A. D. 1992. The effect of prairie management practices on mycorrhizal symbiosis. Mycologia. 84(4):522-527

Bossio, D.A. and Scow, K.M. 1998. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patters. Microbial Ecology. 35:265–278

Brady, N.C. and Weil, R.R. 2008. The nature and properties of soils. 14th ed. Pearson Prentice Hall, N.J. pg.148

Bremmer J.M. 1996. Methods of Soil Analysis, Part 3, Chemical Methods. Chapter 37, Total Nitrogen. Soil Science Society of America. Madison, WI. Pp 1087-89.

Briggs, J.M., Hoch, G.A. and Johnson, L.C. 2002a. Assessing the rate, mechanisms, and consequences of the conversion of tallgrass prairie to *Juniperus virginiana* forest. Ecosystem. 5:578–586

Briggs, J.M., Knapp, A.K. and Brock, B.L. 2002b. Expansion of woody plants in tallgrass prairie: a fifteen-year study of fire and fire-grazing interactions. American Midland Naturalist Journal. 147:287–294

Briggs, J.M., Knapp, A.K., Blair J.M., Heisler, J.L., Hoch, G.A., Lett, M.S. and McCarron, J.K. 2005. Causes and Consequences of Conversion of Mesic Grassland to Shrubland. Bioscience. 55(3):243

Broughton, L.C. and Gross, K.L. 2000. Patterns of diversity in plant and soil microbial communities along a productivity gradient in a Michigan old-field. Oecologia. 125:420–427

Csonka, L.N. 1989. Physiological and genetic responses of bacteria to osmotic stress. Microbiological Review. 53:121-147

Didham, R.K., Tylianakis, J.M., Hutchison, M.A., Ewers, R.M., and Gemmell, N.J. 2005. Are invasive species the drivers of ecological change? TRENDS in Ecology and Evolution. 20:9

Engle, M., Stritzke, J.G. and Claypool, P.L. 1987. Herbage standing crop around eastern red cedar trees. Journal of Range Management. 40(3):237–239

Eom, A.H., Hartnett, D.C. and Wilson, G.W., 2000. Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. Oecologia. 122(3):435-444

Falk-Petersen, J., Bohn, T., and Sandlund, O.T. 2006. On the numerous concepts in invasion biology. Biological Invasions. 8:1409–1424

Ferguson, E.R., Lawson, E.R., Maple, W.R. and Mesavage, C. 1968. Managing eastern red cedar. USDA Forest Service. South For Exp Stn SO-37

Gavlak, R., Horneck, D., Miller, R.O. and Kotuby-Amacher, J. 2003. Soil, Plant and Water Reference Methods for the Western Region. WREP-125, 2nd eds. Fairbanks, AK, USA: University of Alaska, 160–166

Gehring, J. and Bragg, T. 1992. Changes in Prairie Vegetation under Eastern Redcedar (*Juniperus virginiana* L.) in an Eastern Nebraska Bluestem Prairie. American Midland Naturalist. 128:209-217

Grunzweig, J. M., Gelfand, I., Fried, Y. and Yakir, D. 2007. Biogeochemical factors contributing to enhanced carbon storage following afforestation of a semi-arid shrubland, Biogeosciences. 4:891– 90

Herre, E.A., Knowlton, N., Mueller, U.G. and Rehner, S.A. 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. Trends in Ecology and Evolution. Vol. 14:49-53

Hogberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Edblas, A., Hogberg, M.N., Nyberg, G., Ottonsson-Lofvenius, M. and Read, D.A. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789–792

Hogberg, M.N., Hogberg, P. and Myrold, D.D. 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia. 150:590–601

Jastrow, J.D., Millera, R.M. and Lussenhop, J. 1998. Interactions of biological mechanisms contributing to soil aggregate stabilization in restored prairie. Soil Biology and Biochemistry. 30:905–916

Jastrow, J.D. 1987. Changes in soil aggregation associated with tallgrass prairie restoration. American Journal of Botany. 74:1656–1664

Johnson, N.C., Wilson G.W.T., Bowker, J.A. and Miller, R.M. 2010. Resource limitation as a driver of local adaptation in mycorrhizal symbioses. Proceedings of the National Academy of Sciences. 107: 2093-2098

Klemmedson, J.O. and Tiedemann, A.R. 2000. Influence of western juniper development on distribution of soil and organic layer nutrients. Northwest Science. 74:1–11

Levine, J.M., Adler, P.B. and Yelenik, S.G. 2004. A meta-analysis of biotic resistance to exotic plant invasions. Ecology Letters. 7: 975–989

Limb, R. F., Engle, D.M., Alford, A.L and Hellgren, E.C. 2010. Tallgrass prairie plant community dynamics along a canopy cover gradient of Eastern Redcedar (*Juniperus virginiana, L*.) Rangeland Ecology Management. 63: 638-644

Linneman, J. S. and M. W. Palmer. 2006. The effect of Juniperus virginiana on plant species composition in Oklahoma grassland. Community Ecology. 7: 235-244

Low, A.J., 1954. The study of soil structure in the field and in the laboratory. Journal of Soil Science. 5:57-77

McGonigle, T.P., Miller, M. H., Evans, D. G., Fairchild, G. L. and Swan, J. A. 1990. A new method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. New Phytologist. 115:495-501

McKinley, V.L., Peacock, A.D. and White, D.C. 2005. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. Soil Biology and Biochemistry. 37:1946–1958

McKinley, G. A., Takahashi, T., Buitenhuis, E., Chai, F., Christian, J. R., Doney, S. C., Jiang, J.S., Lindsay, K., Moore, J.K., Le Quere, C., Lima, I., Murtugudde, R., Shi, L., and Wetzel, P. 2006. North Pacific carbon cycle response to climate variability on seasonal to decadal timescales, Journal of Geophysics. Res., 111

McKinley, D.C. and Blair, J.M. 2008. Woody plant encroachment by *Juniperus virginiana* in a mesic native grassland promotes rapid carbon and nitrogen accrual. Ecosystems. 11:454–468

Meiners, S. J., Pickett, S. T. A. and Cadenasso, M. L. 2001. Effects of plant invasions on the species richness of abandoned agricultural land. Ecography. 24: 633–644

Miller, R.M. and Jastrow, J.D. 1990. Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. Soil Biology and Biochemistry. 22:579–584

Myers, H. R. 1982. Climatological data of Stillwater, Oklahoma, 1893–1980. Oklahoma Agriculture Experimental Station. Res. Rep. P-321

Nelson, D.W. and Sommers, L.E., 1996. Methods of Soil Analysis, Part 3, Chemical Methods. Chapter 34, Total Carbon, Organic Carbon, and Organic Matter. Soil Science Society of America. Madison, WI. Pp 963-77.

Norris, M.D., Blair, J.M., Johnson, L.C. and McKane, R.B. 2001a. Assessing changes in biomass, productivity, and C and N stores following *Juniperus virginiana* forest expansion into tallgrass prairie. Canadian Journal of Forest Research. 31: 1940–1946

Norris, M.D., Blair, J.M. and Johnson, L.C. 2001b. Land cover change in eastern Kansas: litter dynamics of closed-canopy eastern redcedar forests in tallgrass prairie. Canadian Journal of Botany-Revue Canadienne de Botanique. 79: 214–222

Norris, M.D., Blair, J.M. and Johnson, L.C. 2007. Altered ecosystem nitrogen dynamics as a consequence of land cover change in tallgrass prairie. American Midland Naturalist. 158(2):432-445

Olsson, P.A., Bååth, E., Jakobsen, I. and Söderström, B. 1995. The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. Mycological Research. 99:623–629

Linneman, J. S. and M. W. Palmer. 2006. The effect of Juniperus virginiana on plant species composition in Oklahoma grassland. Community Ecology. 7: 235-244

Pierce, A., and Reich, P.B. 2010. The effects of eastern redcedar (*Juniperus virginiana*) invasion and removal on a dry bluff prairie ecosystem. Biological Invasions. 12: 241-252

Samson, F.B., and Knopf, F.L., 1994. Biological Diversity-Science in Action. Conservation Biology. 8:909-911

Schmidt, T.L., Leatherberry, E.C. 1995. Expansion of eastern red cedar in the lower Midwest. Northern Journal of Applied Forestry. 12(4):180–183

Shah, M.A., Reshi, Z.A. and Khasa, D.P. 2009. Arbuscular Mycorrhizas: Drivers or Passengers of Alien Plant Invasion. Botanical Review. 75: 397-417

Smith, D. L. and Johnson, L.C. 2003. Expansion of Juniperus virginiana L. in the Great Plains: Changes in soil organic carbon dynamics. Global Biogeochemistry Cycles. 17:1062

Smith, D.L. and L.C. Johnson. 2004. Vegetation-mediated changes in microclimate reduce soil respiration as woodlands expand into grasslands. Ecology. 85:3348–3361

Smith, S.E. and Read, D.J. 1997. Mycorrhizal symbiosis. 2nd ed. San Diego, California: Academic Press. 605 p.

Smith, S.D. and Stubbendieck, J. 1990. Production of tall-grass prairie herbs below eastern red cedar. Prairie Naturalist. 22(1):13–18

Tisdall, J.M. and Oades, J.M. 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science. 33:141-163

van der Heijden, M.G.A., Boller, T., Wiemken, A. and Sanders, I. R. 1998. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology. 79:2082–2091.

Van Els, P., Will, R. E., Palmer, M. W. and Hickman, K. R. 2010. Changes in forest understory associated with Juniperus encroachment in Oklahoma, USA. Applied Vegetation Science. 13: 356–368. doi: 10.1111/j.1654-109X.2010.01078

Vitousek, P.M. and Matson, P.A. 1988. Nitrogen transformation in tropical forest soils. Soil Biology and Biochemistry. 20: 361–367

Vogelsang, K.M., Bever, J.D., Griswold, M., and Shultz, P.A. 2004. The use of mycorrhizal fungi in erosion control applications. Final Report for Caltrans. Contract Number 65A0070. California Department of Transportation. Sacramento, California, U.S.A.

Vogelsang, K.M. and Bever, J.D. 2009. Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. Ecology. 90:399-407

Wilson, G.W.T., Rice, C.W., Rillig, M.C., Springer, A. and Hartnett, D.C. 2009. Arbuscular mycorrhizal fungi control soil aggregation and carbon sequestration. Ecology Letters. 12:452-461

Yao, H., He, Z., Wilson, M.J., Campbell, C.D. 2000. Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use. Microbial Ecology. 40:223–237

Yoder, R. E. 1936. A direct method of aggregate analysis of soils and a study of the physical nature of soil erosion losses. Journal of American Society of Agronomy. pp. 337-351

Zelles, L. 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: A review. Biological Fertility of Soils. 29:111–129

Zogg, G.P., Zak, D.R., Ringelberg, D.B., MacDonald N.W., Pregitzer, K.S. and White, D.C. 1997. Compositional and functional shifts in microbial communities due to soil warming. Soil Science Society American Journal. 61:475-481

TABLES

Table 1

Canopy	AM Fungal (%) Colonization*	Root Biomass** (g/cm^3)	Herbaceous Aboveground Biomass** (g/m^2)	Plant Species Richness**
Open	50 a (±1.65)	0.007 a (±0.002)	15.83 b (±3.02)	2.00 b (±0.49)
Closed	51 a (±0.83)	0.008 a (±0.002)	1.35 c (±0.68)	0.83 b (±0.17)
Prairie	41 b (±2.55)	0.003 b (±0.001)	200.86 a (±37.96)	6.00 a (±0.77)

* December 2010, March 2011 and June 2011 combined means

**Data were collected in June 2011 only

Table 2

Canopy	Soil Orangic Carbon* (%)	Total Nitrogen* (%)	pH*
Open	4.85 a (±0.83)	0.31 a (±0.04)	6.2 a (±0.06)
Closed	3.79 a (±0.44)	0.27 a (±0.03)	7.0 a (±0.05)
Prairie	2.17 b (±0.30)	0.24 a (±0.01)	6.3 a (±0.07)

* December 2010, March 2011 and June 2011 combined means

Table 3

Canopy	Month	Soil Aggregation(GMD)*	Bulk Density** (g/cm^3)
Open	Dec	3.92 a (±0.07)	1.14 a (±0.09)
	Jun	2.52 a (±0.16)	n/a
Closed	Dec	4.57 a (±0.17)	1.11 a (±0.07)
	Jun	2.73 a (±0.11)	n/a
Prairie	Dec	2.57 b (±0.12)	1.25 a (±0.11)
	Jun	2.12 a (±0.26)	n/a

n/a(not available) data were not collected for that month
* Data were collected for December 2010 and June 2011 only.
**Data were collected for December 2010 only.

Table 4

Canopy	Photosynthetically active radiation **
Open	1104 b (±5.47)
Closed	992 b (±12.14)
Prairie	1306 a (±1.75)

**Data were collected for June 2011 only.

Table 5

Month	Ambient Temperature (^0C)	Soil Temperature with vegetative cover (^0C)	Soil Temperature without vegetative cover (^0C)	Rainfall (mm)
September	22	24	25	6.60
October	16	18	18	43.94
November	10	11	11	49.28
December	3	6	5	13.46
January	0	3	2	8.13
Feburary	4	6	6	47.50
March	11	11	9	21.08
April	18	17	17	50.29
May	20	20	24	85.34
June	29	27	29	36.83

Monthly mean obtained from mesonet.org.
September 2010 to June 2011.

FIGURES

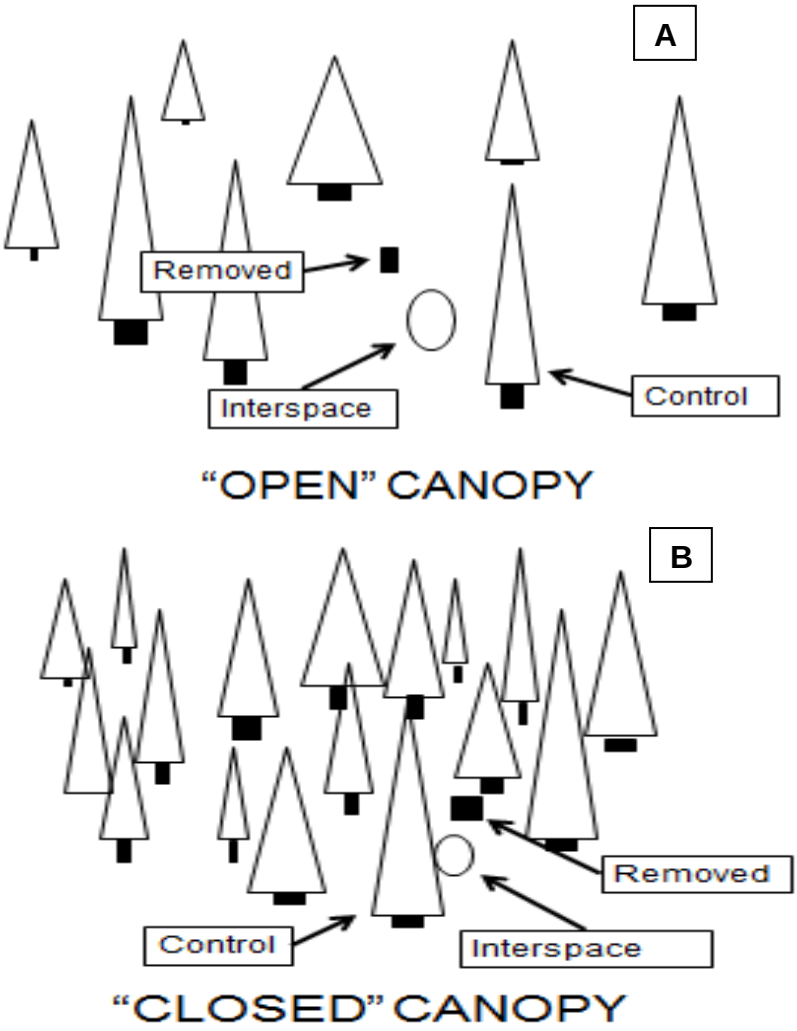


Figure 1 A-B

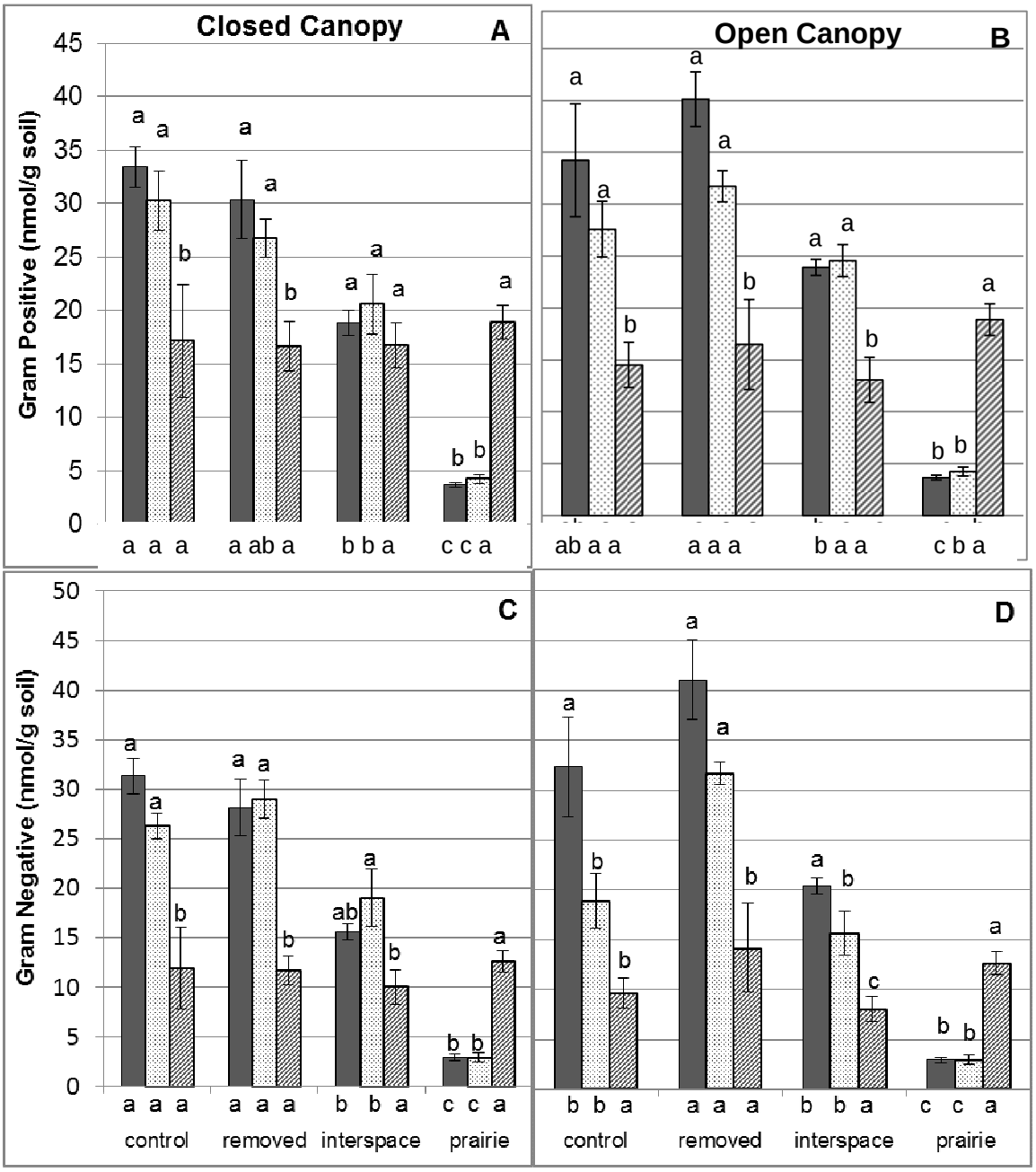

Closed Canopy
A
Open Canopy
B
Gram Positive (nmol/g soil)
45
40
35
30
25
20
15
10
5
0
a a a
a ab a
b b a
c c a
ab a a
a a a
b a a
c b a
C
D
Gram Negative (nmol/g soil)
50
45
40
35
30
25
20
15
10
5
0
a a a
a a a
b b a
c c a
b b a
a a a
b b a
c c a
control
removed
interspace
prairie
control
removed
interspace
prairie

Figure 2 A-D

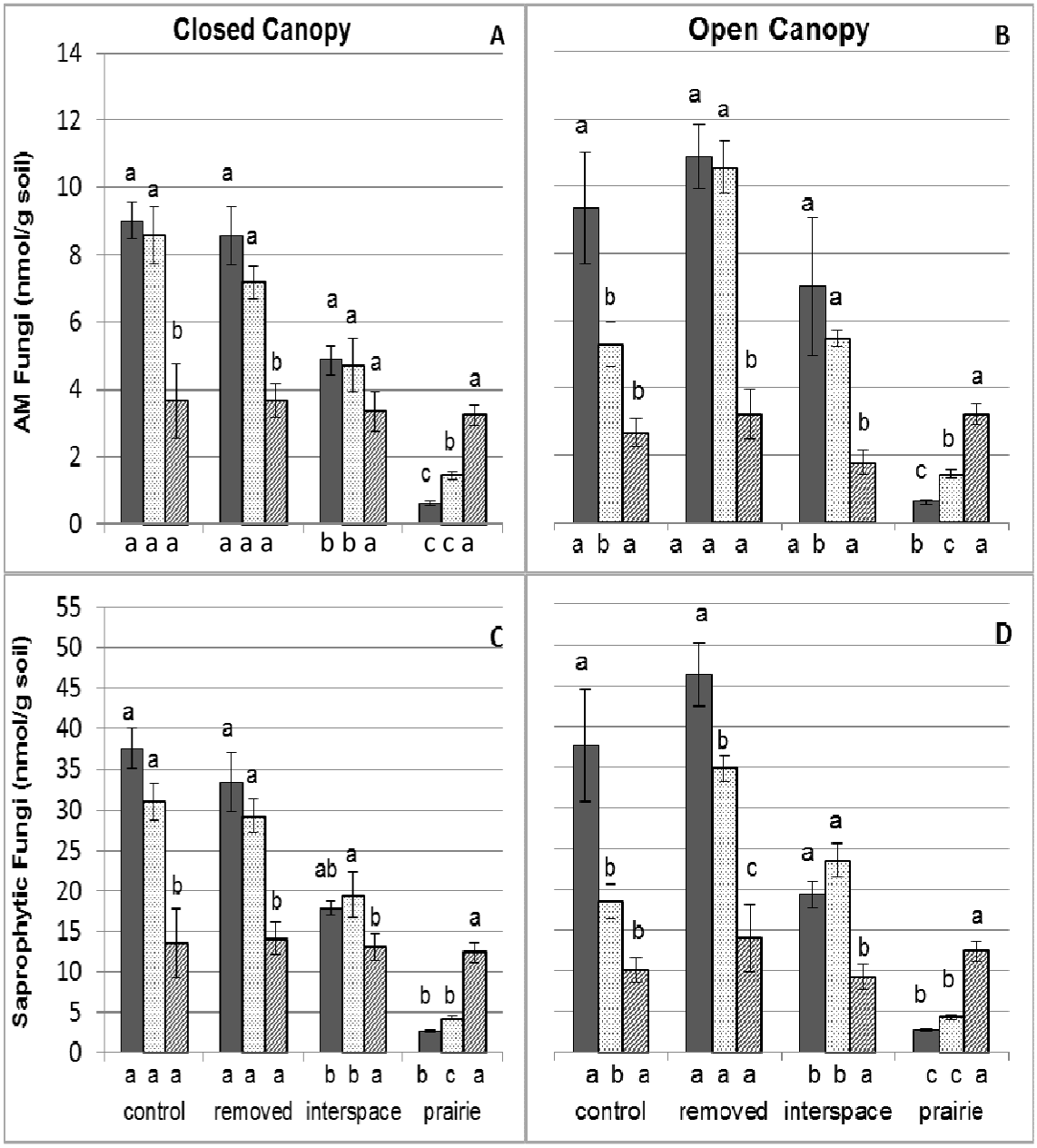

Closed Canopy
A
Open Canopy
B
AM Fungi (nmol/g soil)
Saprophytic Fungi (nmol/g soil)
C
D
control
removed
interspace
prairie

Figure 3 A-D

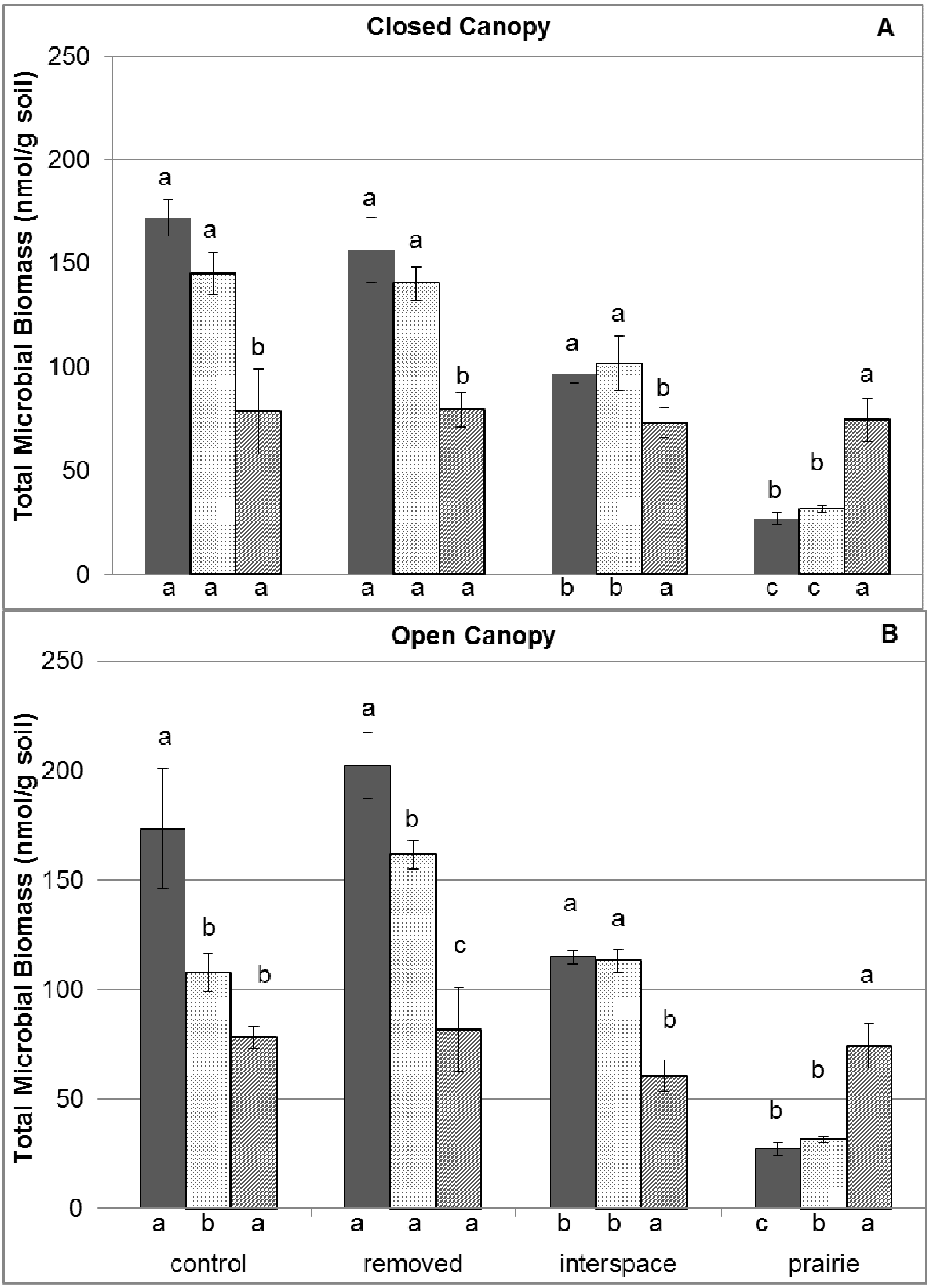
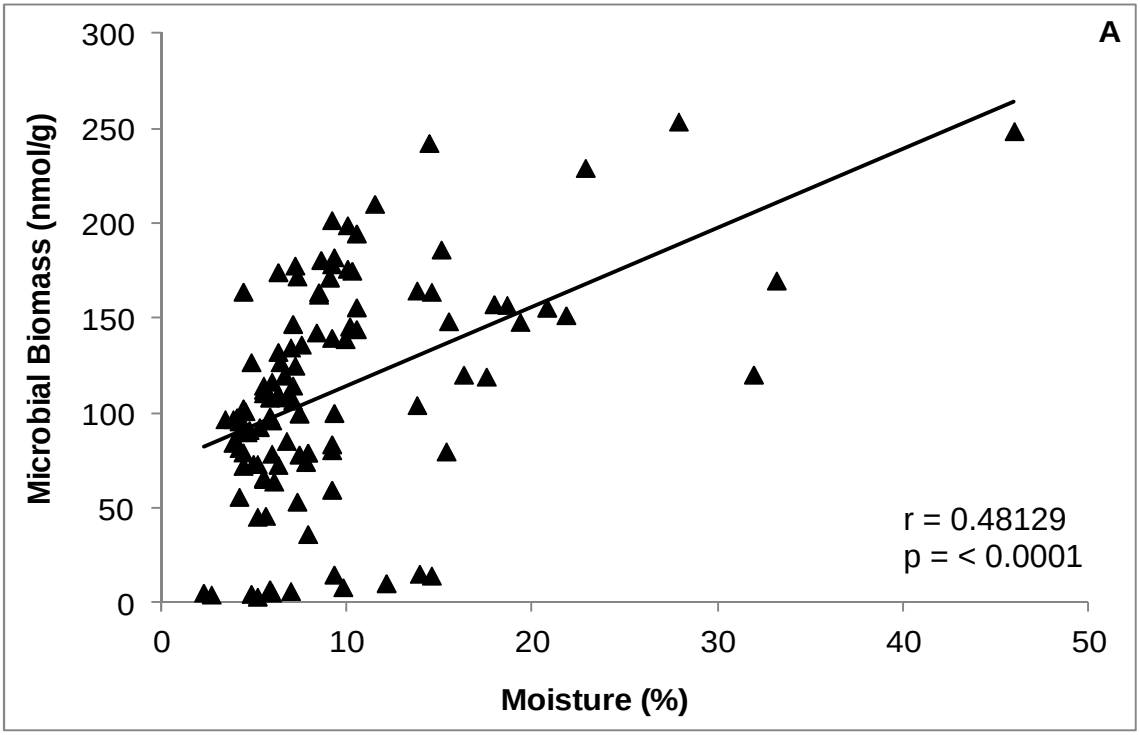

Closed Canopy
A
Total Microbial Biomass (nmol/g soil)
250
200
150
100
50
0
a a a a a a a a a b b a b b b a b a
Open Canopy
B
Total Microbial Biomass (nmol/g soil)
250
200
150
100
50
0
a b a a a a b b a c b a
control
removed
interspace
prairie

Figure 4 A-B


A
Microbial Biomass (nmol/g)
300
250
200
150
100
50
0
0
10
20
30
40
50
Moisture (%)
r = 0.48129
p = < 0.0001

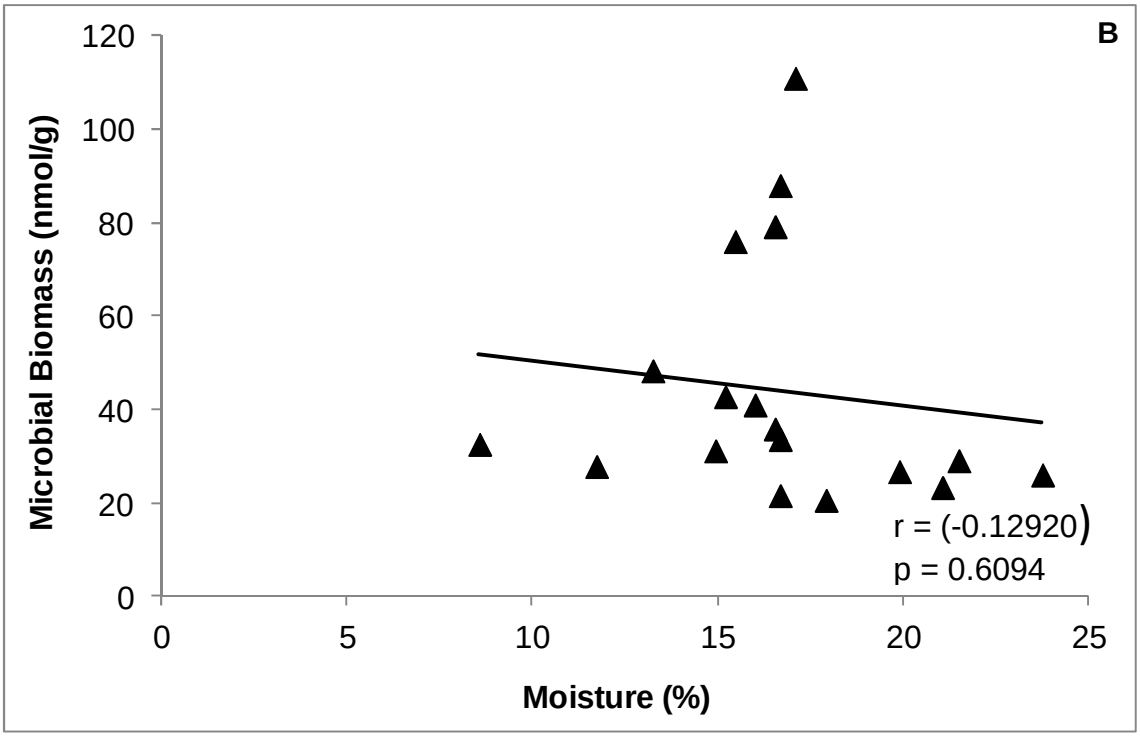

B
Microbial Biomass (nmol/g)
120
100
80
60
40
20
0
0
5
10
15
20
25
Moisture (%)
r = (-0.12920)
p = 0.6094

Figure 5 A-B

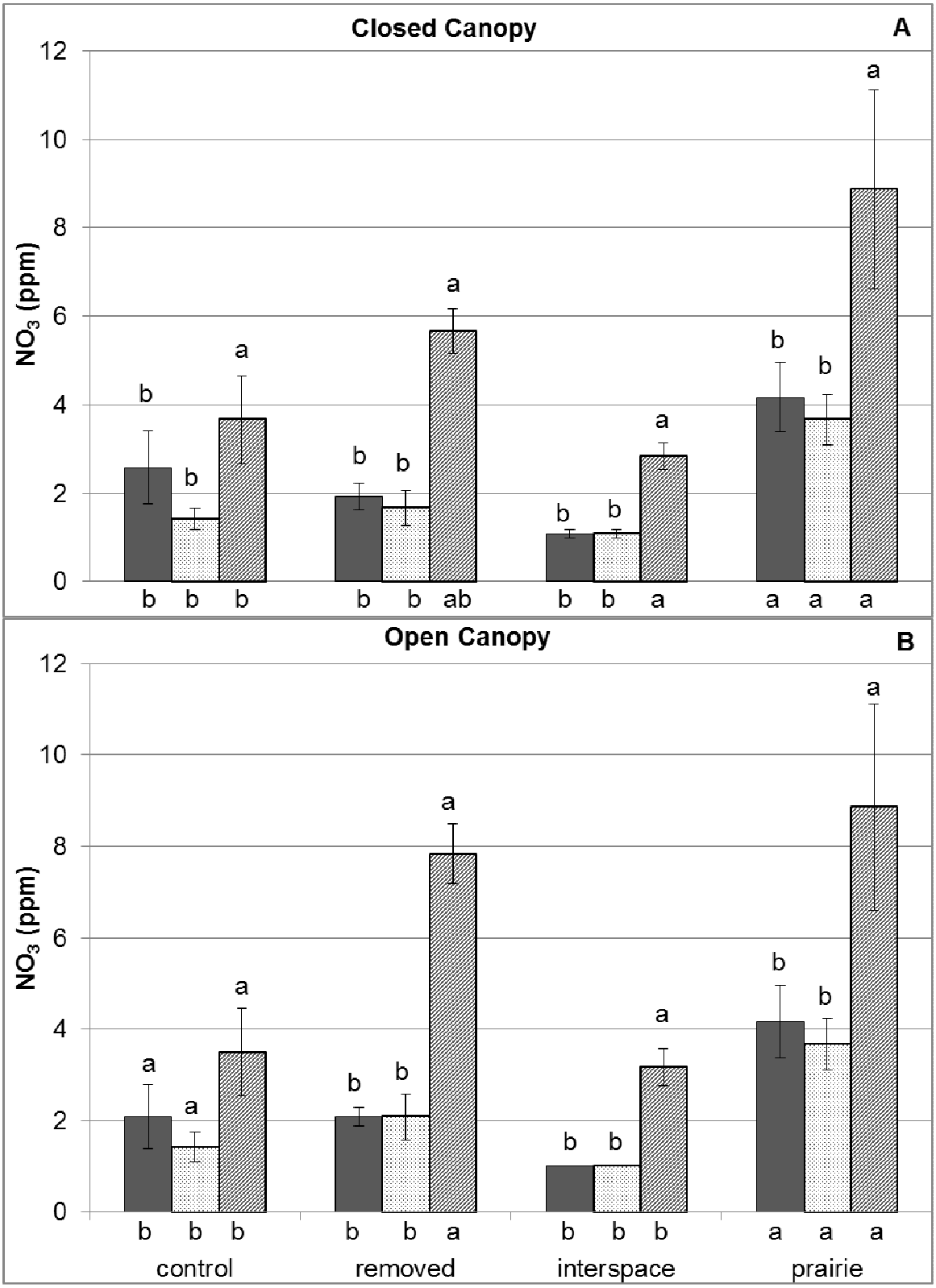
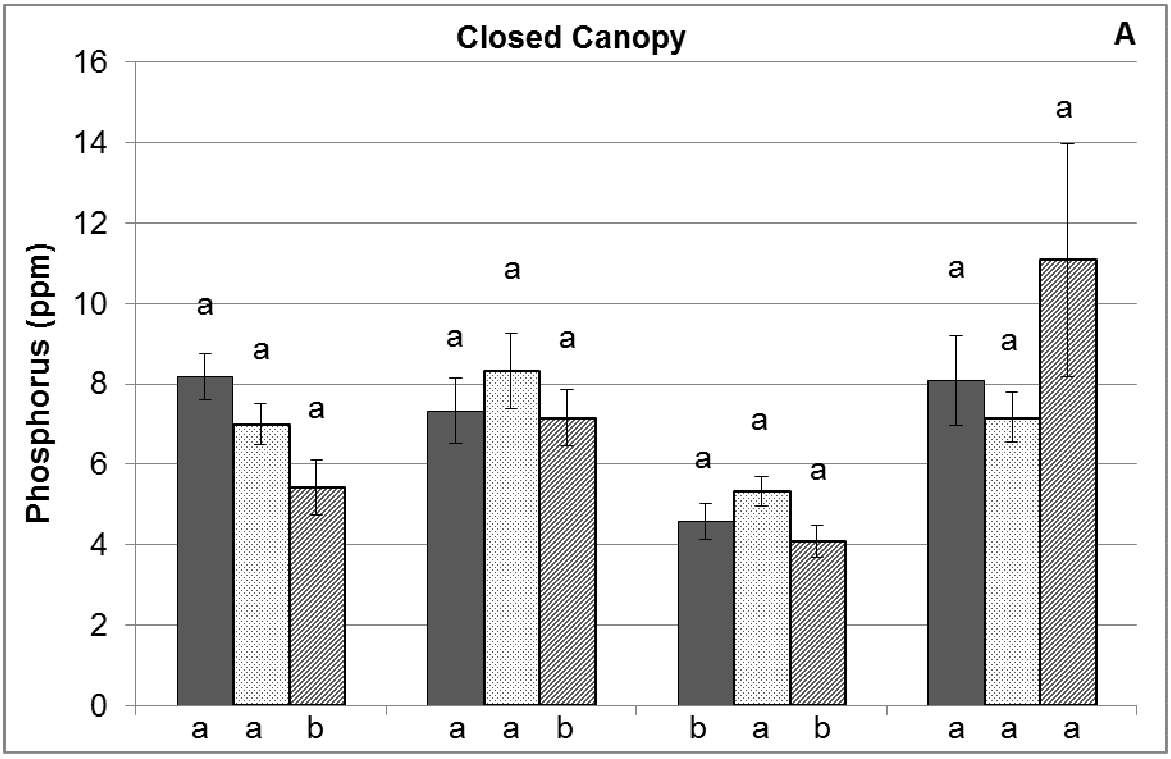

Closed Canopy
A
NO_3 (ppm)
12
10
8
6
4
2
0
b b b
b b ab
b b a
a a a
Open Canopy
B
NO_3 (ppm)
a a a
b b a
b b b
a a a
control
removed
interspace
prairie

Figure 6 A-B


Closed Canopy
A
Phosphorus (ppm)
16
14
12
10
8
6
4
2
0
a a b
a a b
b a b
a a a

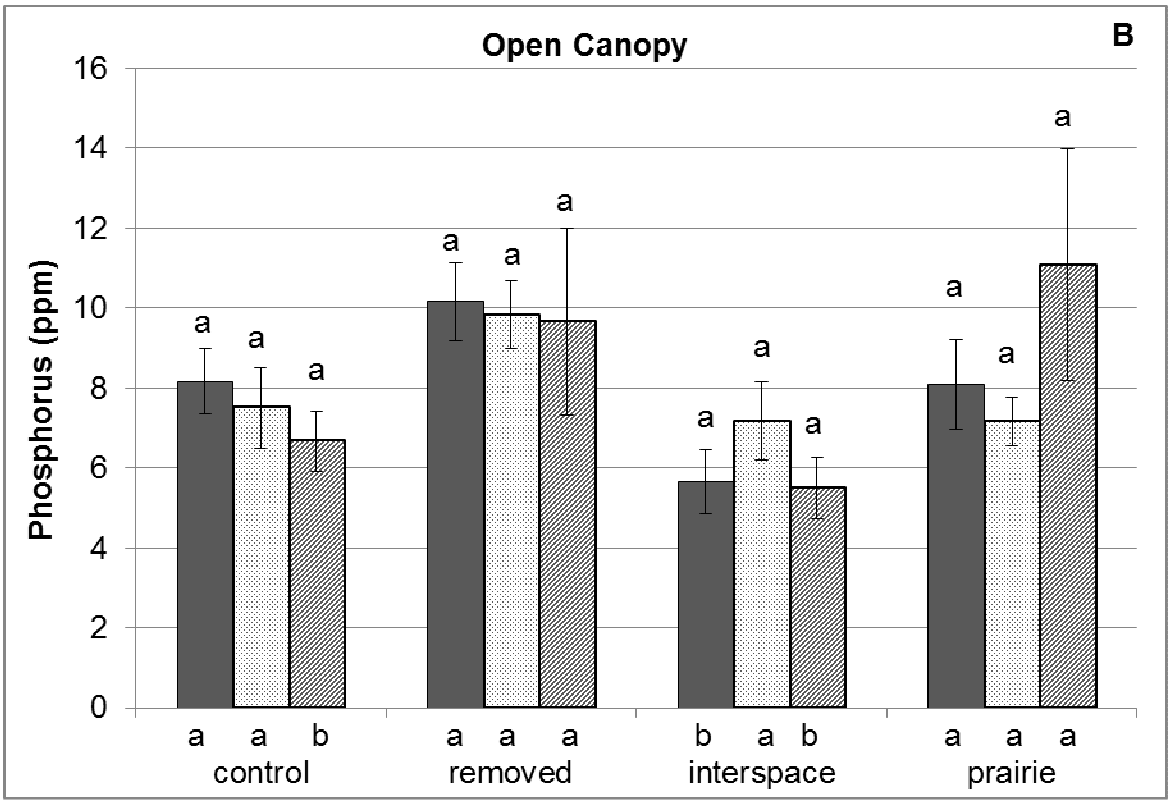

Open Canopy
B
Phosphorus (ppm)
16
14
12
10
8
6
4
2
0
a a b
a a a
b a b
a a a
control
removed
interspace
prairie

Figure7A-B

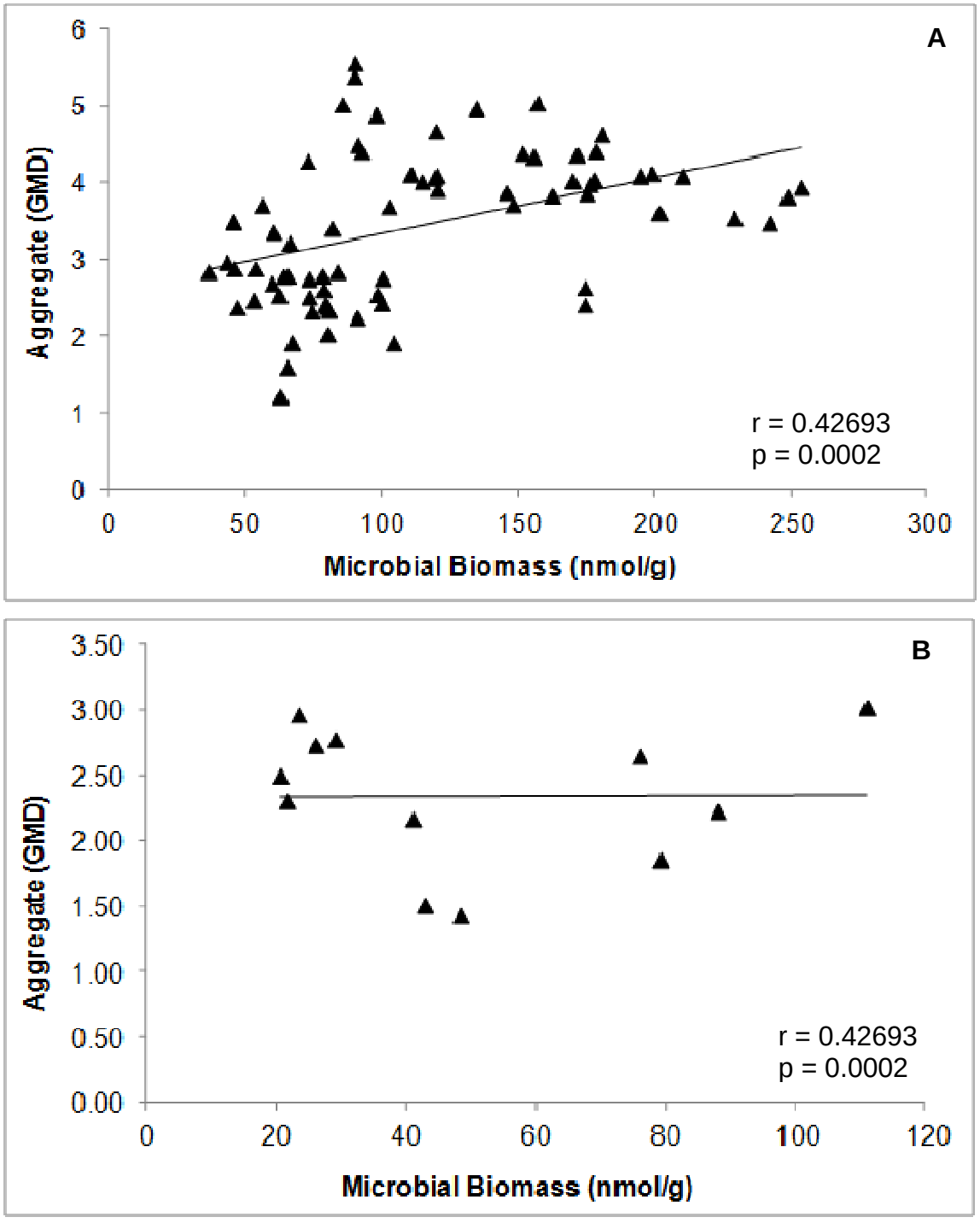

A
Aggregate (GMD)
Microbial Biomass (nmol/g)
r = 0.42693
p = 0.0002
0
1
2
3
4
5
6
0
50
100
150
200
250
300
B
Aggregate (GMD)
Microbial Biomass (nmol/g)
r = 0.42693
p = 0.0002
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
0
20
40
60
80
100
120

Figure 8 A-B

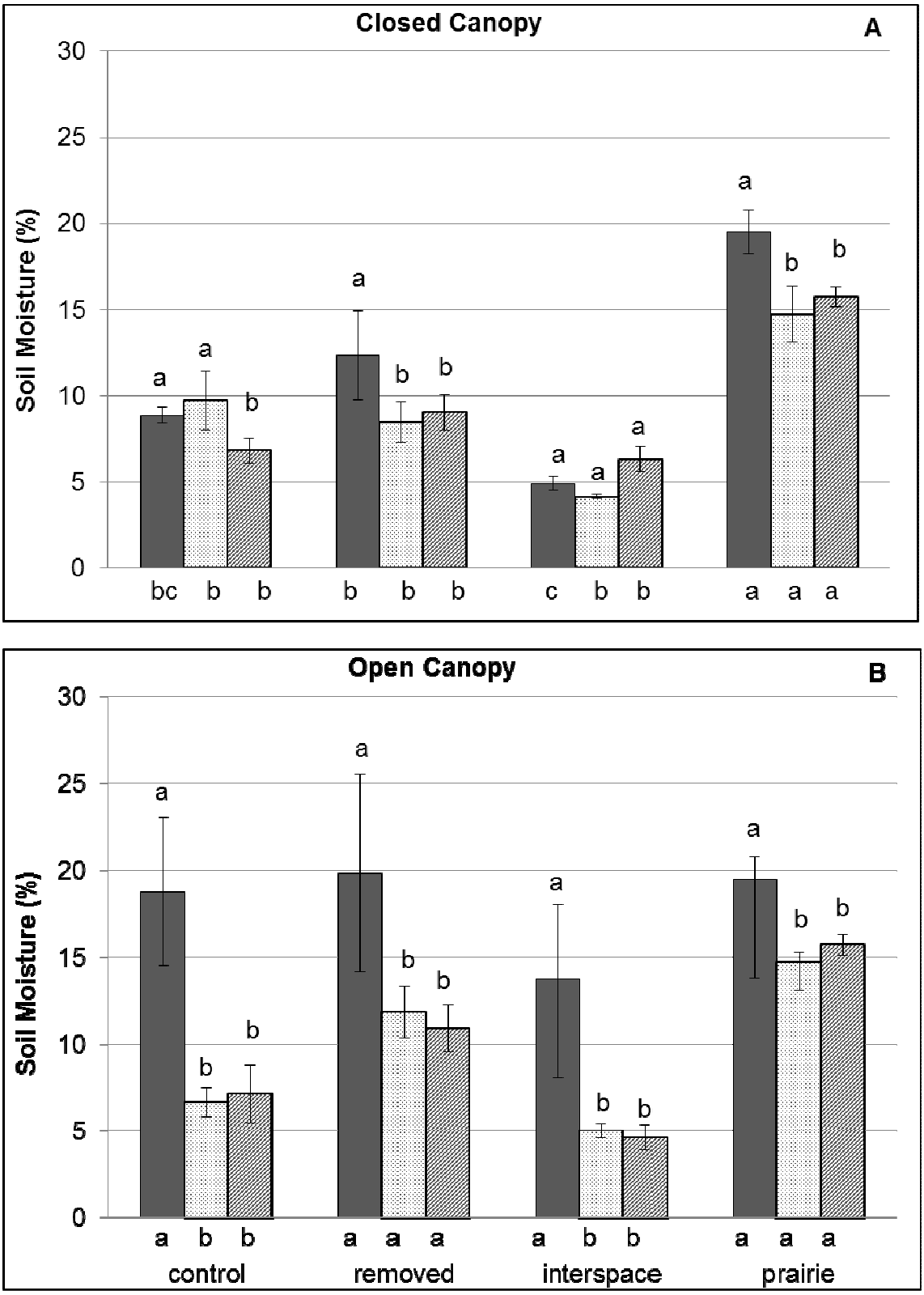

Closed Canopy
A
Soil Moisture (%)
30
25
20
15
10
5
0
a a b
a b b
a a a
bc b b
b b b
c b b
a b b
Open Canopy
B
Soil Moisture (%)
a b b
a b b
a b b
a a a
control
removed
interspace
prairie

Figure 9 A-B

VITA

LEANE COPPICK

Candidate for the Degree of

Master of Science

Thesis: Consequences of *Juniperus virginiana* encroachment into a tallgrass prairie: Soil physical and biological alterations

Major Field: Natural Resource Ecology and Mangement

Biographical:

Education:

Completed the requirements for the Master of Science in Natural Resource Ecology and Mangement at Oklahoma State University, Stillwater, Oklahoma in December, 2011.

Completed the requirements for the Bachelor of Science in Biology at Northeastern State University, Tahlequah, Oklahoma in 2009.

Experience:

Independent Research (funded by INBRE)
Fall 2008, spring and summer 2009 at Northeastern State University

- Identifying potentially new siderophores from several marine fungal strains
- Presented poster presentations in Oklahoma City, Oklahoma, Broken Arrow, Oklahoma and Washington D.C.

Graduate Assistantship (Dept. Plant and Soil Science)
Fall 2009 ending December 2010
Project title: Isolation and characterization of arsenic resistant bacteria
Advisor: Dr. Shiping Deng
Research Assistant (Dept. Natural Resource Ecology and Management)
January 2011ending December 2011
Project title: Consequences of *Juniperus virginiana* encroachment into a tallgrass prairie: soil physical and biological alterations
Advisor: Dr. Gail Wilson

- Investigation of ecosystem functions relative to woody species encroachment in the tallgrass prairie with the ultimate goal of restoration

Name: Leane Coppick Date of Degree: December, 2011

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: CONSEQUENCES OF *JUNIPERUS VIRGINIANA* ENCROACHMENT INTO A TALLGRASS PRAIRIE: SOIL PHYSICAL AND BIOLOGICAL ALTERATIONS

Pages in Study: 51 Candidate for the Degree of Master of Science

Major Field: Natural Resource Ecology and Management

Scope and Method of Study: The invasion of *J. virginiana* into grasslands has multi-scale effects including loss of biodiversity, loss of habitat for wildlife species, and alterations in community-level and biogeochemical functions. Most previous studies focus on aboveground changes, therefore, in this study I assess belowground changes in soil characteristics including soil microbial communities, nutrient availability, moisture, and aggregate stability following establishment of *J. virginiana* into tallgrass prairie. To assess the influence of tree density, I included forested areas with moderate levels of encroachment, as well as sites that are highly encroached, and compare these sites to an adjacent non-encroached native prairie. To assess the impact of *J. virginiana* at the individual tree level, a single *J. virginiana* tree, paired with an uncut control, was cut and removed from each site.

Findings and Conclusions: My results show significant differences in microbial communities, nutrient availability, moisture, and aggregate stability in forested sites compared to the native prairie sites. The removal of an individual *J. virginiana* tree did not significantly affect soil characteristics, compared to a corresponding uncut control tree. Possible explanations for this lack of response could be due to the relatively short (6 month) duration of this study or that there is a continued influence from surrounding J.virginiana trees. Understanding how microbial communities and these plant-fungal associations are influenced by *J. virginiana* invasions may be a critical aspect of the ecology and management of this invading species, as well as the conservation and restoration of native ecosystems.

ADVISER'S APPROVAL: Dr. Gail Wilson_____