URBAN ECOLOGY IN NORTH TEXAS:
NATIVE PLANTS AND NITROGEN DEPOSITION

by

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DISSERTATION

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ABSTRACT

Urban Ecology in North Texas:
Native Plants and Nitrogen Deposition

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Globally, the human population continues to grow and move into urban areas causing a range of effects on local ecosystems. Elevated temperatures, altered hydrology, and higher concentrations of atmospheric pollutants in urban environments create novel conditions for resident organisms. My research focused on one particular aspect of human alteration to urban environments – nitrogen (N) deposition – and its effects on native plants across an urban to rural gradient in the southern Great Plains, North Texas (NTX), USA.

Nitrogen oxides (NO_x) – the product of combustion and precursor to nitrate deposition in precipitation – were significantly positively correlated with impervious surface area, a measure of urban development, in NTX. Dissolved inorganic nitrogen deposition (composed of nitrate and ammonium) was also greater in the developed parts of NTX than in the surrounding rural area. Bulk N deposition in NTX was dominated by ammonium suggesting that fertilizers are likely an important source of N in rural and urban environments.

Native plant responses to these differences in N deposition were subtle. Naturally occurring native post oak trees (*Quercus stellata*) had similar tissue quality (carbon:nitrogen ratio) across the gradient. Little bluestem (*Schizachryium scoparium*) and Texas wintergrass (*Nasella* *leucotricha*), native perennial grasses, grew similarly when planted at six sites along the gradient. However, little bluestem plants taken from urban and rural remnant prairie sites and grown together in a common garden differed in some plant characteristics, suggesting genetic divergence among these populations.

Native plants provide ecosystem services including cooling, carbon sequestration, erosion control, flood mitigation, and human health benefits. Because NTX is one of the most rapidly expanding urban areas, understanding the impacts of anthropogenic N deposition on native plants is crucial to maintaining ecosystem integrity in the southern Great Plains.

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Thank you, truly.

Sincerely,

Michelle L. Green, Ph.D.

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CHAPTER 1
INTRODUCTION

Urban Ecology

With the global human population recently surpassing 7 billion, more of that population is moving to urban centers. Today, more than 54% of people live in urban areas across the globe, and in the United States (US) more than 75% of the population resides in urban areas (United Nations 2014). These numbers are only projected to increase, yet urban areas are among the least understood of all ecosystems (Grimm et al. 2000, Pickett et al. 2001).

Traditionally, ecologists have studied ecosystem processes without incorporating humans as active participants (Grimm et al. 2008a). However, with a growing global population, more than 83% of the earth's surface bears the imprint of our activities (Sanderson et al. 2002). It is now no longer possible to ignore the impact humans have on the Earth's ecosystems, especially in areas of concentrated human activity. The relatively young field of urban ecology began in the 1970s with a workshop hosted by the Institute of Ecology. Ecologists from a variety of disciplines identified urban issues and published the results as a book, The Urban Ecosystem: A Holistic Approach (Stearns and Montag 1974). However, it was not until the 1990s that the focus shifted to ecology *of* cities in the United States, most notably with the beginning of the Baltimore and Phoenix Long Term Ecological Research (LTER) sites in 1997.

While the importance of urban studies has become widely accepted, the definition and measurement of "urbanness" has not. As the field of urban ecology grew, definitions of "urban" were borrowed from sociologists, geographers, and economists (McDonnell et al. 1997). For example, the United Nations defines an urban area in the US as “an agglomeration of 2,500 or more inhabitants, generally having population densities of 1,000 persons per square mile or more” (United Nations 2014). The problem with definitions such as this is that the cut offs are arbitrary and not necessarily ecologically relevant. The term “urban” can be used more broadly to refer to areas under human influence, though this definition is not without problems as it could apply to most of the Earth. Thus far, there is no unified, accepted definition of “urban” (Wu 2014). In urban ecology studies, common measures of “urbanness” include distance from the city center, population density, and physical characteristics such as percent impervious surface area (ISA) and road density (Short Gianotti et al. 2016).

A clear definition of “urban" is important in understanding ecosystem nutrient cycling and the role cities will play in future climate change scenarios. Raciti and colleagues (2012) found that measures of urban C and N pools changed dramatically depending on the metric used to define urban areas. After examining metrics of urban development including population density, land-use category, ISA, and distance to urban core, Raciti et al. (2012) determined that the most promising metrics reflect the degree of human modification to the landscape, e.g. ISA. Distance-based transects fail to take into account the complexity and heterogeneity of fragmented urban areas. US Census population data are inaccurate because census blocks vary widely in area, and population density data are not useful because non-residential land uses have a value of zero. Political boundaries leave out potential areas of ecological interest and are bound by historical legacies not applicable beyond that city. Similar to the results of Raciti et al., Rao and colleagues (2013) found that ISA and proximity to urban core were good predictors of anthropogenic atmospheric nitrogen (N) inputs. The field of urban ecology has yet to settle on a standard, ecologically relevant metric of "urbanness," but it is clear that it must take into account the degree of alteration by humans.

Research at urban LTER sites and in cities across the globe has revealed quite a bit about the effects of human activities on urban ecosystem processes and patterns. First and foremost, urbanization directly transforms landscapes through changes in land use and fragmentation of natural landscapes. These direct changes have affected biodiversity, ecosystem productivity, and biogeochemical cycling through the loss of native biodiversity and introduction of exotic species (Hope et al. 2003). Urbanization has also indirectly affected the urban ecosystem by altering the local climate and atmosphere of cities. Cities are warmer than the surrounding land, most notably at night and in the winter (Jones and Groisman 1990, George et al. 2007); this phenomenon has been dubbed the "urban heat island." The main contributing factors are the high thermal conductivity of buildings, the low albedo of manmade structures, low evapotranspiration, and the heat released directly from buildings and vehicles (George et al. 2007). Cities also have elevated levels of carbon dioxide (CO_2), mostly resulting from the combustion of fossil fuels in vehicles (Idso et al. 1998, Idso et al. 2002, Koerner and Klopatek 2002, Wentz et al. 2002, George et al. 2007). Fossil fuel combustion is also responsible for the release of a cocktail of pollutants including nitric oxide, nitrogen dioxide, ammonia, nitrous acid, carbon monoxide, carbon dioxide, volatile organic compounds, polycyclic aromatic hydrocarbons, metals, and contributes to the formation of ozone (Bignal et al. 2004). Taken together, these conditions result in significant microclimate differences between urban and rural areas (George et al. 2007), which have the potential to affect the growth, tissue quality, and reproduction of native plants. My research explores the effects of the urban environment on native plants with special emphasis on the effects of anthropogenic N additions.

Nitrogen Cycle

While the Earth's atmosphere is made up of 78% N_2, this inert N is not available to plants. In order for N to be useful to plants, the N must be "fixed" or turned into reactive N (Nr) by either nitrogen-fixing bacteria, lightning, or man-made chemical reactions (Canfield et al. 2010). The natural production of Nr (mostly from biological fixation by cyanobacteria) has remained relatively stable at 125 Teragrams of nitrogen per year (Tg N/yr), but human activities have increased global reactive nitrogen production to over 300 Tg N/yr (Galloway et al. 2008). Two-thirds of the anthropogenically created Nr comes from the production of fertilizer and other industrial activities, while the remaining input comes from the increase in cultivation-induced biological fixation and the combustion of fossil fuels (Galloway et al. 2008). Most anthropogenically created N flows through the agricultural food chain, but human activities also generate N molecules that are released directly into the atmosphere (Galloway et al. 2003). These atmospheric N compounds may be deposited to the earth dissolved in precipitation (wet deposition) or directly as particles and gases (dry deposition; Ponette-González et al. *In press*), a process known as N deposition. The deposition of atmospheric N represents an additional source of N to ecosystems and is most pronounced in areas of concentrated human activity (Baker et al. 2001, Groffman et al. 2004).

The two main types of anthropogenic N inputs to the atmosphere are nitrogen oxides (NO and NO_2, collectively NO_x) and reduced forms of N (NH_3 and NH_4^+, collectively NH_x; Galloway et al. 2004). The oxidized forms of N are a byproduct of the combustion of fossil fuels in industry and vehicles and the reduced forms are produced as a result of the volatilization of NH_3 from fertilizer and animal waste (Reis et al. 2009). The deposition of these N compounds occurs primarily as NO_3^- (nitrate) and NH_4^+ (ammonium), which is readily available to plants for incorporation into biomass (Aber and Melillo 2001). Ammonification is the process in which NH_4^+ is formed during the decomposition of biomass. Some soil bacteria oxidize NH_4^+ into NO_3^-, a process known as nitrification. Some NH_4^+ and NO_3^- is immobilized for use by the bacteria, and some is returned to the soil water solution, where NO_3^- can be leached from the system via water transport. Denitrifying bacteria reduce NO_3^- to inert N_2, which then returns to the atmosphere.

Anthropogenic NO_x emissions are not only deposited to the earth via deposition, however. They are also involved in complex chemical reactions that impact plant and animal health. Once in the atmosphere, the photolysis reaction of NO_2 yields NO and atomic oxygen (O), which then reacts with O_2, forming ozone (O3; Fabian et al. 2014). The products of these two reactions (NO and O_3), then react with each other again via the “titration reaction” to yield NO_2 and O_2, thereby removing ozone from the atmosphere. The presence of volatile organic compounds in the atmosphere, however, also allows for the accumulation of ozone in the atmosphere by bypassing the titration reaction in the production of NO_2. In areas where the production of O_3 is limited by VOCs rather than NO_x (such as urban areas), increases in NO_x tend to result in a decrease in O_3. This is because anthropogenic NO_x, emitted largely as NO, goes into the ozone-consuming “titration reaction” which reduces O_3. In addition to this cyclic reaction, NO_x can be removed from the regional atmosphere via the reaction of NO_2 with OH^- to form HNO_3 which is deposited to the earth in precipitation or by the conversion of NO_2 to peroxyacetyl nitrate (PAN) which acts as a temporary reservoir for NO_x as it moves downwind (Barker 1995).

The National Atmospheric Deposition Program (NADP), in collaboration with the National Trends Network (NTN) has been monitoring N deposition across the United States since 1981. Nitrate constitutes the major form of nitrogen in deposition and ranged from 1-15 kg/ha across the United States in 2010 (National Atmospheric Deposition Program 2011). However, the NADP/NTN monitoring sites are specifically located away from urban areas and point sources, resulting in a potential underestimation of the nitrogen deposition in and around cities. Areas downwind of urban centers in the western United States have deposition rates as high as 30-90 kg N/ha/yr (Fenn et al. 2003).

Effects of an Altered Nitrogen Cycle

In ecosystems where nitrogen is limiting, changes in N availability have the potential to affect both plant and animal inhabitants. In plants, increases in available Nr can affect growth, biomass allocation, C:N ratio, and the production of secondary metabolites (Chapin 1980, Reich et al. 2003, Yang et al. 2011c), though the magnitude and direction of these responses is highly variable (Pan et al. 2010). Increases in Nr availability have been shown to negatively affect plant biodiversity, with a particular reduction in the number of native plant species (Vitousek et al. 1997). These changes in plants in turn affect insect herbivore communities. Increases in plant tissue quality (in the form of increased foliar N and decreased C:N ratio) have been shown to have a positive effect on individual insects and insect abundance (Throop and Lerdau 2004). However, both insect and plant species richness can be negatively affected by increased N deposition (Haddad et al. 2000). With the potentially far-reaching effects of N deposition on ecosystem patterns and processes, there is a need for greater understanding of deposition patterns and the resulting ecological effects.

Urban Ecology in North Texas

My research was conducted in North Central Texas, centered around the Dallas-Fort Worth Metroplex (DFW). DFW was identified by Nancy Grimm and colleagues (2008b) as one of seven “megapolitan” regions in the United States that will play a crucial role in understanding ecosystem responses across climatic and societal gradients. It is the largest megapolitan region within the Great Plains ecoregion, and the combination of rapidly growing urban area and undeveloped rural area within close proximity makes it an ideal research site for urban studies. Most of DFW lies within the Cross Timbers and Prairies ecoregion. The prairie component of this ecoregion is the Grand Prairie, a southern extension of the Great Plains that consists mainly of shallow soil underlain by limestone. Late-successional communities are characterized by little bluestem (*Schizachyrium scoparium*), big bluestem (*Andropogon gerardii*), Indiangrass (*Sorghastrum nutans*), switchgrass (*Panicum virgatum*) and various perennial forbs (Dyksterhuis 1946). The Cross Timbers are dominated by post oak (*Quercus stellata*) and blackjack oak (*Quercus marilandica*) with an understory of shrubs and grasses (Griffith et al. 2007). The DFW region began to be settled in the mid-19th century, with ranching as the primary economic activity (Smeins 2004). The first major period of growth in the cities of Fort Worth and Dallas occurred in the late 1880s, though significant growth in the intermediate cities did not come until a half century later (Vision North Texas 2008). Since that time, the DFW area has continued to expand rapidly such that in 2001, more than half the land in Dallas and Tarrant counties had been developed for urban use (Vision North Texas 2008). As a consequence, only small remnant patches of the natural landscape remains.

My dissertation research investigated anthropogenic additions of N into North Texas urban ecosystems and relationships between these inputs and growth of native plants. To do this, I conducted the following experiments and observational studies:

> Chapter 2. A survey of atmospheric NOx, soil N, and plant characteristics of the long-lived post oak tree (*Quercus stellata*) along an urban to rural gradient

Chapter 3. A common garden investigation into urban and rural ecotypes of the dominant prairie plant, little bluestem (*Schizachyrium scoparium*)

Chapter 4. A phytometer experiment in which I quantified bulk N deposition in North Texas and used little bluestem and Texas wintergrass (*Nasella leucotricha*) to measure impacts on plant growth

CHAPTER 2

URBAN DEVELOPMENT IN THE SOUTHERN GREAT PLAINS: EFFECTS OF ELEVATED ATMOSPHERIC NOx ON THE LONG-LIVED POST OAK TREE (*QUERCUS STELLATA*)

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Abstract: Concentrated human activities such as the burning of fossil fuels have resulted in chronic nitrogen (N) additions to urban ecosystems. Anthropogenic N inputs to N-limited ecosystems generally result in increased soil N levels, plant tissue quality (lower C:N ratio), and herbivory. Until now, these relationships remained unexamined in the largest “megapolitan” urban area of the Great Plains ecoregion. This survey investigated the influence of chronic inputs of nitrogen oxides (NO_x) on these relationships using a long-lived native tree - post oak (*Quercus stellata*). Eleven sites across North Texas (NTX), USA were selected for our study conducted in late spring 2014. For each site, a “percent urban development” metric was calculated based on impervious surface area, and data from 16 air monitoring stations were used to calculate distance-weighted estimates of atmospheric NO_x. Post oak trees were sampled for leaf tissue quality, herbivory estimates, and tree size. Soil samples were collected for chemical analysis. The urban development metric that was calculated was a good predictor of atmospheric NO_x levels. Contrary to the authors' expectations, there were no correlations between atmospheric NO_x and soil N. There was, however, a positive relationship between soil N and leaf tissue quality but only when atmospheric NO_x levels were relatively low. Herbivory was not correlated with leaf tissue quality, but percent urban development may have an influence on herbivory rates in other ways. Further investigation is needed to better understand the relationships between human activities and the movement of N through urban ecosystems in the Great Plains.

Keywords: soil nitrogen, leaf tissue quality, C:N, herbivory, urban development, NO_x

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Introduction

With the world population growing and urban areas expanding, more and more native landscape is being transformed to suit human needs. If current trends in population growth and density continue, urban land cover will increase 1.2 million km^2 by 2030, which is triple the urban land area in 2000 (Seto et al. 2012). In urban areas, concentrated human activities result in habitat fragmentation, altered hydrology, elevated temperatures, greater concentrations of carbon dioxide (CO_2) and ozone (O_3), and altered nutrient cycles (Pickett et al. 2011). All of these alterations to the landscape can negatively impact native flora and fauna, though one of the most devastating is the addition of nutrients to a nitrogen (N) -limited landscape (Bobbink et al. 2010, Pan et al. 2010).

Biologically available N is produced naturally (via lightning and biological nitrogen fixation) at a rate of about 125 Tg N per year. Through various activities humans introduce an additional 300 Tg N per year (Galloway et al. 2008). In urban areas, biologically available nitrogen is added primarily through the combustion of fossil fuels. The combustion of fossil fuels in automobiles and industry result in the creation of nitrogen oxide byproducts (NO and NO_2, collectively NO_x). These NO_x compounds are released into the atmosphere and may return to the ground in gaseous form, as dry particles, or most commonly as ions in precipitation - a process known as N deposition. Atmospheric NO_x inputs to urban areas enter an already complex nutrient cycle, further complicated by human activities and the interactions between human activities and climate. This may result in an urban N cycle drastically different from the surrounding rural area.

In N-limited terrestrial ecosystems, plant communities that receive additional N inputs generally exhibit greater overall productivity (Vitousek et al. 1997, Lebauer and Treseder 2008). At the organismal level, increases in N availability increase plant growth rate, reproductive output, tissue quality (lower C:N ratio), and palatability to herbivores (Chapin 1980, Yang et al. 2011b). However, greater nutrient availability can also change competitive relationships among species, resulting in a loss of biodiversity - particularly in native flora (Bobbink and Roelofs 1995, Aronson et al. 2014). Thus nitrogen additions may affect plant species and communities in complex and conflicting ways. Thus far, our understanding of plant responses to N additions mostly comes from experiments in which large N pulses are added. Much less is known about plant responses to the chronic low-level N inputs associated with urban areas (Bedison and McNeil 2009).

Plant responses to N are also mediated (or co-limited) by aspects of climate such as temperature and precipitation (Lebauer and Treseder 2008, Shen et al. 2008, Xia and Wan 2008, Yang et al. 2011a). Thus, plant responses to N additions will vary across different biomes. Most of the U.S. research on plant responses to urbanization has come from two Urban Long-Term Ecological Research (urban LTER): Phoenix, AZ and Baltimore, MD. The Phoenix Metropolitan Statistical Area (MSA), has a population of just over 4 million (U.S. Census Bureau 2011), and is located in the xeric Sonoran Desert ecoregion. Baltimore MSA has a population of just under 3 million (U.S. Census Bureau 2011), and is located in a humid, temperate forest. A handful of coastal cities including Boston, MA and Seattle, WA are also contributing to the breadth of urban ecosystem studies, but there is a distinct paucity of information for urban ecosystems in the Great Plains ecoregion. Understanding interactions between climate and anthropogenic N additions will become increasingly important as climate change brings even more extreme temperatures and weather events to the Great Plains (Melillo et al. 2014).

North Texas, the metropolitan area encompassing the cities of Dallas and Fort Worth (henceforth NTX), occupies over 24,000 square kilometers in the southern Great Plains ecoregion. NTX has a rapidly expanding population of over 7 million (U.S. Census Bureau 2016) and has been identified as an area of urban development ripe for exploring the impacts of urbanization (Grimm et al. 2008b). Despite this, there has been very little investigation into the effects of concentrated human activities on this biome.

We explored the effects of chronic atmospheric NO_x inputs on a long-lived native tree, post oak (*Quercus stellata*), across a gradient of urban development in NTX. By sampling soil and trees located in areas of differing urban development and comparing them to hourly atmospheric NO_x concentrations, we were able to test the following hypotheses:

1. Atmospheric NO_x will increase along a gradient of increasing urban development
2. Increased atmospheric NO_x will be positively correlated with:
    a. elevated soil nitrogen (via N deposition)
    b. increased tissue quality (lower leaf C:N ratio via greater soil N availability)
    c. increased herbivory of leaf tissue (via increased palatability)

To the best of our knowledge, this is the first study to examine the effects of urban N additions in a city of the southern Great Plains.

Methods

Study Area

The area surrounding the Dallas-Fort Worth metropolitan area belongs to the "Cross Timbers Climate Division," a sub-tropical and sub-humid mixed savanna and woodland (National Oceanic and Atmospheric Administration 2016). Post oak (*Quercus stellata*) and blackjack oak (*Quercus marilandica*) dominate the overstory of the Cross Timbers, with an understory of shrubs and grasses. Where there are stream bottoms, trees such as bur oak (*Quercus macrocarpa*), Shumard oak (*Quercus shumardii*), and sugar hackberry (*Celtis laevigata*) dominate. The sites in our study have a fifty-year average of 9.2cm of precipitation (site averages range from 8.4-10.7cm) and a fifty-year mean temperature of 18.3°C with site averages ranging from 17.8-18.8°C. The region is drained by the Trinity River and its forks. Very little of the natural ecoregions remain because of cattle grazing, conversion to cropland, and urbanization (Griffith et al. 2007).

The first major period of population growth in NTX occurred in the late 1880s and a second rapid development period began in the 1960s (Vision North Texas 2008). The pattern of development in NTX is such that the city-centers of Dallas and Fort Worth were the earliest settled and are now the most densely populated. The percentage of land dedicated to urban development decreases in all directions with increasing distance from the city-centers.

Study Species and Experimental Design

Post oaks were chosen as the subject of this study because of their ubiquity and status as the dominant tree in the region. Post oaks are a slow-growing deciduous species, and remnant forests in NTX have an age range of 200-300 years old (Diggs et al. 1999). The post oaks of the NTX region are well adapted to the poor soil and periods of extreme drought that occur.

Survey sites were chosen a priori from publicly accessible parks along and near the Trinity River. Nineteen sites were identified as possible survey sites, and eleven were chosen based on the presence of five or more post oak trees. The sites represented a range of urban development (see section below) with less urban sites on both the eastern and western sides of the metropolitan region (Fig. 2.1).

Between May 28, 2014 and June 13, 2014, five post oak trees were haphazardly chosen from an undeveloped area of each site based on ease of access. For each tree, diameter at breast height (DBH) was measured, and loose litter (Oi layer) was removed from an area near the base of the tree so that a 10cm long x 5cm diameter PVC pipe could be used to obtain a soil sample. The lowest main branch of the tree was identified, and from it, a small branch was removed. To estimate herbivory, five leaves were randomly selected from the branch and photographed on grid paper to be analyzed later. An additional five leaves (with no or minor herbivory) were randomly selected from the same branch for use in quantifying tissue quality (C:N ratio).

To calculate our estimate of herbivory, the leaf photographs were opened in Adobe Photoshop V 14.1.2. The outline of the leaf as it was in the field was traced and the area converted from pixels to cm^2. The remaining leaf tissue was used to hand draw a full leaf shape based on a generalized post oak leaf template scaled to appropriate size. The total area of the estimated original leaf was divided by the actual leaf area and subtracted from one hundred to estimate percent herbivory.

Soil samples were refrigerated at 3°C before being sent to Texas A&M Soil, Water, and Forage Testing Lab (College Station, TX) for analysis of pH, KCl-extractable nitrate-N (NO_3-N) and ammonium-N (NH_4-N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and sodium (Na). Leaf samples were dried in a 50°C oven for one week before grinding and analysis of percent C and percent N using a Perkin Elmer CHN series 2400 analyzer.

Measures of Atmospheric Nitrogen and Urban Development

The Texas Commission on Environmental Quality (TCEQ) has established atmospheric NO_x monitoring sites across North Texas (Fig. 2.1). Because NO_x concentrations (ppbv) are positively correlated with N deposition (Butler et al. 2003, Likens et al. 2005, Elliott et al. 2007, Redling et al. 2013), atmospheric NO_x was used as a proxy for N deposition in this study. Hourly NO_x concentration data were obtained from the TCEQ's on-line database (TCEQ 2015a) for the period of 01 January 2013 to 31 December 2013 (the most recent full year of data prior to the study). Atmospheric NO_x data from 2011 and 2012 were also examined, and matched very closely with 2013, thus the 2013 data are considered to be representative for the region and are the only data presented here. An inverse distance weighting formula was used to calculate the estimated hourly NO_x values in parts per billion by volume (ppbv) for the survey sites based on all available TCEQ air monitoring sites within a 40km radius. The formula was as follows:

$$P_i = \frac{\sum_{j=1}^{G} P_j \,/\, D_{ij}^{n}}{\sum_{j=1}^{G} 1 \,/\, D_{ij}^{n}} \tag{2.1}$$

where (P) represents estimated NO_x values for each survey site (i), G is the number of monitoring stations, Pj is the NO_x values of monitoring station at location j, and Dij is the distance between the monitoring station j, and survey site, i. In this equation n=2 - a constant used by NOAA for determining missing rainfall measurements. For the KSP survey site, only one air monitoring station was within 40km (3km away) and consequently was the only value used. For the MWP site, there were no data from the closest TCEQ monitoring site, therefore, the next closest site (56km away) was used as the sole data source. The 2013 hourly values were averaged into monthly values to examine seasonal trends, and each hour of the day was averaged across 2013 to examine diurnal trends.

The "percent urban development," metric was calculated using the U.S. Department of the Interior's 2011 National Land Cover Database (NLCD; Homer et al. 2015). The NLCD urban land-use categories are calculated based on percent impervious surface area (ISA). The NLCD categories "Developed High Intensity," "Developed Medium Intensity," and "Developed Low Intensity" represent ISA values of 80-100%, 50-79%, and 20-49%, respectively. For a set radius around each site, the area of these categories were summed into one value and divided by the total area of that radius to calculate "percent urban development." This was done for each survey site at a 1k, 3k, 5k, 10k, 15k, and 30k radius to find the best determination of urban development. The same calculations for percent urban development were made for each of the TCEQ monitoring sites.

Statistics

All statistical analyses were conducted using R 3.1.2 (R Core Team 2014).

The percent urban development value for each site was regressed against the estimated atmospheric NO_x. This was repeated for each of the radii listed to evaluate which radius was the best indicator of urban development. This analysis was also conducted at all radii for the measured atmospheric NO_x at the TCEQ monitoring sites to determine the accuracy of our estimated NO_x values.

Site averages were calculated for each variable examined, and percent herbivory was arcsine square-root transformed. A Pearson's R correlation matrix was constructed using R package *psych* (Revelle 2014) to examine relationships between relevant soil characteristics (NO_3-N, NH_4-N, and pH), site characteristics (percent urban development and hourly NO_x) and plant characteristics (DBH, leaf C:N, and percent herbivory). Soil NO_3-N and soil NH_4-N were summed to create the variable "soil N" as an indicator of total available soil N and used in the remainder of the analyses.

For the response variables leaf C:N and leaf herbivory, the effects of multiple predictor variables and their potential interactions were examined. The multiple linear regression was conducted in R and the significance of terms was determined using Type III sums of square from function "Anova" in R package *car* (Fox et al. 2009). For leaf C:N ratio, we started with a model that included NO_x, soil N, and the interaction between NO_x and soil N, as well as the covariates DBH and soil pH. Soil pH and DBH were included as covariates because of their influence on nutrient availability and primary productivity, respectively (Aber and Melillo 2001). We then used automated stepwise model section using exact AIC as the model selection criterion (function "step AIC" in R package MASS; Venables and Ripley 2002). This eliminated nonsignificant terms DBH and soil pH from the model, leaving us with a final model that included only soil N, NO_x, and their interaction.

For leaf herbivory, we used a similar multiple linear regression process as with leaf C:N using the predictor variables leaf C:N, percent urban, and their interaction, along with tree DBH as a covariate. Using the same stepwise process, tree DBH was eliminated. The final model included leaf C:N, percent urban, and their interaction.

Results

NO_x: Spatial and Temporal Patterns

Overall, the relationship between urban development and estimated atmospheric NO_x was positive and linear, though the strength of the relationship varied depending on the radius of the area calculated. At radii of 3k, 5k, 10k, 15k, and 30k, the relationship between NO_x and urban development was highly significant ($p<0.001$), with R^2 values of 0.35, 0.58, 0.69, 0.66, and 0.62 respectively. The strongest relationship was found at the 10k radius (adjusted $R^2=0.69$, $F(1,53)=122$, $p<0.001$; Fig. 2.2), thus we used the percent urban development at the 10k radius as our metric of urban development for the remainder of the analyses.

The measured atmospheric NO_x at the TCEQ monitoring sites was also highly correlated with the percentage of urban development at the 10k radius (adjusted $R^2=0.61$, $F(1,12)=18$, $p<0.001$), confirming the relationships documented for the survey sites.

The overall hourly average of atmospheric NO_x in 2013 for each of the sites ranged from 4 to 12.9 ppbv (Table 2.1). Relative to each other, sites followed the general trend of greater urban development having greater atmospheric NO_x with the exception of two sites: the median atmospheric NO_x value belonged to RLP - the site with the highest urban development - and the fourth highest atmospheric NO_x value belonged to the eighth most urban site – FWN (Table 2.1, Fig. 2.2).

From an hourly perspective, atmospheric NO_x peaked at hour 7, coinciding with morning rush hour traffic (Fig. 3). A second longer lasting, but lower magnitude increase began at 17 hours (coinciding with evening rush hour), peaked around 21 hours, and decreased to the morning low by hour 1. The lowest hourly NO_x values were found during the hottest part of the day from 13-16 hours. The intensity of the daily peaks was for the most part dependent on the percentage of urban development for each site. For example, the diurnal range in hourly atmospheric NO_x averages for EMP, the site at the 1st quartile of urban development (henceforth used as an example of a low urban development site), was 9.4 ppbv while the site at the 3rd quartile of urban development, MCP (henceforth used as an example of a high urban development site), had a range of 14.2 ppbv in hourly average NO_x values. Throughout the day, atmospheric NO_x values at the less urban sites fell below the value of the median urban development site (LLA = 41.8 % urban development), and the more urban sites had values above the median site. However, as noted above, FWN, ranked eight out of eleven in urban development, did not follow this trend, and tended to have one of the highest hourly atmospheric NO_x values (Fig. 2.3).

Seasonally, the highest atmospheric NO_x values occur during the colder winter months, while the lowest occur during the warmer summer months (Fig. 2.4). The seasonal pattern of NO_x levels follows the hourly pattern in that the more rural sites are in the lower half of atmospheric NO_x values while the more urban sites are in the upper half. The one exception again is the FWN site, which had a lower urban development value, but atmospheric NO_x values above those of the median site (Fig. 2.4). The magnitude of seasonal changes in average hourly atmospheric NO_x is again closely related to the percentage of urban development for each site. EMP had an average hourly low of 4.7 ppbv in June and a high of 14.0 ppbv in December, while MCP had an average hourly low of 5.4 ppbv in June and 21.4 ppbv in December (Fig. 2.4).

Soil Characteristics

The average soil pH for all sites was 7.6 ±0.1, and all sites had alkaline soil. Soil pH was not correlated with atmospheric NO_x, soil NO_3-N, or soil NH_4-N. The averages for soil NO_3-N and soil NH_4-N for all sites were 13.2 ±0.4 and 9.3 ±1.4 ppm, respectively. For combined soil N, EMP (low urban) fell below the mean and MCP (high urban) above the mean, however, there were no clear trends across the sites (Table 2.1). Soil Ca, Mg, S, and Na were also measured at each site in addition to pH, but there were no significant relationships between them or with the other measured variables (data not shown). There was no relationship between soil N and atmospheric NO_x across the sites (Table 2.1; Fig. 2.5).

Plant Characteristics

Site averages for DBH ranged from 31 - 71 cm (Table 2.1). The westernmost sites tended to have the smallest diameter trees (Fig. 2.1; Table 2.1), but there was no statistical relationship between longitude and DBH. There was also no relationship between DBH and leaf tissue quality.

Leaf C:N ratio did not vary widely across the study sites. The average for all sites was 26.0 ±0.4 with EMP (low urban) and MCP (high urban) both at a C:N ratio of 27 (Table 2.1). There were no significant correlations between leaf C:N and other measured variables, however, the multiple linear regression revealed a significant interaction between atmospheric NO_x and soil N (Table 2.2). At low levels of atmospheric NO_x, leaf C:N and soil N were negatively correlated, indicating that tissue quality increased with increasing soil N (Fig. 2.6). At high levels of atmospheric NO_x, leaf C:N and soil N were positively correlated, indicating that tissue quality decreased with increasing soil N (Fig. 2.6). We ran the same linear regression model with percent urban development in place of NO_x, and there was no relationship.

Herbivory

The average leaf herbivory was 7 ±0.0%, and the majority of sites had herbivory levels of less than 10%. The two exceptions to this were MTC (13%) and RLP (20%), the two most urban sites (Table 2.1). The representative low urban site had lower percent herbivory than the representative urban site (EMP= 5% and MCP=8%, respectively), however, there were no clear trends in herbivory across the entire gradient. We did not see a relationship between herbivory and atmospheric NO_x or any of the other measured variables (Fig. 2.5).

Discussion

Atmospheric NO_x increased along a gradient of increasing urban development

Hourly atmospheric NO_x levels were significantly positively correlated with urban development at five of the six radii that we examined, though the strongest relationship was found at the 10k radius. The high correlation between the NLCD metric of urban development and atmospheric NO_x indicates that the ISA-based metric of urban development is a good predictor of NO_x concentrations in NTX.

Unsurprisingly, the daily rise and fall of atmospheric NO_x closely followed morning and evening rush hour traffic (Fig. 2.3), as approximately 50% of NO_x emissions in NTX were attributed to vehicular sources in 2015 (TCEQ 2015b). As in other US cities, the decrease in atmospheric NO_x after morning rush hour likely occurred because NO_x is a precursor for the formation of ozone (Qin et al. 2004, Song et al. 2011). As atmospheric NO_x production and solar radiation increase during the day, the photochemical reactions that produce ozone increase and NO_x is consumed in the reaction (Fabian et al. 2014). As the evening rush hour occurred, NO_x

concentrations again increased, though the peak was smaller in the evening due to greater solar radiation and photochemical reactions in the afternoon (Fig. 2.3).

The seasonal variation in atmospheric NO_x in NTX also followed the patterns of NO_x in US and other global cities (Zhang et al. 2003, Vellingiri et al. 2015a, Xie et al. 2016) with lower hourly averages in the summer and the highest hourly averages in the winter (Fig. 2.4). Higher winter atmospheric NO_x relative to summer levels may be attributed to a combination of factors including lower solar radiation and less photochemical activity, increased consumption of fuels, and poorer dispersion conditions (Vellingiri et al. 2015b).

The diurnal and seasonal patterns of NO_x can be seen at each site, though the magnitude of the peaks was much greater at the sites with greater urban development. MTC, the second most urban site, showed the greatest variation in average hourly (Fig. 2.3) and seasonal (Fig. 2.4) atmospheric NO_x, while the range of NO_x values for KSP (the least urban) was 45% smaller for the peak hourly averages and 25% smaller for the peak seasonal average. This suggests that plants at the more urban sites experience a “pulse” of N input during peak atmospheric NO_x seasons rather than a constant low-level input as found at more rural sites.

However, atmospheric NO_x levels in NTX were lower than those documented in other urban areas. In Nanjing, China, hourly NO_x concentrations in 2008 averaged 19 ppbv in the summer season and 35 ppbv in the winter season (Xie et al. 2016), compared to 7 and 20 ppbv, respectively, in 2013 at the NTX site with the highest NO_x concentrations (MTC). In the eastern U.S. (New Jersey), hourly averages in the spring of 2007 peaked around 50 ppbv during morning rush hour (Song et al. 2011), whereas morning rush hour NO_x at MTC peaked at 13 ppbv in the spring of 2013. Though urban atmospheric NO_x concentrations have been on the decline in the past decade (Lu et al. 2015), this decline does not account for the magnitude of the differences between NTX NO_x concentrations in 2013 and other cities in 2007-2008.

Soil nitrogen did not increase with greater NO_x

We hypothesized that the effects of cumulative atmospheric NO_x additions would result in elevated soil N via chronic low-level N deposition. However, our study revealed no correlations between atmospheric NO_x, soil NO_3-N, or soil NH_4-N (Fig. 2.5). This may be the result of the relatively small increase in atmospheric NO_x in urban areas (relative to other major metropolitan areas, discussed above), or it may be that the signal was obscured by the complex deviations and/or transformations that occur in the urban N cycle. We discuss some of these possibilities below.

As previously mentioned, NO_x is consumed by the photochemical reactions involved in ozone production. Tarrant, Dallas, Denton, and six adjacent counties have been in 8 hour ozone non-attainment for the past several decades (TCEQ 2015b), suggesting high levels of NO_x consumption. Thus, it is possible that a large portion of NO_x is being transformed before it can be deposited as biologically available N.

If the atmospheric NO_x does reach the ground in the form of N deposition, it still may not be accessible. The greatest NO_x concentrations occur during winter when the post oak trees are dormant, thus available N may be taken up by perennial shrubs and forbs. Various soil processes such as immobilization and denitrification of N by bacteria may mean that very little of the added anthropogenic N addition is available to the trees. There is also evidence from other US cities that N can move directly from deposition to leaching without any intermediate biological processes (Rao et al. 2013). The fact that our sites are in close proximity to the Trinity River and the sampling occurred just after the annual spring rains raises the possibility that these NTX sites experience rapid NO_3-N leaching. And although we measured soil N during the period of active growth in post oak, the seasonal nature of atmospheric NO_x levels may mean that annual mineralization is better correlated with NO_x.

Effect of soil N on Leaf C:N depends on NO_x

We did not see the same positive relationship between soil N and leaf tissue quality that has been documented in many other ecosystems (Yang et al. 2011b, Phoenix et al. 2012). There was, however, an effect of the interaction between soil N and atmospheric NO_x on leaf C:N. At high levels of NO_x (greater than the median 9.8 ppbv), leaf tissue quality decreased with increasing soil N, counter to expectations; at low levels of atmospheric NO_x (less than the median 9.8 ppbv), tissue quality increased with increasing soil N (Fig. 2.6). These results suggest that at low levels of NO_x, the positive correlation between greater soil N availability and greater tissue quality occurs as expected, but at higher levels of NO_x, the relationship reverses. One explanation is that an undefined characteristic of urbanization concomitant with higher NO_x is negatively impacting this relationship, but this requires additional investigation.

Again, ozone may be obscuring relationships between environmental factors and tree growth. Ozone negatively affects trees in the genus *Quercus* via the disruption of chloroplast membranes (Broadmeadow and Jackson 2000, Wittig et al. 2009). Gregg and colleagues (2003), in an examination of the growth patterns of another deciduous tree (*Populus deltoides*) along an urban gradient, found that tree biomass was strongly negatively correlated with ozone, rather than correlated with NO_x, temperature, or carbon dioxide. Tarrant, Dallas, Denton, and the six adjacent counties have been in 8 hour ozone non-attainment for the past several decades (TCEQ 2015b) with ozone levels very similar to those reported for New York in the Gregg et al. study.

Blanchard (2008) determined that in NTX there were no significant differences in ozone concentrations between upwind and downwind sites, or between weekdays and weekends. Thus it is possible that tree growth in both developed and less developed areas of NTX is being negatively affected by elevated ozone.

Herbivory not correlated with greater NO_x

We did not see a relationship between atmospheric NO_x and herbivory, nor did we see the expected intermediary relationship between leaf C:N and herbivory. However, there was a trend toward greater herbivory with increasing urban development, with the two most urban sites having the greatest levels of herbivory. Raupp and colleagues (2010) offer several thoughts on the possible mechanisms behind elevated herbivory in urban areas: the reduction of native plant diversity in urban environments may contribute to the reduction of natural enemies; elevated temperatures in developed areas may favor an increased population of herbivores; and the (non-native) vegetative complexity may provide more opportunities to hide from predators.

In NTX, chewing insects commonly found on post oak trees include leaf-cutting bees (Megachilidae), katydids (Tettigoniidae), luna moths (*Actias luna*), and other Lepidoptera larvae, some of which have the potential to cause significant damage (Texas A&M Forest Service 2015). Gaining a greater understanding of the interactions between the urban environment in NTX and insect herbivores will be important in preventing economic losses, protecting ecosystem services, and enhancing human health.

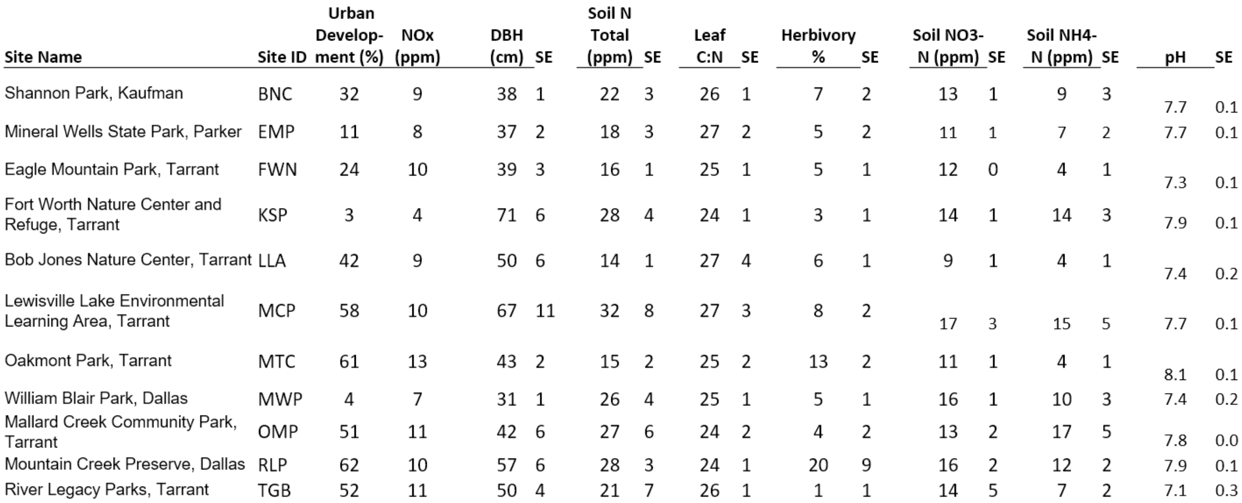
Conclusions

Our results established that atmospheric NO_x is highly correlated with percentage of urban development in NTX. We also examined the relationships between atmospheric NO_x and available soil N, leaf tissue quality, and herbivory of post oak trees during the early active growing season. While we found no relationship between atmospheric NO_x and soil N, leaf tissue quality was positively related to soil N as expected, but only at low NO_x values. This suggests that at higher NO_x values, another undefined characteristic of urbanization is impacting this relationship. Herbivory was not correlated with leaf tissue quality, but the trend toward greater herbivory with greater urban development warrants further investigation.

Studies such as this one, done on a local scale (50-100km), are vitally important in linking ecosystem processes to urbanization patterns (Brazel et al. 2000). Understanding the effects of NTX climate on urban ecosystems (and the feedback of urbanization on climate) will become increasingly important as Texas is predicted to be one of the hardest hit U.S. states in future climate change scenarios (Melillo et al. 2014). The higher temperatures, irregular rainfall, and more dry-days predicted for NTX may result in a reduction of N mobility and greater soil N accumulation as the limited water availability reduces plant uptake and decreases leaching. These changes in climate, combined with the rapidly expanding population of NTX, provide the impetus for further investigation into the effects of anthropogenic N additions on urban ecosystems in the Great Plains.

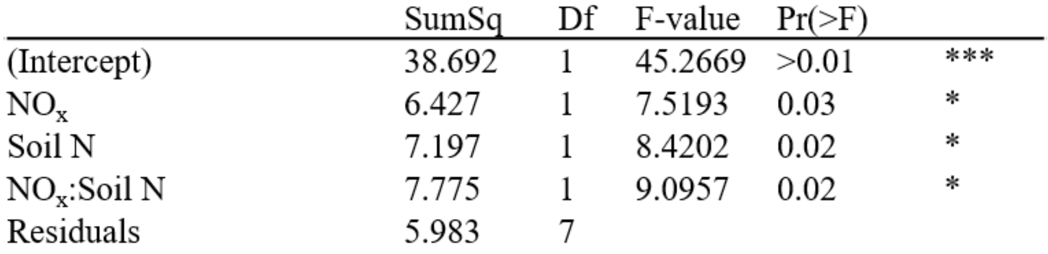
Tables and Figures

Table 2.1 Survey Site names and counties, site IDs, percent urban development, hourly atmospheric NOx averages for 2013, and site averages for measured variables ± standard error. n=5 for each of the variables except NOx, which had an n=12 based on monthly averages.



Site Name	Site ID	Urban Develop-ment (%)	NOx (ppm)	DBH (cm)	SE	Soil N Total (ppm)	SE	Leaf C:N	SE	Herbivory %	SE	Soil NO3-N (ppm)	SE	Soil NH4-N (ppm)	SE	pH	SE
Shannon Park, Kaufman	BNC	32	9	38	1	22	3	26	1	7	2	13	1	9	3	7.7	0.1
Mineral Wells State Park, Parker	EMP	11	8	37	2	18	3	27	2	5	2	11	1	7	2	7.7	0.1
Eagle Mountain Park, Tarrant	FWN	24	10	39	3	16	1	25	1	5	1	12	0	4	1	7.3	0.1
Fort Worth Nature Center and Refuge, Tarrant	KSP	3	4	71	6	28	4	24	1	3	1	14	1	14	3	7.9	0.1
Bob Jones Nature Center, Tarrant	LLA	42	9	50	6	14	1	27	4	6	1	9	1	4	1	7.4	0.2
Lewisville Lake Environmental Learning Area, Tarrant	MCP	58	10	67	11	32	8	27	3	8	2	17	3	15	5	7.7	0.1
Oakmont Park, Tarrant	MTC	61	13	43	2	15	2	25	2	13	2	11	1	4	1	8.1	0.1
William Blair Park, Dallas	MWP	4	7	31	1	26	4	25	1	5	1	16	1	10	3	7.4	0.2
Mallard Creek Community Park, Tarrant	OMP	51	11	42	6	27	6	24	2	4	2	13	2	17	5	7.8	0.0
Mountain Creek Preserve, Dallas	RLP	62	10	57	6	28	3	24	1	20	9	16	2	12	2	7.9	0.1
River Legacy Parks, Tarrant	TGB	52	11	50	4	21	7	26	1	1	1	14	5	7	2	7.1	0.3

Table 2.2 Terms included in the final multiple linear regression for leaf tissue quality C:N * = p<0.05, ** = p<0.01, *** = p<0.001



	SumSq	Df	F-value	Pr(>F)	
(Intercept)	38.692	1	45.2669	>0.01	***
NO_x	6.427	1	7.5193	0.03	*
Soil N	7.197	1	8.4202	0.02	*
NO_x:Soil N	7.775	1	9.0957	0.02	*
Residuals	5.983	7			

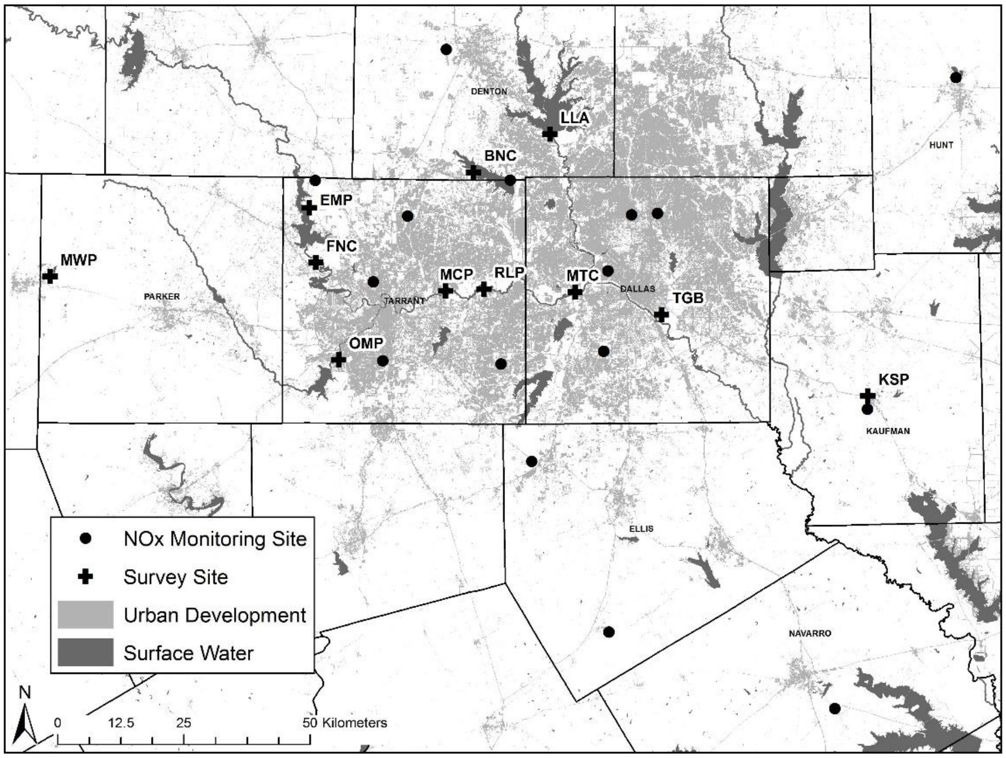


Figure 2.1 Map of the NTX area with urban development in light gray, surface water in dark gray, survey sites (indicated by a plus), and NOx monitoring sites (indicated by a point)

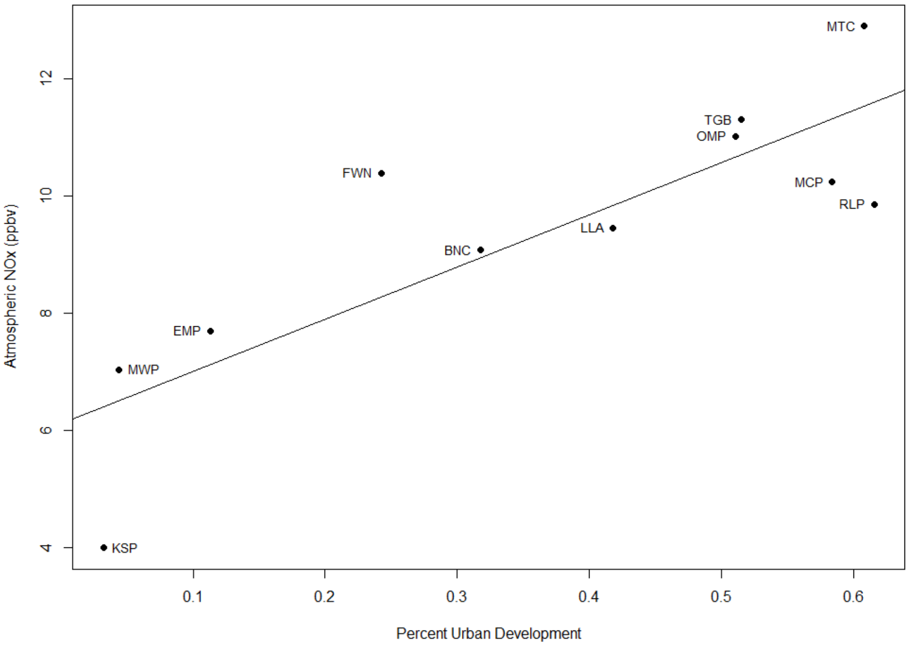


Figure 2.2 Plot of average hourly atmospheric NOx values in 2013 for each of the sites against the calculated percent urban development of that site

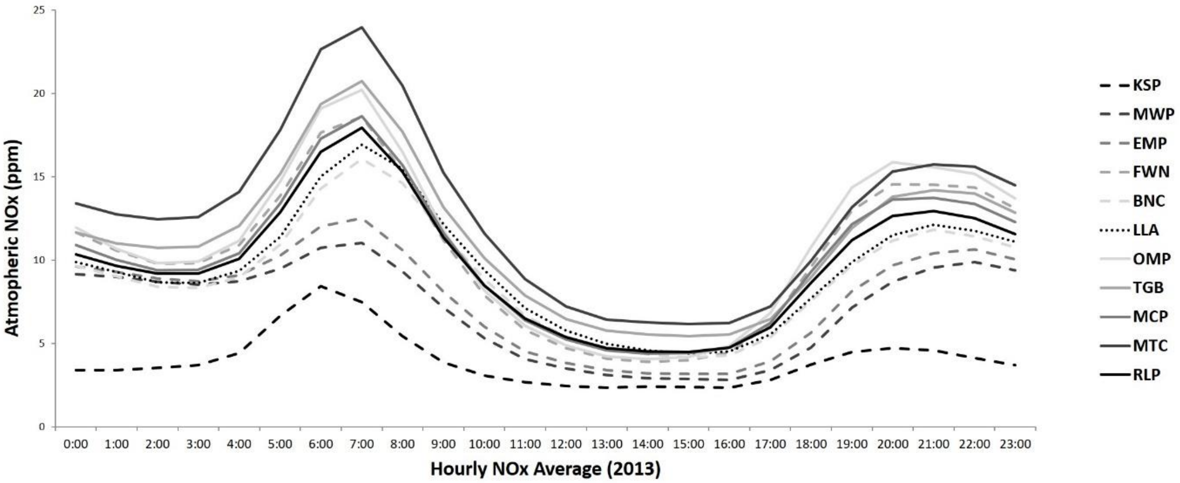


Figure 2.3 Average hourly NOx values in 2013 for each survey site. The dotted line represents the site with the median percent urban development. Solid lines represent urban sites with the

darkest being the most urban. Hashed lines represent sites with less urban development, the darkest being the lowest percent urban development

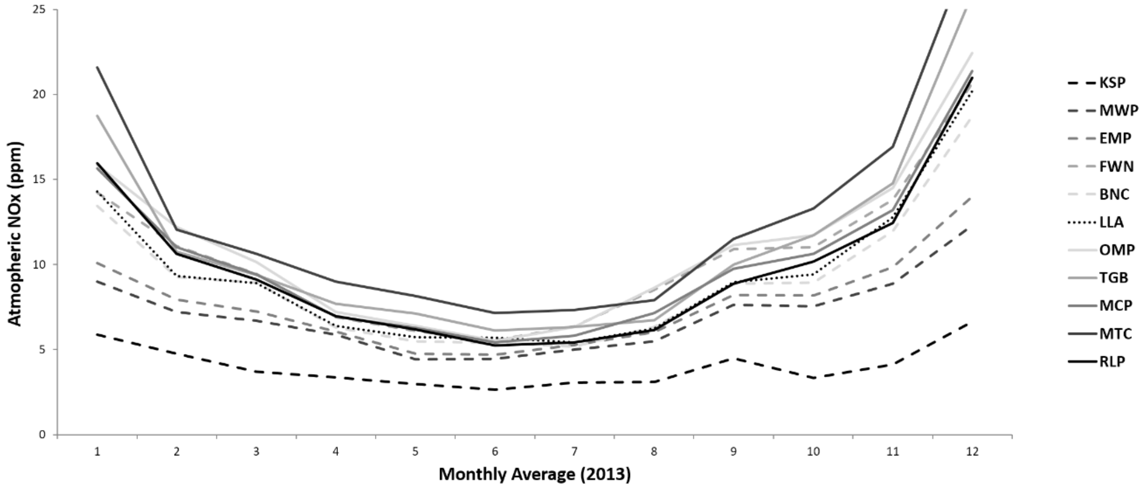


Figure 2.4 Average monthly NO_x values in 2013 for each survey site. The dotted line represents the site with the median percent urban development. Solid lines represent urban sites with the darkest being the most urban. Hashed lines represent sites with less urban development, the darkest being the lowest percent urban development

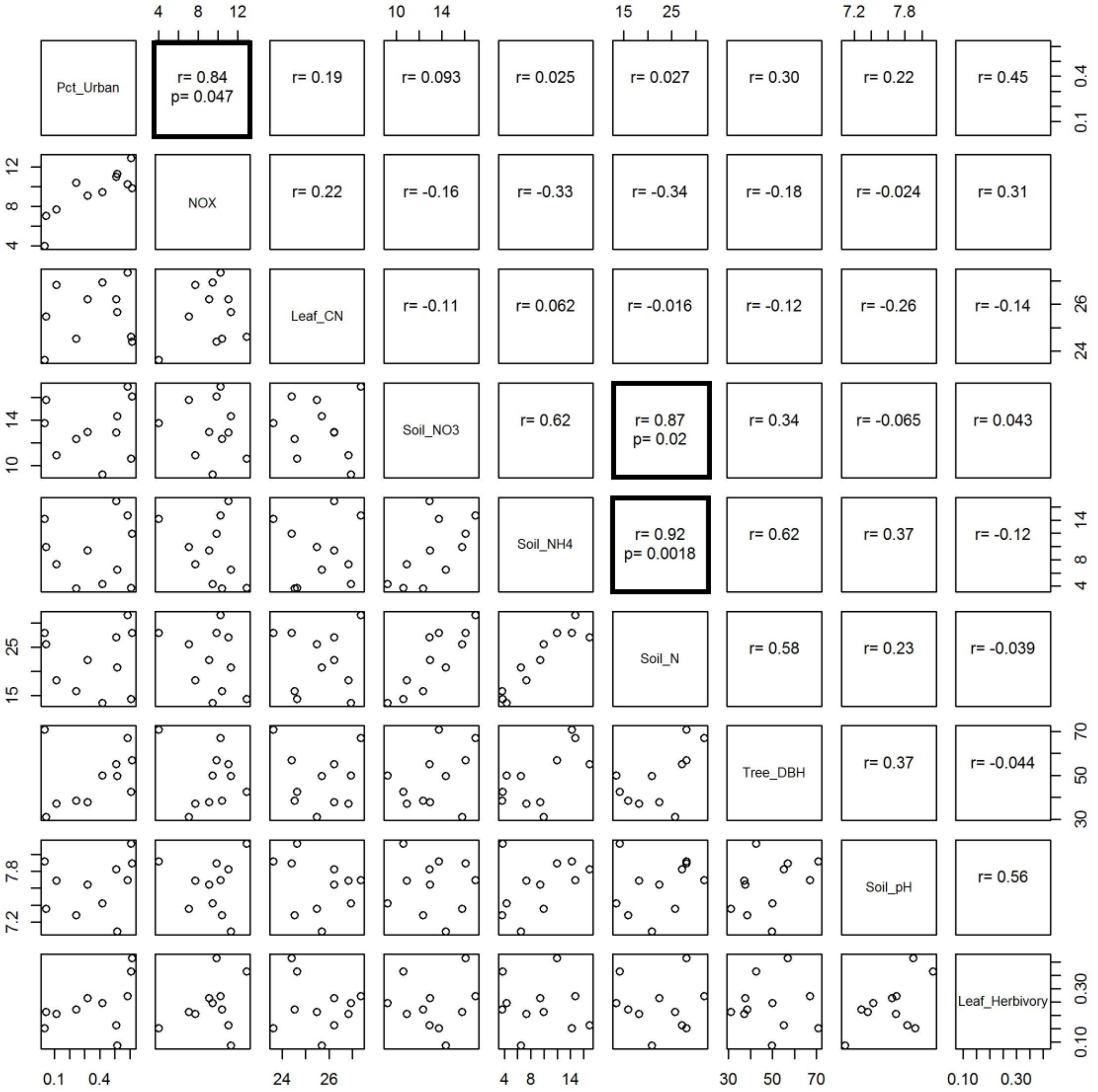


Figure 2.5 Correlation matrix and plot for all measured variables with Holm-adjusted p values. Only p values less than 0.05 are shown and bolded

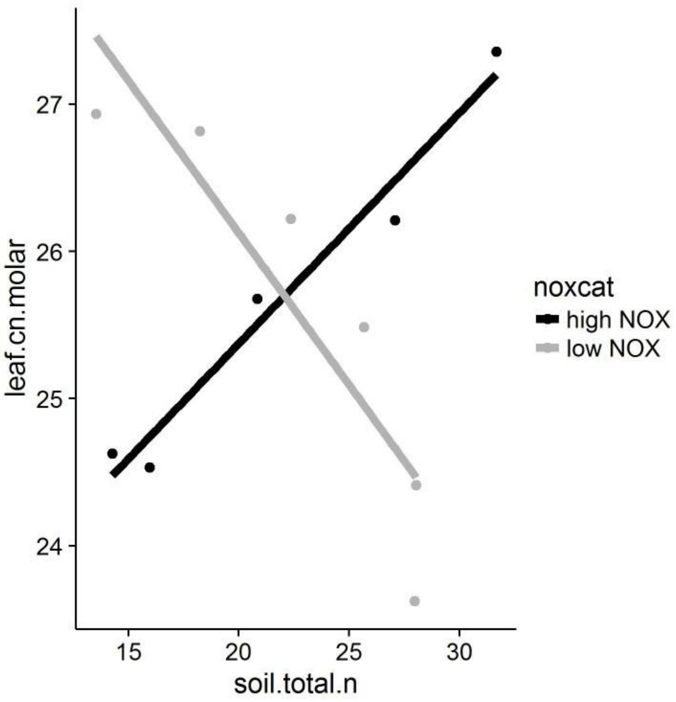


Figure 2.6 Plot of leaf C:N as a function of soil N at two levels of NO_x based on the median concentration. Sites were divided into high-NO_x (greater than the median of 9.8 ppbv) and low-NO_x (less than the median of 9.8 ppbv)

CHAPTER 3

A COMMON GARDEN INVESTIGATION INTO URBAN AND RURAL ECOTYPES OF A DOMINANT PRAIRIE GRASS, LITTLE BLUESTEM (*SCHIZACHYRIUM SCOPARIUM*)

Abstract

Native plant populations in or near urban areas are subject to higher temperatures, elevated carbon dioxide and nutrients, and altered hydrology compared to plants farther away. In the same way that habitat heterogeneity and natural selection can combine to create genetically distinct alpine and subalpine populations of a species, these differences resulting from urbanization may select for distinctly urban populations. I looked for the presence of urban and rural ecotypes - genetically distinct populations within a species whose differences are presumed to be adaptive – in the dominant prairie bunchgrass, little bluestem (*Schizachyrium scoparium*). Four remnant prairie sites (two urban and two rural) in the north central part of Texas, USA were examined for differences in the morphology of *S. scoparium*. Clump size, tiller count, and tiller length differed significantly among the remnant prairie sites. Clumps of *S. scoparium* were also collected from each of the remnant prairie sites, divided into replicates, and grown together in a common garden for two growing seasons to determine if genetic divergence in plant growth and biomass occurred. Vegetative biomass and tiller count were greater in plants from the rural sites than the urban sites. Additionally, plants from the most urban site flowered on average two weeks earlier than the other sites. Though the reproductive tiller lengths and total biomass did not differ significantly among the common garden plants from each site, comparing their characteristics to plants at the origin sites revealed a distinct genetic component to the phenotype, suggesting the potential for continued divergence of these traits in the future. *S.*

scoparium is a dominant grass in the Great Plains and is used often in prairie restorations, residential landscaping, and highway vegetation. Thus, the existence of urban and rural ecotypes is especially important for restoration efforts in which local adaptation plays a key role in the success of the endeavor.

Introduction

North America's tallgrass prairie once extended from the Gulf of Mexico to southern Canada covering 140 million acres (Gardner 2011). The portion of the prairie in North Texas belongs to the Grand Prairie, a southern extension of the Great Plains that consists mainly of shallow soil underlain by limestone. Late-successional communities are characterized by little bluestem (*Schizachyrium scoparium*), big bluestem (*Andropogon gerardii*), Indiangrass (*Sorghastrum nutans*), switchgrass (*Panicum virgatum*) and various perennial forbs (Dyksterhuis 1946). Over the past half century, however, the original twenty million acres of Texas prairie have been reduced to less than one percent (Native Prairies Association of Texas 2016). In the past 150 years of settlement in Texas, the prairie has been lost to agriculture, overgrazing, and, especially in North Texas, urban sprawl.

Prairies are a valuable resource in Texas, providing erosion control, carbon sequestration in soils, water quality protection, non-native species suppression, and habitat for native wildlife (Native Prairies Association of Texas 2016). Over the course of the past few decades, the plight of Texas prairies and the importance of restoring them has been brought to the attention of the public by organizations such as the Native Prairies Association of Texas, a non-profit organization founded in 1986 (http://texasprairie.org). With the help of federal and state government agencies, efforts are being made to preserve intact remnant prairies and to expand restoration efforts.

With the rapidly increasing population of North Texas (now over seven million people), sites slated for preservation and restoration are either surrounded by urban development, or soon will be (U.S. Census Bureau 2016). With concentrated human activity comes intensive changes to the environment. For example, it is now well established that cities are warmer than the surrounding land, most notably at night and in the winter (Jones and Groisman 1990, George et al. 2007). This phenomenon has been dubbed the "urban heat island." The main contributing factors are the high thermal conductivity of buildings, the low albedo of manmade structures, low evapotranspiration, and the heat released directly from buildings and vehicles (George et al. 2007). Cities also have elevated levels of carbon dioxide (CO_2), mostly resulting from the combustion of fossil fuels in vehicles (Idso et al. 1998, Idso et al. 2002, Koerner and Klopatek 2002, Wentz et al. 2002, George et al. 2007). Fossil fuel combustion from vehicles and industry is also responsible for the release of a cocktail of pollutants including nitric oxide, nitrogen dioxide, ammonia, nitrous acid, and volatile organic compounds which contributes to the formation of ozone (Bignal et al. 2004). Taken together, these conditions result in significant microclimate differences between urban and rural areas (George et al. 2007).

Microclimate differences have the potential to affect the growth, tissue quality, and reproduction of native plants. Warmer temperatures have the potential to increase net primary productivity in cooler climates, but in places subject to extensive periods of drought, such as Texas, warmer temperatures could result in reduced water use efficiency and reduced net primary productivity (Polley et al. 2013). Elevated temperatures associated with urban areas are correlated with changes in phenology (the timing of life events) such as advances in bud burst or flowering date and the extension of the growing season (Jochner and Menzel 2015). Elevated CO_2 levels have the potential to affect tissue quality (e.g., carbon:nitrogen ratio), and to increase secondary metabolites used in plant defenses (Yang et al. 2011b). Both of these environmental factors, temperature and CO_2, in turn have the potential to indirectly impact animals influenced by phenology and tissue quality of plants (Throop and Lerdau 2004).

Plants are known for their plasticity in response to the changing environment. Individuals of the same species may display different morphology and phenology depending on the environment in which they live. For example, *S. scoparium*, the dominant perennial grass of the Grand Prairie, has been shown to have wide variation across the continent in tiller morphology, plant height, color, and initial flowering date (Springer 2012). Over time, habitat heterogeneity and natural selection combine to yield genetically distinct populations in many plant species (Hufford and Mazer 2003). In *S. scoparium*, populations of the northern and western US flower earlier and have shorter stature than populations of the southern and eastern US (McMillan 1959).

Ecotypes and the Common Garden Approach

In 1922, the term “ecotype” was coined to refer to populations of a species whose differences in phenotype are caused by genetic differences (Turesson 1922). Today we refer to ecotypes as genetically distinct populations within a species whose differences are presumed to be adaptive (Gurevitch et al. 2006). This becomes important in the field of ecological restoration, as locally adapted populations generally perform better in plots of the same or similar habitat than plants from foreign populations (Leimu and Fischer 2008). Thus, the identification of ecotypes and sourcing from populations adapted to the appropriate environment will go far in improving the chance of a successful restoration.

Investigations into plant ecotypes generally occur across a large geographical range such as that of Europe (Bischoff et al. 2006) or across an elevational gradient (Clausen et al. 1940). Recently, there has been increasing evidence for “microgeographic” adaptation (Bischoff et al. 2006, Leimu and Fischer 2008). In areas such as North Texas, with its rapidly expanding human population, native plant populations might evolve in response to the novel selection pressures that accompany urban development. The combination of factors experienced by plants in developed areas (e.g., pollution, elevated carbon dioxide, higher temperatures, less water availability), may contribute to the creation of urban and rural ecotypes.

Common garden experiments are commonly used to investigate the presence of ecotypes in plants. In a common garden experiment, plants from different populations are grown together under the same environmental conditions. In doing this, the influence of the origin environment is removed, and any differences among populations in the phenology or morphology of the plants are due to genetic control rather than phenotypic plasticity. In the study of *S. scoparium* mentioned earlier (McMillan 1959), plants from different populations in the US were brought back to a common garden to determine the presence of ecotypes. *S. scoparium* from northern US populations flowered earlier than plants from southern US populations, just as was seen in the in situ populations. This indicated that the populations were genetically distinct (ecotypes), and that the flowering times were influenced more strongly by genetics than environment.

Here, four populations of *S. scoparium* were identified in remnant prairies of North Texas that differed in levels of urban development. The sites were located in and around the Dallas-Fort Worth metropolitan area and were isolated from one another by various land uses; two sites had a high level of urban development surrounding them and two were in rural locations with less urban development. The *S. scoparium* populations at the remnant prairie sites were characterized along with plant community and environmental characteristics. Additionally, plants from each population were collected and grown together in a common garden to test for genetically controlled differences in phenotype. We tested the hypothesis that plants from the urban and rural sites are ecotypes of *S. scoparium*. This would be evidenced by the plants exhibiting differences in phenology and morphological traits such as plant height, number of tillers, and reproductive biomass when grown together in a common garden. If these populations are ecotypes, urban populations should be preferentially used in restoration and creation efforts within urban areas to ensure successful establishment of these prairies.

Methods

Origin Sites

Four remnant prairie sites were chosen in North Texas near the Dallas-Fort Worth metropolitan region. These remnant prairie sites represent a range of geographic locations, surrounding urban development, and environmental characteristics (Table 3.1; Figure 3.1). To categorize the sites as "urban" or "rural," a "percent urban development" variable was calculated using the U.S. Department of the Interior's 2011 National Land Cover Database (NLCD; Homer et al. 2015). The NLCD urban land-use categories are calculated based on percent impervious surface area (ISA). The NLCD categories "Developed High Intensity," "Developed Medium Intensity," and "Developed Low Intensity" represent ISA values of 80-100%, 50-79%, and 20-49%, respectively. Using a 10k radius buffer surrounding each site, the area of these categories was summed into one value and divided by the total area of the buffer to calculate "percent urban development." The two prairies surrounded by 40% or greater urban development were categorized as "urban" prairie sites and the two with less than 40% urban development were categorized as "rural sites" (Table 3.1).

Tandy Hills Natural Area (TH), the most urban site, is an indigenous remnant of Fort Worth Prairie located near the center of Fort Worth, TX. It has been designated as a natural area since 1987 and has been managed by the Fort Worth Nature Center and Refuge since 1990. This site is a rare unplowed and undeveloped grassland that has survived into the 21st century (Ecological Communications Coprotation Environmental Survey Consulting 2008).

Lewisville Lake Environmental Learning Area (LL), the second-most urban site, stands out from the other remnant prairie sites in that it is northeast of Fort Worth and is situated at the transition between the Eastern Cross Timbers and the Blackland Prairie. In 1955, the Lewisville Lake Dam was created, and land below the dam was set aside for flood control. The area was left idle for the most part until the early 1990s when a consortium of local, state, and national governmental agencies created the wildlife management area (http://www.llela.unt.edu).

The Fort Worth Prairie Park (PP), the most rural site, is located southwest of the city of Fort Worth and is one of the largest remaining tracts of native tallgrass prairie in North Texas. In 2006, the US General Land Office (GLO) sought to sell the public land to developers, but public outcry led the GLO to partner with the Great Plains Restoration Council to work toward a conservation alternative (http://www.gprc.org). Currently, the land around the park is being developed, and the PP is in a state of limbo as the GLO determines the future of this land.

The Fort Worth Nature Center and Refuge (NC), the second-most rural site, exists on the northwest side of Fort Worth, TX, and was set aside in 1914 to protect the quality of the drinking water in the newly created Lake Worth. In 1964, the land was designated as a wildlife sanctuary and nature preserve. Portions of the NC are remnant patches of the Fort Worth Prairie (http://www.fwnaturecenter.org).

Study Species

Schizachyrium scoparium (Michx.) Nash, known as little bluestem, is a dominant perennial C_4 grass present in all US states except Alaska and Nevada (Steinberg 2002). *S. scoparium* is an important source of forage for livestock in the US, and has been used extensively in prairie restoration projects because of its dominant role in prairie communities (Steinberg 2002). *S. scoparium* is a bunchgrass that grows in clumps of 10-25cm and consists of both sexually reproductive tillers (with flower spikelets) and vegetative tillers that remain leafy (Steinberg 2002).

Common Garden

Four raised beds were constructed in a fenced area at the Fort Worth Nature Center and Refuge (FWNC) for the purpose of this experiment. The raised beds were approximately 30cm deep and filled with 5cm of pea gravel followed by 25cm of a commercially obtained 50/50 sand/local topsoil mix.

Clumps of little bluestem were collected from the origin sites between March 28 and May 17, 2013. A minimum of 16 clumps were collected at each site, and were chosen haphazardly with at least 1.5 m distance between the chosen clumps. The clumps were returned to the lab and divided into individual ramets which had at least 1 live vegetative tiller (up to 3) and healthy roots. The live vegetative tillers of each ramet were trimmed down to 7cm, and the ramets were weighed to ensure that each one's live biomass ranged between 1 and 4g. Ramets were labeled so that each one was associated with a specific clump of little bluestem from an origin site.

Three ramets of each clump were potted into a one-gallon black plastic pot filled with commercially obtained topsoil, such that each pot represented one clump (genetic individual) from one of the origin sites. The plants were placed in the UTA greenhouse and watered *ad libitum* to acclimate them to the pots. At the beginning of June, each pot was thinned so that the two most robust ramets remained in each pot, and the pots were moved to the FWNC. In all, there were 16 pots of plants that originated from LL, 13 pots from NC, 16 from PP, and 14 from TH. The pots were randomly assigned to a raised bed such that each bed contained at most four plants from each origin site for a total of sixteen plants. The pots were placed in the raised beds so that the centers of each pot were 40cm apart, thereby minimizing intraspecific competition for light. The pots were left under shade structures with 50% shade cloth for three days to acclimate. Each pot was watered twice-weekly with 250mL of well water unless it rained within 24 hours of the scheduled watering time.

Beginning on June 14th, 2013, maximum vegetative tiller height and maximum reproductive tiller height as well as the count of vegetative and reproductive tillers were recorded on a weekly basis. Once monthly, a detailed measurement of each vegetative and reproductive tiller was conducted. At each measuring event, the presence or absence of herbivory (chewing only) was recorded. Dates of flowering were also recorded.

The reproductive tillers were clipped at the base of each ramet and harvested during the week of November 1-8. The pots were removed from the raised beds and brought back to the lab at UTA on November 15. At the time of harvest, some fibrous roots had escaped each of the pots; this should not have impacted the experiment much due to the sand/topsoil mixture being the same in all raised beds. Over the course of the next week, the plants were removed from the pots, cleaned of dirt, and separated into roots and shoots. Three live vegetative tillers were clipped from each pot to be used in CHN analysis. Two healthy daughter ramets were removed from the outside edge of the healthiest looking plant in each pot, trimmed to 7cm, and repotted in the same soil to begin the second generation. All remaining plant matter harvested from the first generation was dried in a 50°C oven for at least one week before weighing the separated roots, vegetative tillers, and reproductive tillers. The second generation plants were placed in the UTA greenhouse and watered *ad libitum* for acclimatization.

The second generation plants were placed back in the raised beds at the FWNC on January 18, 2014 while dormant. At this point, plant mortality had reduced the numbers of live plants to 13 LL, 3 NC, 11 PP, and 9 TH. In May, 2014, the pots with two ramets remaining were thinned so that only the most robust plant remained. Measurement of the second generation plants began in June 2014, with monthly detailed measurements of the lengths of each vegetative and reproductive tiller. Reproductive tillers were measured weekly to track flowering phenology. Plants were watered once a week with 500 mL of well water from the FWNC.

The second generation plants were removed from the FWNC and brought back to the lab at UTA on October 4, 2014. The reproductive tillers were clipped at the base of the plant to separate them from the vegetative tillers. The roots were washed and separated from the remaining vegetative tillers. The separated plant matter was dried in a 50°C oven for at least 4 days, at which point the biomass of each plant's roots, vegetative tillers, and reproductive tillers was recorded.

A portion of the roots, vegetative tillers, and reproductive tillers of each second generation plant was ground in a Wiley Mill with a size 60 mesh screen. The %C and %N of the plant tissues was determined using a combustion approach in a Perkin Elmer 2400 CHN Analyzer.

Field Sampling of Origin Sites

When the *S. scoparium* plants were collected from the origin sites, some of the soil was removed from the hole left by the plant at approximately 10cm below the surface. This soil was brought back to the lab and refrigerated at 3°C for approximately 6 months until it was sent to the Texas A&M Soil, Water, and Forage Testing Lab (College Station, TX) for analysis of pH, KCl-extractable nitrate-N (NO_3-N) and ammonium-N (NH_4-N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and sodium (Na).

In the fall of 2013, two twenty-five meter transects were run at each of the origin sites with one by one meter square quadrats laid down every five meters. As the remnant prairie sites were odd-shaped and sometimes small, the orientation of the transect was determined by the direction which would allow us the most measurements. The smallest site, LL, had two transects with two quadrats and two transects with three quadrats. Each site had a total of ten quadrats. Within each quadrat, percent cover was visually estimated for little bluestem, other grasses, forbs, and bare ground. Trees, manure, cacti, and other aberrations were placed in the "Other" category. For every clump of little bluestem within the quadrat, the longest vegetative tiller and longest reproductive tiller were measured, and three tillers of each category were haphazardly selected and brought back to the lab for CHN analysis following the same procedure as the common garden plants.

Plants that were measured at the origin site will henceforth be called origin site plants, while plants that were collected and grown in the common garden will be called CG plants.

Statistical Methods

All statistics were conducted using IBM Corp. SPSS Version 24.0. To test for the effect of raised bed on plant growth, a multivariate analysis of variance (MANOVA) was conducted with box as the independent variable and all measured plant characteristics as the dependent variables. There were no significant differences, thus raised bed was excluded from the remainder of the analyses.

The maximum values of vegetative and reproductive tiller count and length for each plant were used in the analyses to avoid issues with null reproductive or vegetative measurements at the time of harvest, which would significantly lower the sample size. Biomass values were only obtained at the last measurement when the plants were harvested. MANOVAs were conducted on origin site and CG plant characteristics separately with site as the independent factor. When overall MANOVAs were significant, individual ANOVAs were conducted for each dependent variable to reveal significant univariate main effects. Post-hoc analyses of differences among sites were conducted using Tukey's HSD test.

Results

Origin Site - S. scoparium Characteristics

The characteristics of little bluestem differed significantly by origin site when vegetative C:N, reproductive C:N, clump diameter, max reproductive tiller height, and max vegetative tiller height were considered jointly (Wilk's lambda=0.014, F(15,50)=12.39, p<0.01, partial eta squared=0.76). A separate ANOVA was conducted for each dependent variable, revealing significant univariate main effects for vegetative C:N (F(3,22)=31.21, p<0.01, partial eta squared=0.81); reproductive C:N (F(3,22), p=0.013, partial eta squared=0.38); clump diameter

(F(3,22)=13.7, p<0.01, partial eta squared=0.651); max reproductive tiller height (F(3,22)=41.61, p<0.01, partial eta squared=0.85); and max vegetative height(F(3,22)=5.92, p=0.04, partial eta squared=0.45. Generally, plant characteristics did not follow a trend associated with the urban development of the sites, however, the diameters of little bluestem clumps were smaller at the two urban sites compared to the two more rural sites (Figure 3.2). Vegetative and reproductive C:N ratios were significantly lower and vegetative tillers were significantly shorter at PP, the most rural site, but the remaining sites did not show a relationship between urban development and these plant characteristics (Figure 3.2, 3.3). The reproductive tillers were shortest at LL, with no clear relationship to urban development (Figure 3.2).

Origin Site - Community Characteristics

Of the different plant groups and surface cover measured at the quadrat level, the percent cover categories (minus "other," which made up less than 4% of the total) were analyzed by MANOVA to determine the characteristics of the plant communities at each site. Relative percentages for *S. scoparium*, other grasses, forbs, and bare ground, when considered jointly, differed significantly by site-of-origin (Wilk's lambda=0.08, F(12,106)=14.158, p<0.01, partial eta squared=0.57). Separate ANOVAs revealed significant univariate main effects for percent *S. scoparium* (F(3,43)=23.24, p<0.01, partial eta squared=0.62) percent forbs (F(3,43)=4.73, p<0.01, partial eta squared=0.25), and percent bare ground (F(3,43)=49.31, p<0.01, partial eta squared=0.78). The two urban sites had the greatest percentages of bare ground and the least percentages of other grasses, with LL supporting the least *S. scoparium* (Figure 3.4). In contrast, NC, the third least urban site, had a significantly greater percent cover of *S. scoparium* (49%) compared to the other sites.

Common Garden - S. scoparium Characteristics

At the end of the 2014 growing season, the number of plants remaining for each site of origin was 11, 13, 3, and 9, for PP, LL, NC, and TH, respectively. The *S. scoparium* characteristics at the end of the second growing season (2014) differed significantly by site-of-origin when the biomass of roots, vegetative tiller number and length, and reproductive tiller number and length were considered together (Wilk's lambda=0.31, F(21,75)=1.81, p<0.03). Separate ANOVAs revealed significant differences among plants of different origins in maximum vegetative tiller count (F(3,32)=3.8, p=0.03, partial eta squared=0.24) and final vegetative biomass (F(3,32)=5.83, p<0.01, partial eta squared=0.35). Though there was no difference in reproductive biomass or root biomass among the plants of different origins, vegetative biomass was significantly greater in plants from the most rural site (PP) and declined with increasing urban development (Figure 3.5). Maximum vegetative tiller count followed a similar trend with PP, the most rural site, having almost twice the number of vegetative tillers as TH, the most urban site (Figure 3.6). There were no significant differences among the plants in reproductive tiller counts. Regarding the height of the plants, the vegetative tiller lengths were very similar across the sites of origin (overall average of 14cm), while the most urban site had the tallest reproductive tillers (overall average of 60cm; Figure 3.6).

The means (± 1.0 standard error) of tissue C:N ratios were 61(±2), 62(±2), and 50(±1) for roots, vegetative tillers, and reproductive tillers, respectively. Site of origin did not affect above ground or below ground tissue quality. There were no differences in rates of herbivory among sites of origin.

Phenology

In 2014, the dates of first flower for the CG plants ranged from July 29th to October 3rd with only one plant failing to put up any reproductive tillers. Though we saw no significant differences among the CG plants from each site, the average first flowering for TH plants was two weeks earlier than the average first flower for the LL plants (Figure 3.7).

Common Garden vs. Site-of-Origin Characteristics

Correlations between origin site and CG plant characteristics were examined to determine the relative genetic vs. environmental influence on certain plant traits. Overall, the origin site plants had slightly taller maximum vegetative tiller lengths (overall average of 26cm) than the CG plants (overall averages of 21cm). The range in site averages of maximum vegetative tiller length for origin site plants was 12cm while there was essentially no difference in maximum vegetative tiller length for CG plants, with a range of only 5cm (Figure 3.8). Similarly, the range in maximum reproductive tiller length site averages for the origin site plants was much greater (65cm) than the range in site averages for the CG plants (18cm; Figure 3.9). However, the reproductive tillers of origin site plants were shorter (63cm) than the CG plants (92cm). The reproductive tiller lengths of CG plants were positively, though not significantly, correlated with the reproductive tiller lengths of origin site plants (Figure 3.9).

The average vegetative C:N ratio for all origin site plants was smaller than the CG plants (39 and 60, respectively), while the range of site averages for vegetative C:N ratio was greater in the origin site plants than the CG plants (17 and 13, respectively). For reproductive tillers, the overall average C:N ratio was similar for both origin site and CG plants (43 and 49, respectively), and the range in site averages for C:N ratio is once again larger for origin site plants (15) than CG plants (5). In the origin site plants, the C:N ratios of both vegetative and reproductive tillers at PP stood out as being significantly lower than the rest of the sites, but this relationship was not seen in the common garden plants (Figure 3.10).

To look for potential genetic influences on clump size, we examined the relationship between clump sizes of the origin site plants of unknown age and the above-ground biomass of the CG plants. We found that the aboveground biomass of CG plants in 2014 was highly significantly correlated with the diameter of origin site clumps (r=0.97, p=0.02; Figure 3.11).

Discussion

Origin Site Plant and Environmental Differences

The little bluestem plants at the origin sites differed significantly in size and tissue quality. PP, the most rural site, had plants that were shorter in stature and also had significantly greater tissue quality than the other sites, due to a greater % N in tissues. The two intermediate urban/rural sites (NC and LL) had the most striking differences in plant size and clump diameter. NC had the largest clumps, tallest plants, and greatest percent cover of *S. scoparium* while LL had the smallest clumps, shortest plants, and the lowest percent cover of *S. scoparium*.

These morphological differences *as a whole* in the origin site plants *were not* clearly correlated with surrounding urban development. The plants are likely responding less to urban climate influences and more to other environmental influences, particularly soil characteristics. Among the origin sites, the greater the total N in the soil, the greater the clump diameter (Table 3.1, Figure 3.2). Additionally, *S. scoparium* has been shown to have higher productivity in areas with soil depths of 30-45cm when compared to areas of 15cm or less (Dalgarn and Wilson 1975), thus soil depth, which was not measured, may be influencing the size of the plants at the origin sites. The soil characteristics at LL were the least favorable of all the sites, possessing the lowest total N and the lowest pH. *S. scoparium* preferentially grows on well-drained soils with a pH of 7 or slightly above (NRCS 1991). The lower pH at this site may be negatively impacting *S. scoparium* and contributing to the abundance of bare ground (i.e. lack of plant cover).

Common Garden Plant Characteristics

After being removed from the origin site for two vegetatively produced generations, plants from different origins had significant differences in the number and biomass of vegetative tillers. The most urban site had the fewest vegetative tillers and least biomass while the most rural site had the greatest number and biomass of vegetative tillers. These significant differences after two generations in a common garden suggests genetic divergence of these traits among these populations. In contrast to this, phenotypic plasticity is indicated as the cause of variation in tissue quality of the vegetative and reproductive tillers, as C:N ratios were not significantly different in the common garden.

The correlation between plant characteristics in the origin site plants and CG plants can provide some insight on genetic influence as well. The relationship between vegetative tiller length at the CG and origin sites was essentially flat (Figure 3.8), indicating that the differences in vegetative tiller length at the origin sites were due to phenotypic plasticity rather than genetic divergence.

Reproductive tillers, however, showed a positive relationship between length in the common garden and length at the origin sites (Figure 3.9). The fact that the order of the sites from shortest to tallest was maintained from origin site to common garden suggests that there is some genetic influence on the differences in this trait, and that there is potential for future divergence of this trait. Three sites fell above the 1:1 line (Figure 3.9) and the reproductive tillers were taller in the common garden, suggesting that the plants in the origin sites were limited by one or more environmental factors.

Finally, there was a positive relationship between the origin site clump size and above-ground biomass of the CG plants. The fact that the total aboveground biomass was highly correlated with the diameter of the clumps from the origin site might suggest maternal affects, but the initial tillers were standardized for weight, and the data represent the second generation of plants that were grown at the common garden. Thus, it suggests that the overall size of the plant has a strong genetic component and is not merely constrained by neighbors.

Conclusions

In this experiment, a common garden approach was used to investigate the presence of urban and rural ecotypes of the native prairie bunchgrass, *Schizachryium scoparium*. The plants from the rural sites had greater numbers and biomass of vegetative tillers than the urban sites, indicating that there is genetic divergence in the production of vegetative biomass. Though there were not significant differences among sites in the lengths of reproductive tillers or overall biomass, relationships between origin site plants and common garden plants suggested a genetic component to the differences; this indicates that these traits have the potential to diverge under future selection pressures. Additionally, the earlier flowering date of plants from the most urban site suggests the potential for further genetic isolation among populations via phenological differences.

In restoration efforts, the source of plant material is very important to the success of the restoration (Gallagher and Wagenius 2016). With *S. scoparium* frequently used in prairie restorations, residential landscapes, and native highway landscaping projects (Steinberg 2002), the presence of an urban ecotype has potentially far-reaching impacts.

Tables and Figures

Table 3.1 Overview of origin site characteristics

Site Name	Category	Site ID	Lat.	Lon.	Urban %	Annual Rainfall (mm)	Textural Class	Soil pH	Extractable Soil N (ppm)
Fort Worth Prairie Park	Rural	PP	32.5809	-97.4413	17	835	Clay Loam	8.1	31.39
Fort Worth Nature Center and Refuge	Rural	NC	32.8373	-97.4801	24	824	Sandy Loam	8.1	40.53
Lewisville Lake Environmental Learning Area	Urban	LL	33.0605	-96.9908	43	918	Clay Loam	5.9	13.51
Tandy Hills Natural Area	Urban	TH	32.7483	-97.2751	62	847	Clay Loam	8.2	18.88

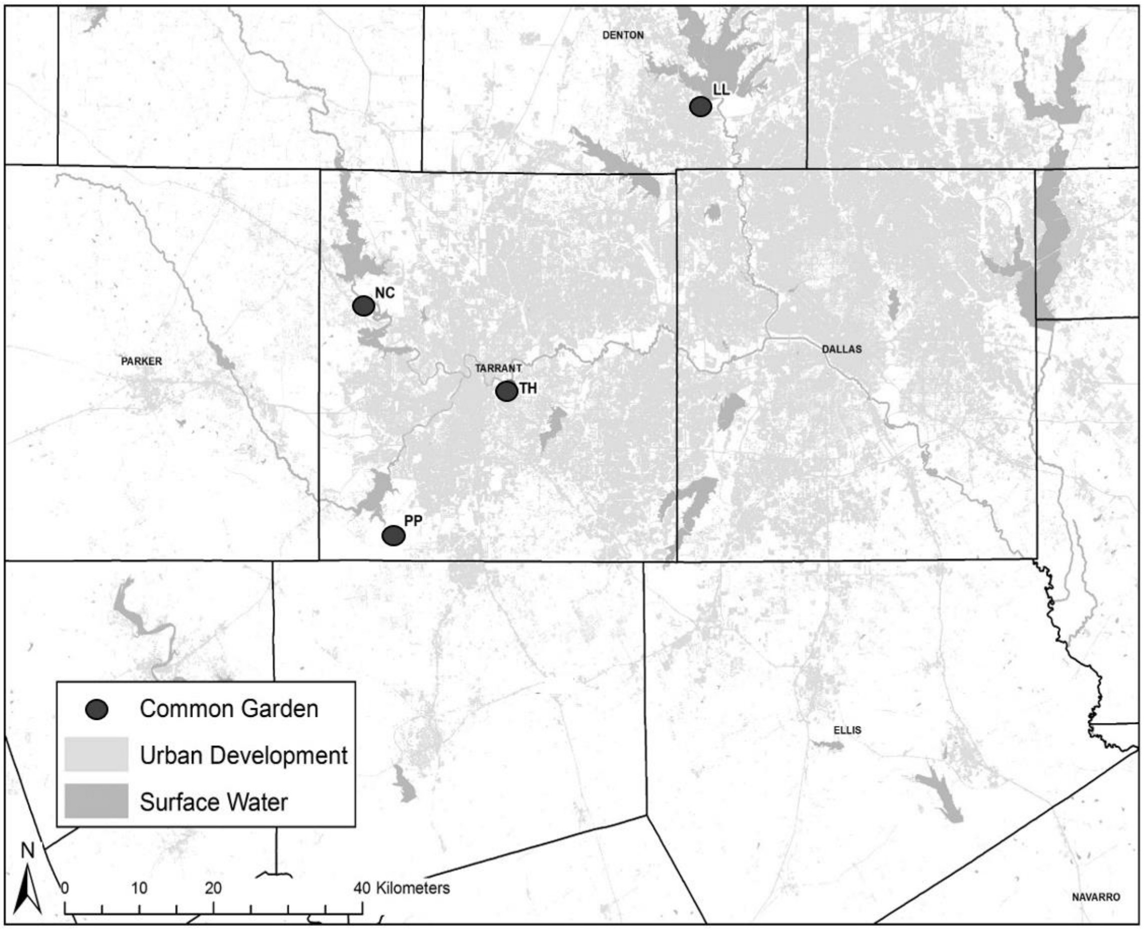


Figure 3.1 Map of North Texas indicating urban development (based on the USDA's NLCD categories of high, medium, and low urban development) and origin sites of little bluestem plants. Site abbreviations in Table 3.1

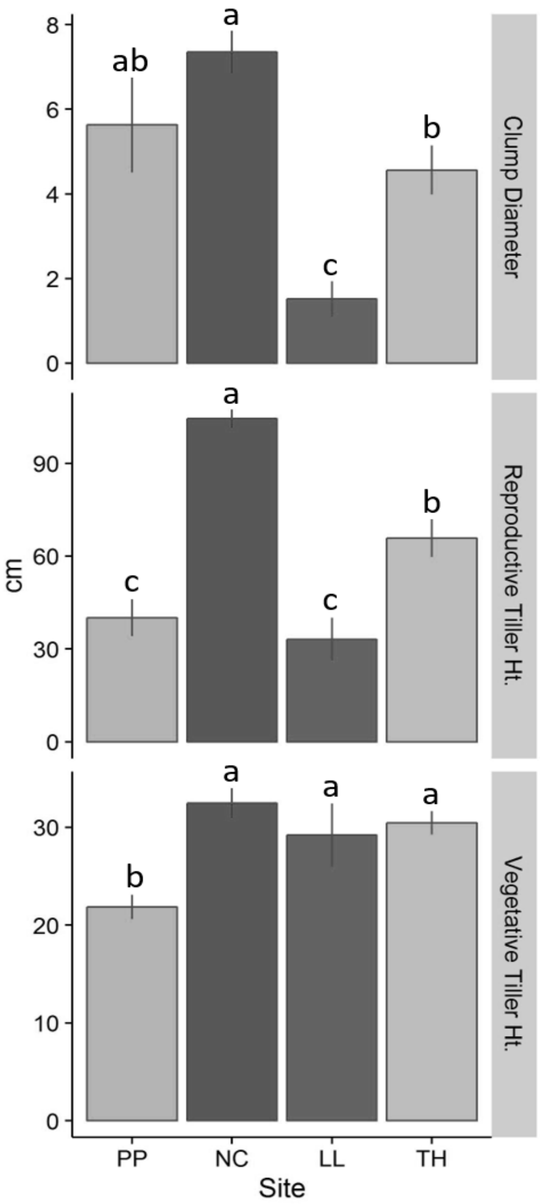


Figure 3.2 Mean and standard error for little bluestem clump characteristics at each of the origin sites (ordered left to right from least to most urban). The diameter of the clump was measured at ground level, and tiller heights represent the tallest vegetative and reproductive tillers of each clump. n= 35, 59, 48, and 44 for PP, NC, LL, and TH, respectively. Site abbreviations in Table 3.1

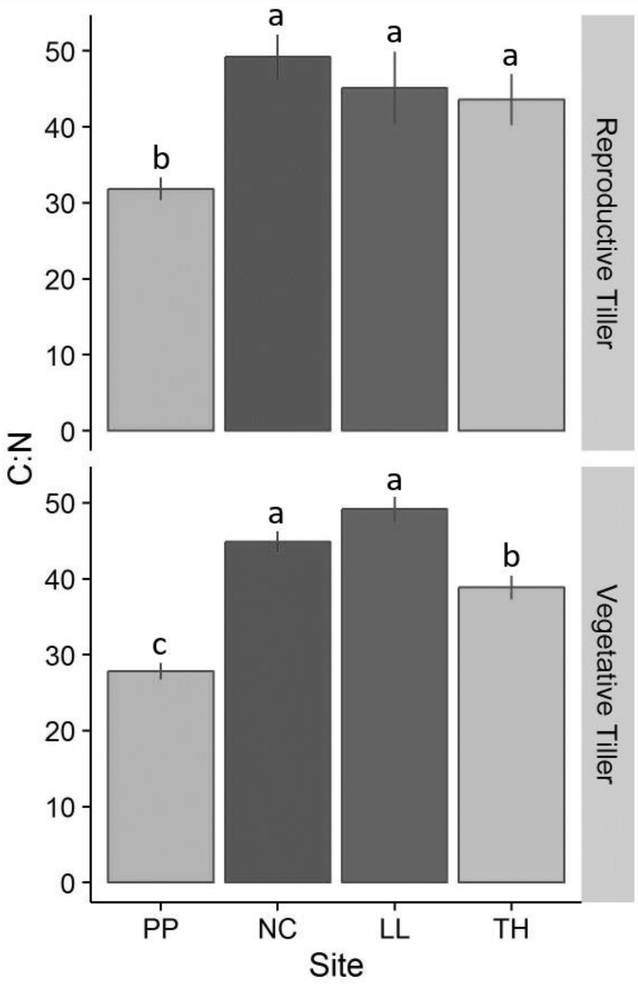


Figure 3.3 Means and standard errors of carbon to nitrogen (C:N) ratios in live reproductive and vegetative tillers of little bluestem at each of the origin sites (ordered left to right from least to most urban). Site abbreviations in Table 3.1.

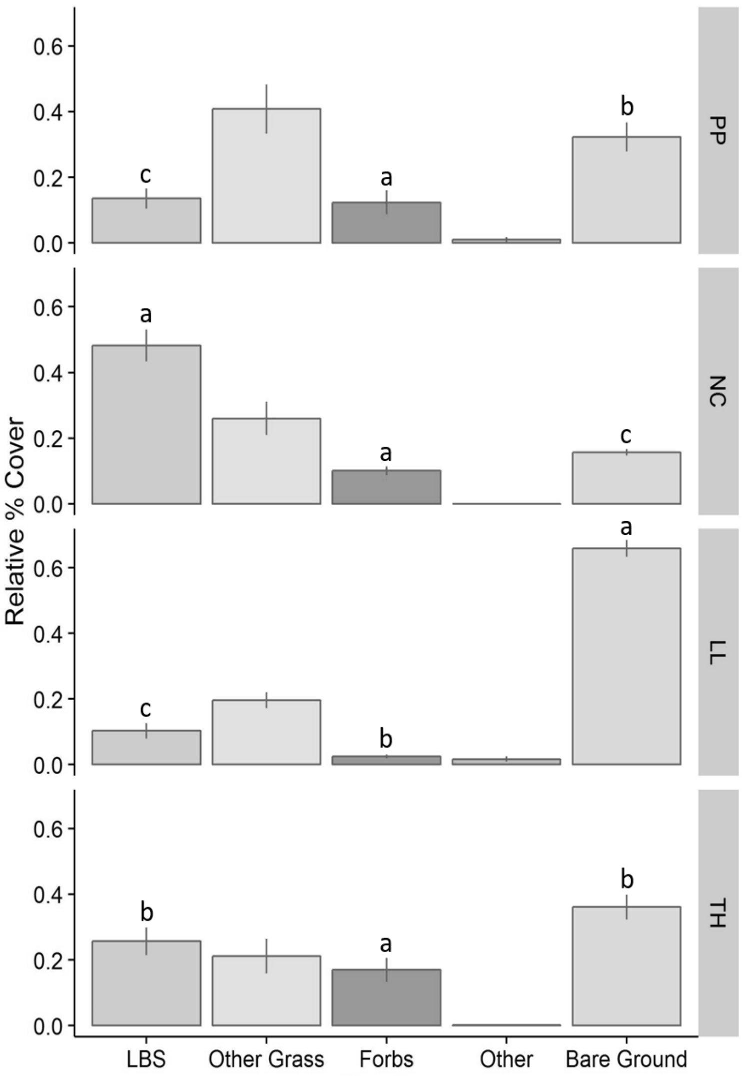


Figure 3.4 Estimated relative percent ground cover averages for 10 one by one meter quadrats at each of the origin sites. Letters represent significant differences (vertically) within each ground cover category. Site abbreviations in Table 3.1

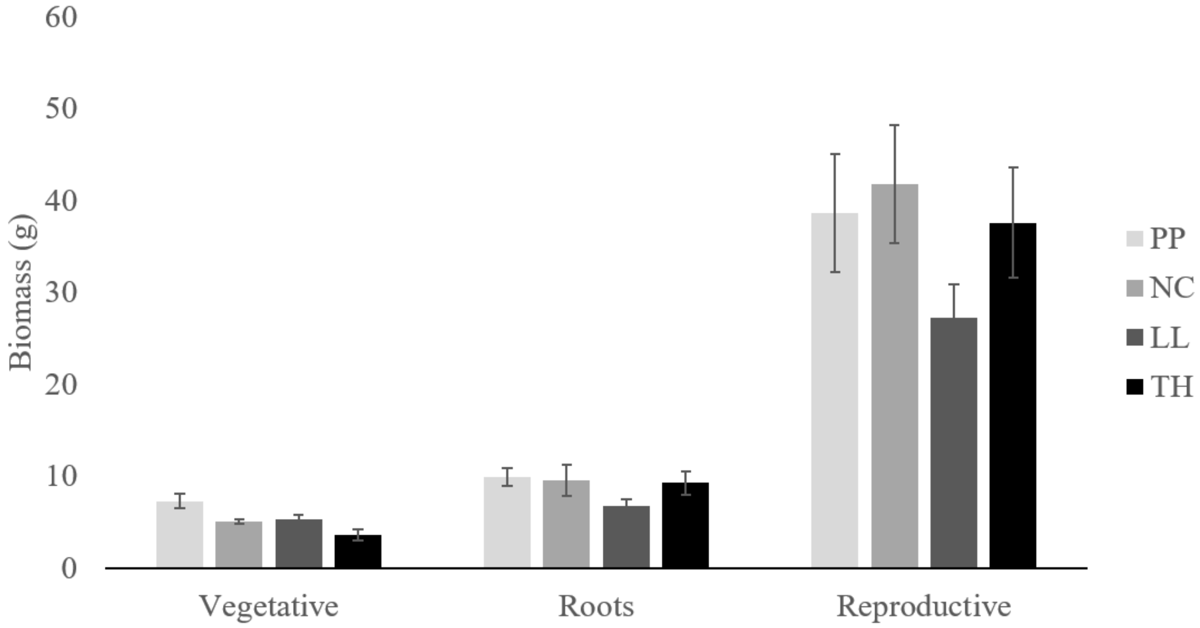


Figure 3.5 Bars represent mean (with standard error) biomass of vegetative tillers, roots, and reproductive tillers of CG plants at the time of harvest in October, 2014. Sites ordered left to right from least to most urban. Site abbreviations in Table 3.1

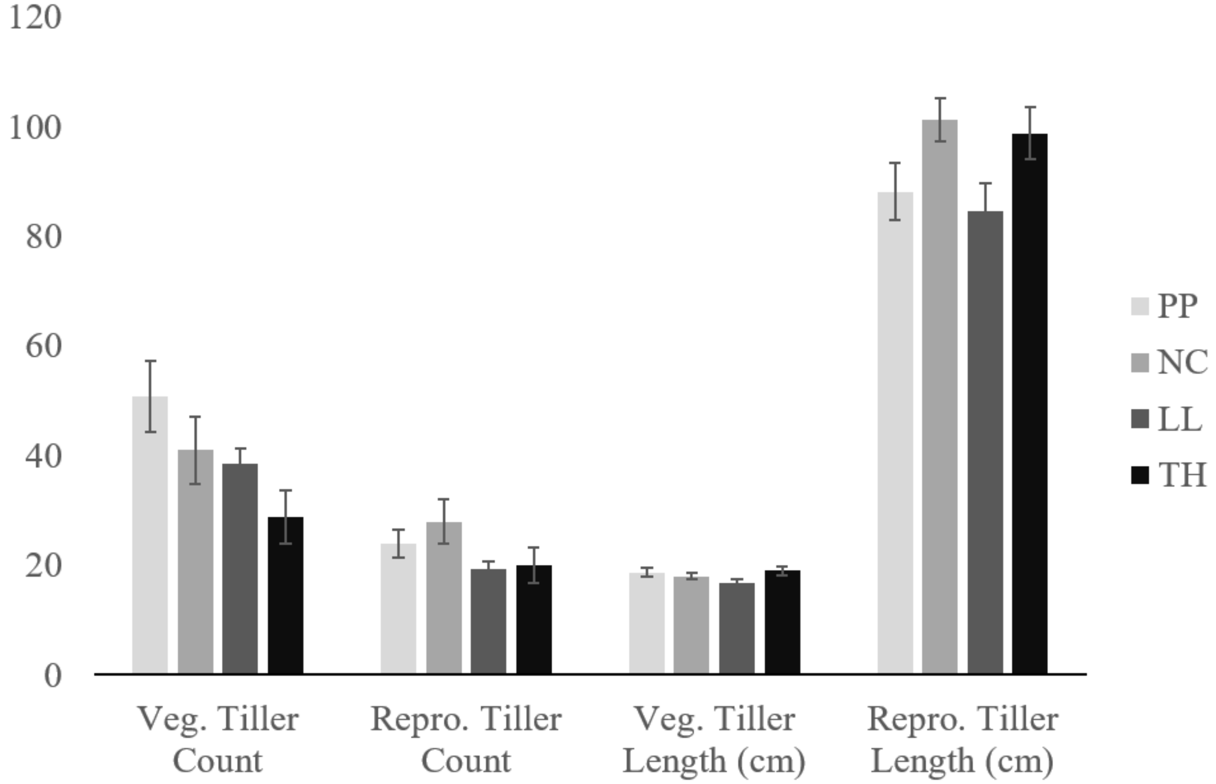


Figure 3.6 Bars represent mean (with standard error) maximum tiller counts and lengths of vegetative and reproductive tillers over the course of the experiment. Site abbreviations in Table 3.1

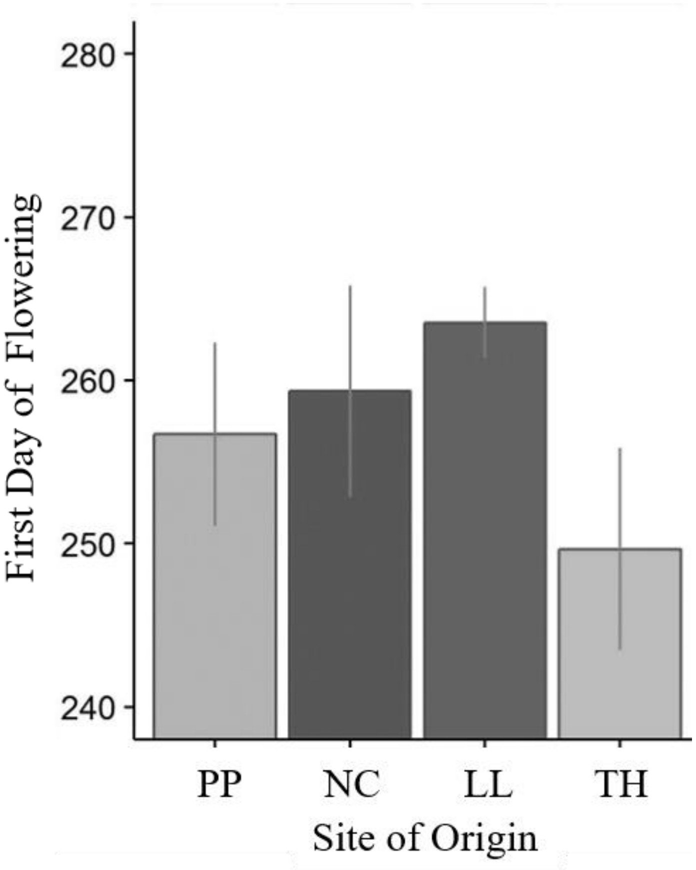


Figure 3.7 Means and standard errors of the first appearance of anthers on the common garden plants. Site abbreviations in Table 3.1

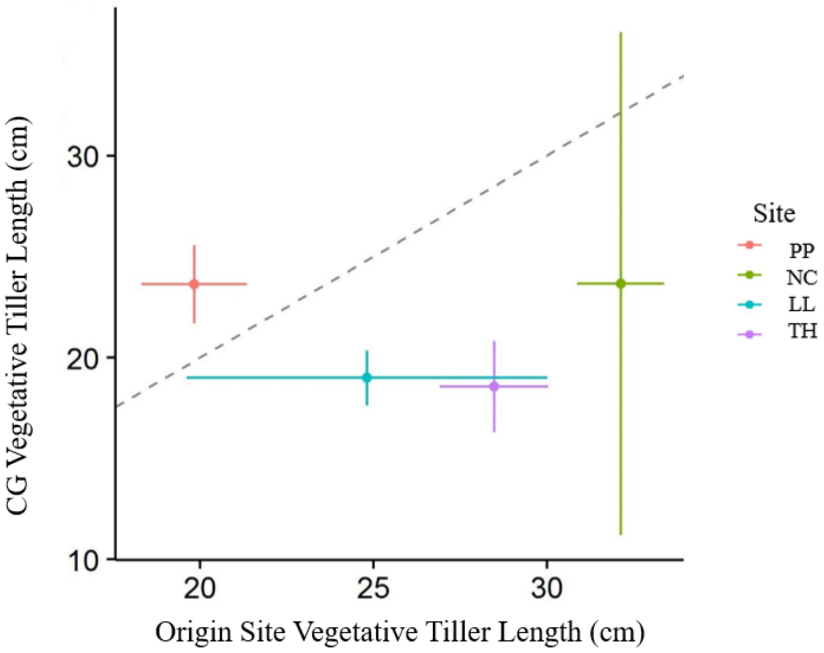


Figure 3.8 Means and standard errors of max vegetative tiller lengths of common garden plants as a function of max vegetative tiller lengths of origin site plants. The dashed line represents a 1:1 ratio. Site abbreviations in Table 3.1

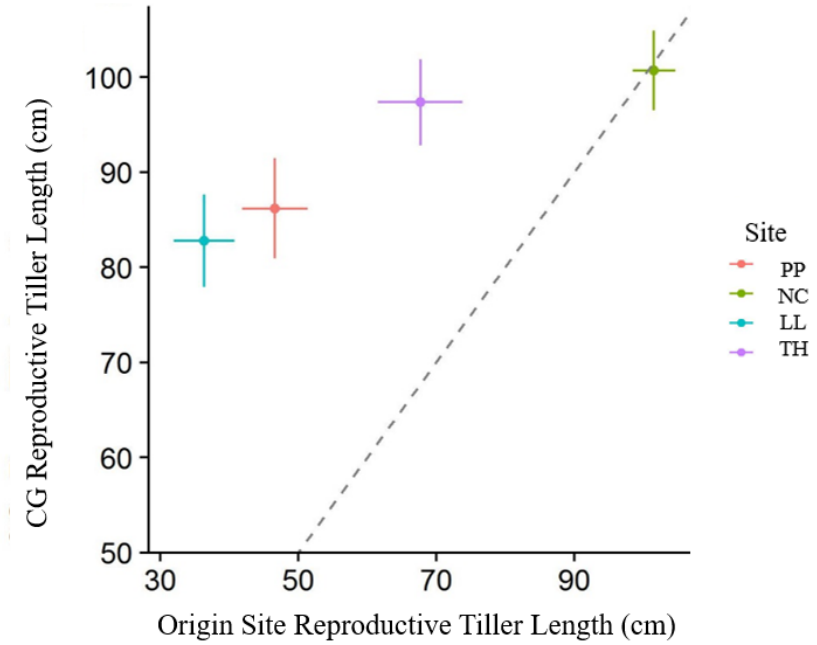


Figure 3.9 Mean and standard error of max reproductive tiller lengths of common garden plants as a function of max reproductive tiller lengths of origin site plants. The dashed line represents a 1:1 ratio. Site abbreviations in Table 3.1

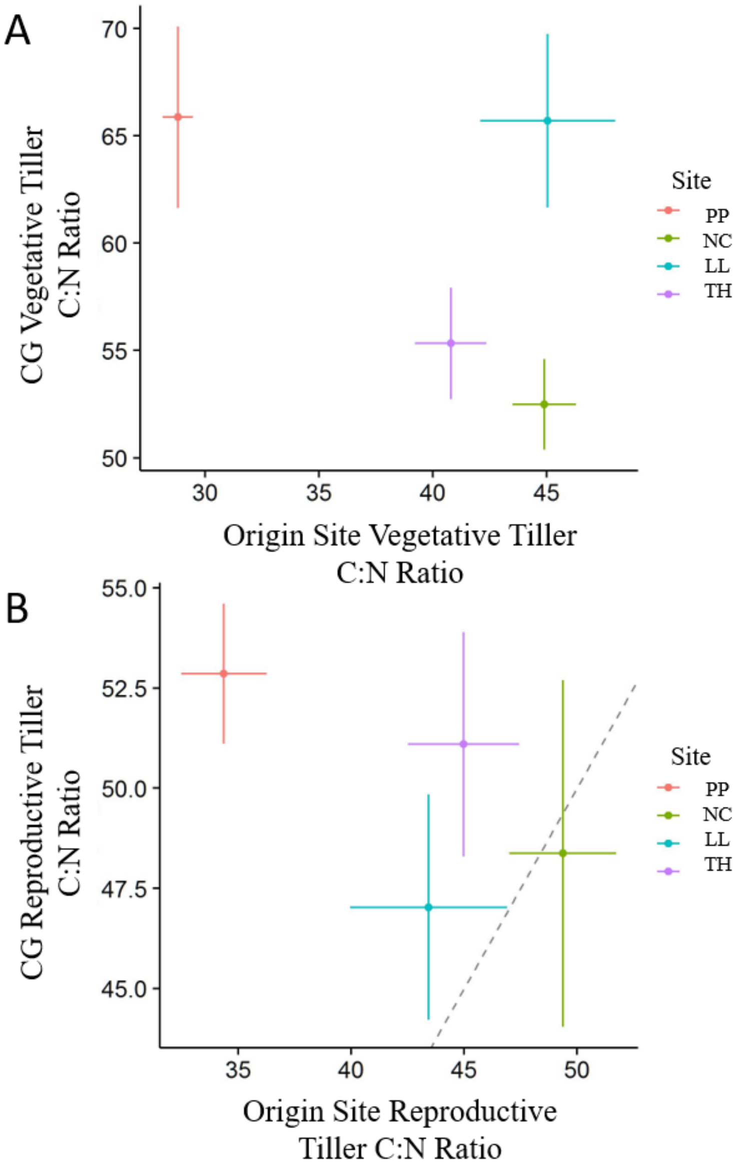


Figure 3.10 Mean and standard error of tissue quality (C:N ratio) of common garden plants as a function of tissue quality of origin site plants for A) vegetative tillers and B) reproductive tillers. Site abbreviations in Table 3.1

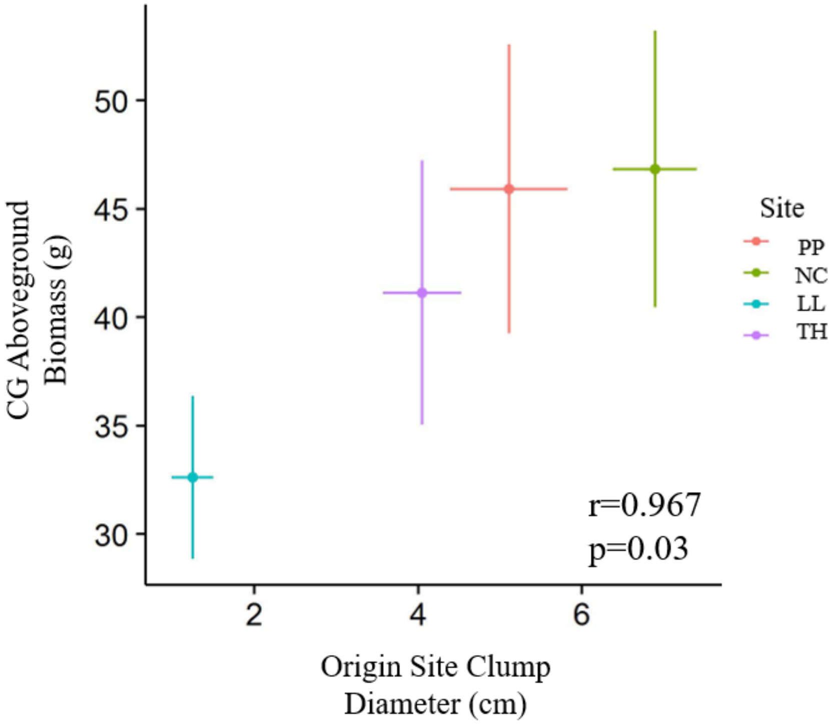


Figure 3.11 Mean and standard error of total aboveground-biomass in the common garden plants as a function of clump diameter in the origin site plants. Site abbreviations in Table 3.1

CHAPTER 4

QUANTIFYING NITROGEN DEPOSITION AND RESPONSES OF NATIVE PRAIRIE GRASSES IN NORTH TEXAS

Abstract

As the human population continues to move into cities, anthropogenic nitrogen (N) additions to urban ecosystems are increasing. The two main types of atmospheric N inputs are nitrate (NO_3^-) and ammonium (NH_4^+), which primarily come from the combustion of fossil fuels and the volatilization of ammonia, respectively. Bulk N deposition was collected at six sites of differing urban development in the Dallas-Fort Worth (DFW) Metroplex along a north-south transect from April 2014 through September 2015. Concurrently, a phytometer experiment was conducted in which the native perennial grasses little bluestem (*Schizachyrium scoparium*) and Texas wintergrass (*Nasella leucotricha*) were grown at each of the sites to measure plant response to N deposition. Bulk N deposition in DFW was compared to that of the rural National Atmospheric Deposition Program (NADP) reference site located outside of the Metroplex. Mean daily deposition for the DFW sites was 14%, 39%, and 30% greater for NO_3-N, NH_4-N, and dissolved organic nitrogen (DIN; NO_3-N + NH_4-N) than at the rural reference site. NO_3-N deposition across the DFW sites ranged from 0.0062-0.01 kg NO_3-N/ha/day and comprised 26-36% of DIN deposited. NH_4-N deposition in DFW ranged from 0.0108-0.0178 kg NH_4-N/ha/day and comprised 62-67% of DIN deposited. There was no relationship between the ratio of NO_3-N:DIN or NH_4-N:DIN and urban development. Plant characteristics such as number and length of tillers and overall biomass differed significantly among the sites, however, there was no relationship between plant growth and bulk N deposition. The lack of response to N deposition in these perennial grass species could be due to deposition levels too low to elicit a response or

due to other effects of urbanization such as elevated ozone. By documenting that N deposition in DFW is higher than in surrounding areas, this research contributes to our understanding of urban N deposition in the southern Great Plains, and adds to the literature suggesting that human activities associated with urban areas result in greater N deposition.

Introduction

Nitrogen (N) is required for all life forms on Earth and is the key nutrient limiting productivity in many undisturbed terrestrial ecosystems (Vitousek and Howarth 1991). The Earth's atmosphere possesses an abundance of N in the form of N_2, however, this inert N is not available to most organisms. In order for N to be incorporated into the global food chain, the N must be "fixed" or turned into reactive N (Nr) by nitrogen-fixing bacteria, lightning, or man-made chemical reactions (Canfield et al. 2010). The natural production of Nr (mostly from biological fixation by cyanobacteria) has remained relatively stable at 125 Teragrams of nitrogen per year (Tg N/yr), but human activities have increased global reactive nitrogen production to over 300 Tg N/yr (Galloway et al. 2008). Two-thirds of the anthropogenically created Nr comes from the production of fertilizer and other industrial activities, while the remaining input comes from the increase in cultivation-induced biological fixation and the combustion of fossil fuels (Galloway et al. 2008). Most anthropogenically created N flows through the agricultural food chain, but human activities also generate N molecules that are released directly into the atmosphere (Galloway et al. 2003). These atmospheric N compounds may be deposited to the earth dissolved in precipitation (wet deposition) or directly as particles and gases (dry deposition; Ponette-González et al. *In press*), a process known as N deposition. The deposition of atmospheric N represents an additional source of nitrogen to ecosystems and is most pronounced in areas of concentrated human activity (Baker et al. 2001, Groffman et al. 2004).

The two main types of anthropogenic N inputs to the atmosphere are nitrogen oxides (NO and NO_2, collectively NO_x) and reduced forms of N (NH_3 and NH_4^+, collectively NH_x; Galloway et al. 2004). The oxidized forms of N are a byproduct of the combustion of fossil fuels in industry and vehicles, and the reduced forms are produced as a result of the volatilization of NH_3 from fertilizer and animal waste (Reis et al. 2009). The deposition of these N compounds occurs primarily as NO_3^- (nitrate) and NH_4^+ (ammonium), which is readily available to plants for incorporation into biomass (Aber and Melillo 2001).

The availability of N is what most often limits plants in temperate terrestrial ecosystems (Lebauer and Treseder 2008). Increases in nitrogen availability can lead to shifts in community structure when species adapted to N-poor soils give way to N-demanding species (Vitousek et al. 1997). Biodiversity, especially in native plants, has almost universally decreased with increases in N availability (Clark and Tilman 2008, Stevens et al. 2010). Nitrogen inputs can also determine the outcome of competition between native and alien invasive species; increased N availability through mechanisms like fertilization and deposition has allowed plants to invade habitats they previously could not inhabit (Rickey and Anderson 2004).

Plants that experience fertilization via N deposition may show changes in resource allocation (e.g., root vs. shoot ratio and reproductive vs. vegetative tissue) or changes in tissue quality in the form of carbon to nitrogen (C:N) ratio (Chapin 1980, Bloom et al. 1985, Reich et al. 2003). Lowered C:N ratio in plant tissues has the potential to affect higher trophic levels by increasing palatability to herbivores (Berner et al. 2005), or by shifting allocation to secondary metabolites that serve as anti-herbivore defenses (Reich et al. 2003).

Thus far, most of our understanding of the response of plants to N additions has occurred in experimental field manipulations (Tilman and Wedin 1991, Wedin and Tilman 1996, Reich et al. 2001, Novotny et al. 2007, Pan et al. 2010), or in growth chambers and greenhouses (Wong 1979, Coleman et al. 1993). Fewer studies have investigated the response of plant growth to N additions in situ along an urban-rural gradient, and of the ones that have, forests have been the focus (Aber et al. 1989, Gregg et al. 2003, 2006, Fang et al. 2011, Searle et al. 2012).

This study aimed to expand our understanding of N deposition effects on plants by quantifying ambient N deposition along an urban to rural gradient in the southern Great Plains and examining the responses of plants native to those grasslands. North Texas, specifically the Dallas-Fort Worth (DFW) area, was identified by Nancy Grimm and colleagues (2008b) as one of seven megapolitan regions in the United States that will play a crucial role in understanding ecosystem responses across climatic and societal gradients. It is the only megapolitan region within the Great Plains ecoregion, and the combination of rapidly growing urban area and undeveloped rural area within close proximity makes it an ideal research site for urban studies.

The National Atmospheric Deposition Program (NADP), in collaboration with the National Trends Network (NTN), has been monitoring wet N deposition across the United States since 1981. However, the NADP/NTN monitoring sites are specifically located away from urban areas and point sources, resulting in a potential underestimation of the N deposition in and around cities (Fenn et al. 2003). The NADP has one monitoring site outside of DFW, but at the time of these experiments, there were no N deposition monitoring efforts near urban areas of North Texas. In this study, bulk deposition collectors, which accumulate wet inputs and coarse particles (>2µm), were used to measure bulk N deposition in the form of NO_3^-, NH_4^+, and dissolved inorganic N (DIN; $NO_3^- + NH_4^+$).

To investigate the effects of N deposition on grassland plants, a phytometer experiment was conducted using two dominant perennial grasses from prairies native to this region, little bluestem (*Schizachyrium scoparium*) and Texas wintergrass (*Nasella leucotricha)*. Phytometer experiments use plants to measure impacts of the local environment by placing plants from one population into multiple location and monitoring their growth (Clements and Goldsmith 1924). In this study it was hypothesized that:

1. Bulk inorganic N deposition would be greater in the DFW Metroplex than at the NADP monitoring site outside of DFW
2. The proportion of bulk NO_3-N deposition would increase while the proportion of NH4-N would decrease with increasing urban development within the DFW Metroplex
3. Plant biomass would increase while root:shoot and tissue C:N ratios would decrease with increasing inorganic bulk N deposition

Methods

Study Area

This research was conducted in the western half of the Dallas-Fort Worth (DFW) Metroplex in Texas, USA, centered around Tarrant County. Most of Tarrant County lies in the Fort Worth Prairie, part of the larger Grand Prairie, and consisting mainly of shallow soil underlain by limestone (Dyksterhuis 1946). The vegetation is dominated by little bluestem (*S. scoparium*) and various perennial forbs (Dyksterhuis 1946). Since settlement of the area in the mid-19th century, the prairie has been converted to agricultural and urban land use, though fragmented remnant patches remain throughout the area.

To calculate “percent urban development” for each site, the U.S. Department of the Interior’s 2011 National Land Cover Database was used (NLCD; Homer et al. 2015). The NLCD urban land-use categories are calculated based on percent impervious surface area (ISA). The NLCD categories “Developed High Intensity,” “Developed Medium Intensity,” and “Developed Low Intensity” represent ISA values of 80-100%, 50-79%, and 20-49%, respectively. For a 10k buffer surrounding each site, the area of these categories were summed into one value and divided by the total area of the buffer to calculate “percent urban development.”

Study Species and Experimental Design

The plants used in this study were two native prairie grasses: Little bluestem, a C_4 warm-season perennial, and Texas wintergrass, a C_3 cool-season perennial. Little bluestem was selected because of its adaptation to the ecoregion and dominance in the native prairie landscape. Texas wintergrass was chosen to serve as a C_3 counterpart to little bluestem, as C_3 and C_4 plants respond differently to N additions (Niu et al. 2008). Plants for this experiment were collected from one population on the perimeter of DFW by a local nursery in Mansfield, TX and grown there for several months.

Six phytometer sites were selected along a north to south transect spanning Denton, Tarrant, and Johnson counties based on space availability and access (Figure 4.1; Table 4.1). Three sites were located on managed property: two community colleges (TCNW and TCNE), and one campus of county services buildings and demonstration garden (TCSO). Two properties were located within designated natural areas (LELA and DENT), and the remaining site was located on five acres of private property with farm animals nearby (ALVA). Average temperature and total rainfall were obtained from the nearest weather station for the period of April 4, 2014 to October 9, 2015 (553 days; Table 4.1). The NADP deposition monitoring site at the Lyndon B. Johnson

National Grasslands (LBJ, 33.41577, -97.62346), 78km NNW of the center of Fort Worth, was used as a reference site for background N deposition levels.

To prevent competition from established plants and minimize weed invasion, black weed barrier was attached to the ground in a 6x7.5m rectangle at each site. A water barrel was installed at each site and filled with city or well water from the site for ease of watering plants. The plants that were obtained from the local nursery were placed in one-gallon black plastic containers filled with topsoil sourced from Mansfield, TX. The plants were randomly assigned to sites such that there were a total of 20 little bluestem and 20 Texas wintergrass plants at each site.

This experiment was conducted with little bluestem twice – once in 2014 and once in 2015. One experiment was conducted with Texas wintergrass that extended from 2014 to 2015. The little bluestem plants grown in 2014 (henceforth LBS 2014) were placed at the sites in October 2013 while dormant, and remained dormant until April 2014, when measurements began. Thus LBS 2014 had an active growing period with measurements from April 2014 to July 2014. The 2015 little bluestem plants (henceforth LBS 2015) were collected from the same nursery in 2015 and planted at the sites in April 2015. The plants began to die off in July, however, thus little bluestem 2015 have an active growing period with measurements from April 2015 to July 2015. The 2015 Texas wintergrass plants (henceforth TW 2015) were placed at the sites in May 2014 and remained until their harvest in May 2015. The Texas wintergrass plants had an effective growing period with measurements from October 2014 to May 2015.

During each group's active growing season, the plants were measured on a monthly basis by counting and measuring each vegetative and reproductive tiller. Once reproductive tillers began appearing, measurements occurred every other week so that a more precise record of flowering phenology could be obtained. For Texas wintergrass, the reproductive tillers and vegetative tillers were so densely packed and indistinguishable at the base that measuring basal diameter became a more accurate and feasible method. To make the switch between tiller number and basal diameter, the basal diameter was measured along with the number of tillers in October 2014 so that the basal diameter could be back calculated.

At their respective harvest times, the plants from each site were brought back to the lab at UT Arlington to remove dirt and separate the plant into roots, vegetative tillers, and reproductive tillers. For Texas wintergrass, the plants were separated into roots and above-ground tillers (both reproductive plus vegetative). The samples were then dried at 52°C for at least 3 days, weighed, and ground using a Wiley Mill in preparation for CHN analysis on a Perkins and Elmer 2400 Series CHN analyzer.

N Deposition Measurements

Bulk DIN (NO_3^- + NH_4^+) deposition was measured at each site using ion exchange resins. Ion exchange resins (henceforth resins) are polystyrene beads that are capable of exchanging ions on the beads for ions in solution (i.e., precipitation). In this experiment, Amberlite IRN 150 Resin (a mixed-bed resin that captures both positive and negative ions) was used. Deposition collectors, modeled after Simkin and colleagues (Simkin et al. 2004, Weathers et al. 2006), consisted of resin-filled 20mL chromatography columns attached to a 20cm diameter funnel with a polywool filter at the neck to prevent debris and insects from falling into the collecting column. Two bulk deposition collectors were placed at each site, approximately three meters apart. Collection began on April 4, 2014, and the columns were replaced at each site with fresh resins on July 11, 2014; October 11, 2014; January 9, 2015; April 10, 2015 and July 13, 2015; resulting in six approximately three month collecting periods. Upon collection, new resin columns were attached to clean collector components and clean funnels with new polywool. Columns were then refrigerated at 4°C.

Samples and blanks were extracted three times in the laboratory with 2 N potassium chloride following the methods of Fenn et al. (2002) and Simkin et al. (2004). Immediately following extraction, samples were refrigerated at 4°C and then shipped to the Cary Institute of Ecosystem Studies where NH_4-N and NO_3-N were determined using a QuickChem Flow Injection Analyzer +8000 Series (Lachat Instruments, Loveland, Colorado). The detection limit of this method is 0.02 mg/L. A series of standards and two blank samples were included with each instrument run.

Calculation of Fluxes

To determine nitrate and ammonium amounts deposited at each site, the values for the two collectors at each site were averaged. During the first collecting period, two funnels (one at TCNW and one at LELA) were knocked off of their PVC stands, resulting in a one- to fourteen-day period in which the resin columns were not accurately collecting bulk deposition. For this reason, these two collectors were excluded from the analyses. Collectors contaminated by bird droppings with evidence of NH_4-N values more than two times greater than the companion collector at the site were also excluded from analyses. This resulted in an additional three collectors being excluded. For periods in which one collector/sample was invalid, the value from the remaining collector/sample was used.

Values below the detection limit were set to half the detection limit for data handling. Bulk NH_4-N and NO_3-N concentrations were converted to fluxes (kg/ha/sampling period) by incorporating collector surface area (324 cm^2). The results of the analyses yielded bulk N deposition values of NO_3-N and NH_4-N in mg/L, which were multiplied by the L of potassium chloride extractant used (0.3 L), converted to kg, and then divided by the area of the funnel in hectares. The total values for the entire experimental period are reported initially in the results. For comparison purposes from then on, the amount of N deposition is reported on a per-day basis.

Given differences in precipitation among the study sites, precipitation-normalized N deposition values - the deposition rate (kg/ha/day) divided by the amount of precipitation in mm at each site for the same time period – were calculated.

Statistical Methods

A multivariate analysis of variance (MANOVA) was conducted to examine differences among sites in plant biomass (root, vegetative, and reproductive) and root:shoot ratio. A separate MANOVA was conducted to examine differences in tissue quality (C:N ratios of roots, vegetative, and reproductive tillers). This was repeated for each species and growing season, with the exception of TW 2015, which had only root C:N and above ground (vegetative and reproductive combined) C:N for tissue quality. For all MANOVAs, root:shoot ratio was arcsine square root transformed, and the remaining plant characteristics were log-transformed. For ease of interpretation, untransformed values are presented in graphs and text. Following each MANOVA, individual ANOVAs were conducted on significant characteristics with Dunnett's C used for all post-hoc tests.

Correlations between plant characteristics and NO_3-N, NH_4-N, and DIN deposition were conducted using Spearman's Rho. To account for multiple comparisons between DIN and root:shoot, vegetative tiller C:N, reproductive tiller C:N, root C:N, and total biomass (5 comparisons), the standard alpha level of 0.05 was divided by 5 to achieve an adjusted alpha level of 0.01. Correlations between N deposition and precipitation were examined using Spearman's Rho. The same correlations were conducted between plant characteristics and precipitation-normalized deposition as well.

Results

Bulk N Deposition in North Texas

Bulk deposition at the LBJ reference site for a collection period spanning 25 February 2014 to 1 October 2015 totaled 3.6 kg NO_3-N/ha/583 days, 5.9 kg NH_4-N/ha/583 days, and 9.5 kg DIN/ha/583 days. Bulk deposition averaged across all DFW phytometer sites for a similar, but slightly shorter collecting period (4 April 2014 to 9 October 2015), was 4.1 ± 0.3 kg NO_3-N/ha/553 days, 8.1 ± 0.7 kg NH_4-N/ha/553 days, and 12.2 kg DIN/ha/553 days. Mean daily deposition for the urban sites was 14%, 39%, and 30% greater for NO_3-N, NH_4-N, and DIN than at the rural reference site.

N deposition varied by season and was greatest in the spring, which included May, the rainiest month in North Texas. There was a one-fold decrease in NO_3-N and a six-fold decrease in NH_4-N from Spring 2015 to the Summer 2015 with a similar decrease at the reference site (Figure 4.2). Deposition rates were generally higher at the DFW sites compared to the reference site during all sample seasons, with the exceptions of Summer 2014 in which NO_3-N deposition was greater at the reference site, and Spring 2015 in which both NO_3-N and NH_4-N deposition were greater at the reference site (Figure 4.2).

Bulk N Deposition and Urban Development

NO_3-N deposition across the DFW sites ranged from 0.0062-0.01 kg NO_3-N/ha/day and comprised 26-36% of DIN deposited. NO_3-N deposition in DFW was similar across sites with the exceptions of TCNW and TCNE, which had deposition rates approximately 50% and 32% greater than the other sites, respectively (Figure 4.3). The proportion of NO_3-N deposited was not related to urban development.

NH_4-N deposition in DFW ranged from 0.0108-0.0178 kg NH_4-N/ha/day and comprised 62-67% of DIN deposited. There was a positive but nonsignificant relationship between urban development and NH_4-N deposition (Figure 4.3).

Phytometer Results

LBS 2014

There were significant differences among sites in plant biomass when root, vegetative, and reproductive biomass as well as root:shoot ratio were considered jointly (Wilk's lambda=0.25, F(20, 157)=4.0, p<0.001, eta squared=0.29). Separate ANOVAs were conducted for each dependent variable, revealing significant univariate main effect for root biomass (F(5,50)=4.7, p=0.001, eta squared=0.32); vegetative biomass (F(5,50)=4.2, p=0.003, eta squared=0.30); reproductive biomass (F(5,50)=8.7, p<0.001, eta squared=0.47); and root:shoot ratio (F(5,50)=3.1, p=0.017, eta squared=0.24).

Total plant biomass at TCNW was almost double the average for all sites (Figure 4.4). TCNW had the lowest proportion of biomass allocated to roots (15%) and vegetative tillers (21%) and the greatest proportion allocated to reproductive tillers (64%). DENT, the site with the lowest overall biomass had the greatest allocation to root biomass (29%). LELA, the site with the second least overall biomass, had the lowest allocation to reproductive tillers (28%) and the highest to vegetative tillers (50%). However, there was no clear relationship between urban development and overall biomass or biomass allocation.

There were no differences among sites in the C:N ratio of LBS roots (average C:N=94) or reproductive tissue (average C:N=75). Vegetative tiller C:N ratios were significantly different among sites (F(5,50)=3.3, $p<0.001$), with TCNW having a significantly greater C:N ratio than LELA due to having 33% less N, though there were no trends along an urban gradient.

There were no significant relationships between bulk DIN deposition (kg/ha/day) and any plant characteristics, nor were there any relationships between precipitation-normalized bulk DIN deposition and plant characteristics.

LBS 2015

When root, vegetative, and reproductive biomass, as well as root:shoot ratio were considered jointly for the 2015 season of LBS (Wilk's lambda=0.3, F(16, 83)=2.5, p=0.004, eta squared=0.26), there were significant differences among sites in root biomass (F(4,30)=4.7, p=0.005, eta squared=0.08) and vegetative biomass (F(4,30)=5.8, p=0.001, eta squared=0.44). Specifically, TCNW had significantly greater root biomass (average±1.00 s.e. = 3.38±0.34g) than TCSO (1.69±0.13g), and significantly greater shoot biomass (4.73±0.38) than TCSO (3.05±0.20g). The reproductive biomass did not differ significantly among sites, and the overall average was 0.81±0.16g. The root:shoot ratio across all sites was 0.57±0.03g.

The biomass for the 2015 LBS plants was much lower than that of the 2014 season. The average plant biomass in 2015 was 6.71g compared to 60.14g in 2014. The reduction in biomass was seen in all parts of the plants in 2015: roots were only 21% of the 2014 biomass, vegetative tillers were 20% of 2014 averages, and reproductive tissues were a mere 3% of last year's production.

Tissue quality of roots, vegetative tillers, and reproductive tillers did not differ among sites. The average C:N ratios for all sites were 78, 63, and 83 for root, vegetative, and reproductive tissues, respectively.

An examination of the relationship between DIN deposition (kg/ha/day) and plant characteristics including biomass, root:shoot ratio, and C:N ratio of roots, vegetative tillers, and reproductive tillers revealed that root C:N ratio was significantly inversely correlated with DIN deposition (Rs=-0.87, p=0.019). The same correlations using precipitation-normalized DIN deposition revealed no significant relationships.

The 2015 little bluestem plants were subject to the greatest monthly amount of rain on record for DFW in May 2015. Initially, this presented the unique opportunity to examine little bluestem responses to N deposition at normal and above-average precipitation levels. However, the plants experienced flooded pots for extended periods in May and much of the above-ground biomass died back. As the sites began to dry out towards the end of June, some little bluestem experienced a regrowth, but the plants were severely stunted. This is evidenced by the fact that the final total biomass for plants in 2015 was an order of magnitude lower than in 2014. This is likely the result of the artificial growing conditions restricting drainage, rather than the precipitation itself. As a result, the data from the 2015 LBS season are not considered further in this investigation.

TW 2015

Considered together, there were significant differences among sites in root biomass, above ground biomass (vegetative and reproductive biomass together), and root:shoot ratio (F(10,160)=0.471, p<0.001, eta squared=0.31) for Texas wintergrass. Individual ANOVAs revealed significant differences among the sites in above ground biomass (F(5,81)=7.71, p<0.001, eta squared=0.32), root biomass (F(5,81)=11.38, p<0.001, eta squared 0.41), and root:shoot ratio (F(5,81)=3.85, p=0.004, eta squared=0.19). The average root biomass (±1.0 s.e.) was 2.98±0.17 g, with TCNW having significantly greater biomass than the four less urban sites (Figure 4.5). TCNW also had an above ground biomass that was 78% greater than the overall average for all sites (Figure 4.5). The average root:shoot ratio of TW was 0.063±0.003, with TCSO having the largest root:shoot ratio of 0.081.

C:N ratios differed among sites (Wilk's lambda=0.46, F(10,148)=6.7, p<0.001, eta squared=.31), and individual ANOVAs revealed significant differences among sites in root C:N ratio (F(5,75)=10.0, p<0.001, eta squared=40), and above ground C:N ratio (F(5,75)=4.7, p=0.001, eta squared=0.23). Below ground tissue quality differed significantly between the two least urban sites, ALVA (40.1±3.0) and DENT (51.9±2.0). ALVA also had the lowest above ground tissue quality (31.2±1.7) while DENT had the highest (42.0±1.8).

There were no significant relationships between DIN deposition (kg/ha/day) and plant characteristics, however. Using precipitation normalized DIN deposition also did not reveal any significant relationships.

Discussion

N Deposition in DFW vs. LBJ Reference Site

The NADP monitoring site nearest DFW is approximately 80km NNW of the center of the city of Fort Worth and has been collecting data since 1983. The NADP monitoring sites were set up to measure regional trends in deposition and are located specifically outside of local pollution sources, i.e. away from urban areas. Thus, NADP measurements of N deposition generally underestimate local and regional values as a result of urban areas, but serve as useful estimates of background levels of deposition (Bettez and Groffman 2013). This research represents the first time that N deposition has been quantified within the highly urbanized areas of NTX.

Seasonal precipitation differences between DFW and LBJ resulted in the reference site sometimes having greater bulk N deposition when examining individual seasons (Figure 4.2). However, the bulk N deposition for the entire experimental period (18 months) was consistently higher (kg N/ha/day) in DFW than at the LBJ reference site. This held true for both absolute N deposition rates and precipitation-normalized rates (Figure 4.3). This indicates that though there may be greater seasonal pulses outside of the urban area, in the long-term, the urban sites are receiving greater rates of N deposition, supporting the first hypothesis.

DFW N Deposition and Urban Development

Rejecting the second hypothesis, there was no increase in the proportion of NO_3-N with increasing urban development, nor was there a decrease in NH_4-N with greater urban development. This is in contrast with other parts of the US such as the Mississippi River Basin in which the highest rates of wet deposition were found downwind of electric utility plants and urban areas, and the highest rates of wet deposition of NH_4^+ were near intensive agricultural activities (Lawrence et al. 2000). The majority of N deposition in DFW was in the form of NH_4-N (63-71%), regardless of whether the site was more urban or rural. Again, this is in contrast with other locations such as the state of Connecticut in the northeastern US which had 66% NO_3-N (Luo et al. 2003), and in California where urban areas received proportionally more NO_3-N deposition than rural areas (Tulloss and Cadenasso 2015).

Recently, it has been suggested that due to factors including the increased regulation of NO_x, lack of regulation of NH_3, and the increased need for fertilizer and livestock, DIN deposition from the late 2000s on is composed largely of NH_4^+ rather than NO_3^- (Du et al. 2014, Li et al. 2016). While the greater proportion of NH_4^+ to NO_3^- in North Texas is in contrast to the studies above, it may be that the bulk N deposition data for North Texas are actually just a reflection of the more recent shift towards reduced N deposition.

Plant Growth and Relationship with N Deposition

Both Texas wintergrass and little bluestem showed significant differences among sites in the size, biomass allocation, and tissue quality of the plants. However, the greatest differences in plant growth resulted from differences between plants at one site (TCNW) and the other sites. TCNW did not have significantly greater N deposition than the other more urban sites, yet that site produced double the biomass of both species compared with the other sites (Figures 4.4 – 4.5). The plants at this site were likely being inadvertently watered or fertilized. The soil within the pots and surrounding the plot was wetter than at the other sites, and the plants were more robust throughout the growing period, even when rain was scarce (personal observations). Although the direct N and water inputs at this site were not quantified, the data from this site may be a good indication of what one might expect from the fertilization of prairie grasses in years when water is abundant.

In grasslands, N additions have been shown to increase primary productivity (Tilman and Wedin 1991, Heggenstaller et al. 2009, Phoenix et al. 2012), and little bluestem, specifically, has responded with greater dry mass (Bush and Van Auken 2010). However, this study found no correlations between rates of bulk N deposition and above- or below-ground biomass in little bluestem or Texas wintergrass. Thus, the third hypothesis is rejected.

One possible reason for the lack of agreement between this study and the Bush and Van Auken study is that the simulated rate of N deposition in their study was 100 kg N/ha/yr, an order of magnitude greater than N deposition rates in North Texas. For the most part, studies that have examined the relationship of N deposition to grassland species have either focused on changes in biodiversity (Clark and Tilman 2008, Stevens et al. 2010) or have used predicted values of future N deposition rates that are unrealistic compared to current levels (Zavaleta et al. 2003, Heggenstaller et al. 2009, Pan et al. 2010, but see McPhee et al. 2015 for rates as low as 20 kg N/ha/yr).

To the best of the author’s knowledge, only one study has examined the response of grassland productivity in response to ambient N deposition. In a global meta-analysis of forty-two plots in the Nutrient Network Global Research Cooperative (http://www.nutnet.unm.edu) with N deposition ranging from <1.00 to 35.91 kg N/ha/yr, Stevens and colleagues found that each additional unit of N deposition (kg N/ha/yr) corresponded to a 3% increase in above-ground net primary production (2015). Thus, it is possible to see responses of grassland species to ambient N deposition, but this study did not find any response to N deposition in little bluestem or Texas wintergrass.

The lack of relationship between measured N deposition and plant growth could also be because of an underestimation of bulk N deposition. This study did not take into account dry deposition or organic N deposition, which can exist in gas, particulate, and dissolved phases can comprise a large fraction (up to 30%) of total deposition (Cape et al. 2011), and should be explored in future studies.

Calculating the precipitation-normalized N deposition values was a way to control for the effects of rainfall on plants and isolate N deposition. However, there are still factors such as ozone that vary with N deposition along an urban gradient that should be investigated in future studies. Additionally, it has been found that N deposition in the northeastern US has an interannual variation of as much as 20% (Butler et al. 2003). This eighteen-month study, therefore, provides a snapshot of N deposition in North Texas, but would benefit from additional years of measurement in future studies to establish an overall pattern.

N Deposition in DFW vs. Other US Cities

Bulk N deposition rates in DFW fall in between those of Baltimore, MD and the state of Connecticut. Bulk deposition measured in Baltimore Metropolitan Statistical Area, MD, from June 2010 to June 2011 ranged from 6.3(±.0.2) to 7.0(±.2) kg N (NO_3-N + NH_4-N)/ha/yr (Bettez and Groffman 2013). In DFW, four years later, a similar timespan of July 2014 to July 2015 yielded DIN deposition rates ranging from 7.3 to 10.0 kg DIN/ha/yr, depending on site. Another study of Baltimore in 2005 estimated wet N deposition (NO_3-N, NO_2-N, and NH_4-N) to be 6.6, 8.4, and 11.8 kg/ha/yr at urban, suburban, and rural sites respectively (George et al. 2007). In Connecticut, the 1997-1999 average wet deposition flux of total N was 12.47kg N/ha/yr (Luo et al. 2003). Despite the fact that the population of North Texas is more than two times larger than the state of Connecticut and the Baltimore metropolitan region, the deposition rates are in the same range, suggesting the importance of factors in addition to population that may be influencing the amount of N reaching the ground surface, i.e. land use, meteorological patterns, and topography.

Summary

Bulk N deposition was measured for the first time within the DFW Metroplex and was found to be greater than the wet N deposition collected at a rural reference site located 80 km from the center of Fort Worth, supporting the first hypothesis. Contrary to the second hypothesis, there was no correlation between the form of N deposited and urban development. Rather, NH_4-N was the dominant form of bulk deposition in DFW, regardless of level of urban development. Finally, though plant growth and tissue quality were significantly different among the sites, there were no relationships between plant growth and N deposition. Other factors of the urban environment could be preventing grass growth responses to the added N, or the added N could be too low to result in growth responses. Future research in urban areas should help untangle the environmental conditions altered by human activity and determine how they are affecting native plant growth.

Tables and Figures

Table 4.1 Site characteristics including percent urban development, mean temperature, and total precipitation during the course of the experiment - April 4, 2014 through October 9, 2015

Site Name	ID	Latitude	Longitude	% Urban Development	Precipitation Mean (mm)
Alvarado Private Property	ALVA	32.396214	-97.239501	4	1430
Clear Creek Natural Heritage Area	DENT	33.260611	-97.064954	14	1971
Lewisville Lake Environmental Learning Area	LELA	33.062995	-96.98849	42	1538
Tarrant County College District Northwest Campus	TCNW	32.831501	-97.391725	46	1155
Tarrant County Resource Connection	TCSO	32.674371	-97.308242	58	1122
Tarrant County College District Northeast Campus	TCNE	32.850975	-97.190621	64	1380

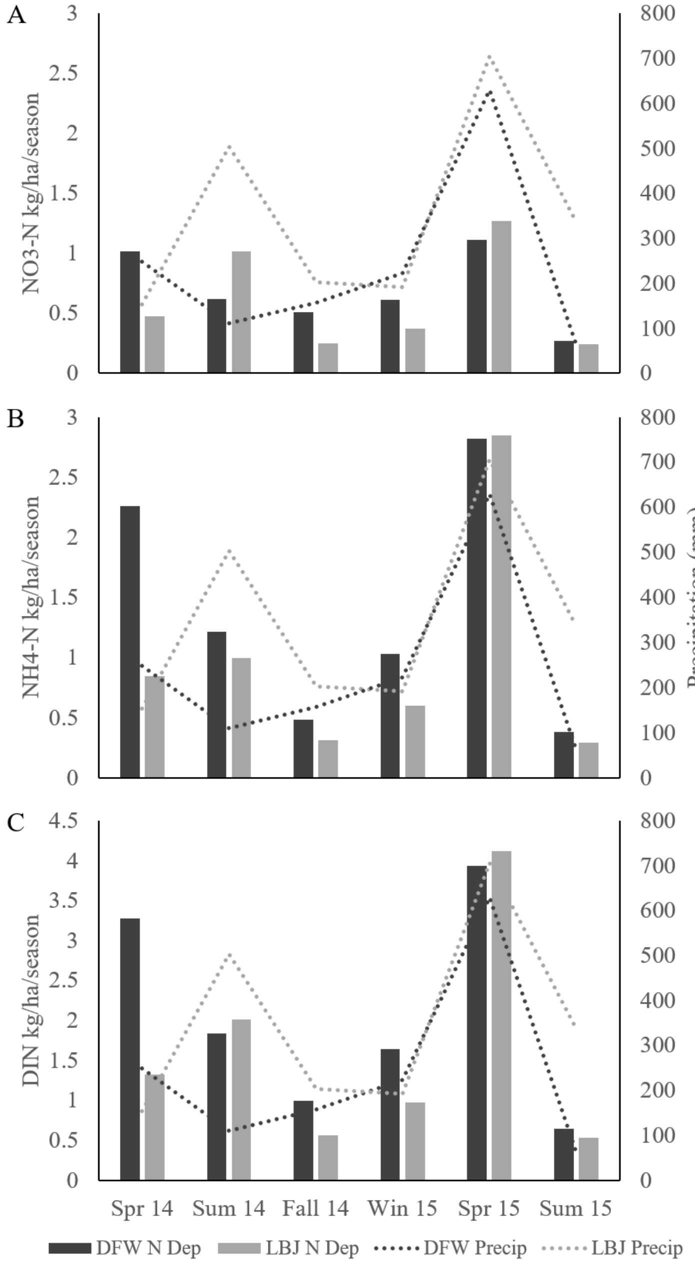


Figure 4.1 N deposition (dark solid bar) and precipitation (dark dotted line) by season for the DFW experimental sites. Beginning dates for DFW collection periods are as follows (left to right): 4 Apr 2014, 11 Jul 2014, 11 Oct 2014, 9 Jan 2015, 10 Apr 2015, 13 Jul 2015; end date 9 Oct 2015. N deposition (light solid bar) and precipitation (light dotted line) by season for LBJ reference site. Beginning dates for LBJ collection periods are as follows (left to right): 25 Feb 2014, 3 Jun 2014, 2 Sep 2014, 2 Dec 2014, 3 Mar 2015, 2 Jun 2015; end date 1 Sep 2015

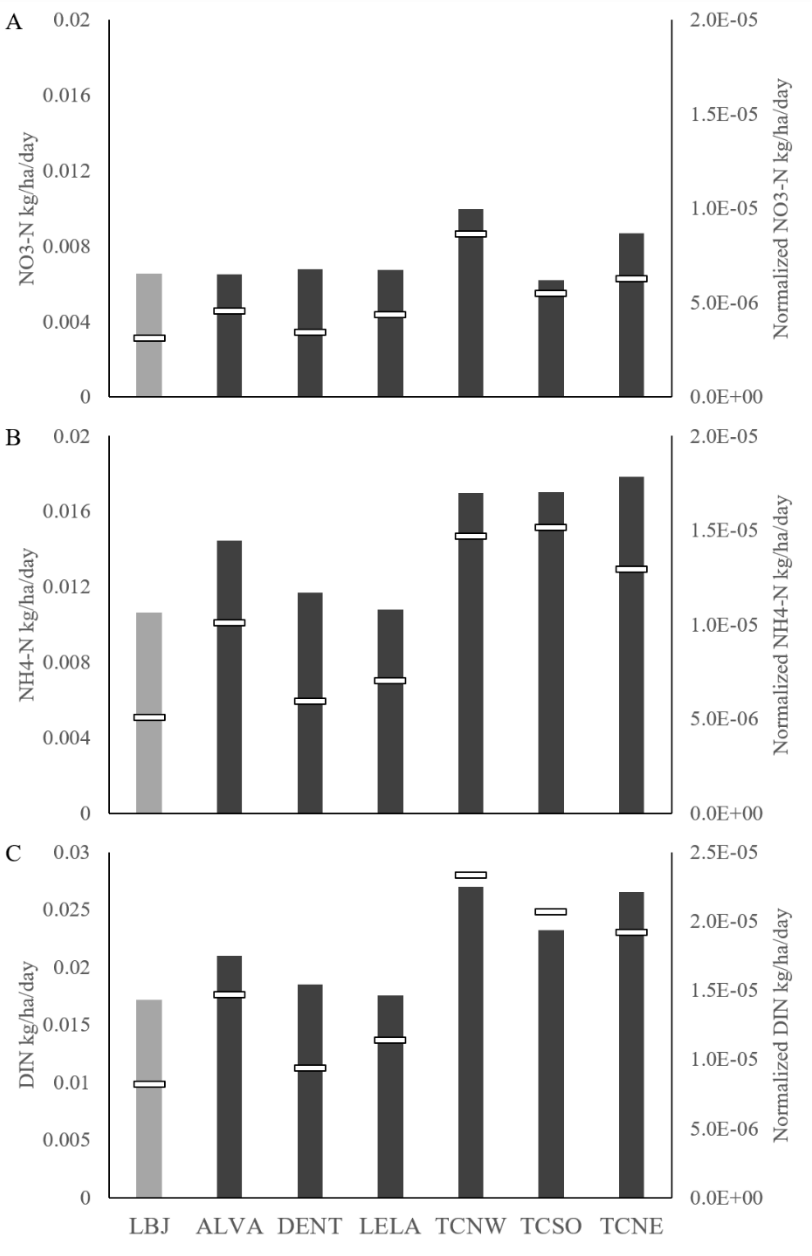


Figure 4.2 Solid vertical bars indicate absolute N deposition in kg/ha/day for A) NO_3-N, B) NH_4-N, and C) DIN. The light gray vertical bar indicates the NADP reference site at the LBJ National Grasslands. The dark gray vertical bars indicate DFW sites from least to most urban. Precipitation-normalized N deposition is represented by white horizontal bars and the secondary axis. Site abbreviations as in Table 4.1

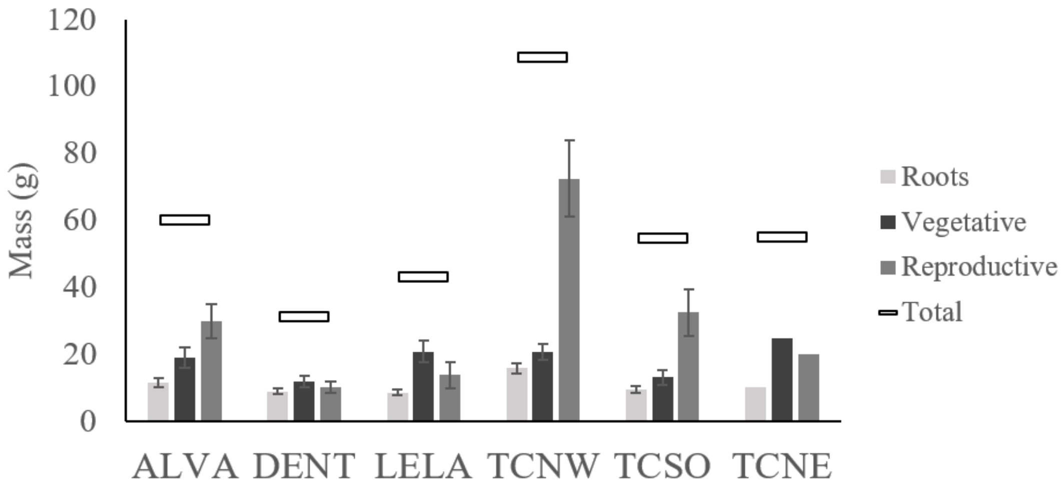


Figure 4.3 Dried biomass of little bluestem at the end of the 2014 season. Bars represent site averages with standard error bars. The total biomass average is represented by horizontal white bars. Site abbreviations as in Table 4.1

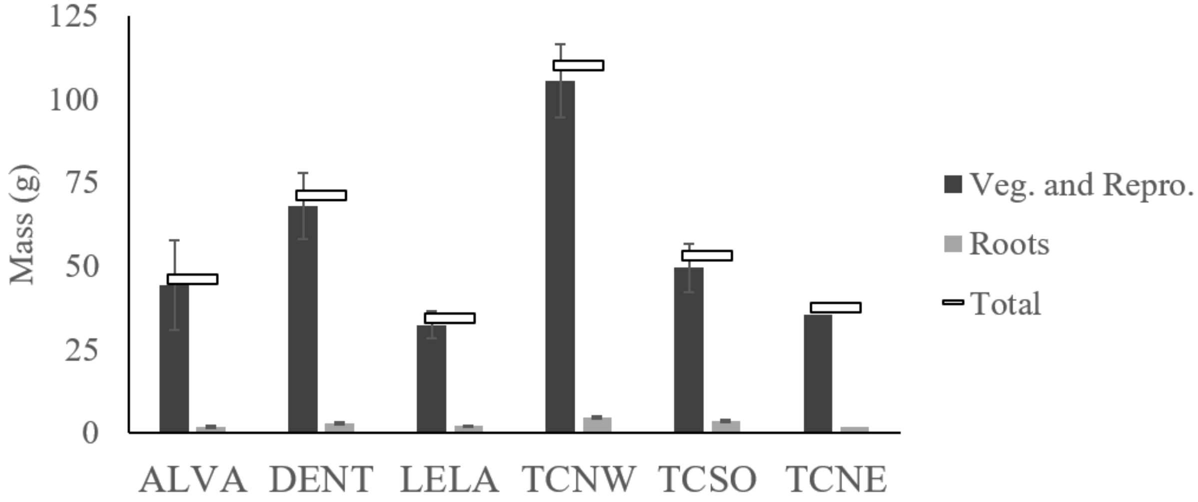


Figure 4.4 Dried biomass of *N. leucotricha* at the end of the 2015 season. Bars represent site averages with standard error bars. The total biomass average is represented by horizontal white bars. Site abbreviations as in Table 4.1

CHAPTER 5

BROADER IMPACTS

In 1990, McDonnell and Pickett emphasized the importance of integrating humans into ecological studies and called for scientists to take advantage of the “natural experiments” occurring as a consequence of urbanization. The Dallas-Fort Worth metropolitan area is an excellent place in which to conduct urban ecology research. The combination of the rapidly expanding human population and the under-studied environment of urban plains ecosystems provides the opportunity to expand our understanding of human influences on ecosystems.

Understanding how species that are characteristic of the Fort Worth prairie respond to urban conditions is critical for conservation and restoration efforts. If the characteristics exhibited by plants in urban areas are the result of adaptation to the urban environment, it will be of vital importance to preserve seeds from these ecotypes. Restoration efforts should use seeds from plants that are genetically equipped to handle the urban or near-urban environment to ensure success now and into the future.

Understanding the response of native plants to urban fertilization can aid in making predictions about shifting community structure and the outcome of competition with invasive species. In ecosystems where N is limiting, studies have established that greater N availability increases growth (Lebauer and Treseder 2008). However, N saturation occurs when biological functions no longer increase in response to additional N. Nitrogen saturation can lead to community changes when species adapted to N-poor soils (such as those found in the Fort Worth Prairie) give way to N-demanding species (Vitousek et al. 1997). In the UK, formerly N-poor heathlands have given way to N-rich grasslands (Aerts and Berendse 1988). Because N deposition has changed the community structure of the heathlands to reflect the surrounding ecosystems, it has resulted in the reduction of both alpha and beta-diversity. Similar scenarios have been seen throughout the world, resulting in the general conclusion that increased N deposition leads to a decrease in plant species diversity (Vitousek et al. 1997, Stevens et al. 2010). Nitrogen availability is also important in the interactions between native and alien invasive species. The Environmental Constraints Hypothesis proposes that in the past, limiting resources have prevented the invasion of some species (Galatowitsch et al. 1999). Increased N availability through mechanisms like fertilization and deposition has allowed plants to invade habitats they previously could not inhabit (Rickey and Anderson 2004). With the potentially far-reaching effects of N deposition on ecosystem patterns and processes, there is a need for greater understanding of deposition patterns and the resulting ecological effects.

My research has implications for herbivory of native plants, which is important in understanding interactions with higher trophic levels. In plants, increases in Nr can affect growth, biomass allocation, C:N ratio, and the production of secondary metabolites (Chapin 1980, Yang et al. 2011c). Changes in foliar N in turn have the potential to affect insect herbivore communities. Increases in plant tissue quality (in the form of increased foliar N and decreased C:N ratio) have been shown to have a positive effect on individual insects and insect abundance . However, both insect and plant species richness have been shown to be negatively affected by increased N deposition (Haddad et al. 2000, Throop and Lerdau 2004).

Finally, studying the responses of native flora and fauna to urban climates is important in the context of global change. Cities are not only highly impacted by elevated CO_2, N deposition and higher temperatures, they are also the drivers of these changes in the world’s climate. The higher temperatures, elevated CO_2, and increased N availability found in urban areas match the predicted global increases in the next 100 years (IPCC 2014), making urban areas and important natural laboratory for studying the effects of global change.

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