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Stephen F. Austin State University, cassey.edwards@yahoo.com

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BRYOPHYTE ASSEMBLAGES ON AMERICAN HORNBEAM (CARPINUS CAROLINIANA) AND THE SURROUNDING ARBOREAL COMMUNITIES WITHIN THE FOREST OF EAST TEXAS

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BRYOPHYTE ASSEMBLAGES ON AMERICAN HORNBEAM (*CARPINUS
CAROLINIANA*) AND THE SURROUNDING ARBOREAL COMMUNITIES
WITHIN THE FOREST OF EAST TEXAS

By

CASSEY EDWARDS, B.Sc.

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Stephen F. Austin State University

In Partial Fulfillment

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Bryophyte Assemblages on American Hornbeam (*Carpinus caroliniana*) and the
surrounding arboreal communities within the forest of East Texas

By

Cassey Edwards

APPROVED:

Dr. Don Pratt, Thesis Director

Dr. James Van Kley, Committee Member

Dr. Jeremy Stovall, Committee Member

Dr. Josephine Taylor, Committee Member

Pauline M. Sampson. Ph. D.
Dean of Research and Graduate Studies

ABSTRACT

The bryophytes, the non-vascular plants, include the mosses (Bryophyta with approximately 13,000 species), the liverworts (Marchantiophyta with approximately 5,000 species), and the hornworts (Anthocerotophyta with approximately 150 species). Initial observations indicated that the midstory tree *Carpinus caroliniana* Walter (American hornbeam) has a bryophyte composition with an unusually high diversity of leafy liverworts. Voucher samples were taken from the four intercardinal directions (Northeast, Northwest, Southwest, and Southeast) from six *Carpinus* trees and their nearest neighbor that was neither *Pinus* nor another *Carpinus* at each of six sites across East Texas. Percent coverage, species richness, Shannon's diversity and Pielou's evenness were computed. Non-metric Multidimensional Scaling (NMDS) was used to determine bryophyte community and site variability. Percent Coverage, Shannon's Diversity Index, and Pielou's Evenness indicate that *Carpinus* trees have a significantly higher leafy liverwort component than that of Non-*Carpinus* trees. The two sites in the Davey Crockett National Forest had been recently burned, the burn reaching the trees that were sampled in one of the sites but not the other. Although confounded, the two Davey Crockett sites indicated potential effects of prescribed burns on bryophyte diversity that would need further investigating.

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CHAPTER 1 –GENERAL INTRODUCTION AND LITERATURE REVIEW

Bryophytes (the nonvascular plants) include the mosses, hornworts, and liverworts. The Bryophytes grow in almost all terrestrial and freshwater environments; they have a global distribution, and are found in most all ecosystems, excluding those located on permanent ice (Gignac, 2001). Thought to be mainly dependent upon the surrounding shade and humidity of their microclimate provided by the forest canopy (Slack, 1977), bryophytes are recently being discovered to be influenced by multiple environmental factors: humidity (Ellis, 2015; Glime, 2017; Philips, 1951), precipitation (Callaghan & Ashton, 2008 ;Vanderpoorten & Engels, 2002), temperature (Gignac & Dale, 2005; Tuba, Slack, & Stark, 2011) , forest canopy cover and light (Hallingback & Hodgetts, 2000; Pentecost, 1998; Weibull & Rydin, 2005), phorophyte (host tree) bark (Phillips, 1951; Studlar, 1982), and soil chemistry (Gustafsson & Eriksson, 1995). Bryophytes are sensitive to slight changes within the available nutrients and possible pollutants within the surrounding water, soil, and atmosphere. All possible micro-environmental factors influencing bryophyte communities can all be affected by anthropogenic disturbances, natural disturbance, forest age, composition, structure and overall forest management practices (McCune, 1993; McGee & Kimmerer, 2002; Király, Nascimbene, & Ódor, 2013). These

sensitivities make bryophyte assemblages plausible for assessing ecological conditions, making them ideal ecological indicators (Ceschin et al., 2012; Fernandez et al., 2006; Davies, 2007) in most any environment bryophytes can be located. To further expand the usefulness of bryophytes as ecological indicators more understanding about the local bryophyte community is needed. Presently little research has been published on bryophytes (Andreasena et al., 2001) , and even less has been completed on the bryophyte communities of East Texas other than the studies of Huson (2007) and Kruse and Davison (2011).

LITERATURE REVIEW

Bryophytes

The term bryophyte refers to the nonvascular plants, including: mosses (Bryophyta), liverworts (Marchantiophyta), and hornworts (Anthocerotophyta) (Hallingback & Hodgetts, 2000; Goffinet & Shaw, 2008) .

Bryophytes are among the most ancient of land plants, and are estimated to be well over 400 million years old (Hallingback & Hodgetts, 2000). The number of bryophyte studies have been limited, and only recently have been of interest due to their possible usefulness as ecological indicators (Ceschin et al., 2012; Fernandez et al., 2006; Davies, 2007; Jackson L., 2000) ; only a fraction of the bryophyte species has been identified and described. It is estimated that there are approximately 19,000 bryophyte species, of those approximately 13,000 belong to the Bryophyta (the mosses), approximately 5,000 belong to the Marchantiophyta (the liverworts), and approximately 150 belonging to the Anthocerotophyta (the hornworts) (Hallingback & Hodgetts, 2000; Goffinet & Shaw, 2008). The bryophyte divisions can be identified using a mix of gametophyte and sporophyte characters (Table 1.). The distinguishing features of the bryophytes include: the lack of vascular tissues, a gametophyte dominant

life-cycle, a short-lived sporophyte that is dependent to semi-independent upon the gametophyte, growth of the gametophyte is by a single apical cell.

To help with field identification a moist environment is necessary. Water is an important part of a bryophytes lifecycle. Not only is water important for identification purposes, it is a necessary requirement in their process of fertilization, with most species not producing the necessary gametophyte until the water requirements have been met. Due to this water requirement bryophytes become easier to identify during their reproduction stage when moist (Schofield, 2001) (Hallingback & Hodgetts, 2000) (Goffinet & Shaw, 2008). Although harder to identify it is possible to identify certain species of bryophytes even when relatively dry.

Bryophytes only reaching up to 10^{-2} cm in height form their own community within the communities of the vascular plants creating “a world all its own” (Goebel 1930; Mägdefrau & Smith, 1982). Bryophytes tend to live in tightly grouped clusters, rarely reaching more than a few centimeters in height. Bryophyte species can inhabit a variety of substrates: rocks, soil, or even other plants. However, most bryophytes tend to prefer certain substrate types. Those that are restricted to growing on the bark of trees are called epiphytic bryophytes, also known as phorophytes. The epiphytic habitat can be divided into three to four well-marked regions: the tree base, up to one meter above the soil, the

trunk, and the crown. The crown of the tree can be broken into two different regions; large branches, and small branches and twigs (Goffinet & Shaw, 2008).

Mosses

Mosses, phylum Bryophyta, are the largest group within the bryophytes consisting of approximately 13,000 species (Goffinet & Shaw, 2008). The mosses can be distinguished by a leafy stem, with entire leaves bearing a mid-rib (costa). The leaves come to a point, and are typically longer than they are wide, with most spiraling along the stem. (Table 1). Young sporangia are covered by a calyptra, which is the remains of the venter, the swollen region of the archegonium containing the egg. The capsules of mosses open by an operculum, the cap covering the tip of the capsule, to reveal a hygroscopic peristome, a single to double ring of teeth inside the mouth of the capsule, aiding in the dispersal of spores (Table 1) (Goffinet & Shaw, 2008; Reese, 1984; Evert & Eichhorn, 2013; Glime, 2017; Malcolm, 2006) (Malcolm, 2006)(Goffinet & Shaw, 2008) (Reese, 1984) (Evert & Eichhorn, 2013) (Glime, 2017).

Liverworts

Liverworts, phylum Marchantiophyta, are the second largest group in the bryophytes, consisting of approximately 5,000 species (Goffinet & Shaw, 2008). Liverworts can be either thalloid, a flat plate of tissue (Malcolm, 2006), or leafy (Table 1). Cellular organelles known as oil bodies are unique to the liverworts,

and can be used as unique identification structures (Table 1). The capsules lack stomata (Table 1), cuticle, and columella, the central column of sterile tissue within the sporangium (Malcolm, 2006), common among the mosses and hornworts. The capsule of the sporophyte, has hygroscopic elaters, the sterile cells that are interspersed within the spores (Malcolm, 2006), (Table 1), whose movements break up the spore mass aiding in the spores dispersal. The sporophyte generation is short lived (Goffinet & Shaw, 2008; Evert & Eichhorn, 2013; Reese, 1984; Schofield, 2001; Glime, 2017). Liverworts can be divided into two categories; those with a thallose body and those with a leafy body. The thallus consists of a flat, sometimes branching, body that is not divided into leafy segments. Leafy liverworts are dorsiventrally flattened with the leaves in two major rows, with a possible third smaller row of under-leaves. Leaves may be lobed, toothed, or folded and never bear a costa (Table 1). The epiphytic liverwort species tend to be leafy in appearance and are grouped as leafy liverworts.

Hornworts

Hornworts, phylum Anthocerotophyta, is the smallest of the bryophyte group consisting of approximately 150 species (Goffinet & Shaw, 2008). Like other bryophytes hornworts are poikilohydric, water requirement is completely dependent upon the environment, (Green & Lange, 1995) and inhabit moist

habitats. Hornworts are the only land plant including the bryophytes that still retain the pyrenoids (Table 1 (Goffinet & Shaw, 2008). Similar to liverworts, hornworts are thalloid and like the mosses can contain a thickened midrib. Hornworts sporophytes lacks a seta, the stalk of the sporophyte; the sporangium splits longitudinally, pseudoelaters along the sporangium wall aids in the spore dispersal upon drying. The sporophytes, arising from the basal meristem (Table 1), are typically tall structures that form a resemblance of horns. Unlike the other bryophytes the sporophyte of the hornwort are semi-independent to the gametophyte generation (Goffinet & Shaw, 2008; Glime, 2017; Evert & Eichhorn, 2013; Reese, 1984) .

Table 1. Characteristics of the Bryophyte Divisions, based on physical characteristics for both the gametophyte and sporophyte generation.

	Marchantiophyta	Anthocerotophyta	Bryophyta
Gametophyte Characters			
Growth Form	Thallose or leafy	Thallose	Leafy
Leaf Arrangement	2 + 1	NA	Whorled
Leaf Shape	Rounded or lobed	NA	Lanceolate or linear
Costa	Absent	NA	Present
Oil Bodies Present	Present	Absent	Absent
Protonema well developed	No	No	Yes
Pyrenoids Present	Absent	Present	Absent
Sporophyte Characters			
Basal Meristem in Sporangium	Absent	Present	Absent
Calyptra well developed	No	No	Yes
Stomata	Absent	Present	Present
Elaters Present	Present	Pseudo-elaters	None
Seta Absent	Present	Absent	Present
Sporophyte semi-independent	Dependent	Semi-independent	Dependent

Epiphytic Bryophytes of East Texas

Many mosses and liverworts are phorophytes, epiphytic species growing on the surface of the bark of trees. Trees present a wide variety of micro habitats based on light intensity, relative humidity, and other conditions. These conditions are also influenced by the proximity of other trees, as well as the chemical and physical nature of the tree bark (Mägdefrau & Smith, 1982).

Huston (2007) identified 84 bryophyte species in her study of the East Texas bryophyte communities. Fifteen of these genera were classified as being epiphytic. The phorophytes were found on both rough and smooth barked tree species, and included representatives of both the mosses and the leafy liverworts (Table2.). These genera form a list of expected genera for this study.

Table 2. Epiphytic bryophyte genera of East Texas (Huston 2007). Genera followed by a * were recorded as the most frequent genera occurring on trees in East Texas.

Mosses	Liverworts
<i>Clasmatodon</i>	<i>Cheilolejeunea</i>
<i>Forstroemia</i> *	<i>Frullania</i> *
<i>Hyalohymenium</i>	<i>Leucolejeunea</i>
<i>Homalotheciella</i>	<i>Metzgeria</i>
<i>Leucodon</i> *	<i>Porella</i> *
<i>Schlothemia</i>	<i>Radula</i>
<i>Sematophyllum</i>	<i>Rectolejeunea</i>
<i>Thelia</i>	

The bryophytes on most trees in our region consist of a mix of multiple moss species with occasional leafy liverworts. Two exceptions to this expectation have been observed. Preliminary observations indicate that southern yellow pines in east Texas (*Pinus spp.*) lack bryophytes (except along the base, close to the soil) and *Carpinus caroliniana* Walter (American hornbeam) trees have a bryophyte composition that is very rich in leafy liverworts (Figure 1).

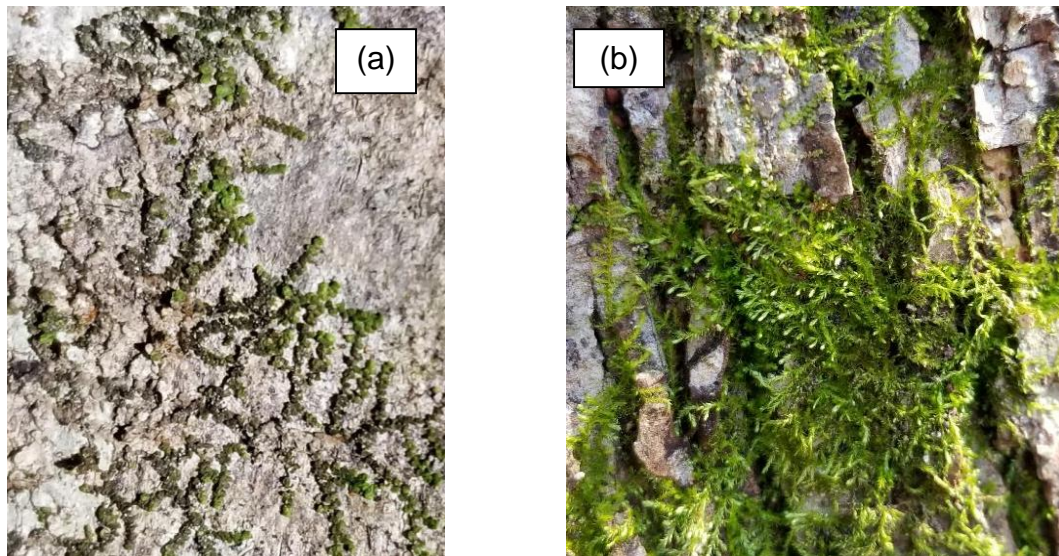


Figure 1. (a) *Frullania* sp. located on *Carpinus caroliniana*. (b) *Leucodon julaceus* intermixed with *Frullania* sp. located on the neighboring *Ulmus americana*.

Carpinus caroliniana, (*C. caroliniana*) is a native understory tree species. This deciduous tree has leaves arranged alternately along the stem, $\frac{3}{4}$ to $4\frac{3}{4}$ inch long, with an elliptical blade shape and doubly- serrated margin. The bark is gray, smooth, and thin; the longitudinal fluting typical of the trunk resembles a

flexed muscle. American hornbeam is an understory species primarily occurring in bottomland mixed-hardwood forests. Usually in a transition from mesic to wet area, lakes, swamps, rivers, and streams, with some gradients along bottomlands. Sites have abundant soil moisture on moderately to well drained soils, although trees can grow on semi-flooded sites (Nesom & Briggs, 2003).

Host specificity of bryophytes on a tree species is not necessarily unexpected, especially in this case considering the distinctive nature of *C. caroliniana* bark. Schmitt and Slack (1990) found host specificity between the Adirondack Mountains and Southern Appalachian Mountains and suggested that host specificity should be tested for all trees at a site.

CHAPTER 2 - PRELIMINARY DATA

A preliminary study was conducted during January 2018 to determine appropriate bryophyte sampling strategy for the proposed research. The study was conducted in the Hunts Woods and Gala Mize Gardens located East of the Stephen F. Austin State University campus along the Recreational Trails in Nacogdoches, Texas (31.620590,-94.636455). The desired objectives for this study were to:

- Determine if bryophyte abundance measures differ significantly between a 5cm quadrat with a 1cm grid (36 points) and a 0.5cm grid (121 points).
- To determine if species richness, the number of species per unit area, varies between the four intercardinal directions.

Materials and Methods

Sampling was conducted on 20 *C. caroliniana*, American hornbeam, located in Nacogdoches, Texas. Trees were randomly chosen along the stream bank and trail of the Hunts woods recreational trails area. Quadrates measuring 5 cm² were printed on clear copier transparency film with a 1 cm grid (36 points) and 0.5 cm grid (121 points). Grids were placed at breast-height (1.3 m) on trees measuring ≥ 6.5 cm in diameter along the intercardinal directions (NE, SE, SW,

and NW). It was necessary to have a diameter measuring $\geq 6.5\text{cm}$ to prevent any overlap in the quadrates when sampled. Samples were misted prior to placing the quadrats to improve visibility and to assist with identification. Abundance was recorded using the points intercept method as the number of points across the quadrat touching a bryophyte for both grid sizes. Quadrats were visually inspected using a hand lens and the number of genera observed recorded for each. Species genera was recorded as the number of points intersected within the quadrate.

Data analysis

Abundance patterns were analyzed by converting points data to percentages to allow for comparisons between the 36 and 121 point quadrats and separate ANOVAs were performed for the 36 and 121 point data sets. T-tests were conducted between 36 and 121 point data set pairs for each intercardinal direction.

Species diversity was calculated using the Shannon's Diversity index (H) accounting for richness, evenness and species abundance present. The proportion of species i relative to the total number of species (p_i) is calculated, and then multiplied by the natural logarithm of this proportion ($\ln p_i$). The resulting product is summed across species, and multiplied by -1 (Beals, Gross, & Harrell, 2000):

$$H = - \sum_{i=1}^S p_i \ln(p_i)$$

Species evenness is the similarity in species abundance in a surrounding community (Zhang, et al., 2012). Evenness or Shannon's equitability can be calculated by dividing H by H_{max}. H_{max} is $\ln S$ (the log of the total number of species present) (Beals et al., 2000).

$$Evenness = \frac{H}{H_{max}} = H/\ln(S)$$

Shannon's Diversity Index was calculated using each grid size (36-points and 121-points) for each intercardinal direction (northeast, southeast, northwest, southwest), yielding eight sets of measurements per *C. caroliniana*.

Results

Average abundances values of sampled bryophytes varied from a low of 1.81% to a high of 53.84% (Figure 2). ANOVAs of species abundance were statistically significant for both the 1 cm (36 point) and 0.5 cm (121 point) grids (Table 3). T-tests of bryophyte abundance were not statistically different between the 36 and 121 point grids on the NE, SE, and NW side of the trees (Table 5). Abundance on the SW side of the trees was found to statistically differ between the 36 and 121 point grids. Abundance and evenness varied by grid

size and intercardinal direction (Table 4). Only two bryophyte genera, *Porella* and *Frullania*, both leafy liverworts, were identified on the 20 trees sampled. Of the two genera *Porella* spp. was more common along the northern side of the tree with *Frullania* spp. being more common along the Eastern and Western side of the tree with *Porella* spp. being absent along the Southern side (Figure 3).

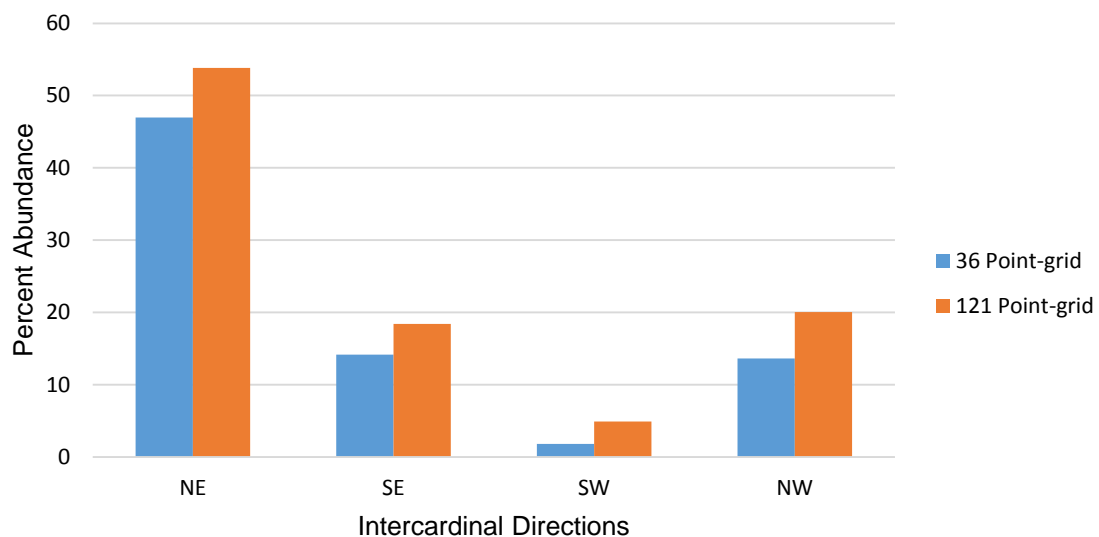


Figure 2. Average abundance values for bryophytes sampled across 20 *Carpinus caroliniana* located in Nacogdoches Texas. Sample comparison between the 36 grid sample and the 121 grid sample for the Northeast, Southeast, Southwest, and Northwest sampling locations. Samples collected at 1.3m (breast height) of each tree measuring > 6.5cm in diameter.

Table 3. The comparison between the 36-point grid and 121-point grid quadrat for the intercardinal directions. Data were collected in January 2018 in the Hunts Woods and Gala Mize Gardens located East of the Stephen F. Austin State University campus along the Recreational Trails in Nacogdoches, Texas on *Carpinus caroliniana*.

Quadrat	df	F	P-value	F crit
36 Points	3	19.57931	1.67E-09	2.72
121 Points	3	17.38483	1.09E-08	2.72

Table 4. The total number of points for each of the 20 *Carpinus* trees sampled covered by *Porella* spp. and *Frullania* spp. using the 36 and 121 grid points. Shannon's diversity (H) and evenness for each sample.

Species	36NE	36SE	36SW	36NW	121NE	121SE	121SW	121NW
<i>Porella</i>	284	25	0	58	1065	112	0	207
<i>Frullania</i>	80	85	13	51	439	360	118	113
Total	364	110	13	109	1504	472	118	320
H	0.53	0.54	0.00	0.69	0.60	0.55	0.00	0.65
Evenness	0.760	0.773	0.000	0.997	0.871	0.791	0.000	0.937

Table 5. The t-test two sample assuming equal variance for the comparison of the 36-grid points and 121-grid point of the 5cm² quadrat for the Northeast, Southeast, Southwest, Northwest sample areas. Data was collected in January 2018 in the Hunts Woods and Gala Mize Gardens located East of the Stephen F. Austin State University campus along the Recreational Trails in Nacogdoches, Texas on *Carpinus caroliniana*.

	Observation	df	P(T<=t) two-tail
Northeast	20	38	0.4765
Southeast	20	38	0.4194
Southwest	20	38	0.0141
Northwest	20	38	0.3982

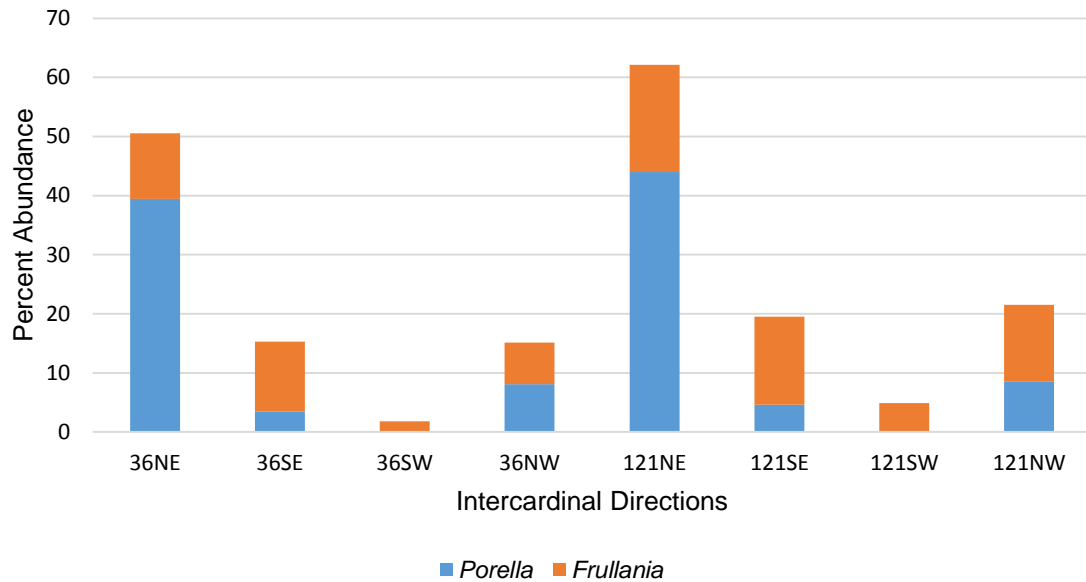


Figure 3. The percent abundance of *Porella* spp. and *Frullania* spp. present along the northeast, southeast, southwest, and northwest sampling locations for the 20 *Carpinus caroliniana* samples. Comparison between the 36-point grid and the 121-point grid when sampling bryophytes. Samples collected at 1.3m (breast height) of each tree measuring > 6.5cm in diameter.

Discussion

ANOVAs indicate that our method is indicating statistically significant differences between abundance patterns between the intercardinal directions.

Differences in T-tests indicate that the 0.5 cm (121 point) grid records the presence of bryophytes that were missed by the systematic sampling with the 1 cm (36 point) grid quadrat in cases where bryophyte abundance is scarce.

Abundance data indicates that a 5 cm² quadrat with a 0.5 cm grid provides statistically significant results.

Richness measures show that bryophyte diversity is differently partitioned around the *Carpinus* trunk. *Porella* is found on the more shaded and wetter NE and NW sides, and *Frullania* is found on all sides of the stem, including the hotter and drier SW side where *Porella* is absent. The richness study indicates that our sampling procedure is sufficient to find differences in patterns of species richness and evenness.

CHAPTER 3 - INTRODUCTION AND OBJECTIVES

Bryophytes (the nonvascular plants) are a species rich group of Embryophytes that includes the divisions Bryophyta (the mosses), Marchantiophyta (the liverworts), and Anthocerotophyta (the hornworts). Bryophyta and Marchantiophyta are respectively the second and fourth largest divisions of Embryophytes. Bryophytes have a global distribution and grow in almost all terrestrial and freshwater environments-except permanent ice (Gignac, 2001). Common growth substrates include bare rock, soil, and tree bark. Studies indicate that bryophyte growth is influenced by multiple environmental factors: humidity (Ellis, 2015) (Glime, 2017) (Philips, 1951), precipitation (Callaghan & Ashton, 2008) (Vanderpoorten & Engels, 2002), temperature (Gignac & Dale, 2005) (Tuba, Slack, & Stark, 2011), forest canopy cover and light (Hallingback & Hodgetts, 2000) (Pentecost, 1998) (Weibull & Rydin, 2005) (Jagodzinski et al. 2017), soil chemistry (Gustafsson & Eriksson, 1995), phorophyte (host tree) bark (Phillips, 1951) (Studlar, 1982), and bark pH and water capacity (Jadodzinski et al. 2017). Trees are capable of providing large variety of micro habitat and micro environments can also vary along the different aspects of the same tree (Phillips, 1951; Studlar, 1982).

While studies of vascular plant communities and diversity are common, studies of bryophyte diversity and communities and their association to their vascular plant communities are fairly rare (Andreasena et al. 2001) (Lovadi et al. 2012). A recent methods paper compared three epiphytic bryophyte sampling techniques and came to the conclusion that placing four equally spaced quadrats on a tree yielded high quality data (Lovadi et al. 2012). Historically, ecological studies of epiphytic bryophytes have found evidence for both host specificity (Studlar, 1982; Schmitt & Slack, 1990) and a lack of host specificity (Phillips, 1951; Palmer, 1986).

Within East Texas, a recent checklist of the Big Thicket bryophytes found 179 species of bryophytes, but the study only lists the species and provides no data as to growth substrate for the species, nor does it include community or diversity studies of the bryophytes (Kruse and Davison 2011). Huston (2007) provided an analysis of bryophyte communities for several ecosystems across East Texas. Initial observation within a highly managed urban environment of bryophytes on *C. caroliniana*, a common East Texas understory tree with a distinctive smooth bark, indicated an unusually high level of leafy liverwort to moss ratio on the bark of that tree species compared to the bryophyte composition on the bark of other species. Based on these initial observations the objectives of this study are to:

1. Describe the epiphytic bryophyte species diversity trees of *C.caroliniana* using standard ecological measures such as percent coverage, species richness, Shannon's diversity index, Pielou's evenness, and Non-metric Multidimensional Scaling (NMDS) at six locations within East Texas
2. To analyze for differences in species composition between *Carpinus* to that of the next closest tree species.
3. To test for possible differences between the sample sites.

The Null hypothesis for objectives 2&3 is that there will be no distinguishable differences between the bryophyte composition of *C.caroliniana* and that of the *Non-Carpinus* sampled, or between the sites sampled.

CHAPTER 4 – MATERIALS AND METHODS

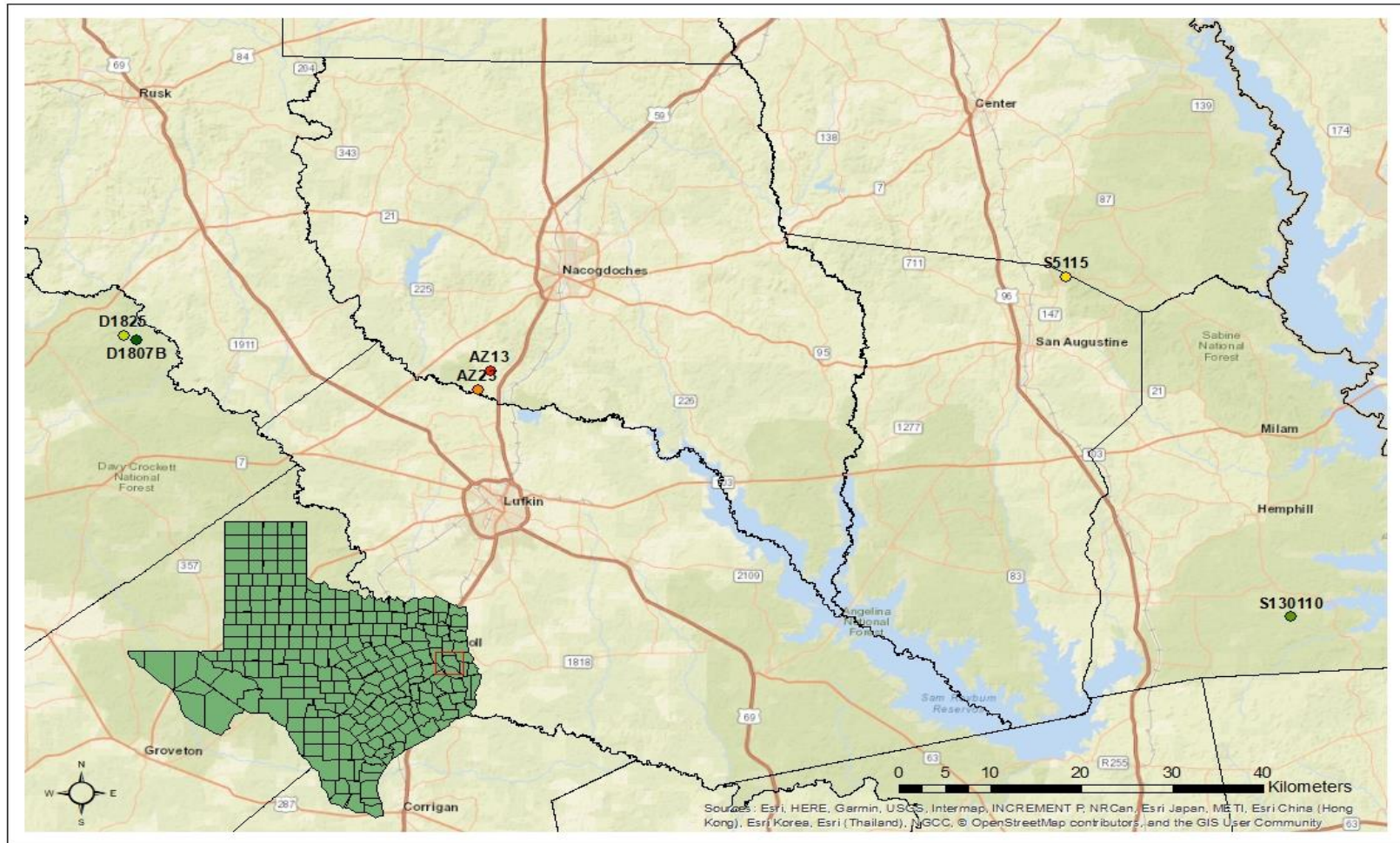
Site Selection:

Six study sites were chosen using the Ecological Classification System from Dr. Van Kley (VanKley et al. 2007; VanKley and Turner, 2009) and the Texas Vegetation Classification data: two from the Alazan Wildlife Management Area, two from the Davey Crockett National Forest, and two from the Sabine National Forest (Table 6). The six sites were chosen based on the abundance of *Carpinus* trees (Table 6), to cover a range of ecosystem types and a large geographic range across East Texas. Soil data was obtained using Web Soil survey (USDA, 2017).

Table 6. Sites chosen using the Ecological Classification System from Dr. VanKley and the Texas Vegetation Classification data. Sites chosen were from Alazan WMA, Davey Crockett National Forest, and Sabine National Forest based on the *Carpinus* density per hectare (D/Ha). Easting and Northing based on the UTM coordinate system zone 15R.

Site	Site Code	<i>Carpinus</i> D /Ha	Latitude	Longitude	Community
Alazan	AZ13	210	31.49504	-94.7378	Wet-Mesic forest at base of slope
Alazan	AZ23	80	31.47309	-94.7506	Mesic slope ("long Island")
Davy Crockett	D1807B	170	31.52909	-95.1621	Wet-mesic gently sloping
Davy Crockett	D1825	110	31.52343	-95.1468	Mesic to dry-mesic lower slope
Sabine	S5115	100	31.60699	-94.0733	Steep mesic midslope of ravine
Sabine	S13010	140	31.22823	-93.8097	Wet-mesic stream floodplain

Figure 4. Map of research locations across East Texas. Sites chosen using the Ecological Classification System from Dr. VanKley and the Texas Vegetation Classification data. Easting and Northing based on the UTM coordinate system zone 15R.



Alazan Bayou Wildlife Management Area is a 2,063 acre forest and wetland managed by Texas Parks and Wildlife Department located in southern Nacogdoches County off FM 2782. The management area consists of both open grassy areas and bottomland hardwood forest. Sites were chosen based on the density of *Carpinus*, so both sites are in the bottomland hardwood forest area.

- AZ13 is a mesic occasionally flooded mixed hardwood forest with 0 to 2 percent slope. The soil survey of Nacogdoches County, determined that AZ13 is frequently flooded Mantachie series, fine- loamy. Siliceous, active. Acid, thermic Fluventic Endoaquepts soils. Trees consist of *Quercus nigra* L., *Ulmus Americana* L., and *Liquidambar styraciflua* L. are the main overstory tree species, while *Carpinus caroliniana*, *Acer rubrum* L., and *Ilex opaca* Aiton are the main mid-story tree species. The ground layer is covered with leaf litter with minimal herbaceous species.
- The soil survey of Nacogdoches County, determined that AZ23 (Long Island) is frequently flooded Mantachie soil series. AZ23 is a wet-mesic forest along the slope to the seasonally flooded bottom. *Quercus phellos* L., *Quercus nigra*, *Quercus alba* L., and *Liquidambar styraciflua* dominate the overstory, and *Carpinus caroliniana*, *Cornus florida* L., *Ulmus americana*, *Acer rubrum*, *Ostrya virginiana* (Mill.) K. Koch, and *Ilex opaca*

form the dominant mid-story species. The ground layer is sparse, lacking an herbaceous understory with few woody understory species present.

The majority of the open ground is being covered with leaf litter.

Davey Crockett National Forest is 160,000 acres with a mix of forest and recreation areas located in Cherokee, Houston, Angelina and Trinity County. The sites D1807B and D1825 are located within the boundaries of Houston County off State Hwy 21W. Both sites are located along stream banks within a mixed pine forest consisting of both *Pinus taeda* L. and *Pinus echinata* Mill. At both locations the surrounding pine forest was recently burned between January and February of 2018. The soil survey of Houston County, determined D1807B and D1825 contained Hainesville fine sand soil, Thermic, coated Lamellic Quartzipsamments, with a 0 to 2 percent slope.

- D1807B, a mesic to dry-mesic forest, is located off forest service road 526. Trees consist of *Nyssa sylvatica* Marshall, *Liquidambar styraciflua*, and *Quercus nigra* dominating the overstory, with *C. caroliniana*, *Acer floridanum* (Chapm.) Pax, and *Ilex opaca* dominating the mid-story. The ground stratum is sparse, lacking an herbaceous understory, with minimal leaf litter due to recent burning.

- D1825, a wet-mesic forest, is located off forest service road 518. Trees consist of *Quercus alba*, *Liquidambar styraciflua*, and *Quercus nigra* in the overstory, with *C. caroliniana*, *Ostrya virginiana*, *Acer floridanum*, and *Betula nigra* L. in the mid-story. Ground layer is sparse, mostly lacking an herbaceous understory, minimal *Osmundastrum cinnamomea* L. present.

The Sabine National Forest is 160,656 acres in the eastern most part of East Texas located in Sabine, San Augustine, Shelby, Jasper and Newton counties.

- Site S5115 is located in San Augustine County from HWY 147. The soil survey for site S5115 contained Tonkawa fine sand, Thermic, coated Typic Quartzipsamments, with slopes ranging from 0 to 15 percent. S5115 consist of a steep mesic mid-sloping ravine with the presence of *Fagus grandifolia* Ehrh, *Nyssa sylvatica*, and *Quercus alba* in the overstory, with *C. caroliniana* *Ilex opaca*, *Ilex vomitoria* Aiton and *Acer floridanum* within the mid-story; adjacent to a small seep area at a mid-slope throughout this mixed pine, *Pinus echinata*, and hardwood forest.

- Site S130110 is located in Sabine county from HWY 87.
S130110 is a wet-mesic forest along a stream floodplain, surrounded by a *Pinus taeda* forest. The soil survey for site S130110 contained Dreka loam, fine-silty, siliceous, active, nonacid, thermic Fluvaquentic Epiaquepts, with 0 to 1 percent sloping, frequently flooded. Trees consist of *Quercus falcata*, *Nyssa aquatic L.*, and *Quercus nigra*, *Fagus grandifolia*, and *Magnolia grandiflora L.* dominating the overstory, with *C. caroliniana*, *Magnolia grandiflora*, *Acer rubrum*, and *Ilex opaca* dominating the mid-story

Data Collection

Sampling was conducted on 30 *C. caroliniana* trees and their nearest neighboring tree at each of the sampling sites. The nearest neighboring tree was determined by measuring tree distance from the sampled *Carpinus* and sampling the closest individual that was neither a *C. caroliniana* or *Pinus* spp. Transects 100 meters in length were installed at each sampling location. *C. caroliniana* were chosen up to 5m from either side of the transect. Multiple transects were used when necessary to locate 30 *Carpinus*.

Quadrates 5 cm² were printed on clear copier transparency film with a 0.5 cm grid (121 points). Grids were placed at breast-height (1.3 m) on trees measuring ≥ 6.5 cm in diameter along the intercardinal directions (NE, SE, SW, and NW) as per the recommendations of Lovadi et al. (2012) Method A. All bryophyte samples were misted prior to placing the quadrats to improve visibility and to assist with identification to the genus level.

Percent Coverage

Percent coverage was measured on all 30 *Carpinus* trees and their nearest neighbor at all sites. Tree species data, were recorded for all trees for possible unique host for the epiphytic bryophyte species. Tree diameters were measured at breast height to verify no overlaps in grid quadrats when measuring bryophyte coverage. The sampled quadrats were visually inspected using a hand lens and

the number of points intersected by a bryophyte along each of the intercardinal directions for each tree were recorded. Total bryophyte data were categorized into leafy liverwort and moss taxa. The number of points were converted to percent coverage by taking the number of points transected out of the total number of points in the grid per site, tree type, intercardinal aspect, and bryophyte type. The coverage data were tested for Heteroscedasticity and patterns of variation were analyzed in SAS University Edition 9.4 (2017, SAS Institute, Cary, North Carolina) using N-Way ANOVA with four variables including: site; tree type (*Carpinus* and other); bryophyte type (leafy liverwort and moss); and intercardinal aspect; and for all interactions. Pairwise Tukey's comparisons were calculated in SAS University 9.4 to determine if means from ANOVA's ran were significantly different from the others bases of the main effects and all possible interaction types.

Species Richness

At each of the six sample locations voucher specimens were collected from every fifth *Carpinus* and its nearest neighbor tree along all four intercardinal directions yielding specimens from six *Carpinus* trees and their six nearest neighboring trees. Care was taken to include representatives of all bryophyte species that could be observed in the field within the voucher sample. Voucher

specimens were placed in acid free envelopes and taken to the lab for species identification. Species were identified using Mosses of the Gulf South (Reese, 1983) and Flora of North America (Flora of North America Editorial Committee, eds., 2014) for mosses, Liverworts of the Mid-Gulf Coastal Plain (Breil, 1970) for the leafy liverworts, and using a compound light microscope (Nikon Eclipse E200) and dissecting scope (Olympus SZ30) (Figure 5). The voucher collections were examined, and all species present were identified. Total species richness was calculated as the total number of species present for each site and each tree category (*Carpinus* vs *Non-Carpinus*). Total species richness was measured for total bryophyte species and subcategorized by leafy liverworts and mosses.

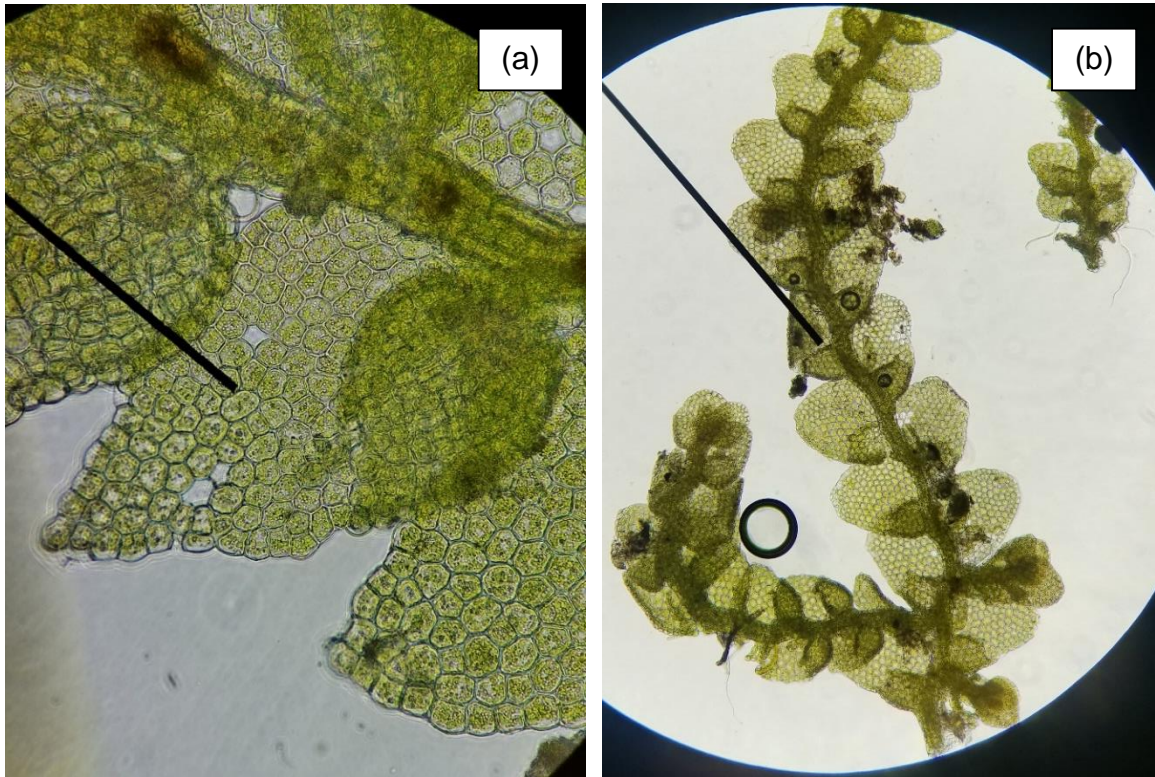


Figure 5. Microscopic images necessary for species identification of leafy liverworts (a) *Cololejeunea minutissima* spp. *minutissima* (b) *Lejeunea laetivirens*.

Patterns in average species richness were explored using N-Way ANOVA in SAS University Edition 9.4 (2017, SAS Institute, Cary, North Carolina). Data were entered as the number of species observed on each aspect of each tree at each site for total bryophyte species and then by separate leafy liverwort and moss components. N-Way ANOVA was calculated for four variables (aspect, bryophyte type, tree type, and site) and for all interactions in SAS University Edition 9.4. Tukey's comparisons were used to determine statistically significant comparisons with the data set. In order to avoid double sampling of bryophyte

species that were found on two or more aspects of a single tree, the number of species per tree at each site was recalculated. Patterns of N-Way ANOVA were run separately for total bryophytes per tree, leafy liverworts per tree, and mosses per tree for the variables tree type and site and for the interaction. Tukey's comparisons were used to determine statically significant comparisons.

Shannon's Diversity Index

Species richness values were obtained by site and tree type for total bryophyte species, leafy liverwort species, and moss species (Table 7) Field observations of percent coverage on trees from which voucher specimens were taken were broken into liverwort and moss categories. However, a high degree of uncertainty is associated with these values for two reasons. 1) species identifications could not be obtained in the field; and, 2) cryptic species that were not observed in the field were found in the voucher collections.

To determine relative abundance values, the number of quadrat points intersected by a moss or leafy liverwort as determined in the percent coverage study was divided by the estimated frequency of the relevant species based on voucher specimen identifications. Data were recorded as number of points out of 121. Rare cryptic species were assigned a value of one to three points based on how frequently they appeared in the sample. Because bryophyte species often overlay each other multiple species were allowed to share a quadrat point.

Species diversity was calculated using the Shannon's Diversity index (H) by site, tree type, and intercardinal aspect. Species diversity was measured for accounting for species richness and abundance. Shannon's Diversity index was chosen because it was demonstrated in a recent study to be the most sensitive index to rare species (Morris et al. 2014). This index uses the proportion of species i relative to the total number of species (p_i) multiplied by the natural logarithm of this proportion ($\ln p_i$). The resulting product is summed across species, and multiplied by -1 (Beals, Gross, & Harrell, 2000):

$$H = - \sum_{i=1}^S p_i (\ln p_i)$$

A mathematical property of Shannon's Diversity is that the maximum value of H increases with species richness (S). However, Shannon's Diversity can be scaled to a number between 0 and 1 using Pielou's Evenness, which can be calculated by dividing H by H_{\max} . H_{\max} is $\ln S$ (the log of the total number of species present) (Beals, Gross, & Harrell, 2000).

$$Evenness = \frac{H}{H_{\max}} = H / \ln S$$

Non-Metric Multidimensional Scaling

Non-Metric Multidimensional Scaling (NMDS) was performed using relative bryophyte abundances of bryophyte species (as estimated above) for all trees at all aspects at all sites yielding a 288 row by 28 column matrix representing 288 tree aspects (4 aspects x 12 trees x six locations= 288) and 28 bryophyte species and subspecies. All aspects or trees with fewer than two ($N=2$) observations were removed to eliminate rows that summed to 0 as per the data requirements of NMDS. A total of 117 rows were retained. The same removal criterion ($N=2$) observations was used for bryophyte species abundances to eliminate columns that summed to 0. A total of 19 species were retained. The removal process resulted in a matrix that was 117 rows by 19 columns.

NMDS was performed on PC-ORD 6.08 software using Sorensen (Bray-Curtis) distance measure (PC ORD v.6.08 22). Number of axes k was set to two, with 1000 runs of real data. All other parameters were set at default. NMDS results were displayed in a two-dimensional scatter plot.

CHAPTER 5 – RESULTS

Total Species Richness

A total of 28 bryophyte species were identified (Table 7), of which 15 species were leafy liverworts and the remaining 13 species were mosses. Of the bryophytes present, 11 leafy liverwort and 9 moss species were found on *C. caroliniana*, 13 leafy liverwort species and 12 moss species were found on the nearest neighboring non-pine non-*Carpinus* tree (Table 9). Richness varied across sites from a low of 9 species at S13010 to a high of 17 species at AZ23 (Table 7; Figure 6). Site AZ23 was particularly rich in leafy liverworts (Figure 6). Bryophytes were classified as unique or common based on patterns of presence and absence. Species that were found at only one site or on only one tree type were classified as unique. Common species were found at all sites and on all tree types. Nine bryophyte species were classified as unique. The mosses *Campyliadelphus chrysophyllus* and *Platygyrium repens* were identified only from the samples collected from AZ13. The leafy liverworts *Jamesoniella autumnalis* and *Rectolejeunea maxonii*, and the moss *Cryphaea nervosa* were identified only from the samples collected from AZ23. The moss *Hypnum imponens* was identified only from samples located from D1807B. The moss *Isopterygium tenerum* was identified only from the samples collected from D1825. The moss *Thelia hirtella* was identified only from the samples collected from S5115. The

leafy liverwort *Radula australis* was identified only from the samples collected from S13010.

Table 7. Bryophyte species identified across all sites samples. Bold red numbers represent a species being unique to a site or tree type. Data was collected spring 2018 from *C. caroliniana* and Non-Carpinus across six sites in East Texas. Species were identified in the lab using cell structure characteristics.

	Bryophyte Species	In Study	Carpinus	Non-Carpinus	AZ13	AZ23	D1807B	D1825	S5115	S13010
Leafy Liverwort	<i>Cololejeunea minutissima</i>	1		1					1	
	<i>Cololejeunea minutissima</i> <i>ssp. minutissima</i>	1		1					1	
	<i>Cololejeunea minutissima</i> <i>ssp. myriocarpa</i>	1	1	1		1		1		
	<i>Frullania brittoniae</i>	1	1	1	1	1	1		1	
	<i>Frullania eboracensis</i>	1	1	1	1	1	1	1	1	1
	<i>Frullania inflata</i>	1	1	1	1	1	1	1	1	1
	<i>Frullania kunzei</i>	1		1	1	1				
	<i>Frullania squarrosa</i>	1	1	1			1	1	1	
	<i>Jamesoniella autumnalis</i>	1	1	1		1				
	<i>Leucolejeunea conchifolia</i>	1	1	1	1	1				
	<i>Leucolejeunea uncioloba</i>	1	1	1	1	1	1	1	1	1
	<i>Porella pinnata</i>	1	1	1		1		1	1	1
	<i>Porella platyphylla</i>	1	1			1		1		
	<i>Rectolejeunea maxonii</i>	1	1			1				
	<i>Radula australis</i>	1		1						1
	Total	15	11	13	6	11	5	7	8	5

Table 7 Continued. Bryophyte species identified across all sites samples. Bold red numbers represent a species being unique to a site or tree type. Data was collected spring 2018 from *C. caroliniana* and Non-Carpinus across six sites in East Texas. Species were identified in the lab using cell structure characteristics.

	Bryophyte Species	In Study	Carpinus	Non-Carpinus	AZ13	AZ23	D1807B	D1825	S5115	S13010
Moss	<i>Campyliadelphus chrysophyllus</i>	1		1	1					
	<i>Campylophyllum hispidulum</i>	1	1	1	1	1	1	1	1	1
	<i>Clasmatodon parvulus</i>	1	1	1	1	1	1	1	1	1
	<i>Cryphaea glomerata</i>	1	1	1	1			1		1
	<i>Cryphaea nervosa</i>	1	1			1				
	<i>Forsstroemia trichomitria</i>	1	1	1	1	1		1	1	
	<i>Homalotheciella subcapillata</i>	1	1	1		1			1	
	<i>Homomallium adnatum</i>	1	1	1			1	1	1	
	<i>Hypnum imponens</i>	1		1			1			
	<i>Isopterygium tenerum</i>	1		1				1		
	<i>Leucodon julaceus</i>	1	1	1	1	1	1	1	1	1
	<i>Platygyrium repens</i>	1		1	1					
	<i>Thelia hirtella</i>	1	1	1					1	
	Total	13	9	12	7	6	5	7	7	4
	All Bryophytes Total	28	20	25	13	17	10	14	15	9

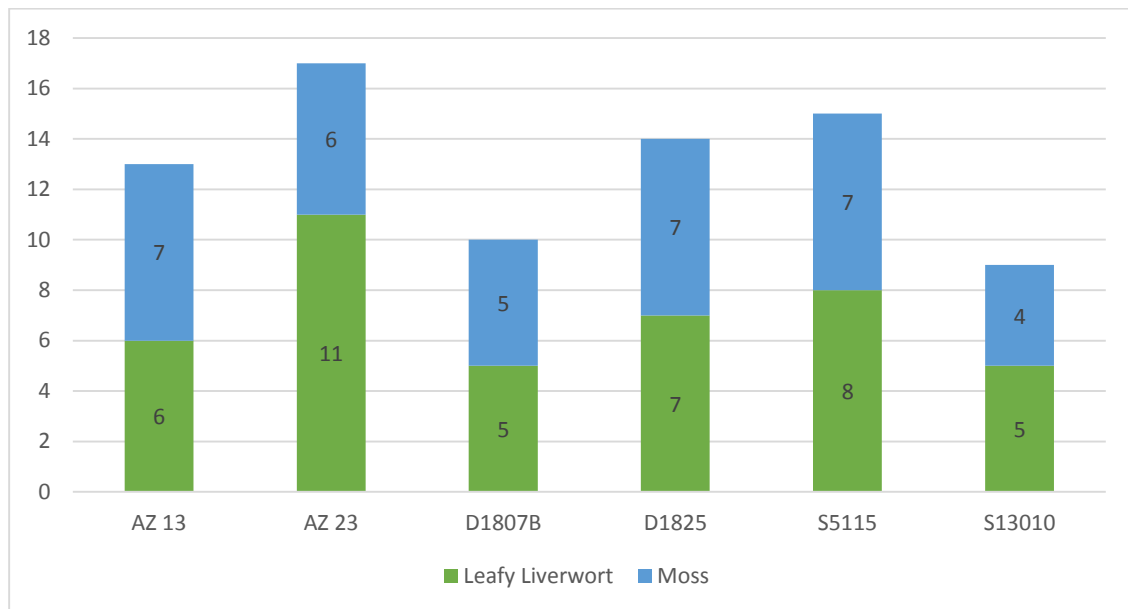


Figure 6. Total species richness across all sites sampled. Numbers represent the total species of Leafy liverworts and Mosses for each site. Data was collected spring 2018 from *C.caroliniana* and *Non-Carpinus* across six sites in East Texas.

Of the twenty eight sampled bryophytes, three bryophyte species were found only on *C. caroliniana*: The leafy liverworts *Porella platyphylla* and *Rectolejeunea maxonii*, and the moss *Cryphaea nervosa*. Eight Bryophyte species were only identified from samples collected from *Non-Carpinus* trees include: the leafy liverworts *Cololejeunea minutissima*, *Cololejeunea minutissima* spp. *minutissima*, *Frullania kunzei*, and *Radula australis*, and the mosses *Campyliadelphus chrysophyllus*, *Hypnum imopnens*, *Isopterygium tenerum*, and *Platygyrium repens*.

A total of six bryophyte species were extremely common and found across all sites and all tree species including three leafy liverworts: *Frullania eboracensis*, *Frullania inflata*, *Leucolejeunea uncioloba*; and three mosses: *Campylophyllum hispidulum*, *Clasmatodon parvulus*, and *Leucodon julaceus*.

ANOVA of Average Species Richness

The N-Way ANOVA model of average species richness for All bryophytes ($\text{Pr}>\text{F}=0.0001$), Moss ($\text{Pr}>\text{F}=0.0453$), and Leafy liverworts ($\text{Pr}>\text{F}=0.0131$) were statistically significant (Table 8). Of the variables ran for bryophyte species richness site ($\text{Pr}>\text{F}= 0.0035$), tree categories ($\text{Pr}>\text{F}= 0.0012$), and aspect ($\text{Pr}>\text{F}< 0.001$) were statistically significant (Table 9). Of the mixed interactions Site by tree categories ($\text{Pr}>\text{F}= 0.0497$), site by aspect ($\text{Pr}>\text{F}= 0.0102$), and site by bryophyte species type ($\text{Pr}>\text{F}= 0.0011$) interactions were statistically significant (Table 9). When broken into mosses and leafy liverworts categories the mosses site by tree interaction ($\text{Pr}>\text{F}= 0.0217$) were significant, and Leafy liverworts site ($\text{Pr}>\text{F}= 0.0046$) and tree ($\text{Pr}>\text{F}= 0.0143$) were significant (Table 10).

Table 8. N-Way ANOVA model for All Bryophytes by plot for species richness of bryophytes by site, tree category, bryophyte type, and aspect. ANOVA ran using SAS University edition 9.4. Data was collected spring 2018 from *C.caroliniana* and *Non-Carpinus* across six sites in East Texas

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	123.16	1.30	2.37	<.0001
Error	480	262.67	0.55		
Corrected Total	575	385.83			

Table 9. The Pr>F values for each variable and interactions for species richness data for each tree for each ANOVA tested. Species richness by site, tree category, bryophyte type, and aspect. ANOVA ran using SAS University edition 9.4. Data was collected spring 2018 from *C.caroliniana* and *Non-Carpinus* across six sites in East Texas.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	5	9.78	1.96	3.58	0.0035
TreeSPP	1	5.84	5.84	10.67	0.0012
Aspect	3	28.62	9.54	17.43	<.0001
BryophyteSPP	1	0.34	0.34	0.62	0.4308
Site*TreeSPP	5	6.12	1.22	2.24	0.0497
Site*Aspect	15	17.01	1.13	2.07	0.0102
Site*BryophyteSPP	5	11.28	2.26	4.12	0.0011
TreeSPP*Aspect	3	3.48	1.16	2.12	0.0969
TreeSPP*BryophyteSPP	1	2.01	2.01	3.67	0.0561
Aspect*BryophyteSPP	3	1.20	0.40	0.73	0.5334
Site*TreeSPP*Aspect	15	12.98	0.87	1.58	0.0748
Site*TreeSPP*BryophyteSPP	5	4.78	0.96	1.75	0.122
Site*Aspect*Bryophyte SPP	15	8.26	0.55	1.01	0.4473
TreeSPP*Aspect*BryophyteSPP	3	1.95	0.65	1.19	0.3135
Site*TreeSPP*Aspect*BryophyteSPP	15	9.51	0.63	1.16	0.3015

Average species richness varied significantly by site. Of all sample locations AZ23 had the highest species richness a LS mean of .93 with a total of 17 species, S13010 had the lowest species richness a LS mean of 0.50 with a total of 9 species identified. There were statistically significant interactions between sites and tree species, sites and bryophyte types, and sites and aspects. Tukey's pairwise comparisons of the site by tree interaction are complex (Figures 7 and 8). Four comparisons were statistically significant. *C. caroliniana* sampled at AZ23 was significantly richer in species than that of the *Non-Carpinus* samples in D1825 ($P > F_{0.0189}$) and S13010 ($P > F_{0.0008}$) (Figure 7). The *Non-Carpinus* sampled at AZ23 had a significantly more species than the *Non-Carpinus* sampled in S13010 ($P > F_{0.0116}$) (Figure 7). *C. caroliniana* sampled at D1825 had significantly more bryophyte species than the *Non-Carpinus* samples in D1825 ($P > F_{0.0189}$) and S13010 ($P > F_{0.0008}$) (Figure 7). The *Non-Carpinus* trees sampled at S13010 had significantly less species richness than the *C. caroliniana* sampled in S5115 ($P > F_{0.0189}$) (Figure 7).

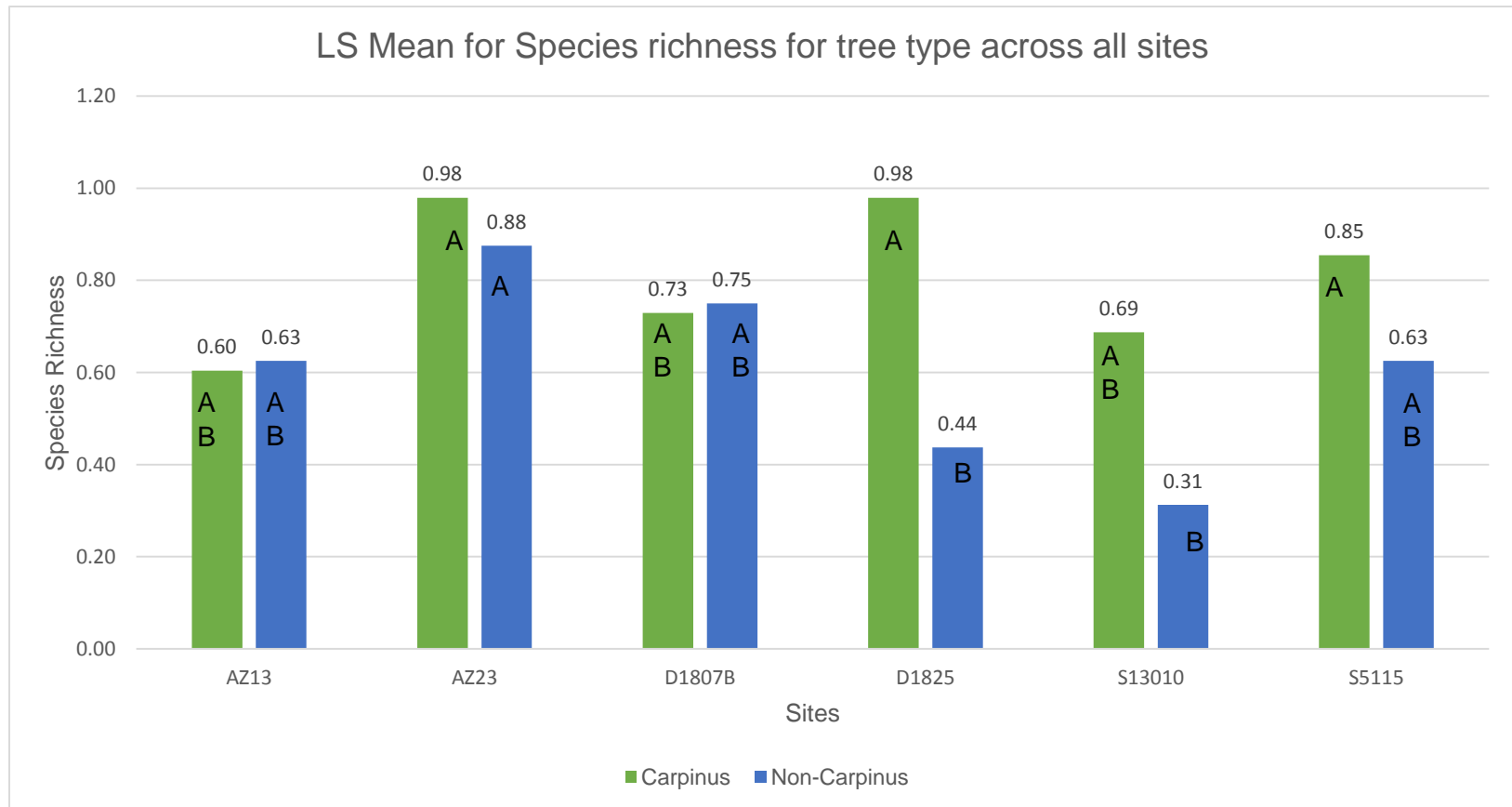


Figure 7: The Least Square Means for Species Richness across all bryophytes for all sites divided between tree type. Data was collected spring 2018. Tukey's pair wise comparison with C representing (C.Carpinus) and NN representing (Non-Carpinus) Using SAS University edition 9.4

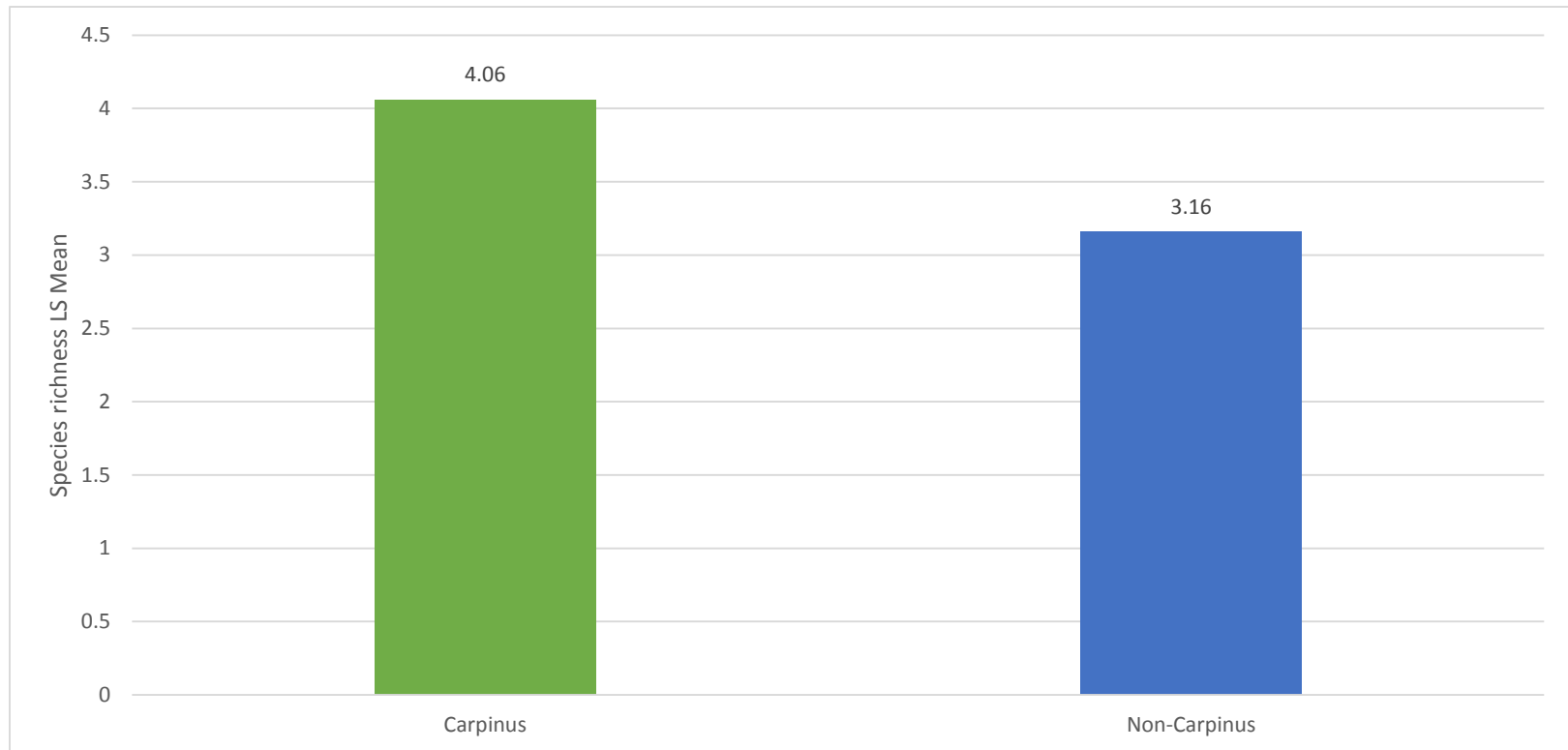


Figure 8. The Least Square Means for Species Richness for tree type for all bryophytes across all sample sites. Data was collected spring 2018. Using SAS University edition 9.4

The site by aspect interactions was statistically significant (Table 9; Figure 9). Tukey's comparisons show that the Northeast aspect of site AZ23 for all sampled trees was significantly richer in bryophyte species than AZ23NW, D1807BNW, D1807BSE, D1825SE, D1825SW, S13010NW, S13010SE, S13010SW, S5115NW, S5115SE, and S5115SW (Figure 10). The Northwest aspect of S13010 for all sampled trees was significantly lower than AZ13NE, AZ23NE, AZ23SE, D1807BNE, D1825NE, D185NW, S13010NE, and S5115NE (Figure 10).

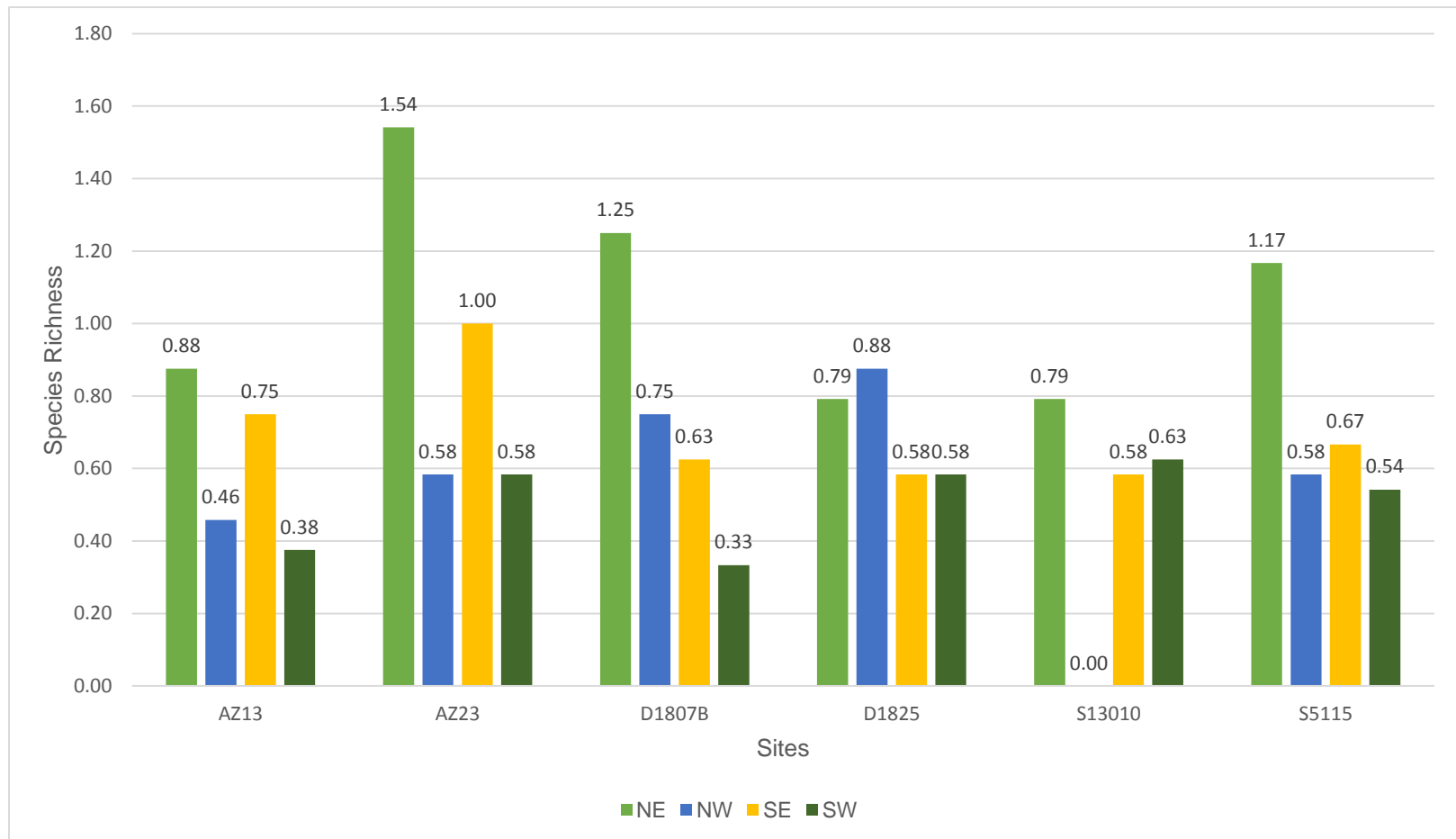


Figure 9. The Least Square Means for Species Richness across all bryophytes for all sites for each aspect. Data was collected spring 2018. Using SAS University edition 9.4

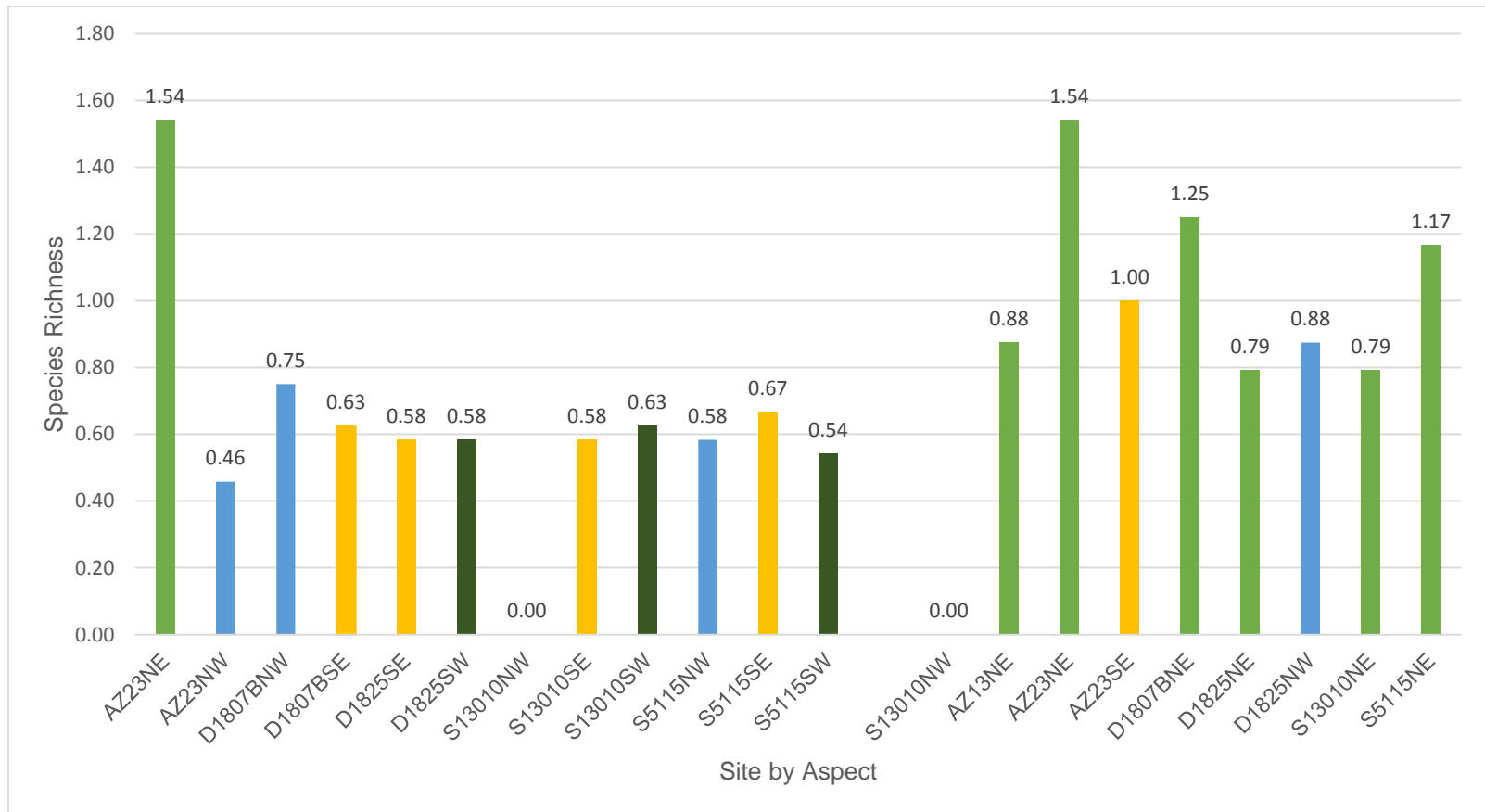


Figure 10. The Least Square Means for Species Richness across all bryophytes for all sites for each aspect. Data was collected spring 2018. Tukey's pair wise comparison of site by aspect with NE representing Northeast, NW representing Northwest, SE representing Southeast, and SW representing Southwest. Comparison ran using SAS University edition 9.4

Tukey's pairwise comparisons of the bryophyte by site interaction (Figure 11) shows that the leafy liverworts richness at AZ23 is significantly higher than that of the leafy liverworts of D1825 and S13010 (Figure 11). The moss richness of D1825 is significantly higher than the leafy liverworts of D1825 and S13010 (Figure 11).

ANOVA of Average Species Richness for tree categories

The N-Way ANOVA model of average species richness for All bryophytes ($Pr>F=0.0087$), Moss ($Pr>F=0.0453$), and Leafy liverworts ($Pr>F=0.0131$) were statistically significant (Table 10). Of the variables ran for bryophyte species richness site ($Pr>F=0.0264$), tree categories ($Pr>F=0.0245$), were statistically significant (Table 11). When broken into leafy liverworts and mosses categories the Leafy liverworts site ($Pr>F=0.0046$) and tree ($Pr>F=0.0143$) variables were significant and the mosses site by tree interaction ($Pr>F=0.0217$) was significant (Table 11).

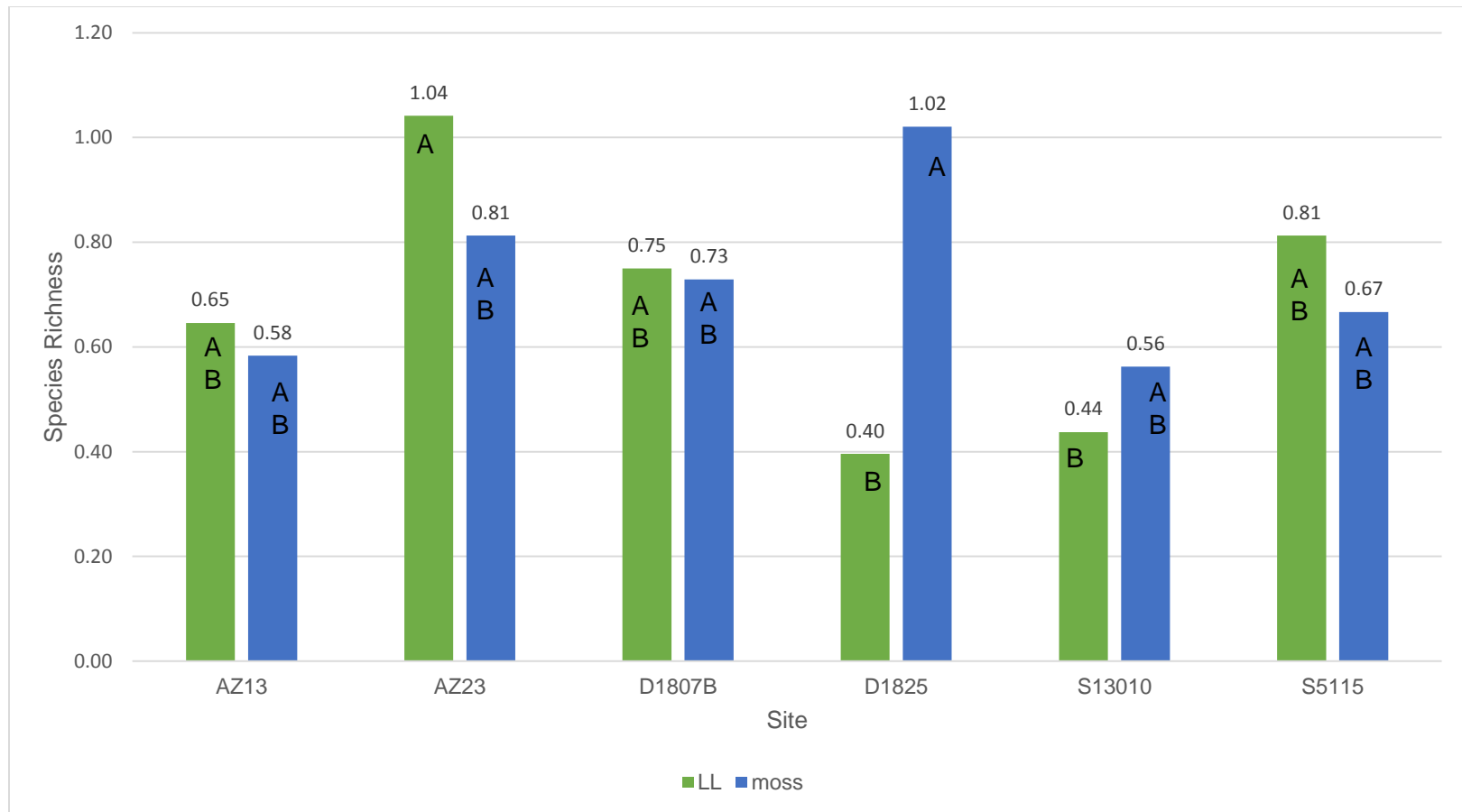


Figure 11. The Least Square Means for Species Richness across all bryophytes for all sites for each tree. Data was collected spring 2018. Using SAS University edition 9.4 Tukey's pair wise comparison of site by aspect with LL representing Leafy liverworts and Moss

Table 10. N-Way ANOVA models for All Bryophytes, Leafy liverworts, and Moss by tree for species richness of bryophytes by site and tree category. ANOVA ran using SAS University edition 9.4. Data was collected spring 2018 from C.caroliniana and Non-Carpinus across six sites in East Texas

	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
All Bryophytes by tree	Model	11	76.78	6.97	2.61	0.0087
	Error	60	160.33	2.67		
	Corrected Total	71	237.11			
Leafy Liverworts	Model	11	33.94	3.09	2.46	0.0131
	Error	60	75.33	1.26		
	Corrected Total	71	109.28			
Moss	Model	11	27.61	2.51	1.99	0.0453
	Error	60	75.67	1.26		
	Corrected Total	71	103.28			

Table 11. The Pr>F values for each variable and interactions for species richness for all bryophytes, for leafy liverworts, and for mosses for each tree for each ANOVA tested. Species richness by site and tree category. ANOVA ran using SAS University edition 9.4. Data was collected spring 2018 from C.caroliniana and Non-Carpinus across six sites in East Texas.

	Source	DF	Type III SS	Mean Square	F Value	Pr > F
All Bryophytes	Site	5	36.78	7.36	2.75	0.0264
	Tree	1	14.22	14.22	5.32	0.0245
	Site*Tree	5	25.78	5.15	1.93	0.1027
Leafy Liverworts	Site	5	23.94	4.79	3.81	0.0046
	Tree	1	8	8	6.37	0.0143
	Site*Tree	5	2	0.4	0.32	0.8999
Moss	Site	5	8.61	1.72	1.37	0.25
	Tree	1	0.89	0.89	0.7	0.4045
	Site*Tree	5	18.11	3.62	2.87	0.0217

Tukey's pairwise comparison showed that site AZ23 was statistically different from site S13010 in species richness for bryophytes (Figure 12). The Tukey's pairwise comparison also showed that the species richness on *Carpinus* was statistically different than the *Non-Carpinus* sampled (Figure 13).

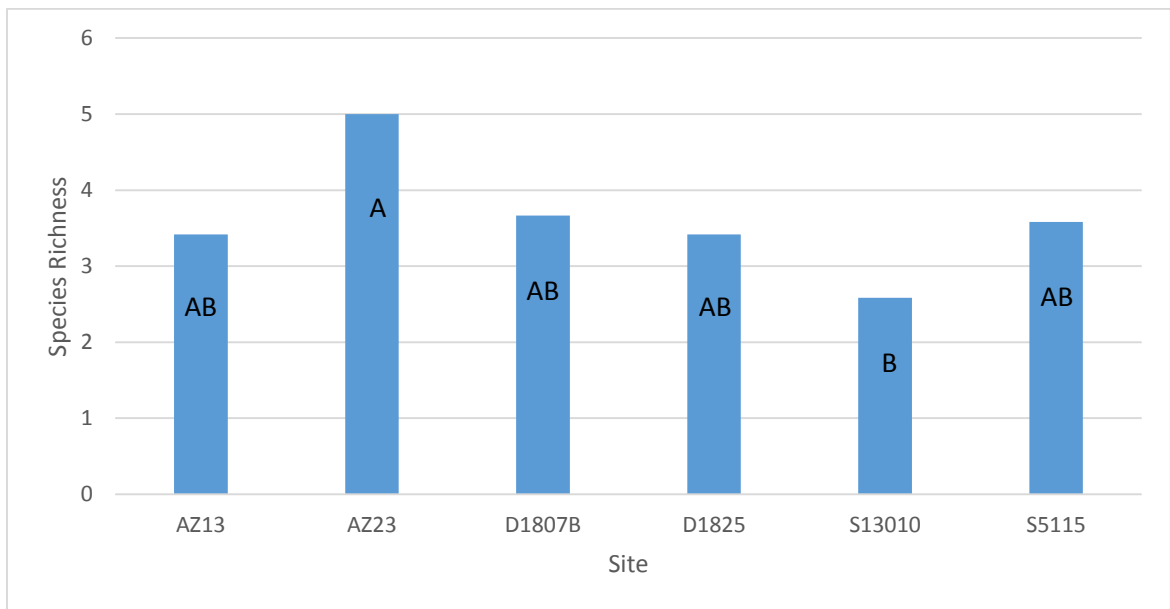


Figure 12: The Least Square Means for Species Richness across all sample locations for all bryophytes for all sites. Data was collected spring 2018. Using SAS University edition 9.4 Tukey's pair wise comparison with bryophyte species richness at AZ23 being different from S13010.

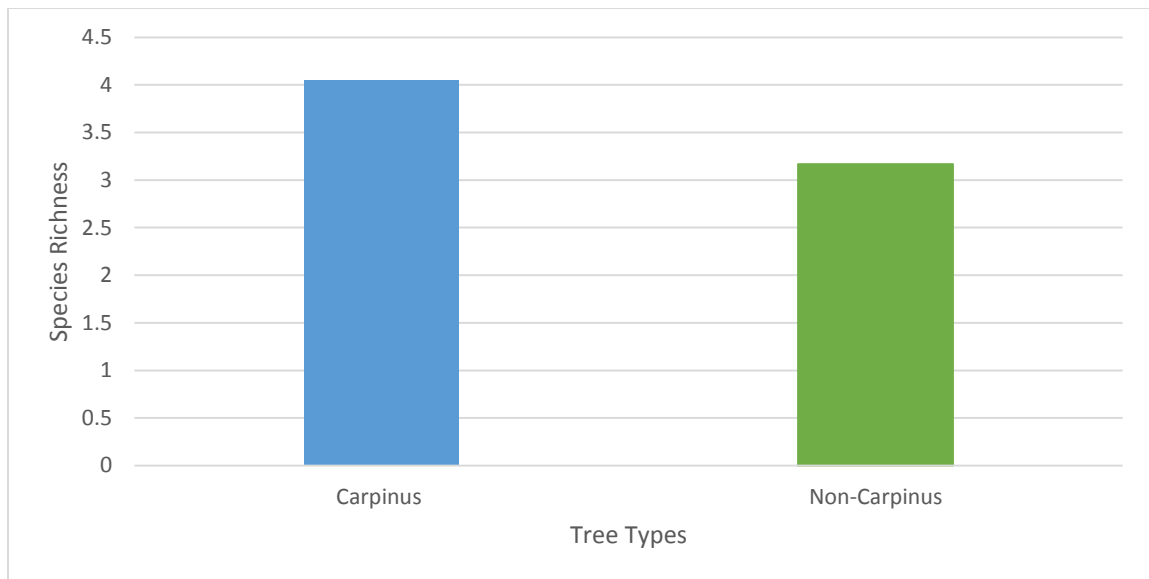


Figure 13: The Least Square Means for Species Richness across all sample locations for all bryophytes between tree type. Data was collected spring 2018. Using SAS University edition 9.4 Tukey's pair wise comparison with bryophyte species richness for *C.caroliniana* being different than the Non-Carpinus sampled.

The N-Way ANOVA ran for species richness for leafy liverworts grouped by tree was statically significant for site ($Pr>F=0.0046$) and for the tree category type ($Pr>F=0.0143$). The Tukey's pairwise comparison showed that site AZ23 was statistically different from site D1825 and S13010 in species richness for leafy liverworts sampled (Figure 14). The Tukey's pairwise comparison showed that the leafy liverwort richness on *Carpinus* was statistically different that the *Non-Carpinus* sampled (Figure 14).

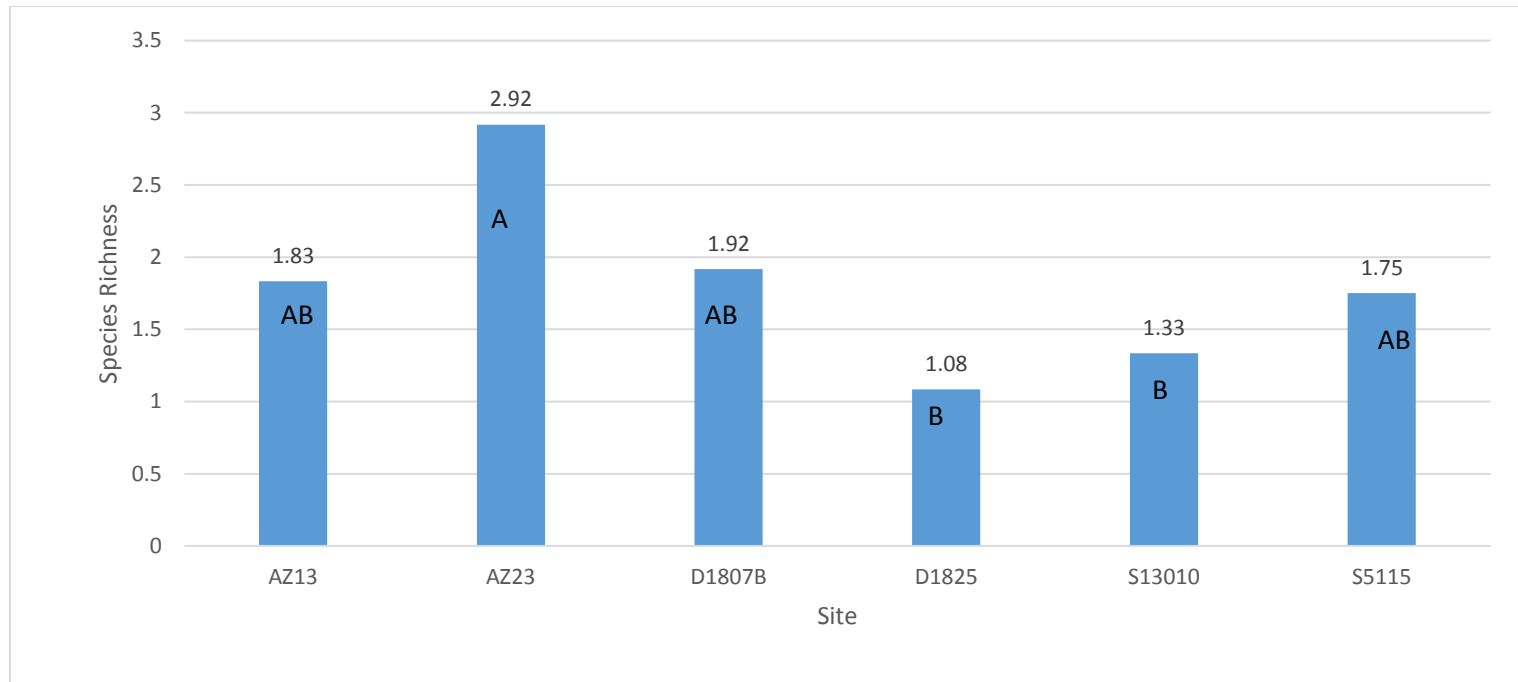


Figure 14: The Least Square Means for Species Richness across all sample locations for Leafy liverworts for all sites. Data was collected spring 2018. Using SAS University edition 9.4 Tukey's pair wise comparison with bryophyte species richness at AZ23 being different from D1825 and S13010.

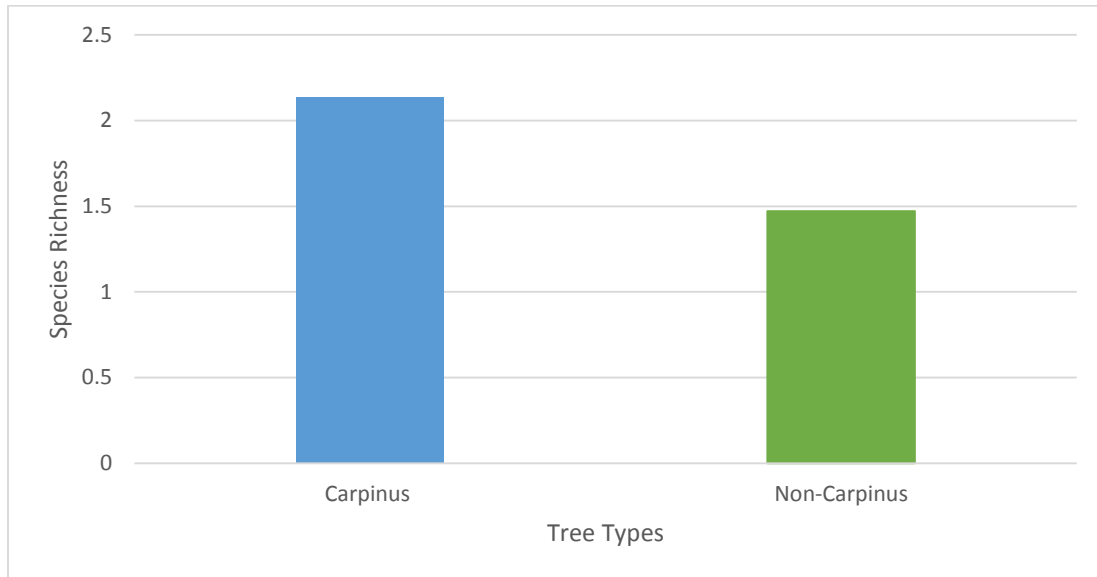


Figure 15: The Least Square Means for Species Richness across all sample locations for Leafy liverworts between tree type. Data was collected spring 2018. Using SAS University edition 9.4 Tukey's pair wise comparison with bryophyte species richness for *C.caroliniana* being different than the Non-Carpinus sampled.

The N-Way ANOVA ran for species richness for mosses grouped by tree was statically significant for the site*tree interaction ($Pr>F=0.0217$). The Tukey's pairwise comparison showed that site D1825 tree type *Carpinus* was statistically different from site S13010 tree type *Non-Carpinus* in species richness for bryophyte sampled (Figure 15).

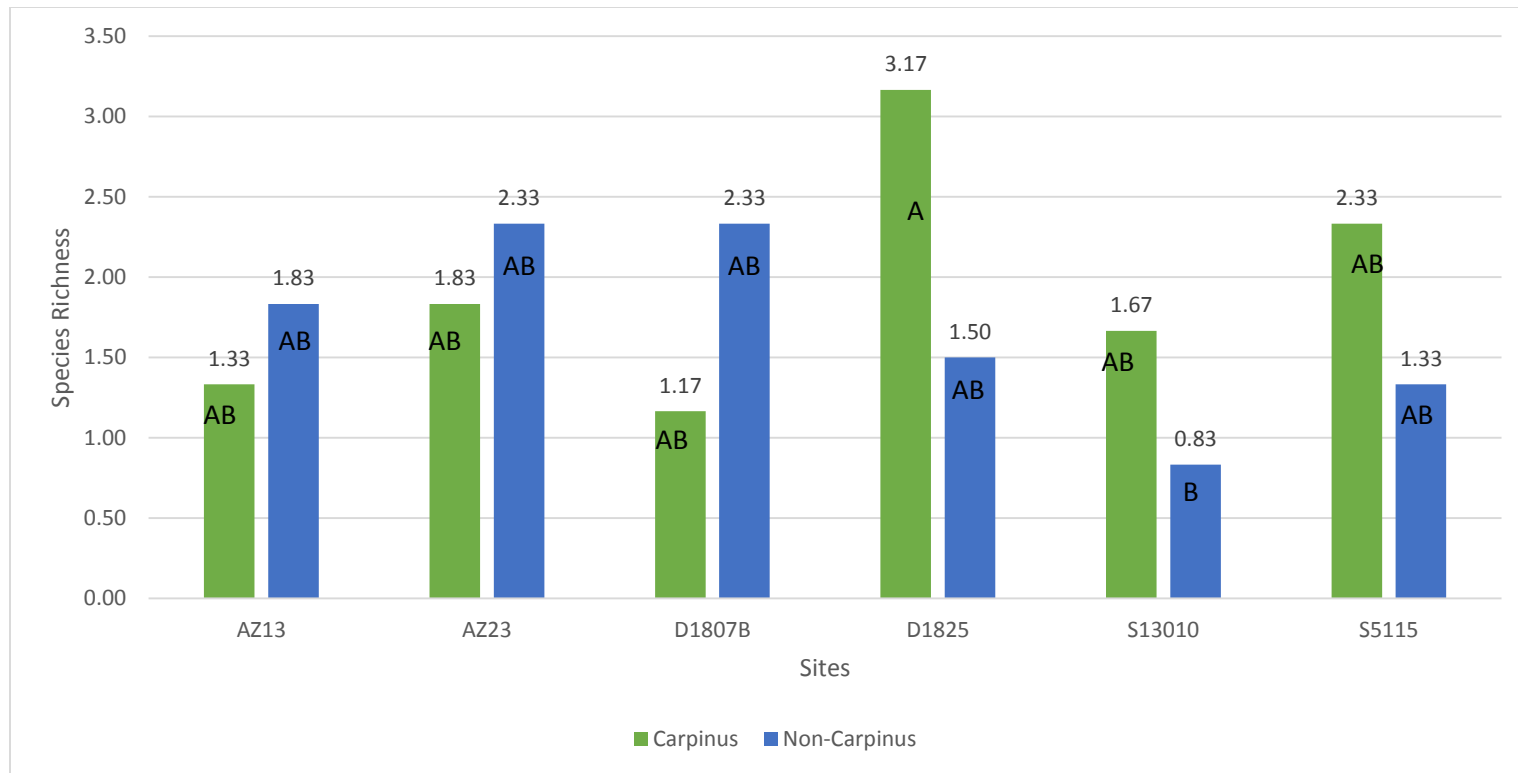


Figure 16: The Least Square Means for Species Richness of moss across all sample locations for site by tree type. Data was collected spring 2018. Using SAS University edition 9.4 Tukey's pair wise comparison with bryophyte species richness for D1825 *C.caroliniana* being different than the S13010 Non-Carpinus sampled.

Shannon's Diversity

Shannon-Weiner species diversity indices evenness were calculated separately for all bryophytes, by leafy liverworts, and by mosses for all sites (Table 12), by tree species (Table 12), and for all four aspects (Table 12). AZ23 had the highest diversity and evenness across all sampling locations, with D1807B having the lowest diversity and evenness. Site D1825 had the highest leafy liverwort diversity ($H = 1.79$) and evenness (0.92) and site S13013 had the lowest leafy liverwort diversity ($H = 1.12$) and evenness (0.54). Site AZ13 had the highest moss diversity ($H = 1.32$) and evenness (0.68) while site D1807B had the lowest moss diversity ($H = .71$) and evenness (0.44) (Table 12).

Table 12. Shannon-Weiner indices for All bryophytes. Leafy liverworts, and Mosses across all sites sampled. Data was collected spring 2018. Total species abundance for each of the voucher trees sampled.

		AZ13	AZ23	D1807B	D1825	S13010	S5115
All Bryophytes	Diversity (H')	2.2	2.52	1.38	2.21	1.83	1.9
	Richness (S)	13	17	10	14	15	9
	Hmax=LN(S)	2.56	2.83	2.3	2.64	2.71	2.2
	H/Hmax (Even)	0.85	0.89	0.6	0.83	0.68	0.86
Leafy Liverworts	Diversity (H')	1.26	1.73	1.18	1.79	1.12	1.19
	Richness (S)	6	11	5	7	8	5
	Hmax=LN(S)	1.79	2.40	1.61	1.95	2.08	1.61
	H/Hmax (Even)	0.70	0.72	0.73	0.92	0.54	0.74
Moss	Diversity (H')	1.32	1.14	0.71	1.04	0.94	0.97
	Richness (S)	7	6	5	7	7	4
	Hmax=LN(S)	1.95	1.79	1.61	1.95	1.95	1.39
	H/Hmax (Even)	0.68	0.64	0.44	0.53	0.48	0.70

Carpinus caroliniana trees had higher diversity values ($H = 2.14$) and evenness (0.71) in comparison to the *Non-Carpinus* samples. *C. caroliniana* trees also had the highest diversity 1.20 and evenness 0.5 of the leafy liverworts collected, where as *Non-Carpinus* had the highest diversity 1.27 and evenness 0.51 of the mosses collected (Table 13).

Table 13. Shannon-Weiner indices for All bryophytes, Leafy liverworts, and Mosses for *Carpinus* and *Non-Carpinus* samples. Data was collected spring 2018. Total species abundance for each of the voucher trees sampled.

		<i>Carpinus</i>	<i>Non-Carpinus</i>
All Bryophytes	Diversity (H')	2.14	2.04
	Richness (S)	20	25
	$H_{max} = \ln(S)$	3.00	3.22
	H/H_{max} (Even)	0.71	0.63
Leafy liverworts	Diversity (H')	1.2	0.78
	Richness (S)	11	13
	$H_{max} = \ln(S)$	2.40	2.56
	H/H_{max} (Even)	0.50	0.30
Moss	Diversity (H')	0.94	1.27
	Richness (S)	9	12
	$H_{max} = \ln(S)$	2.20	2.48
	H/H_{max} (Even)	0.43	0.51

Of each aspect sampled the Northeast had the highest diversity 2.28 and evenness 0.73 of all the bryophyte collected. The northeast aspect also had the highest diversity for both the leafy liverworts 1.09 and mosses 1.19, as well as evenness of leafy liverworts 0.44 and mosses 0.5 that were collected (Table 14).

Table 14. Shannon-Weiner indices for All bryophytes, Leafy liverworts, and Mosses across each sampled aspect. Data was collected spring 2018. Total species abundance for each of the voucher trees sampled.

		Northeast	Southeast	Southwest	Northwest
All Bryophytes	Diversity (H')	2.28	1.97	2.00	2.00
	Richness (S)	23	19	16	20
	Hmax=LN(S)	3.14	2.94	2.77	3.00
	H/Hmax (Even)	0.73	0.67	0.72	0.67
Leafy liverworts	Diversity (H')	1.09	0.98	0.84	0.95
	Richness (S)	12	10	8	11
	Hmax=LN(S)	2.48	2.30	2.08	2.40
	H/Hmax (Even)	0.44	0.43	0.40	0.40
Moss	Diversity (H')	1.19	0.99	1.16	1.05
	Richness (S)	11	9	8	9
	Hmax=LN(S)	2.40	2.20	2.08	2.20
	H/Hmax (Even)	0.50	0.45	0.56	0.48

Percent Coverage

The N-Way ANOVA model preformed for percent cover is statistically significant ($Pr>F=0.0001$) (Table 15). Of the variables site ($Pr>F=0.0106$), aspect ($Pr>F=<0.0001$), and bryophyte species type ($Pr>F=0.0021$) were statistically significant (Table 16.). Of the mixed interactions, the tree by bryophyte species type interaction ($Pr>F=0.0012$) was statistically significant (Table 16).

Table 15. The N-Way ANOVA model ran for percent abundance of bryophytes by site, tree category, bryophyte type, and aspect. ANOVA ran using SAS University edition 9.4. Data was collected spring 2018 from *C.caroliniana* and *Non-Carpinus* across six sites in East Texas.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	44912.317	472.7612	1.72	0.0001
Error	480	132132.4818	275.276		
Corrected Total	575	177044.7988			

Table 16. The Pr>F values for each variable and interactions for percent abundance sampling data for each ANOVA tested. For percent abundance by site, tree category, bryophyte type, and aspect. ANOVA ran using SAS University edition 9.4. Data was collected spring 2018 from *C.caroliniana* and *Non-Carpinus* across six sites in East Texas.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	5	4164.60933	832.92187	3.03	0.0106
Tree Category	1	40.58357	40.58357	0.15	0.7012
Aspect	3	14584.94008	4861.64669	17.66	<.0001
Bryophyte Type	1	2621.97601	2621.97601	9.52	0.0021
Site*Tree Category	5	1861.60179	372.32036	1.35	0.241
Site*Aspect	15	5073.38956	338.22597	1.23	0.2458
Site*Bryophyte Type	5	2685.20218	537.04044	1.95	0.0846
Tree Category*Aspect	3	944.46887	314.82296	1.14	0.331
Tree Category*Bryophyte Type	1	2911.68755	2911.68755	10.58	0.0012
Aspect*Bryophyte Type	3	18.16982	6.05661	0.02	0.9956
Site*Tree Category*Aspect	15	3300.723	220.0482	0.8	0.6788
Site*Tree Category*Bryophyte Type	5	986.12083	197.22417	0.72	0.6113
Site*Aspect*Bryophyte Type	15	2728.77512	181.91834	0.66	0.8232
Tree Category*Aspect*Bryophyte Type	3	706.62841	235.5428	0.86	0.464
Site*Tree Category*Aspect*Bryophyte Type	15	2283.44086	152.22939	0.55	0.9096

Mean percent coverage by site varied from 5.14 at AZ13 and 12.84 at S5115 (Figure 17). Tukey's pairwise comparisons indicated that AZ13 was significantly different from AZ23, D1825, and S5115 but was not different from D1807B or S13010. The Northeast aspect has a significantly higher percent coverage than that of the other aspects with the LS mean of 18.50 (Figure 17). Tukey's pairwise comparisons indicated that the Northeast aspect was significantly different from the Southeast, Southwest, and Northwest aspects. The difference between leafy liverwort (8.01%) and moss (12.27%) percent abundance was statistically significant (Figure 18).

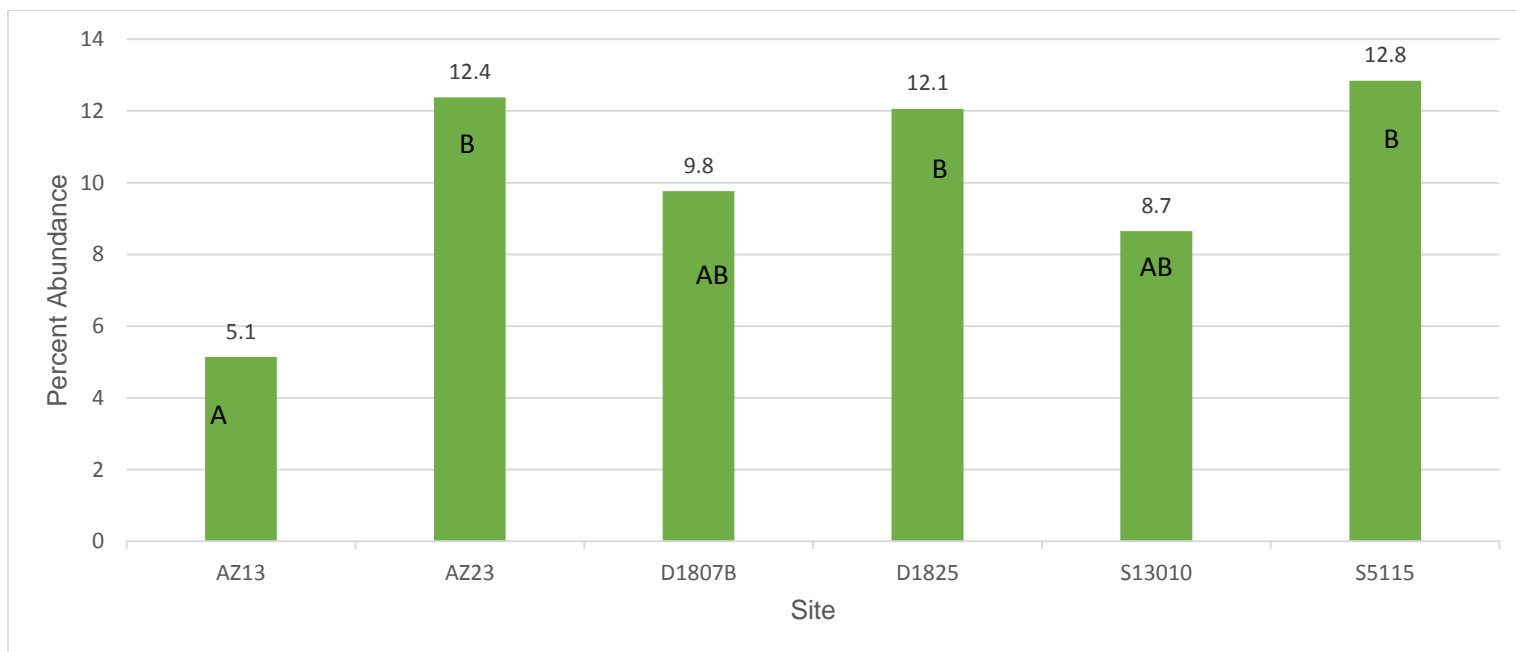


Figure 17. The Least Square Means for the percent coverage across all sample locations for bryophyte percent abundance for the twelve sample trees across each site. Tukey's pair wise comparison with AZ13 being different from AZ23, D1825, and S5115. Using SAS University edition 9.4. Data was collected spring 2018 from *C.caroliniana* and Non-Carpinus across six sites in East Texas.

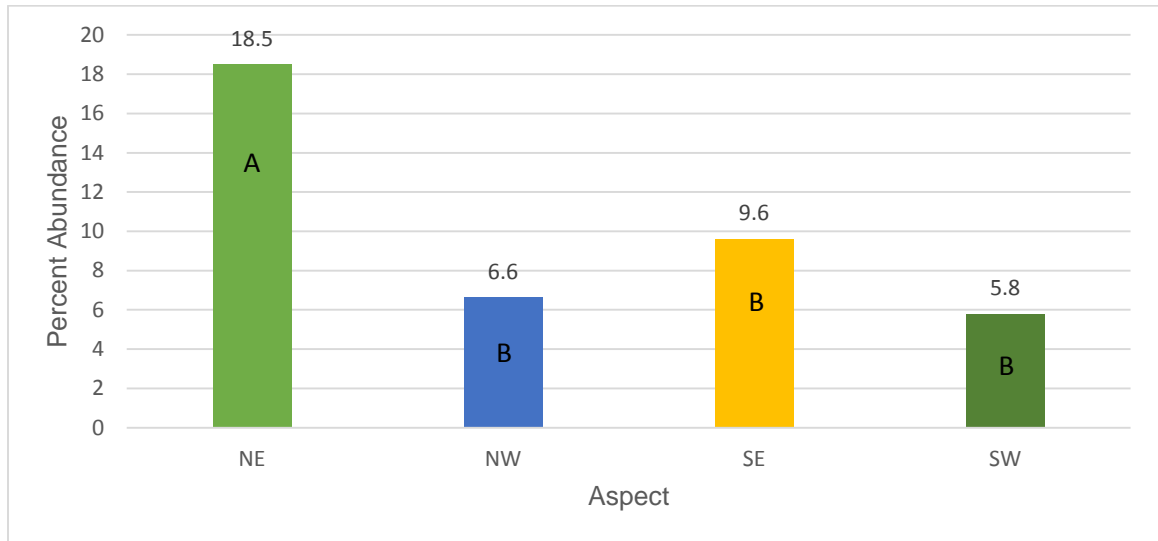


Figure 18. The Least Square Means for the percent coverage across all sample locations for bryophyte percent abundance for the twelve sample trees across each sampled aspect. Data was collected spring 2018 from *C.caroliniana* and Non-Carpinus across six sites in East Texas. Tukey's pair wise comparison with the Northeast aspect being different from the other sampled aspects Using SAS University edition 9.4.

The interaction of tree type by bryophyte species type were significantly different. Tukey's pairwise comparisons indicate that percent coverage of leafy liverworts is significantly higher than that of mosses on *C.caroliniana* (Figure 19). Percent coverage of the moss on the *Non-Carpinus* trees is significantly higher than that of moss on *C.caroliniana* (Figure 19.).

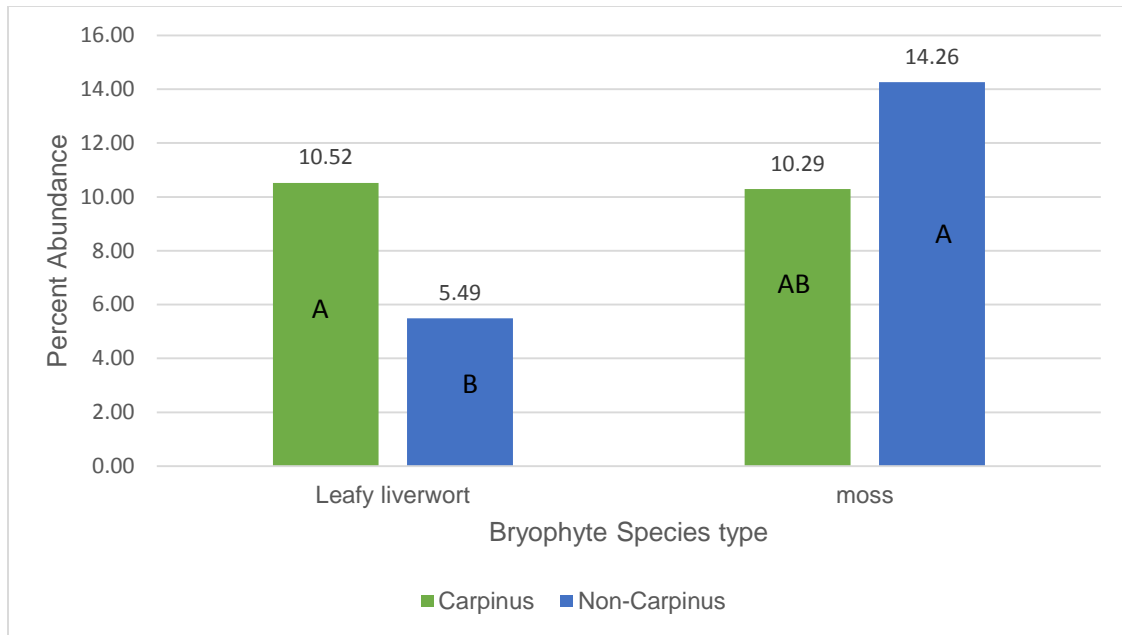


Figure 19. The Least Square Means for the percent coverage across all sample locations for bryophyte percent abundance for the twelve sample trees. Samples were categorized form Carpinus and Non-Carpinus, leafy liverworts and mosses. Tukey's pair wise comparison with AZ13 being different from AZ23, D1825, and S5115. Using SAS University edition 9.4.

Non-metric multidimensional scaling (NMDS)

Non-metric multidimensional scaling (NMDS) of bryophyte relative abundances revealed a single continuum along the X and Y axes accounting for 77.20% of the variation within the original data set; 48.79% on axis one and 28.42% on Axis two. Results were coded by tree type and site. Sampled *C. caroliniana* have a greater tendency towards *Porella pinnata* and *Leucolejeunea uniciloba*, whereas the *Non-Carpinus* had a tendency towards *Leucodon julaceus* and *Isopterygium tenerum* (Figure 20).

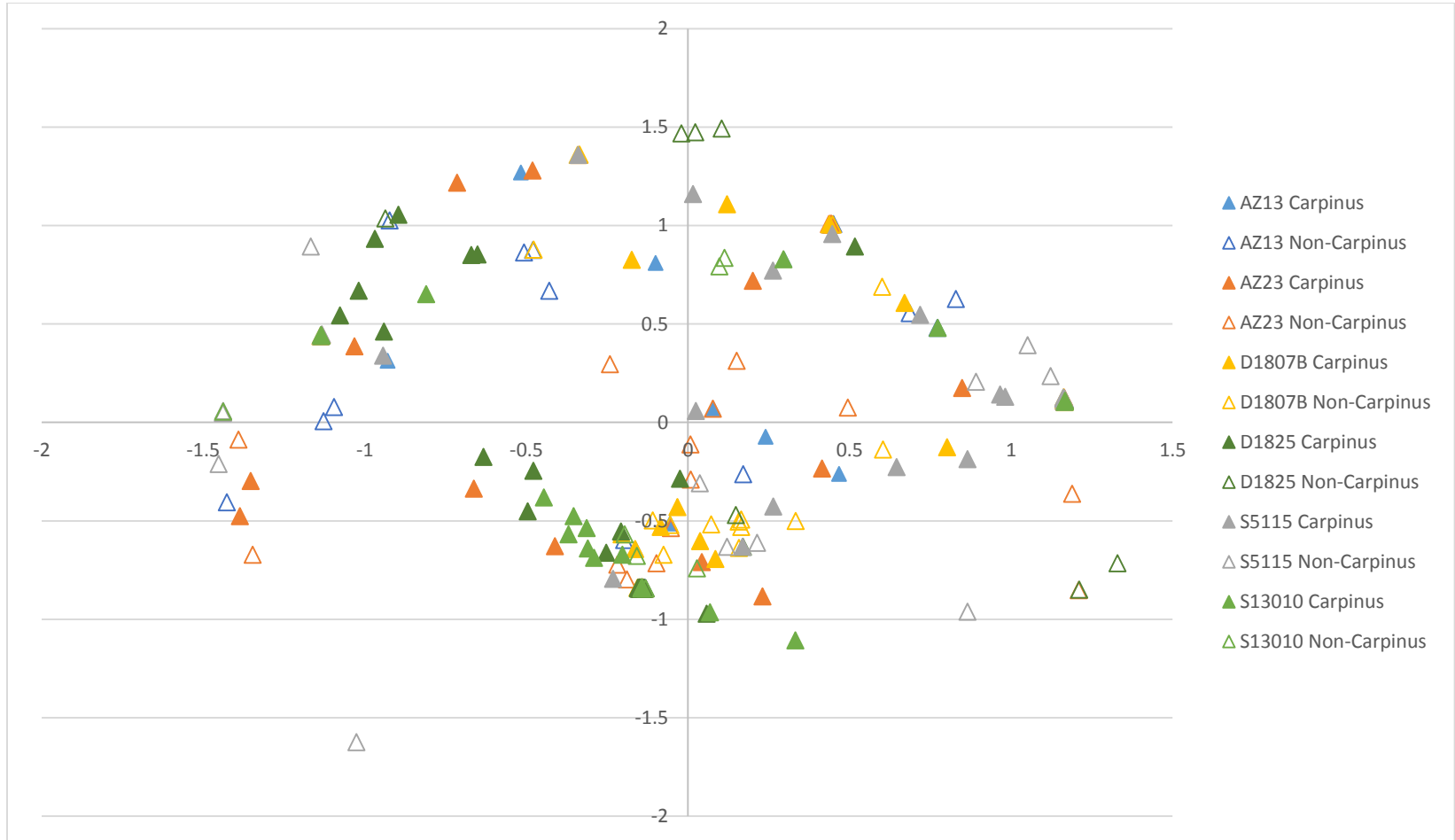


Figure 20. Non-metric multidimensional scaling of bryophyte relative abundance for each bryophyte species for Carpinus and Non-Carpinus and each sampled aspect. NMDS was ran on PC-ORD 6.08 software using Sorensen (Bray-Curtis) distance measure. Number of axes k was set to two, with 1000 runs of real data. Data was collected from six sample locations across East Texas Spring 2018.

CHAPTER 6 – DISCUSSION

The main goals of this research were to determine if *C. caroliniana* has a uniquely different bryophyte composition compared to that of the surrounding *Non-Carpinus* tree species, and to determine if epiphytic bryophyte communities vary across sample sites. This research is based off of initial observations that individuals of *C. caroliniana* seemed to have a uniquely rich leafy liverwort composition compared to that of the surrounding trees.

The epiphytic bryophyte composition of *C. caroliniana* differs from that of the surrounding *Non-Carpinus* trees. In support of our initial observations that *C. caroliniana* trees have a significantly greater leafy liverwort composition than their nearest neighbors, I found that the percent coverage of leafy liverworts is statistically greater than the percent moss coverage on individuals of *C. caroliniana* (Figure 19). Our data also indicate that *Non-Carpinus* trees have a significantly larger percent moss coverage than their *Carpinus* neighbors (Figure 19). Total species richness indicated that surrounding *Non-Carpinus* tree species had a higher number of leafy liverwort species ($S=13$) than *C. caroliniana* ($S=11$) (Table 7). However, species richness data alone can be misleading as a guide to species diversity because richness fails to account for relative abundance (Morris et al., 2014). Accordingly, *C. caroliniana* trees have higher Shannon's diversity (which accounts for abundance) and evenness for total bryophytes and leafy liverworts than *Non-Carpinus* trees, while *Non-Carpinus* trees have a higher

diversity and evenness of mosses than does *Carpinus* (Table 13). While *C. caroliniana* does have a uniquely diverse leafy liverwort composition (Tables 7 and 1) *C. caroliniana* trees had no diagnostic, fixed, host-specific bryophyte species (Table 7), rather they differed from the Non-*Carpinus* neighbors primarily in terms of the relative abundance of a common set of species (Appendix A).

The bark of *C. caroliniana* is distinctively smooth and furrowed. While our study included some other smooth barked species (*Acer rubrum*, *Ilex opaca*, and *Fagus grandifolia*) sampling of these species was limited. The hypothesis that smooth barked trees will have a higher coverage of leafy liverworts remains to be tested.

Past research in North American epiphytic bryophyte communities indicated that some regions epiphytic bryophyte community differed based on host tree and site (Studlar 1982; Schmitt and Slack 1990), whereas other regions show little variation based on host and site (Phillips 1951). Our data support the presence of a single epiphytic community at our sampling sites. Non-metric Multidimensional Scaling (NMDS) indicates the sites we investigated share a similar bryophyte community (Figure 20). The variation represented in the NMDS study forms a single continuum that does not form discrete groupings by tree species, site, aspect, or any other biologically recognizable variable. The epiphytic bryophytes across all sample sites had a similar community type,

Leucodon julaceus being the most abundant species identified, interspersed with *Frullania* spp., *Leucolejeunea unciloba*, *Clasmatodon parvulus*, and *Campylophyllum hispidulum*. Other less common species were identified throughout this community, the significance of which would require greater sampling to determine if site and/or species patterns are occurring. As our original research question related primarily to the composition of epiphytic bryophytes on *C. caroliniana*, our site selection was confined to the wet mesic forested river and stream banks and slopes of East Texas in which that species is abundant. These results apply only to this particular ecosystem.

Schmitt and Slack (1990) suggested that all epiphytic bryophytes be tested for host tree specificity. While our data indicate a preference of leafy liverworts for *C. caroliniana*, some other bryophyte species sampled showed a potential host specificity based on tree species or site. The moss *Isopterygium tenerum* was collected only at D1825 from *Betula nigra* trees. This may indicate the possibility of a host specificity; it is also possible that regional flooding trends may have influenced the growth habit of this otherwise ground dwelling species, a hypothesis that can be tested with greater sampling from the tree at more sites with a variety of flooding regimes. *Jamesoniella autumnalis* was only identified from samples collected from AZ23 on both *C. caroliniana* and *Quercus nigra*. AZ23 is a site in which standing water was present, it also had the highest species richness (S= 17, Table 7) of our sampled sites, confirming to Huston's

observation (Huston 2007) that the greatest bryophyte diversity was found in areas with streams and standing water. It can be hypothesized that *J. autumnalis* may also be found at other locations that resemble the flooded bottomland hardwood depression of AZ23. *Thelia hirtella* was sampled at only one location, S5115, on *C. caroliniana*, *Nyssa sylvatica*, and *Quercus marylandica*. *Thelia hirtella* is morphologically distinctive and was also identified at S13010, but was not represented in our voucher specimens. Extended sampling could help determine the possibility for host and site specificity for this moss.

Huston (2007) listed the most common epiphytic bryophyte species as *Porella pinnata*, *Forstroemia trichomitria*, *Leucodon julaceus*, and *Leucolejeunea* sp. In our study *Leucodon julaceus*, *Frullania* sp., *Leucolejeunea uncioloba*, *Clasmatodon parvulus*, and *Campylophyllum hispidulum* were the most common and most abundant species sampled. *Porella pinnata* and *Forstroemia trichomitria* were present within our data set, but were not as common as Huston's study indicated (2007).

Huston (2007) also found two epiphytic liverworts and 2 epiphytic mosses not found in our study. We collected 3 leafy liverworts and 7 mosses not included in Huston's epiphytic bryophyte list (Huston 2007). Differences between findings are likely due to sampling and habitat differences between the two studies. Our

sampling focused heavily on the wet mesic stream banks and slopes in which *C. caroliniana* grows, while Huston included other ecotypes and plant communities. For an accurate assessment of the entire epiphytic community of bryophytes within East Texas the epiphytic community structure needs to be investigated for other ecotypes.

Prescribed burning appears to have an effect on bryophyte diversity. Samples collected across Davy Crockett sites were exposed recently to prescribed burns. Site D1807B had fires that reached into the sampling site with trunks and bryophytes showing evidence of the burn. Although surrounded by the prescribed burn, site D1825 did not show evidence of fire along the trees we sampled. Trees at site D1825 had a higher bryophyte richness, diversity, and evenness than those sampled at D1807B (Tables 7 and 12).

The average species richness interaction varied significantly between site and bryophyte type within the Davy Crockett sites. The moss richness of site D1825 is statistically higher than its leafy liverwort richness, but the moss richness of D1825 is not higher than the leafy liverwort richness of D1807B (Figure 11). However, D1825 has a higher overall bryophyte, leafy liverwort, and moss richness, diversity and evenness (Table 12; appendix A) when compared to the samples collected from D1807B. Species abundances differ markedly between the sites. *Frullania brittoniea*, *Frullania eboracensis*, *Frullania inflata*,

Clasmatodon parvulus, and *Homomallium adnatum* have higher abundances at site D1807B. *Cololejeunea minutissima* ssp. *myriocarpa*, *Porella* sp. *Cryphaea glomerata*, *Forsstroemia trichomitria*, and *Isopterygium tenerum* had a lower abundances or were completely absent from D1807B.

Sampling at the Davy Crockett sites coincided with prescribed burning. Site D1825 had fire surrounding, but not within, the location. Site D1807B had fire reaching within the sampling location, scorching the trees and bryophytes. Unfortunately the burn and site variables are confounded and the significance of burning could not be determined from our sample set. The effect of prescribed burning on bryophyte composition in east Texas forest remains to be tested.

Prescribed fire has been used across many sites to help increase vascular plant species diversity. Our data indicate that burning may have a complex effect on epiphytic bryophyte communities. Variables may include proximity to the burn (whether the burn reaches into the sampling area) and length of time elapsed since the burn. Other variables may include height of the sample along the tree, host tree species, and vascular plant community type. More studies need to be conducted comparing bryophyte diversity of burned sites to non-burned sites in order to determine the effects of burning on epiphytic bryophyte communities.

Conclusion

In conclusion, *C.caroliniana* has a greater cover and species richness for leafy liverworts than that of the *Non-Carpinus* sampled. The *Non-Carpinus* trees sampled have a greater coverage and richness of mosses than that of the *C.caroliniana* sampled. To determine if bark type (rough vs. smooth) has an effect on bryophyte community composition more samples would need to be collected. To determine any host specificity to bryophytes within east Texas more studies would need to be conducted to ensure tree species across many community types were sampled. The effect of prescribe burn on epiphytic bryophyte communities needs to be tested within the forest regions of east Texas.

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APPENDIX

Appendix A. Total bryophyte species abundance for each site. Data was collected spring 2018 from *C.caroliniana* and *Non-Carpinus* across six sites in East Texas. Species were identified in the lab using cell structure characteristics.

	AZ13	AZ23	D1807B	D1825	S5115	S13010
comi	0	0	0	0	25	0
comi2	0	1	0	0	0	0
comy	0	0	0	47	0	0
frbr	9	16	24	0	0	0
freb	95	206	117	72	2	54
frin	82	60	187	39	99	51
frku	14	1	0	0	0	0
frob	0	0	0	0	0	0
frsq	0	0	10	23	13	0
jaau	0	18	0	0	0	0
lela	0	0	0	0	0	0
lecl	0	0	0	0	0	0
leco	14	33	0	0	0	0
leun	2	107	28	97	132	190
popi	0	266	0	80	0	24
popl	0	44	0	14	0	0
rema	0	7	0	0	0	0
raau	0	0	0	0	0	9
cach	18	0	0	0	0	0
cahi	25	139	18	28	20	104
clpa	172	19	104	55	0	72
crgl	26	0	0	71	0	40
crne	0	6	0	0	0	0
fotr	3	156	0	63	0	0
hosu	0	21	0	0	36	0
hoad	0	0	70	21	0	0
hyim	0	0	1	0	0	0
iste	0	0	0	55	0	0
lebr	0	0	0	0	0	0
leju	136	494	738	808	131	536
plre	4	0	0	0	0	0
thhi	0	0	0	0	337	13

VITA

While completing her Bachelor degree at Stephen F. Austin State University at Nacogdoches, Texas Cassey immersed herself into the study of Forestry. Being provide all opportunities for success she was able to graduate University Scholar in May of 2016 with her Bachelor of Science in Forestry. Entering directly into the Graduate School of Stephen F. Austin State University she was able to further expand her experience with instructing undergraduate laboratory class by being a Teaching Assistant in the department of Biology. Upon graduation in December of 2018 she will continue towards obtaining her PhD in Forestry at Stephen F. Austin State University, expanding upon the current knowledge and understanding of the bryophyte community of east Texas.

Permanent Address: 516 E. Lamar
 Palestine, Texas 75801

Style manual designation APA

This thesis was typed by Cassey Edwards.