CHANGES IN LEAF AREA AND NUTRIENT CONTENT OF

CELTIS SPP. ACROSS A PRECIPITATION

GRADIENT IN TEXAS

by

Allison Taylor Bordini, B.S.

A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the
Master of Science
with a Major in Biology
August 2017

Committee Members:

Susan Schwinning, Chair

Michael Huston, Co-Chair

David E. Lemke

ACKNOWLEDGEMENTS

I would first like to thank my committee and advisors who helped me on my academic journey and aided in the completion of my thesis. I would also like to thank La Vaun Wilson from CE Elantech and Dan Millikin for teaching me how to use the Flash EA 1112, and for fixing it when I had one of my many issues. I would also like to thank the Durenberger Scholarship for Women in Science and the Christmas Mountains Research Fund for funding my education and my project. I would also like to thank my friends and family, especially my parents, in helping me finish my masters work. Finally, I'd like to thank my dog, Colette, for always being there for me when I needed her most.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure **Page**

ABSTRACT

Nitrogen is a key nutrient for cell and protein function in both plants and animals, and the ratio of carbon to nitrogen in leaves is a good indicator of nutrient quality within an ecosystem. Studies have shown that an increase in precipitation increases leaf size and the C/N ratio. While quantity increases, the quality of forage potentially decreases. I examined whether this prediction held across the precipitation gradient of Texas. I also tested whether soil mineral nutrients showed a unimodal relationship across the same gradient, as asserted by Albrecht's curve. I sampled leaves from three species of *Celtis* (Family: Cannabaceae) and took soil samples under their canopies along US Interstate 10 from El Paso County to Orange County, spanning a mean annual precipitation range of 24 – 156 cm. I measured leaf area (LA), specific leaf area (SLA) and C/N ratio using a Flash EA 1112 C/N Analyzer. I determined the relative concentrations of soil mineral nutrients using Mehlich-3 analysis. Of the three species, *Celtis reticulata* had the widest distribution and showed a significant change in LA, SLA, and the C/N ratio with changes in precipitation. Leaf size and C/N ratio increased with precipitation. No significant statistical trends could be attributed to the relative concentrations of the nutrients P, Na, K, Mg, Ca and Mn and heavier elements Fe, Cu, Zn, and Al in soil. My results showed that, in general, leaf characteristics are highly variable but that a large sample size can exhibit significant trends with precipitation, specifically larger but less nutritious foliage with precipitation. The decrease in nutritional quality may be due to the dilution of leaf nitrogen as leaf size increases.

I. INTRODUCTION

The food webs of terrestrial ecosystems are governed by the productivity and nutrient quality of plants (White 1993). The main factors affecting plant growth and species composition are the climate and soil (Diamond *et al*. 1987; Eskelinen and Harrison 2015). Climate, especially precipitation, directly affects plant productivity by providing water necessary for photosynthesis, but also indirectly through effects on soil fertility (Girardin *et al*. 2010; Jenny and Leonard 1934).

In the 1950s, William Albrecht studied the effects of a long-term precipitation pattern on available mineral and organic nutrients in grassland soils and their effect on plant nutrient quality. He hypothesized that a unimodal relationship exists along a precipitation gradient, with optimal nutrient conditions occurring at the median level of precipitation. Too little or too much precipitation becomes detrimental to leaf nutrient quality, he argued, despite the increase in leaf area with precipitation (Albrecht 1957). In this study, I tested this hypothesis across a precipitation gradient in Texas by quantifying leaf areas and leaf C/N ratios directly, as well as by measuring the relative elemental content of numerous soil nutrients and heavy metals.

Precipitation directly affects the available water plants need for photosynthesis and growth. Studies have shown that, in general, precipitation increases total plant biomass in ecosystems (Girardin *et al*. 2010; Huston and Wolverton 2009). Above-ground biomass is reduced in conditions of lower precipitation as plants allocate available resources to extending below-ground root structure as an adaptation to water scarcity (McDonald et al. 2003; Shao et al. 2008). Along with an overall reduction in leaf area per plant, leaf morphology also changes along precipitation gradients and between years. For example, in drier years, plants often produce smaller, thicker leaves, fewer leaves, and produce fewer fruits. A decrease in a plant's total leaf area, whether from reductions in leaf size or number, leads to a decrease in transpiration (Abrams *et al*. 1994; Wright *et al.* 2001). Plants in areas of limited water availability also adapt by growing thicker, waxier leaves to provide a stronger barrier to water loss, thus reducing specific leaf area (SLA) (Dimmit 1985; Xu *et al.* 2009). Specific Leaf Area (SLA) is the ratio of leaf area to dry mass, and has been used to characterize the water use and survival strategies of plants (Hoffmann *et al.* 2005). Most species tend to have a decreased SLA when growing in areas that have predictable, seasonal drought (Marron *et al.* 2001).

Plants can be negatively affected by precipitation through the dissolution and leaching of nutrients from the geological parent material that forms the soil (Diamond *et al* 1987; Huston 2012). Soils in warm, wet, tropical regions and other areas receiving higher amounts of precipitation tend to have lower nutrient concentrations due to more intense weathering and nutrient leaching than their more temperate or drier counterparts (Brady and Weil 2002; Huston and Wolverton 2009; Sanchez 1976).

Soil Chemistry and Mineral Nutrients

Soil nutrient content is influenced by the relationship between the parent material and weathering caused by climatic conditions (Diamond *et al*. 1987). Weathering is necessary for soil formation and breaking down minerals into water-soluble forms, for facilitating cation exchange between soil and plant, and increasing the rate of nutrient cycling (Deng and Dixon 2002).

Nitrogen is necessary for basic cell function and the manufacturing of proteins and amino acids essential for the herbivores feeding on the plant and plays a key role in the photosynthetic processes of the plant itself (Reich *et al*. 1997). Soluble forms of nitrogen enter the soil through the decomposition of organic material or by bacteria that fix nitrogen from the atmosphere into forms that can be used by host plants. These processes are accelerated by precipitation (Galloway *et al*. 2004).

While higher amounts of rainfall may increase the rate of nitrogen cycling, high rainfall can also cause nitrogen to leach as either anion (e.g., NO_3^-) or cation (e.g., NH_4^+). Anaerobic conditions created in poorly drained, wetter environments may reduce the breakdown of nutrients into usable forms and limit the decomposition of organic material (Schuur and Matson 2001). Excessively wet soils may release methane and gaseous forms of nitrogen and typically have low concentrations of oxygen and a diminished rate of decomposition (Girardin *et al*. 2010; Silver *et al*. 1999). Austin and Vitousek (1998) also found wetter ecosystems to have a more closed nitrogen cycle, while drier sites could replenish sources of nitrogen into their soils. These ecosystem processes influence levels of usable soil nitrogen and overall plant growth.

Phosphorus, like nitrogen, is one of the most important mineral nutrients in plant health, contributing to DNA structure and protein synthesis, yet its availability is often the limiting factor in the growth of plants, particularly in highly weathered soils. Phosphorus is usually found in parent material derived from igneous rock and is less available in sandy, quartz-based soils. Phosphorus is low in highly weathered areas, such as the tropics, because of loss through leaching and combination with aluminum and iron, making it unavailable to the plant (Brady and Weil 2002; Harris 2002).

Soils in areas with rapid weathering also tend to have lower levels of key mineral nutrients, such as the cations Ca^{2+}, K^{+}, Mg^{2+}, Mn^{+} and other key micronutrients. As the key mineral nutrients leach out and become unavailable to plants, they are often replaced by H^{+} ions, increasing the acidity of the soil (Huston and Wolverton 2009).

Potassium is a mineral nutrient important for environmental stress tolerance (especially drought tolerance) in plants, decreasing cellular osmotic water potential and water loss. Potassium also improves the fruit and flower quality of an individual plant and activates enzymes for metabolism, starch synthesis, and photosynthesis (Brady and Weil 2002). Potassium is derived from micas and K feldspar weathering. It can be reintroduced to the soil through the decomposition of animal waste. In areas of excess rainfall, potassium is often lost to leaching, as it does not bind to other elements as readily as phosphorus (Thompson and Ukraincyzk 2002).

Sodium cations are important for chlorophyll formation, the absorption of water, and in maintaining turgor pressure. Similar in size, sodium ions are often absorbed instead of the very beneficial potassium ions, and sodium becomes toxic to plants in large quantities (Pardo and Quintero 2002).

Manganese plays a role as a catalyst in photosynthesis, nitrogen metabolism, and nitrogen assimilation. Manganese is usually in a Mn^{2+} oxide form and originates from basalts, gabbro, and limestones. It is mostly found in younger soils, as it is soluble and easily leached, and helps to activate enzymes like decarboxylase, dehydrogenase, and oxidase in plants. In soils, manganese selectively adsorbs heavy metals (such as Pb, Co, and Ba) that could be toxic to plants in high concentrations, making them unavailable (Brady and Weil 2002, Dixon and White 2002).

Calcium is important in forming structures such as cell walls in plants. Studies have shown that in the eastern United States, increased rainfall contributed to an increase in carbohydrates and a decrease in food quality of plants due to the loss of calcium and other essential nutrients lost from the soil (Albrecht 1957). Originating from limestone, calcium cations tend to be found in areas with younger, less weathered soils (Schulze 2002). Calcium is often found with magnesium and a loss in both leads to a decrease in pH. These more acidic conditions can increase aluminum toxicity in an ecosystem (Brady and Weil 2002; Doner and Grossl 2002).

Copper is important for oxidase enzymes, photosynthesis, and the metabolism and assimilation of nitrogen, while zinc is used for proteinase and peptidase formation as well as growth hormones, starch and seed production, and seed maturation (Brady and Weil 2002). Both copper and zinc are usually found in association with, and adsorbed by, manganese.

The leaching of soluble mineral nutrients leads to the dominance of larger, less mobile mineral nutrients (such as iron and aluminum). At higher concentrations, these heavy metals are eventually not adsorbed by other mineral nutrients, and while necessary for plant growth, they become less beneficial and more toxic to plants in an ecosystem (Schulze 2002; Huston and Wolverton 2009; Sanchez 1976). For example, excess aluminum can cause loss of cell function at the cell membrane or in binding with ATP, making it unusable (Ferris 1989; Viola *et al.* 1980, as cited by Huang *et al.* 2002). Iron is very important in the process of nitrogen fixation, chlorophyll formation, and the creation of peroxidase, catalase, and cytochrome oxidase enzymes (Brady and Weil 2002), but high concentrations, both aluminum and iron fixate usable forms of phosphorus, making it unusable to the plant (Bigham *et al.* 2002). This interaction ultimately inhibits plant growth and contaminates food sources for herbivores (Huang *et al.* 2002; Huston and Wolverton 2009).

Aluminum is the third most abundant element on Earth, forming oxides, hydroxides, and oxyhydroxide minerals (Huang *et al.* 2002). The most common aluminum mineral, gibbsite [$Al(OH)_3$], is often found in very weathered soils in tropical ecosystems, such as in Oxisols and Andisols (Schulze 2002). Gibbsite is important in the absorption of key metals, such as Cu, Zn, and Pb, making them unavailable to the plant. However, bonding and cation and anion exchange with Al require a more acidic pH.

Iron is the fourth most abundant element on Earth and is easily oxidized and easily weathered. Mineral forms of iron, goethite (FeOOH) and hematite (Fe_2O_3), are often found in association with each other. Iron compounds originate from ferromagnesian silicates and are some of the least stable compounds when weathered (Bigham *et al.* 2002).

Albrecht's Curve

William Albrecht summarized this concept of soil properties changing across a precipitation gradient, suggesting that as precipitation increases, there is an increase in potential organic matter, useful clay content, and effectiveness of water (Figure 1). However, too much precipitation may cause the leaching of mineral nutrients, an increase in the unfavorable forms of clay, and an increase in acidity. Since soil is affected by the underlying geological parent material as well as the climate of a region, Albrecht's curve provides a simple model of how soil nutrient content is affected by climate (Albrecht 1957). This relationship between water and soil properties may also be seen at a smaller geographic scale, as differences in topography affect water drainage, soil moisture, nutrient cycling, and, in turn, the nutrient quality of leaf material (Garten *et al.* 1994). This may lead to a decrease in plant nutrient quality and productivity with an increase in precipitation, despite the increase in leaf quantity.

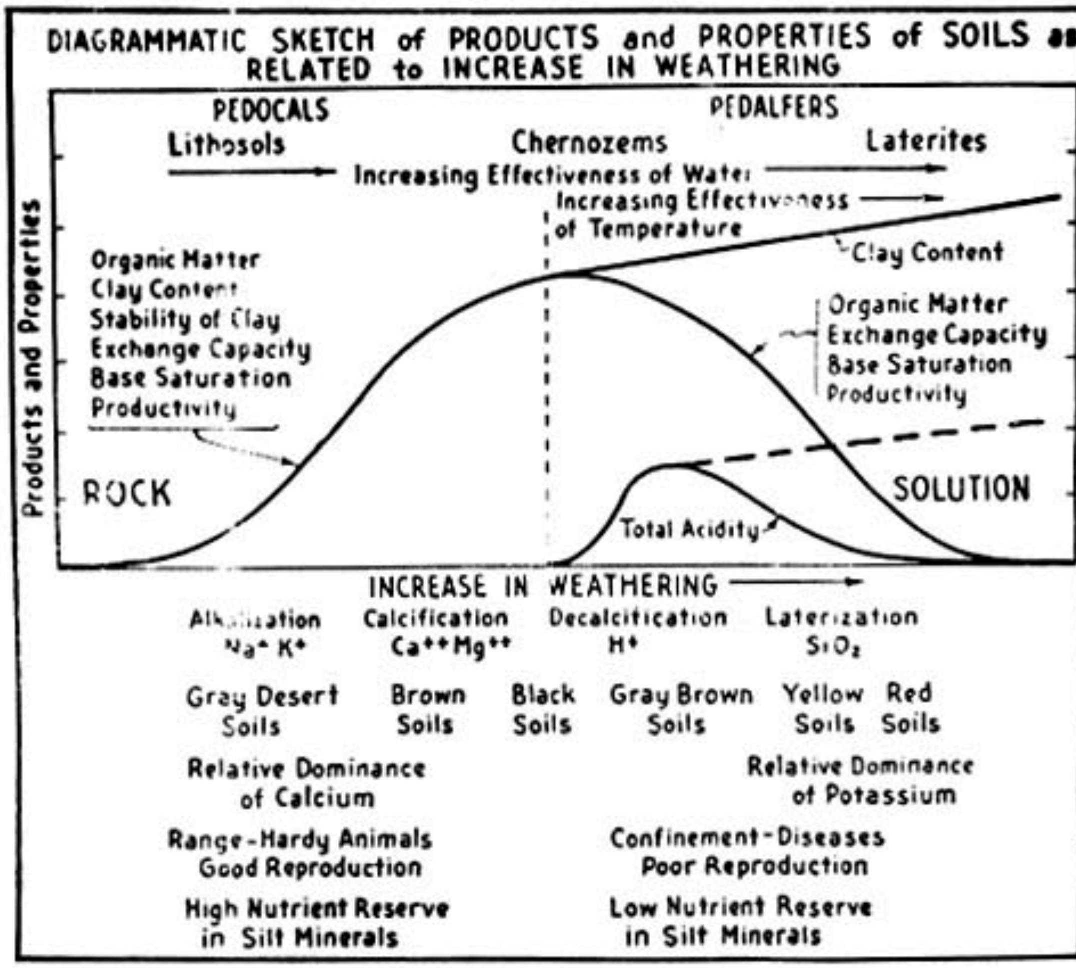

Figure 1: Diagrammatic representation of the development of soil under increasing forces of weathering by rainfall and temperature. Originally printed in Soil Fertility and Animal Health (Albrecht 1957), Albrecht's theoretical curve depicts the change in organic matter, available mineral nutrients, and exchangeable base pairs, as well as the change in pH with an increase in weathering.

Net primary productivity (NPP) is the rate (e.g., g m $^{-2}$ day $^{-1}$) at which plants convert soil nutrients and carbon dioxide into plant structure (Huston and Wolverton 2009). This productivity sequesters carbon within ecosystems and provides the energy and nutrients required by primary consumers (Gillman *et al*. 2015). Analyzing productivity can aid in interpreting the health and biodiversity of an ecosystem (Gillman *et al*. 2015). Areas with higher levels of precipitation (e.g., near the equator) have long been thought to be more productive ecosystems due to the increase in woody growth and leaf tissue matter that comes with this increase in water resources. However, studies have shown that while warmer, wetter regions tend to have more biomass and constant productivity year-round, they tend to have less fertile soils than their drier and/or cooler counterparts for reasons outlined in the previous section (Huston and Wolverton 2009; Cunningham *et al*. 1999). One indicator of the productivity and nutrient cycling rates of an ecosystem is the ratio between carbon and nitrogen concentrations, which is an indicator of the quality of the plant tissue available to herbivores (Vinton and Burke 1997). Regions of higher productivity due to high precipitation, high nitrogen availability or both, tend to have leaves with low C/N ratios (Barbosa *et al*. 2014; Stiling and Cornelissen 2007). Stressful environments also tend to increase CN ratios as leaves increase their content of carbon-rich compounds to reduce water loss or deter herbivory (e.g., tannins, phenols, and fibrous material) (Cunningham *et al.* 1999). These leaf characteristics feed back on ecosystem processes in numerous ways, including decomposition rates and secondary productivity (Grace *et al*. 2016).

Study Area and Geologic History

As the second largest state in the United States, Texas stretches over 1300 km and almost 14 degrees of longitude at its greatest width (roughly -107°W to -94.5°W). This long expanse of land, coupled with Texas' proximity to the Gulf of Mexico and its varying range of elevations from sea level to over 2600 meters, have created a diverse climate from Chihuahuan Desert in West Texas (mean annual precipitation of less than 24.5 centimeters) to the humid subtropical climate to the east, receiving over 125 centimeters of rainfall annually (Diamond *et al*. 1987).

Texas also has a long and complex geologic history, with exposed Precambrian-age rocks dating at least 1.1 billion years in age in the Llano Uplift of central Texas, where exposed Cretaceous-age rocks dating back 144 million years surround the southern, eastern, and western edges of the Llano Uplift. Tertiary volcanic material is found in West Texas and Quaternary alluvium and sediments occur in both west Texas and along the coastline of the state dating back 58 million years and 2 million years, respectively (Bureau of Economic Geology, Appendix A). The mineralogy of the parent material influences the soil types found in a region, the mineral nutrients available, and the vegetation that grows in that region (Brady and Weil 2002). The wide range of precipitation values and the complex geology of Texas provide an interesting and extensive study area for assessing changes in soil mineralogy and corresponding plant nutrient composition. Some studies have assessed changes in net primary productivity, plant nutrient quality, and soil mineral nutrients with changes in water resources within labs and across short ranges of precipitation (Austin and Vitousek 1998; Aranibar *et al.* 2004; Schuur and Matson 2002; Castro-Diez *et al.* 1997). However, no studies have been done over such a long climate gradient as found in Texas to assess the relationships among precipitation, soil mineral nutrient quality, and leaf nutrient quality.

Texas follows a similar gradient in precipitation as the entire continental United States, with some parts of southern California receiving a similar mean annual precipitation as the Chihuahuan Desert and the Atlantic coast receiving a similar mean annual precipitation as the eastern portion of the state of Texas. This provides an opportunity to assess how plants and ecosystems are affected by changes in precipitation on this scale.

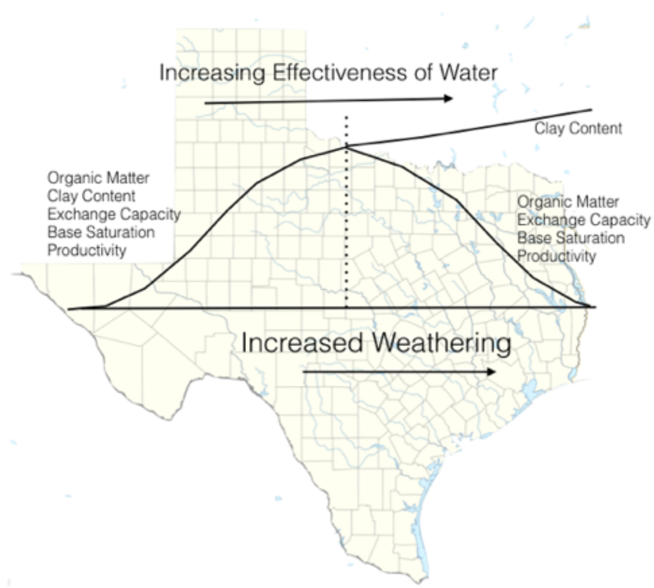

Figure 2: Albrecht's Curve depicted across the state of Texas. This representation shows the same concepts as Albrecht's original design and how this fits with the patterns of precipitation across the state of Texas.

Study Species

Three species of hackberry trees (Genus: *Celtis*, Family: Cannabaceae, formerly placed in the Ulmaceae) occur across Texas' rainfall gradient and were therefore used to study the effects of soil chemistry and climate on the nutrient content and leaf area of plants. The species *Celtis ehrenbergiana*, *C. laevigata*, and *C. reticulata* have ranges in central Texas. This overlap allows for comparison of their nutrient content under common conditions, which will help interpret and standardize any patterns observed across the 1300 km gradient. The species are deciduous and leaf out in late April or early May, allowing for sampling in June. Species of *Celtis* are often found in many different ecosystem types, and can greatly vary in characteristics in response.

Celtis ehrenbergiana (desert or spiny hackberry) is a shrub growing up to about 3 meters. Branches are a white-gray with thorns measuring 3 – 25 mm. Leaves are thick and oblong, measuring about 2 – 3 cm in length. Desert hackberry inhabits gravelly or well-drained sandy soils in deserts, canyons, mesas, washes, brushlands, and grasslands in south central to southwest Texas (Flora of North America; Figure 3a).

Celtis laevigata (sugarberry) is a large tree growing up to 24 meters. The bark is warty and light gray in color. The leaves of *C. laevigata* are 5 – 10 cm long and up to 5 cm wide. Leaves are smooth and lance shaped, tapering to a point. Sugarberry is found in sandy loam or rocky soils along streams, in bottomlands, and in woodlands common throughout central to east Texas (USDA; Figure 3b).

Celtis reticulata (netleaf hackberry) grows as a tree or shrub up to 16 meters tall. The bark is gray with warts and ridges. Leaves are ovate and 2 – 7 cm in length, mostly entire, and pubescent and rough. Netleaf hackberry is found on dry hills of limestone or basalt on ravine banks and rocky outcrops of sandy soils throughout most of Texas (Flora of North America; Figure 3c).

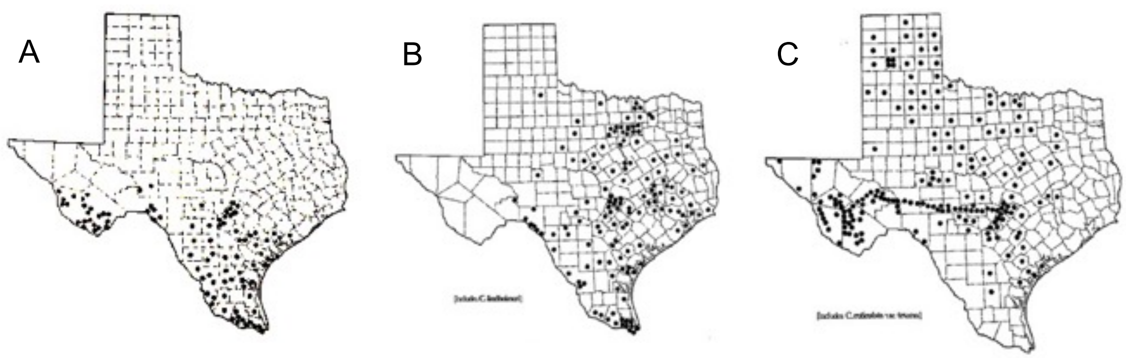

Figure 3: Occurrences of each studied species in counties of Texas. A) *Celtis ehrenbergiana;* B) *C. laevigata;* and C) *C. reticulata*.

<u>Objectives and Hypotheses</u>

The objective of this study was to (1) determine the relationship, if any, between leaf size (average leaf area, average leaf length, average leaf width, and SLA) of the three hackberry species (*C. laevigata, C. ehrenbergiana,* and *C. reticulata*) and the recent precipitation (three and twelve months prior to sampling) for each sampling location, (2) assess the ratio between carbon and nitrogen in relation to recent precipitation by species, and (3) analyze the soil chemistry and composition through particle size analysis and soil chemistry analysis for any patterns in soil nutrients related to long-term average precipitation and their relation to the leaf nutrient quality.

I hypothesize that (1) the leaf area, length, width, and SLA will increase with recent precipitation for each species. This increase is due to the increase in available water resources for the plant in wetter environments and an evolutionary strategy for water conservation in drier environments.

(2) There will be an increase in the C/N ratio as recent precipitation increases. This increase is due to a dilution of nitrogen and an increase in the amount of carbon structures. Plants with a higher C/N ratio are less nutritious because the amount of nitrogen available is relatively less than the amount of carbon in the leaf.

(3) In general, smaller, more mobile soil mineral nutrients (P, Ca^{2+}, K^{+}, Mg^{2+}, Mn^{+}, and N) will show a unimodal relationship similar to Albrecht's Curve, with the highest amounts of these nutrients in central Texas, where average rainfall is at an optimal amount, aiding in weathering of organic matter without excess leaching of important nutrients. Relative concentrations of heavier, less mobile minerals (Zn, Al, Fe, and Cu) will increase with an increase in precipitation due to their lack of mobility and the leaching of other, more beneficial mineral nutrients.

II. METHODS

Sample Collection and Preparation

I traveled along the Interstate Highway-10 corridor across the state of Texas from El Paso County to Orange County, Texas. Hackberry samples were collected every 30 km, and were also collected at state parks near IH-10 by use of the State Parks Research Permission granted by Mr. David Riskind on March 31, 2016 (Village Creek, Palmetto, Government Canyon, South Llano River, Balmorhea, and Franklin Mountains) and at the Christmas Mountains in the Chihuahuan Desert. Sample locations are shown in Figure 4. All samples were collected from May 7, 2016 to June 26, 2016. However, *Celtis ehrenbergiana* was difficult to find, at least in part due to an extremely dry summer in the Trans-Pecos region. Many plants did not leaf-out during the summer, and only produced leaves, flowers, and abundant fruits after heavy rains in August and September (M. Huston, pers. obs.), thus accounting for a low sample size.

Twenty leaves were collected at each sampled tree, selecting branches with the newest leaf growth and leaves with no evidence of herbivory to best represent this season's growth. Leaves were stored in paper bags and were labeled with the specific GPS coordinate of the sampled tree, the temperature, and cloud cover or precipitation at the time of sampling. Once transported back to the lab, leaf samples were rehydrated in plastic bags with a moist paper towel and refrigerated at 3°C for five days. Leaves were flattened on paper and scanned for leaf length, width, and area analysis in ImageJ (Version 1.51g, 2016). Leaves were then dried in an oven for 48 hours at 75°C and weighed in batches of twenty. They were then ground to a fine powder for C/N analysis. Specific leaf area (SLA) was calculated by dividing the average leaf area for a given sample by the average dry mass per leaf.

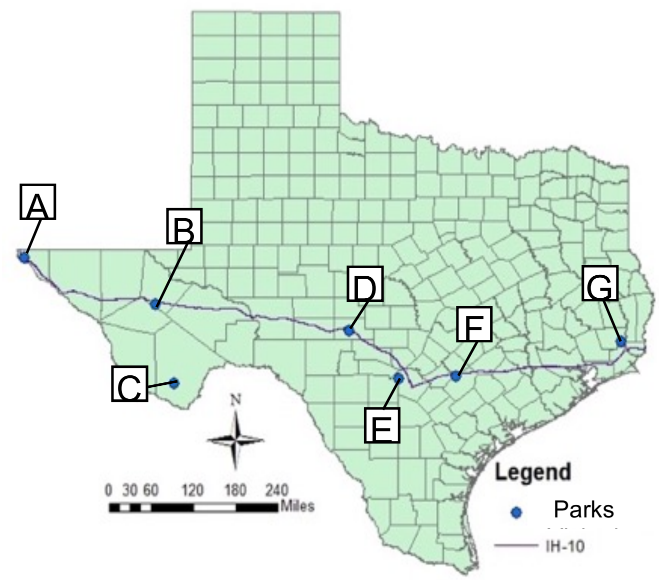

Figure 4: Locations visited for sampling. Thick line represents the route of IH-10 in Texas, with the sites where samples were taken. A represents Franklin Mountains State Park, B represents Balmorhea State Park, C represents the Christmas Mountains Research Station, D represents South Llano River State Park, E represents Government Canyon State Park, F represents Palmetto State Park, and G represents Village Creek State Park.

Dried, ground leaves were weighed into 30 mm tin cups for use in the CE Elantech Flash EA 1112 Series C/N Analyzer. Each tin cup had about 25 mg of ground leaf material, and the ends of the tin cup were folded in for sealing the sample. Tin cups were dropped into the Flash EA 1112, combusted in the left furnace at 950°C through a steel reactor and passed through a filter in the right furnace at 840°C. The right furnace held a quartz tube with quartz wool at the top and bottom and reduced copper between the quartz wool. The reduced copper filters the gas moving through and separates oxygen from nitrogen, converting nitrogen oxide to nitrogen gas. The adsorption filter used quartz wool and granulated magnesium perchlorate to filter out water vapor to dry the gas before analysis by the conductivity detector. The carrier gas used was helium set at 140 ml/min with a helium reference set at 100 ml/min. Oxygen gas was set at 250 ml/min. Total carbon and nitrogen were assessed using a Thermal Conductivity Detector (TCD) that differentiated between carbon dioxide (CO_2) and nitrogen gas (N_2) that formed throughout the burning process of the cycle. The total time of the cycle run for each sample was 420 seconds.

Soil samples were also taken at each sampling site by removing a volume of soil approximately 20 cm deep, starting from the surface, and 8 centimeters in diameter at three randomly selected locations surrounding the hackberry sampled. Soil was stored in plastic bags labeled with the same information as the leaf samples at the same site. Once returned to the lab, soil samples were dried in an oven for 48 hours at 75°C to remove excess water from the soil. Samples were then ground and passed through a series of sieves measuring 16 mm to 25 μm. Particles of different size classes were individually weighed for particle size analysis. Those that were 2 mm and larger were categorized as rocks, those between 0.05 mm and 2 mm were categorized as sand, and those that were smaller than 0.05 mm were categorized as silt.

Dried and ground soil samples were sent to the Iowa State University Soil and Plant Analysis Laboratory in Ames, Iowa. Soil mineral nutrients (P, K, Na, Mg, Ca, Mn, Fe, Cu, Zn, and Al) were extracted using the Mehlich-3 soil extraction method. For the Mehlich-3, 2 grams of each soil sample were added to 200 mL of Mehlich-3 extracting solution and shaken at 200 EPM for 5 minutes at 24°C. The solution was then filtered, and 2 mL were added to a test tube with 8 mL working solution (25 mL acid molybdate stock in 800 mL distilled water, 10 mL ascorbic acid stock solution, 165 mL distilled water). Mineral nutrients were read on an inductively coupled plasma (ICP) instrument to determine the mg/kg of each element within the sample (Frank *et al.* 1998). Total carbon and nitrogen were read on the Flash EA 1112 C/N Analyzer using the same methods and parameters used for the leaf C/N analysis.

Climate and Soil Parameter Estimates

Precipitation prior to the growing season (February-April 2016, hereafter known as recent precipitation) was obtained for each sample location from the PRISM Climate Group website Mean annual precipitation (1981-2010) was calculated using weather station data from NOAA, using the station closest to the sampling location.

Using their precise GPS coordinates, each sample's soil community type was found using the Natural Resource Conservation Service soil maps (USDA). This information provided a baseline for potential levels of each mineral nutrient and provided a general soil texture analysis for further examining water retention. Samples gathered at the site were sorted and the fractions weighed to calculate the ratio of rocks to the rock, silt, and sand total within each sample. This allowed direct assessment of the water storage capacity of the soil at each sample site.

Statistical Analysis

Statistical analyses were conducted in R (R Development Core Team 2016, Version 3.3.1). I first performed an ANOVA to assess if there were differences in C/N and SLA among the three species of hackberry. To assess this, I only analyzed samples between -102.00 and -96.00 decimal degrees West Longitude to limit the analysis to plants receiving a similar amount of rainfall to ensure plants in extreme wet and extreme dry conditions did not affect my analysis in the similarities between species. This ANOVA was performed to determine if all three species could be treated as one for statistical analysis of C/N and to potentially provide insight into how each species is affected by changes in precipitation.

Regression analysis was used to determine how carbon, nitrogen, and the C/N ratio changed with a change in recent precipitation. Linear regression analysis was also used to determine the effect of recent rainfall on leaf area, leaf length, leaf width, and SLA, performing separate analysis for each species since *Celtis laevigata*, *C. reticulata,* and *C. ehrenbergiana* vary in their leaf size and shape. Leaf C/N and soil C/N were compared using a one-way ANOVA to determine if soil carbon and nitrogen have a direct effect on the leaf carbon and nitrogen amounts.

Soil analysis used all samples, regardless of hackberry species, along the longitudinal gradient to assess how each mineral nutrient changed with precipitation. Both linear and non-linear (quadratic) regression analysis were conducted on the effect of mean annual precipitation (average calculated from 1981-2010, NOAA). The mineral nutrients P, K, Na, Mg, Ca, Mn, Fe, Cu, Zn, and Al were quantified as mg per Kg soil, while C and N were quantified as percentage of total soil mass. If there was a significant correlation between rainfall and a specific nutrient, I then assessed which soils may be more suitable for plant growth based on concentrations of important elements and which of these nutrients followed the concepts described by Albrecht's Curve. The soil community type for each sample was found using geographic location and maps from the Natural Resources Conservation Service. This information allowed me to qualitatively analyze soil community types for each sample while considering the mineral nutrient data from soil samples collected in the field. Specific information on parent material, particle texture, and drainage was also found through NRCS in separate county assessments. Finally, the particle analysis of rock-to-total sample (rock, silt, sand) calculations for each sample was used to compare differences in the water retention capabilities of soils sampled across the gradient. Samples with a larger amount of rock, thus a higher percentage for our particle size calculation, will be less able to hold water than their siltier and sandier counterparts. Soils with higher percentages of silt and sands (and lower %rock) will have higher water-holding capabilities.

III. RESULTS

Total soil and leaf samples for each species is presented in Table 1. The ANOVA compared C/N and SLA for species found between -102°W to the -96°W, as this range covered the median conditions represented in the state. This geographic range contained 26 samples of *Celtis reticulata*, 26 samples of *C. laevigata*, and 2 samples of *C. ehrenbergiana*. It is important to note that *C. laevigata* was found only in the easternmost portion of Texas (-93.8 to -99.0 decimal degrees W) where average annual rainfall is at least 20 cm, whereas *C. reticulata* was found from slightly east of central Texas (-97.8 decimal degrees W) to the very westernmost sampling locations (-106.5 decimal degrees W).

Table 1: Total samples taken for each species. Column for total samples represents all samples taken before removal of outliers.

Species	Total Samples	Samples Within Median Longitudinal Range (-102°W to the -96°W)	Outliers Removed
Celtis laevigata	35	26	3
Celtis reticulata	40	26	3
Celtis ehrenbergiana	3	2	0

The ANOVA between species and C/N ratio showed significant difference between species ($F_{2,48}=3.519$, $p=0.038$). Tukey's HSD was used as a post-hoc to further examine where these differences fell and showed a significant difference between *C. reticulata* and *C. ehrenbergiana,* with *C. reticulata* having a significantly higher average C/N ratio (p=0.047; $\bar{x} = 15.96$ and 9.972, respectively). I used ANOVA to compare the average SLA (cm^2 g-1) of each species, which also showed significant differences ($F_{2,48}$=10.62, p<0.001). A Tukey's HSD post-hoc test revealed a difference between *C. reticulata* and *C. laevigata,* with *C. laevigata* having a significantly larger average SLA (p<0.001; $\bar{x} = 150.6$ and 245.9, respectively). Because there was a significant difference between the species for both parameters, I proceeded with my data analysis treating each of the species individually for all other parameters.

With only three sampled individuals, *Celtis ehrenbergiana* leaf traits did not show any significant correlation with recent precipitation (Table 2; Figure 5).

Table 2: Statistical analysis results of changes for *C. ehrenbergiana* across a precipitation gradient for accumulated rainfall three months prior to sampling. Sample size was n=3. Larger sample size is needed for testing of assumptions and proper statistical analysis. General trend of the three samples taken shows an increase in leaf area with an increase in precipitation.

	F Ratio	p-value	R^2	Estimated Slope
Foliar Area (cm^2)	$F_{1,1}$=9.885	0.1960	0.908	0.010
Foliar Length (cm)	$F_{1,1}$=13.44	0.1695	0.931	0.001
Foliar Width (cm)	$F_{1,1}$=0.662	0.5653	0.398	0.001
SLA (cm/g)	$F_{1,1}$=0.0007	0.9834	0.0006	-0.039
Foliar C/N Ratio	$F_{1,1}$=0.0316	0.8881	0.0306	0.005

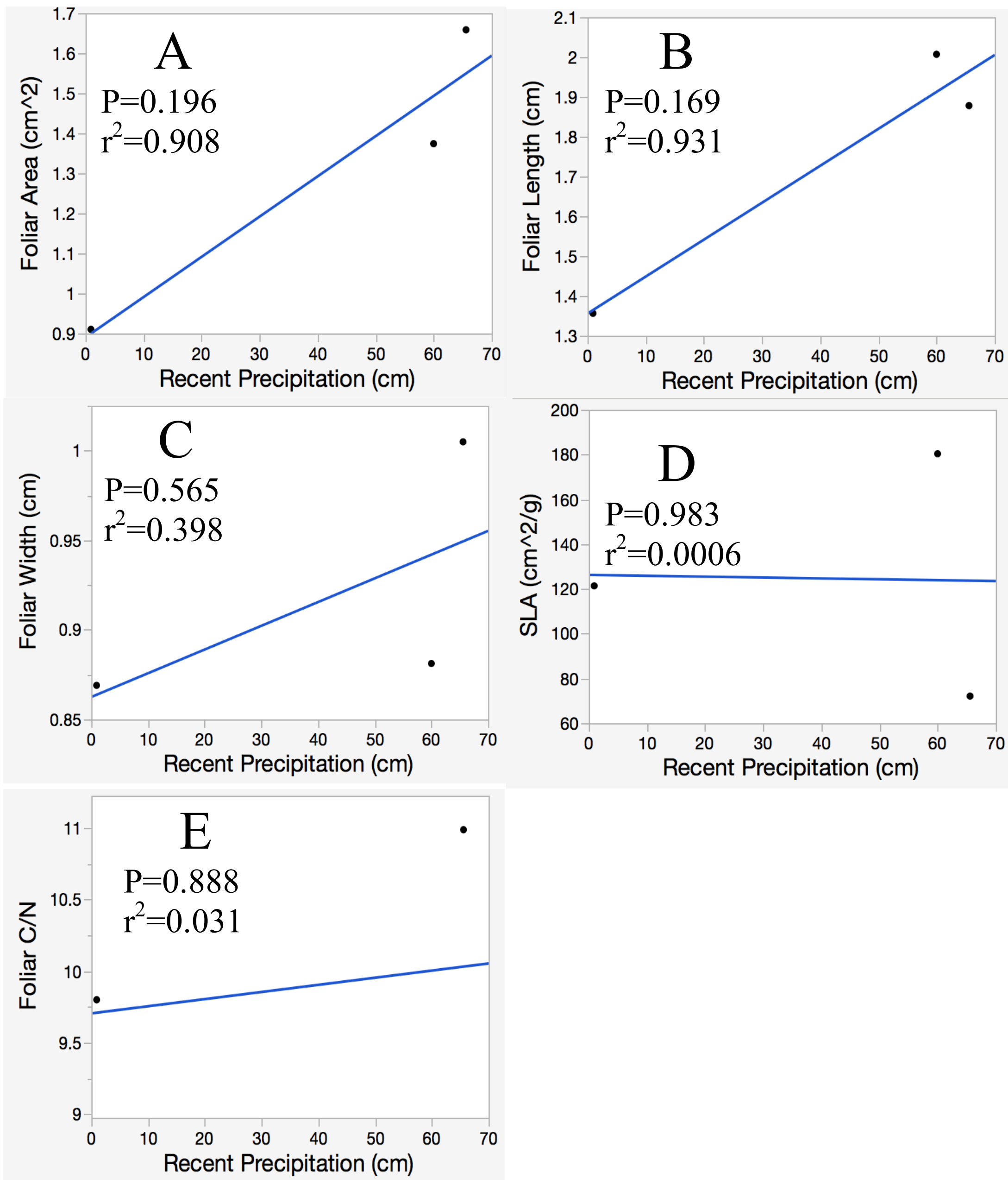

Figure 5: Changes for C. ehrenbergiana across a precipitation gradient for recent rainfall. Change in foliar area (A), foliar length (B), foliar width (C), SLA (D), and C/N (E) shown here, however small sample size does not allow for accurate statistical analysis.

Celtis laevigata showed a significant response to recent precipitation only for the C/N ratio ($F_{1,31}$=4.840, p=0.035). The C/N ratio increased by 0.121 with every centimeter of rainfall (Table 3; Figure 6).

Table 3: Statistical analysis results of changes for *C. laevigata* across a precipitation gradient for accumulated rainfall three months prior to sampling Sample size was n=32 and showed a significant change in the C/N ratio with precipitation.

	F Ratio	**p-value**	**R^2**	**Estimated Slope**
Foliar Area (cm^2)	$F_{1,31}$=0.260	0.553	0.011	-0.062
Foliar Length (cm)	$F_{1,31}$=0.976	0.331	0.031	-0.023
Foliar Width (cm)	$F_{1,31}$=0.004	0.950	0.0001	-0.0007
SLA (cm^2/g)	$F_{1,31}$=2.753	0.107	0.082	-2.755
Foliar C/N Ratio	$F_{1,31}$=4.840	0.035	0.131	0.121

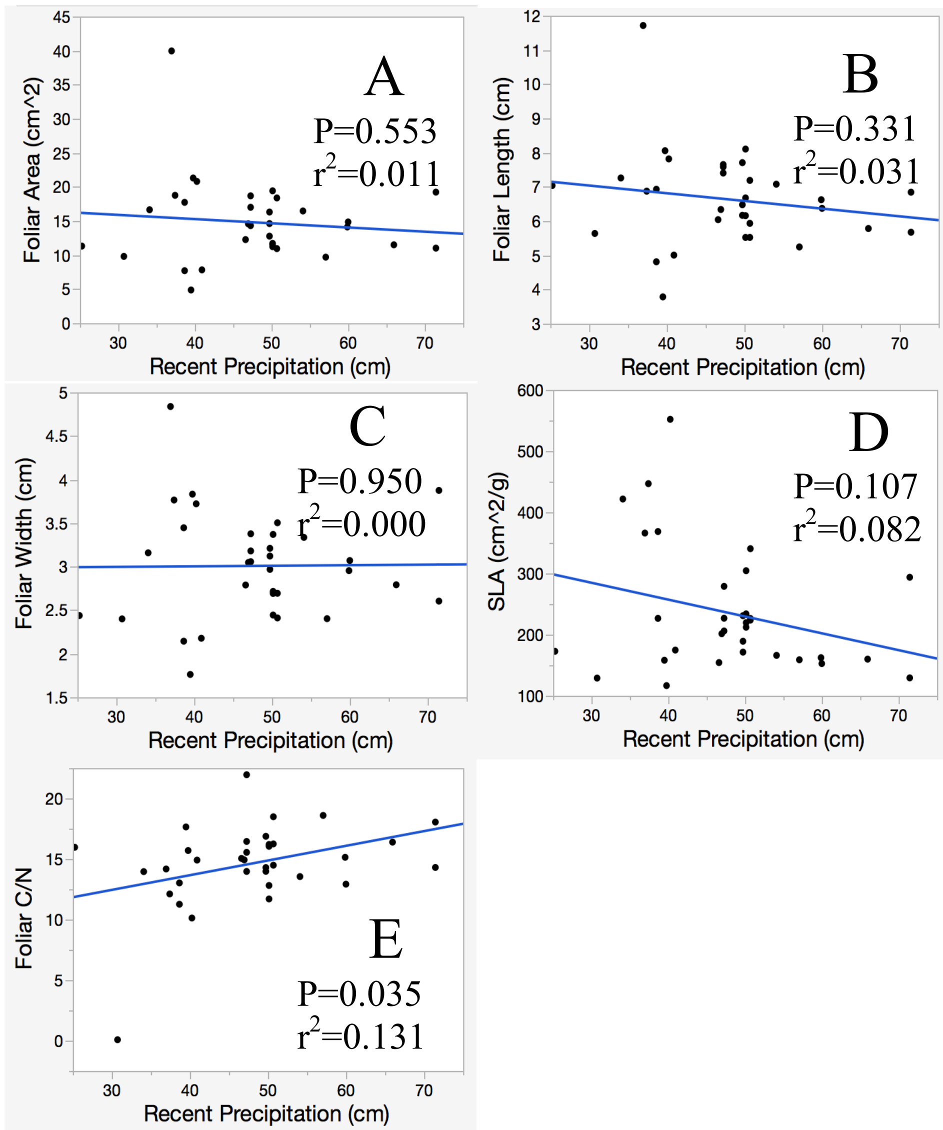

Figure 6: Changes for C. laevigata across a precipitation gradient for recent rainfall. Change in foliar area (A), foliar length (B), foliar width (C), SLA (D), and C/N (E) shown here. Statistical significance was found only for C/N ratio, but had high variability (r=0.131).

Celtis reticulata showed significant responses to recent precipitation for foliar area ($F_{1,36}$=8.947; p=0.005), foliar length ($F_{1,36}$=9.953; p=0.003), foliar width ($F_{1,36}$=5.138; p=0.029), SLA ($F_{1,36}$=20.55; p<0.001), and C/N ratio ($F_{1,36}$=8.346; p=0.006; Table 4). Foliar area increased linearly at a rate of 0.110 cm per centimeter increase in rainfall, leaf length increased linearly at a rate of 0.035 cm per centimeter of rainfall, foliar width increased linearly at a rate of 0.015 cm per centimeter of rainfall, and SLA increased linearly by 1.629 cm^2/g per centimeter of rainfall. The C/N ratio increased by 0.118 with every centimeter or rainfall (Table 4; Figure 7).

Table 4: Statistical analysis results of changes for *C. reticulata* across a precipitation gradient for accumulated rainfall three months prior to sampling. Sample size was n=37 and showed a significant change in leaf area, length, width, SLA, and C/N ratio with precipitation.

	F Ratio	**p-value**	**R^2**	**Estimated Slope**
Foliar Area (cm^2)	$F_{1,36}$=8.947	0.005	0.187	0.110
Foliar Length (cm)	$F_{1,36}$=9.953	0.003	0.203	0.035
Foliar Width (cm)	$F_{1,36}$=5.138	0.029	0.116	0.015
SLA (cm^2/g)	$F_{1,36}$=20.55	0.0001	0.345	1.629
Leaf C/N Ratio	$F_{1,36}$=8.346	0.006	0.176	0.118

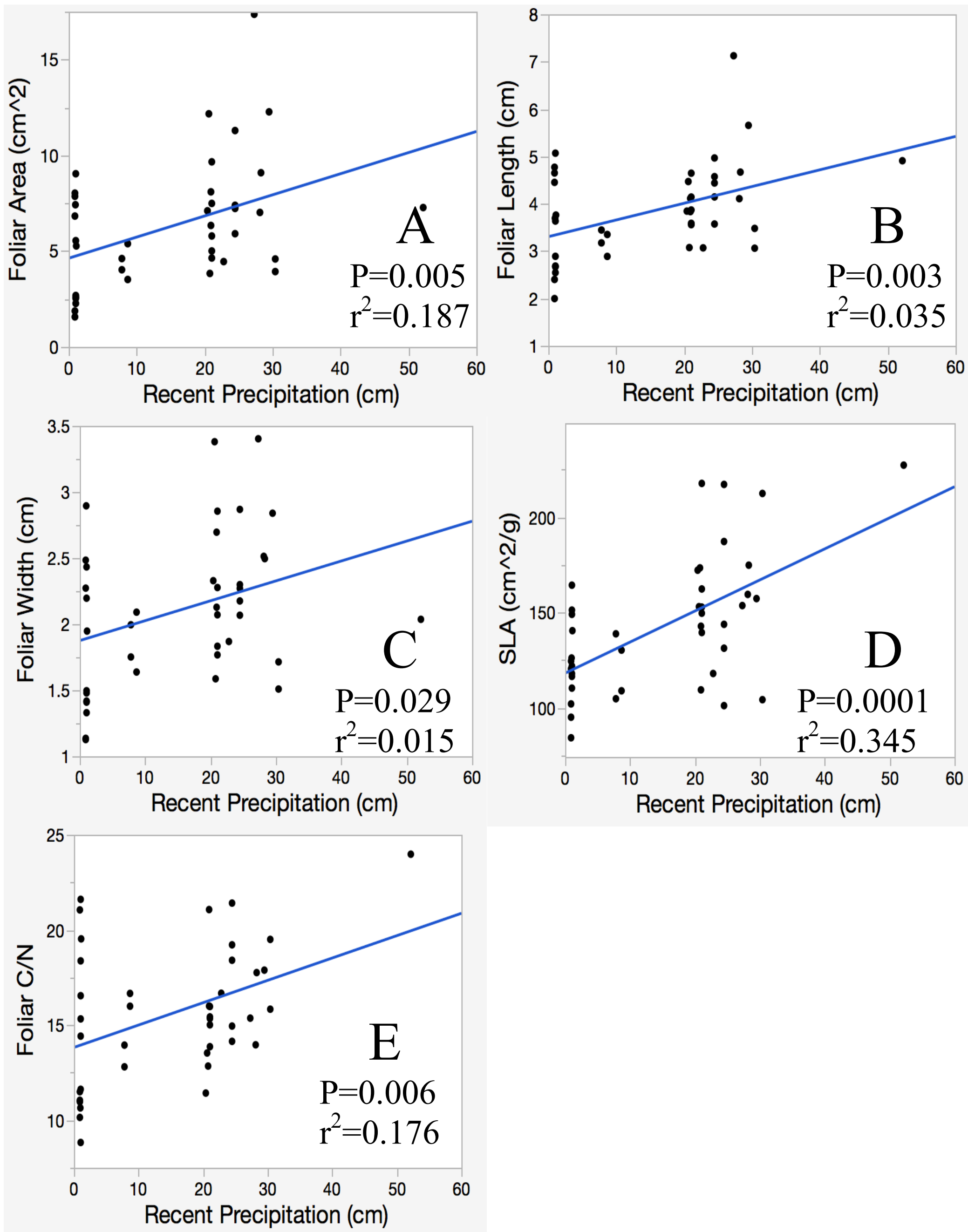

Figure 7: Changes for C. reticulata across a precipitation gradient for recent rainfall. Change in foliar area (A), foliar length (B), foliar width (C), SLA (D), and C/N (E) shown here. All relationships showed statistical significance.

The relative concentrations of nutrient elements (P, K, Na, Mg, Ca, Mn, Fe, Cu, Zn, and Al) were measured using the Mehlich-3 method, reported as mg/kg, while C and N were measured using the Flash EA 1112 C/N and were reported as a percentage of the total soil sample weight. Linear and quadratic regressions were tested for all relationships.

For the effect of mean annual precipitation (1981-2010) on soil mineral nutrients (Figure 8), significant linear relationships were found for potassium ($F_{1,76}$=21.572; p<0.0001), calcium ($F_{1,76}$=15.449; p=0.0002), manganese ($F_{1,76}$=4.120; p=0.046), iron ($F_{1,76}$=54.342; p<0.0001), and copper ($F_{1,76}$=4.151; p=0.045). Zinc was not significant due to a non-significant intercept estimate (p=0.115). Carbon, nitrogen, and the C/N ratio did not show any significant linear relationship with mean annual precipitation (Figure 9).

Quadratic regression showed significance for calcium ($F_{1,76}$=11.251; p<0.0001) and iron ($F_{1,76}$=48.022; p<0.0001), as seen in Figure 10. Potassium and copper were non-significant due to non-significant slope (coefficient of x for potassium, p=0.312; coefficient of x and x^2 for copper, p=0.184 and p=0.139, respectively). Carbon, nitrogen, and the C/N ratio did not show any significant quadratic relationship with mean annual precipitation (Figure 11). All statistical results and parameter estimates are detailed in Appendix B.

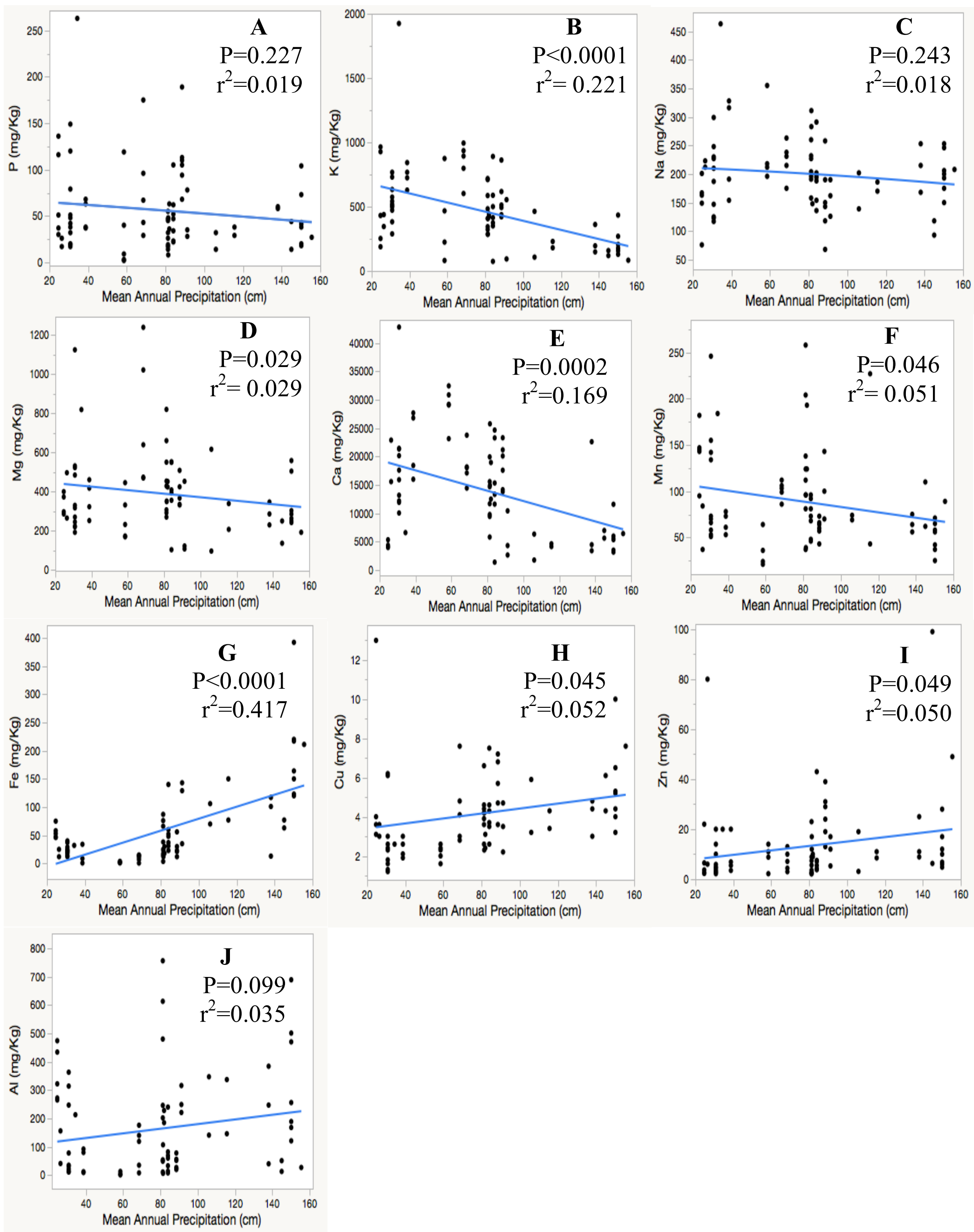

Figure 8: Linear regression of soil minerals with mean annual precipitation (1981-2010). Significance was found for potassium (B), calcium (E), manganese (F), iron (G), and copper (H). No significance was found for phosphorus (A), sodium (C), magnesium (D), zinc (I), and aluminum (J).

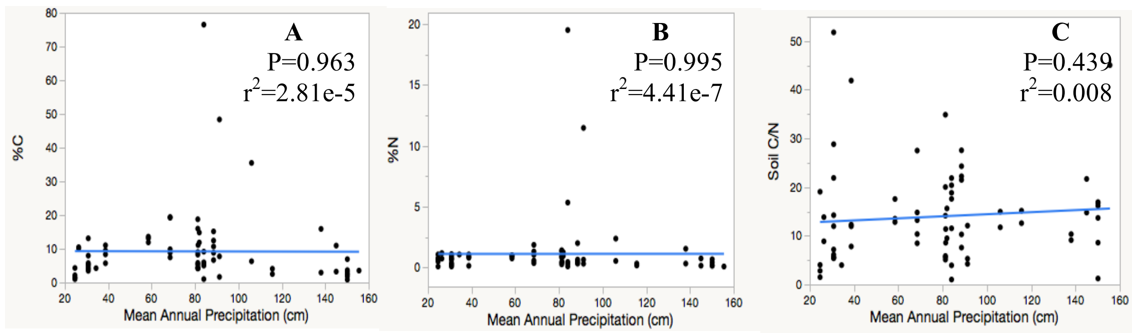

Figure 9: Linear regression for %C, %N, and C/N ratio of soil when assessing change with mean annual precipitation (1981-2010). No relationship showed any significance.

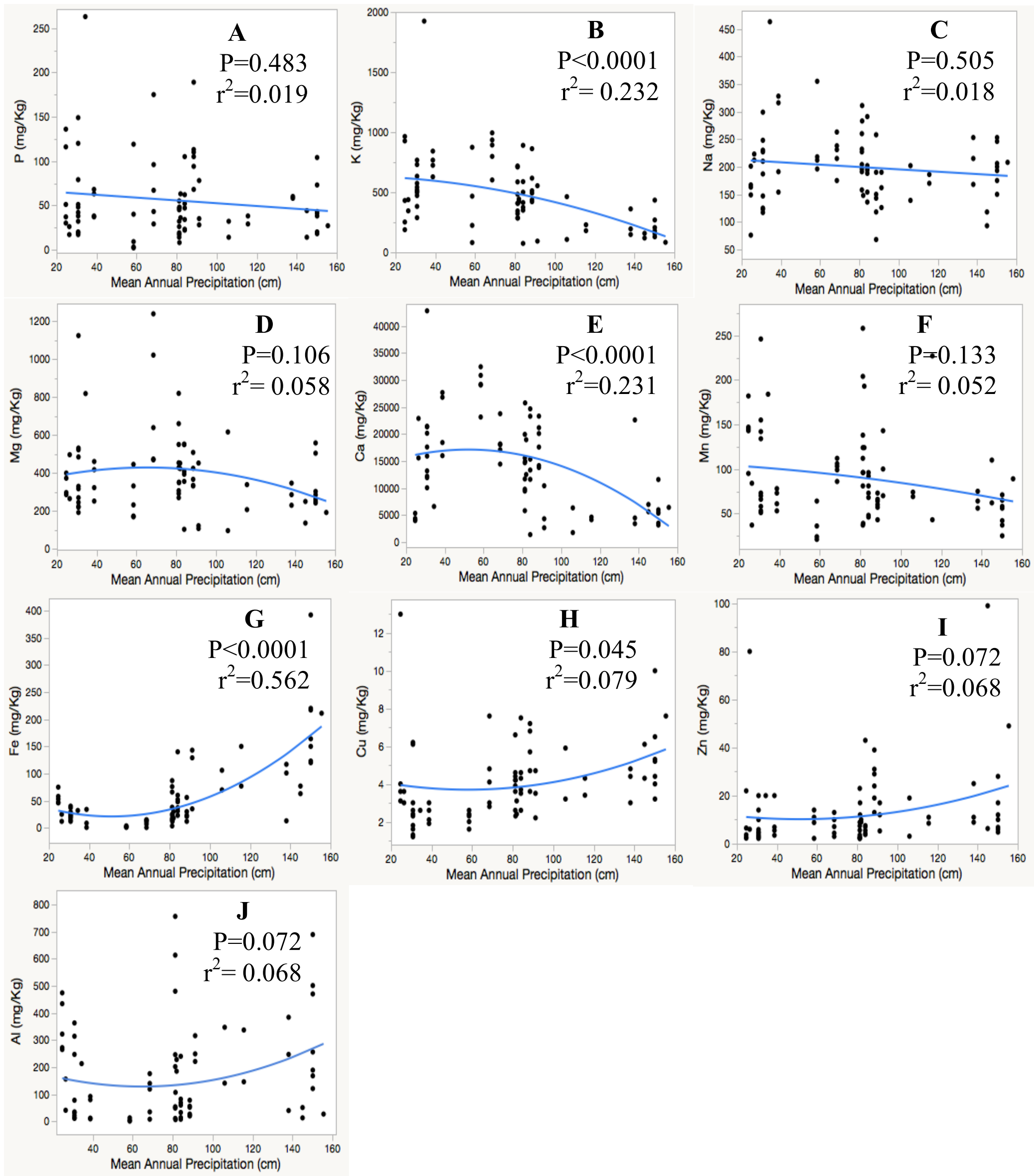

Figure 10: Quadratic regression of soil minerals with mean annual precipitation (1981-2010). Significance was found for calcium (E) and iron (G). No significance relationship was found for phosphorus (A), potassium (B), sodium (C), magnesium (D), manganese (F), copper (H), zinc (I), and aluminum (J).

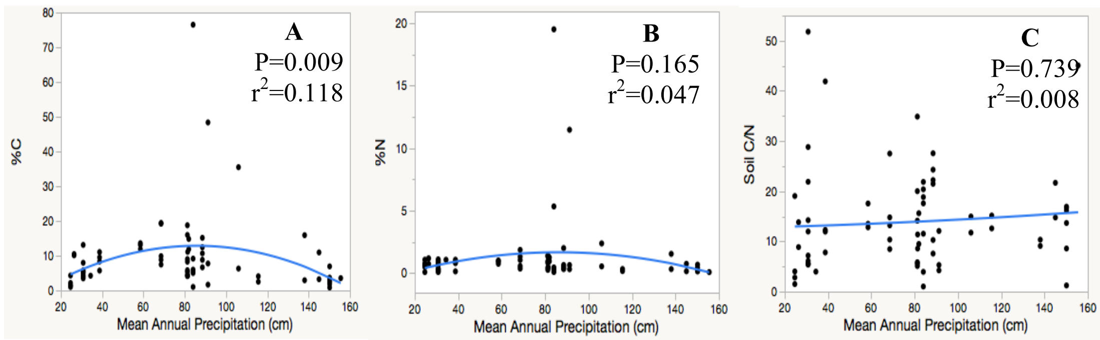

Figure 11: Quadratic regression for %C, %N, and C/N ratio of soil when assessing change with mean annual precipitation (1981-2010). No relationship showed any significance.

Linear and quadratic relationships were also analyzed for each element using longitude (decimal degrees) as the independent variable. Phosphorus (mg/Kg) had no significant response to change along the longitudinal gradient ($F_{1,77}$=1.201; p=0.277). Potassium showed a significant linear relationship ($F_{1,77}$=15.07; p=0.0002), with K decreasing at a rate of 35.339 mg•Kg^{-1}•D_{dec}. Calcium also showed a significant linear relationship ($F_{1,77}$=6.579; p=0.0123), decreasing at a rate of 710.225 mg•Kg^{-1}•D_{dec}. Iron ($F_{1,77}$=25.934; p<0.001) and zinc ($F_{1,77}$=6.329; p=0.014) both showed a significant linear relationship, increasing at a rate of 9.546 mg•Kg^{-1}•D_{dec} and 1.267 mg•Kg^{-1}•D_{dec}, respectively. When assessing changes in %C, %N, and the C/N ratio, no significant relationship was found. Linear regressions are shown in Figure 12 and Figure 13.

Quadratic relationships were only significant for calcium ($F_{2,77}$=26.277; p<0.0001), iron ($F_{2,77}$=51.406; p<0.0001), copper ($F_{2,77}$=11.964; p<0.0001; r^2=0.242), and aluminum ($F_{2,77}$=7.379; p=0.0012). The regression for calcium was unimodal, with the highest point at around -101 decimal degrees W. No significant relationship was found for sodium ($F_{2,77}$=2.229; p=0.115), magnesium ($F_{2,77}$=2.022; p=0.140), and the C/N ratio ($F_{2,77}$=0.981; p=0.380). Iron, copper, and aluminum all had a similarly shaped relationship, with the highest peaks at our easternmost (-92 decimal degrees W) and westernmost (-108 decimal degrees W) sampling extremes. All quadratic regressions are shown in Figure 14 and Figure 15. All individual graphs for each element and the C/N ratio across the longitudinal and precipitation gradients may be found in Appendix B.

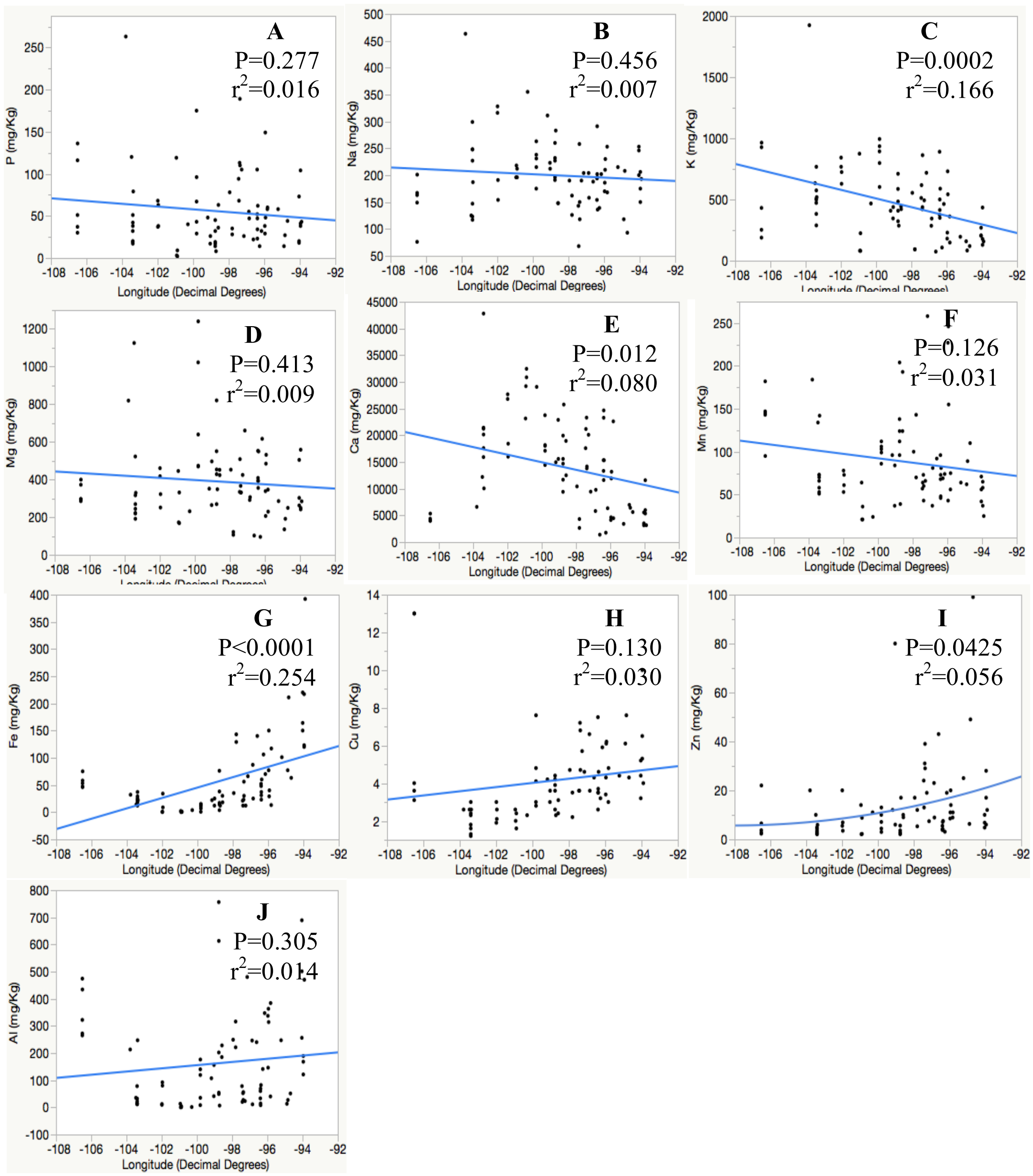

Figure 12: Linear regression of soil minerals with longitude (decimal degrees). No significant relationship was found for phosphorus (A), sodium (B), magnesium (D), manganese (F), copper (H), and aluminum (J). Significance was found for potassium (C), calcium (E), iron (G), and zinc (I).

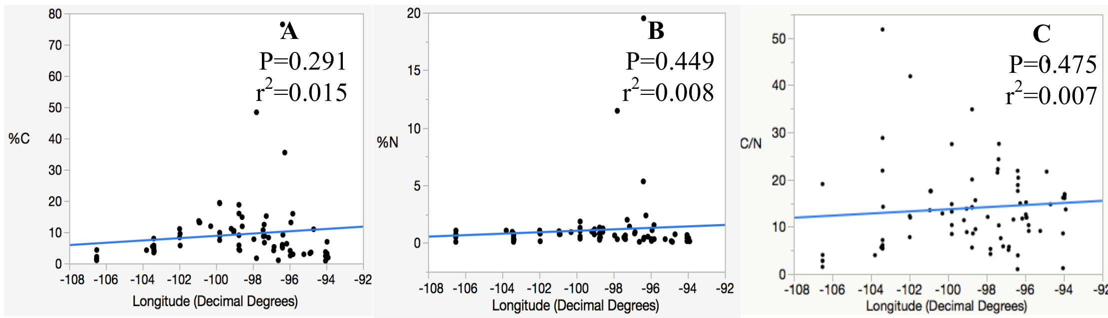

Figure 13: Linear regressions for soil %C, %N, and the C/N ratio when assessing change with longitude (decimal degrees). No relationship showed any significance.

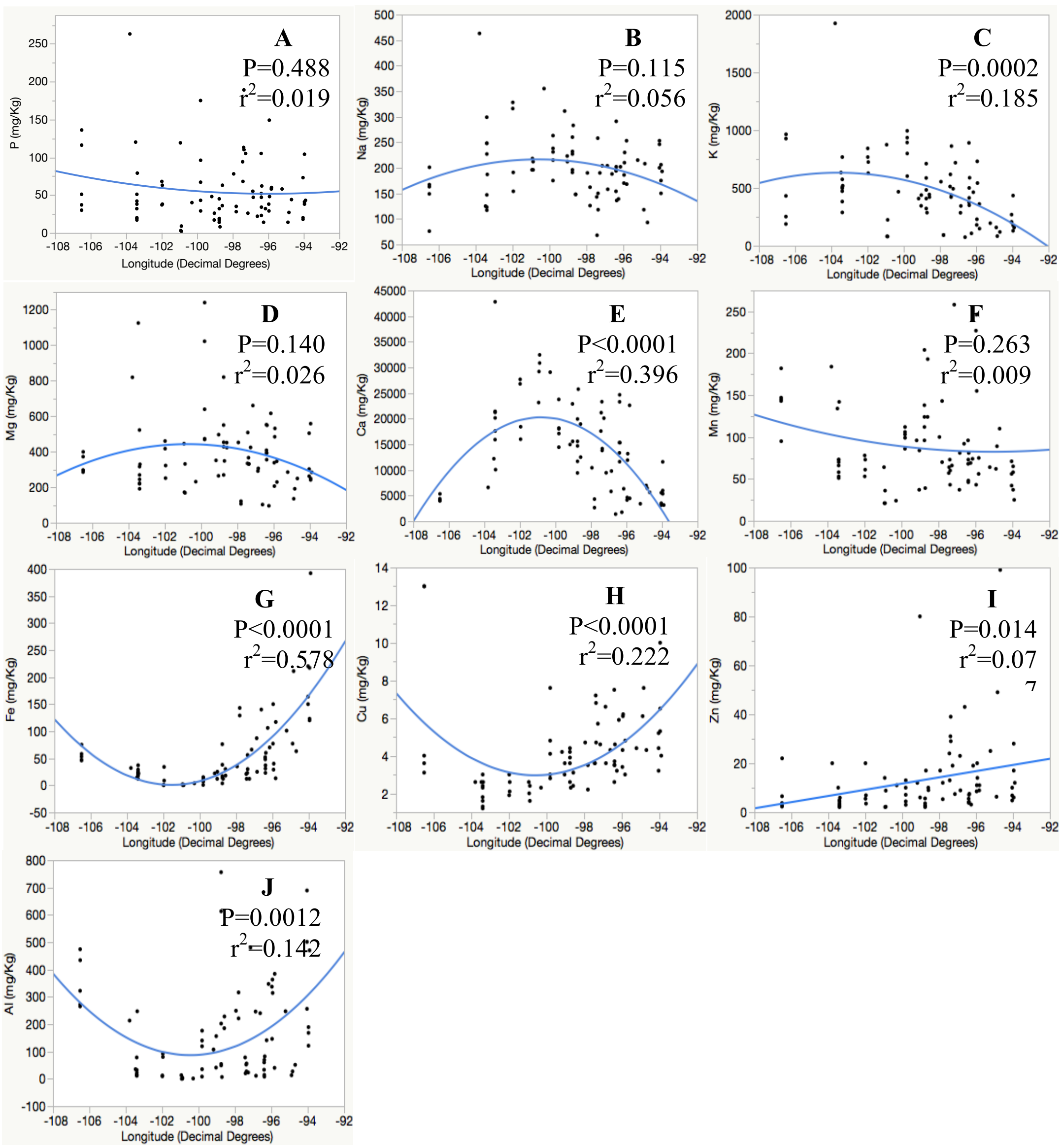

Figure 14: Quadratic regression for soil minerals with longitude (decimal degrees). No significant relationship was found for phosphorus (A), sodium (B), potassium (C), magnesium (D), and manganese (F). Significance was found for calcium (E), iron (G), copper (H), and aluminum (J).

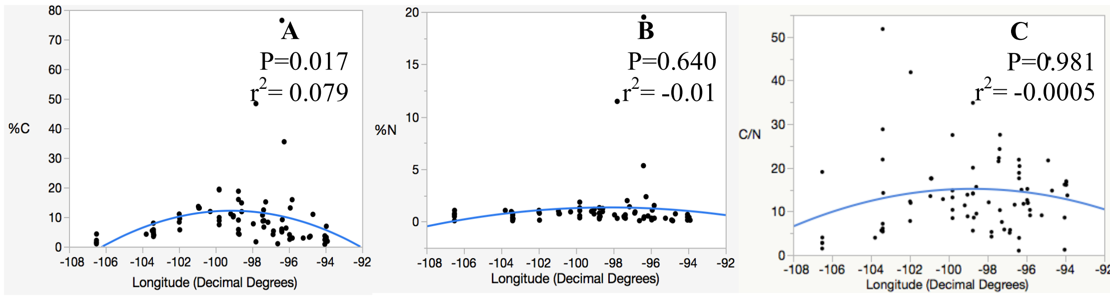

Figure 15: Quadratic regressions for soil %C, %N, and the C/N ratio with longitude (decimal degrees). No relationship showed any significance.

Soil types and qualities by county are summarized in Table 7. However, no published quantitative data on mineral nutrient amounts were found for these counties. Samples in Austin, Chambers, Colorado, Fayette, Gonzalez, Harris, and Waller counties had a loamy, sandy soil that was moderately to poorly drained. Because of their saline condition, one can assume a higher Na and Cl level in these sampling locations. Samples in Orange, Liberty, Bexar, and Kimble counties had shallow, clayey, well-drained to moderately drained soils on top of a limestone parent material. Because of this limestone, one can expect higher levels of Ca and Mg in these soils. Samples from El Paso and Kerr counties had well-drained, calcareous stony loam on top of a limestone parent material. Samples taken in El Paso were on very steep inclines, leading to a lower water storage within the soils. Samples from Guadalupe, Sutton, Pecos, and Reeves counties were alkaline clays. These areas were moderately well-drained silty, calcareous loams. The larger mineral nutrients (Fe, Mn, Zn, Cu, and Co) require an acidic environment to be broken down into soluble forms and transferred from soil to plant. Based on this information, we can infer that these counties will have lower levels of these larger mineral nutrients. Finally, the parent material in Brewster County is a combination of igneous, metamorphic, and sedimentary rock. Brewster County has rolling topography, ranging from very shallow to deep soils. The bedrock is igneous with some limestone, and the soil is well-drained and very gravelly.

Table 5: Potential dominant soil associations within sampled counties. Table depicts parent material and geologic formation as well as potential mineral nutrients available based on parent material.

County	Geologic Formation	Material/Quality	Drainage/Depth	Source
Austin	Lissie	Clayey and loamy soils; marine sediments	Moderately well drained, somewhat poorly drained	Na+ and Cl-; Marine Sediments (quartz)
Bexar	Undivided Navarro and Taylor	Clayey soils over chalk and marl	Moderately deep	Limestone (CaCO3)
Brewster	Cretaceous undivided	Very gravelly and gravelly soils, on uplands	Very well drained, rolling terrain (deep and shallow)	Igneous Bedrock, limestone
Chambers	Alluvium	Loamy, sandy soils; Saline, High runoff	Somewhat poorly drained	Na+ and Cl-
Colorado	Lissie;Fleming and Oakville	Loamy, sandy soils; Saline	Moderately well drained, somewhat poorly drained	Na+ and Cl-
El Paso	Wolfcampian Series	Stony, steep soils; surface layer of pinkish-grey calcareous stony loam	Moderately well drained	Limestone (CaCO3)
Fayette	Lissie; Fleming and Oakville	Fine, sandy and loamy soils on upland savannah	Well drained and moderately well drained	Na+ and Cl-
Gonzales	Lissie; Fleming and Oakville	Loamy, clayey, weathered from shale of cretaceous; fine sandy loam	Moderately well drained	Shale, quartz, calcite (CaCO3)
Guadalupe	Clairborne, Fleming and Oakville	Loamy, clayey alluvium of quaternary age	Moderately well drained	Mixed sources (Alkaline clay)
Harris	Lissie	Loamy, sandy soils; Saline	Poorly drained, slowly permeable	Na+ and Cl-
Kendall	Trinity Group	Loamy, gravelly, and clayey soils	Shallow to very shallow	Limestone (CaCO3)
Kerr	Fredericksburg; L. Washita Groups; Trinity	Residuum weathered from limestone	Well drained, very little water storage	Limestone (CaCO3)
Kimble	Fredericksburg and L. Washita Groups	Fine, silty, and clayey alluvium	Well drained, slowly permeable	Limestone (CaCO3)
Liberty	Alluvium	Loamy, clayey deposits from igneous, metamorphic	Somewhat poorly drained	Mixed
Orange	Beaumont	Loamy, clayey deposits from igneous, metamorphic, and sedimentary rock	Somewhat poorly drained	Mixed (Alkaline clay)
Pecos	Austin, Eagle Ford, Woodbine	Fine silty clay and loam	Well drained	Calcite, gypsum
Reeves	Quaternary Undivided	Clayey alluvium	Moderately well drained	Calcareous, slightly alkaline to neutral
Sutton	Fredericksburg and L. Washita Groups	Clayey alluvium, gravelly	Moderately well drained	Calcareous, slightly alkaline
	Fredericksburg and L. Washita Groups	Silty clay loam	Well drained	Calcareous limestone
Waller	Lissie	Loamy, sandy soils; Saline	Somewhat poorly drained	Na+ and Cl-

No statistically significant relationship was found between soil and foliar C/N ratio ($F_{1,77}$=2.629; p=0.109), which remained high despite trends in soil C/N.

There was a quadratic relationship between longitude and the rock: total soil sample ratio ($F_{2,77}$=11.237; p<0.0001; r^2=0.231), showing that samples in west Texas tended to have rockier soils than in east Texas, which are more clay and silt based soils (Figure 16).

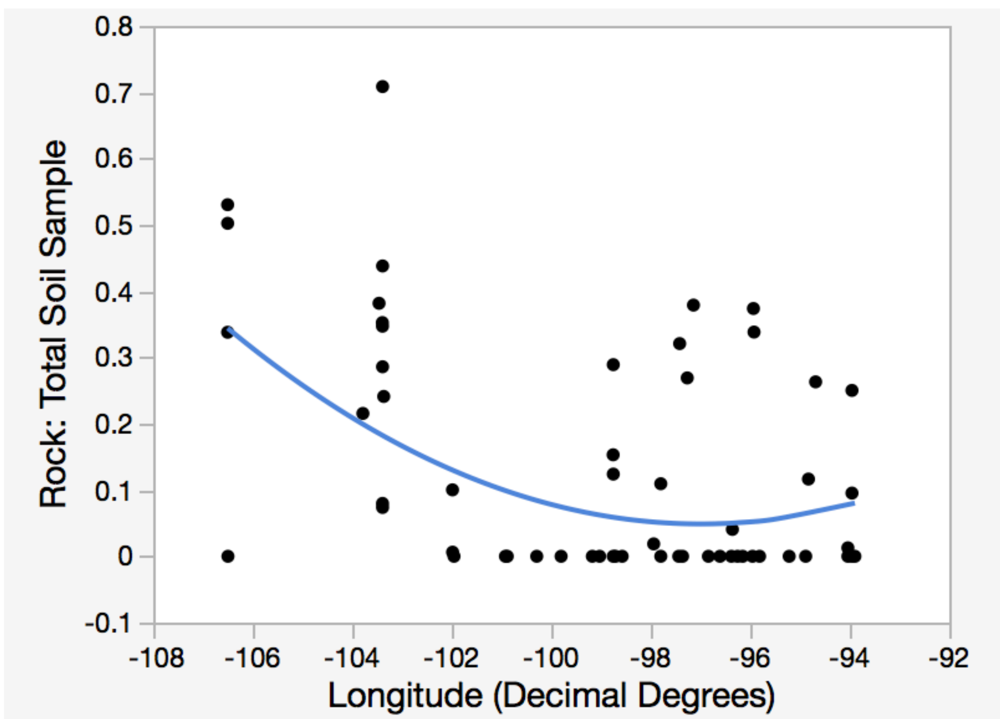

Figure 16: Rock: Total soil sample change across Texas. Total soil sample is the sum (mg) of the rock, sand, and silt present in the sample. Samples in west Texas were rockier, and soils became less rocky as sampling moved east.

IV. DISCUSSION

Due to the small sample size of C. *ehrenbergiana* (n=3), it was not possible to test for violation of any assumptions for analysis and consequently it is not possible to definitively conclude statistical significance for this species.

Change in C/N ratio showed the predicted positive relationship with recent rainfall in both *C. laevigata* and *C. reticulata*, but changes in leaf size (area, length, and width) and SLA correlated with recent rainfall only in *C. reticulata*. The significant increase in area, length, and width for *C. reticulata* is consistent with the results of previous studies that drier seasons or locations will have a decrease in leaf area and the number of leaves and fruits produced as a means of water storage and water conservation (Abrams *et al*. 1994; Wright *et al.* 2001). The results for *C. reticulata* support the hypothesis that leaf size (area, length, and width) will increase with an increase in precipitation. While linear regressions for these three measurements of size showed that there was an increase in leaf size with an increase in water resource, a low r^2 value was calculated for area (r^2=0.187), length (r^2=0.203), and width (r^2=0.116) for *C. reticulata*. While the hypothesis is still supported, high variability may make future predictions of changes in leaf size difficult.

Specific Leaf Area (SLA) also increased linearly with recent precipitation for *C. reticulata* at a rate of 1.629 cm^2/g per centimeter increase in precipitation, indicating thinner leaves with increased precipitation and following the finding of some studies that areas with predictable, drier conditions will have a lower SLA than areas or seasons of increased rainfall (Marron *et al.* 2001). Studies have cited both decreasing area and increasing leaf thickness as plant responses to drought conditions, with plants in drier conditions having smaller leaves or thicker leaf structures to slow water loss and transpiration (Wright *et al.* 2001). Samples of *C. reticulata* in drier conditions had a larger mass distributed over a smaller area of leaf, indicating more leaf tissue per area (cm^2; Figure 7D). The calculation of SLA depends both on leaf area as well as leaf thickness, therefore a lack of significant change in leaf area for *C. laevigata* may reflect in the final total SLA. The findings of an increased SLA for *C. reticulata* support the hypothesis that SLA will increase with increased recent precipitation.

Celtis laevigata requires a semi-humid climate, which limits the range of this species to the eastern-most portion of Texas. Samples of *C. reticulata* occurred in a wider range of precipitation (0.127 cm - 49.12 cm recent precipitation for *C. reticulata*; 25.25 cm - 71.45 cm recent precipitation for *C. laevigata*). The climate range from arid to semi-humid for *C. reticulata* allows for more varied conditions of study and the potential for more change in both leaf size (area, length, and width) and in the SLA calculation.

Significant increases in C/N with recent precipitation were seen in both *C. laevigata* and *C. reticulata*, and both species showed a similar increase in the slope as the centimeters of recent precipitation increased (0.121 and 0.118, respectively). Many studies suggest that the increase in C/N with precipitation may be due to the increase in carbon with little change in nitrogen content as the leaf area increases with precipitation (Cunningham *et al*. 1999; Veteli *et al.* 2002). While this similar, significant increase in C/N and leaf area was seen in *C. reticulata*, the lack of a significant change in leaf area and SLA for suggests that the increase in C/N in *C. laevigata* with recent precipitation is potentially due more to a decrease in available soil nitrogen and less to an increase in carbon. This result partially supports the second hypothesis that the C/N ratio did increase with the increase in recent precipitation and that nitrogen concentration was decreased throughout the leaf. Because *C. laevigata* did not exhibit a similar change in leaf area or SLA with the increase in C/N, I cannot determine if the general decrease in nitrogen is due to a dilution of available nitrogen as carbon content increases or because of a loss of nitrogen due to lack of nitrogen sources in the soil, but I can conclude that leaf material in areas of higher precipitation does have a decrease in nutrient content in the form of nitrogen.

The C/N ratio is important in assessing the nitrogen available in plants for both plant growth processes and the quality as food for herbivores. Nitrogen can be the limiting factor of an ecosystem (White 1993). Higher C/N ratios are considered less nutritious, as they have less nitrogen per amount of leaf material. Samples taken in locations of higher precipitation were less nutritious than those sampled in drier locations, despite the plants from drier areas typically having a smaller leaf area.

It is also well-known that plants increase leaf carbon in various forms (waxy cuticles, thick cell walls, surface hairs) to reduce water loss in dry environments (Garten et al. 1994) and increase carbon content to protect leaves and twigs from herbivores (toxic compounds, thorns) in environments where plant growth is slow and plants must protect leaves to survive, typically areas with low rainfall and/or low soil nutrient content (Coley et al. 1985).

Potassium, calcium, and manganese all showed a significant, linear relationship with a negative slope with change in mean annual precipitation. Micas, the main parent source of potassium, is found in west Texas where precipitation is lowest and due to the high mobility of potassium ions (Sterrett 1923). Potassium, calcium, and manganese cations are often leached in areas of high rainfall due to their electric charge, therefore finding less in areas of more precipitation here is due in part to the parent material and in part to the leaching from precipitation (Thompson and Ukraincyzk 2002). Potassium is critical for functions related to drought survival, making its presence in the drier areas of the state favorable for the plants inhabiting that region (Brady and Weil 2002). Calcium also showed a significant, quadratic relationship with a peak in a median amount of rainfall similar to Albrecht's Curve.

While there is no historical data on how iron and copper amounts have changed, iron and copper showed a significant, linear relationship with a positive slope with change in mean annual precipitation. Both iron and copper are important in the production of chlorophyll and undergoing photosynthesis in small quantities, however large concentrations have been known to be toxic to the plant (Brady and Weill 2002). Iron also fixes phosphorous into unusable forms for the plant, which can be detrimental for plant growth and function. Iron and copper are very immobile ions. As other minerals leach through the soil, iron and copper remain at the surface and increase in concentration (Bigham *et al.* 2002). Though areas of higher rainfall do have more water resources to undergo photosynthesis, these areas may eventually have levels of iron that are not suitable for plant growth.

Using the longitudinal gradient in soil elemental analysis reflects the long-term effects of climate and parent material on the changes in total soil mineral nutrients. Of the key mineral nutrients tested, only calcium showed a strong unimodal relationship similar to Albrecht's Curve, with a peak at around -101 decimal degrees W (around Sutton County). Parent material for most of the state is marine sediments and limestone ($CaCO_3$), allowing for sources of calcium to be available. Some precipitation is necessary for weathering parent material without leaching the calcium from the soil, and calcium may be leached once precipitation exceeds amounts usable in the ecosystem (Doner and Grossl 2002). Higher amounts of available calcium will provide nutrients key for forming structures in plants, such as the cell walls, but too much leaching has the potential to be detrimental to the plant (Brady and Weil 2002).

The significant increase of the more toxic elements in the soil (iron, copper, aluminum, and zinc) in east Texas follows predictions from Albrecht's Curve, because leaching of smaller, more mobile cations and mineral nutrients out of the surface soils will leave higher concentrations of heavy minerals at the upper layers. Unlike Albrecht's Curve, I found a second peak in amounts of iron, copper, and aluminum in soil samples of west Texas. This may be explained by the parent material in the Trans-Pecos region. Chalcocite and chalcopyrite are two potential parent sources of copper, almandite is a potential source for iron-aluminum silicate, biotite is a potential source of iron, and albite and orthoclase are two potential sources for aluminum. These known parent material types are formed in igneous and metamorphic rock in west Texas, and thousands of years of physical and chemical weathering have created the sandy, silty, and sometimes clayey soil sources found in the Chihuahuan deserts of west Texas (Girard 1964). While no previous measurements have been found on historical levels of these nutrients, one can see a general trend that exists and conclude that soils in east Texas are higher in these heavier mineral nutrients. While these minerals are required by plants in small amounts, excess iron, copper, aluminum, and zinc can be toxic to plants. The level at which toxicity occurs and whether that point is reached here is not known, however we can predict that this area has the potential to increase toxicity with continued weathering.

While not significant, sodium (Figure 14B) and magnesium (Figure 14D) all showed a similar, unimodal trend seen in Albrecht's Curve. Sodium, used in regulating osmotic potential and maintaining chlorophyll, and magnesium, used in the production of chlorophyll and activating enzymes, are important as cations for plant function (Pardo and Quintero 2002; Evert and Eichhorn 2013). Soils in east Texas are typically saline clayey soils. The unimodal relationship we see may represent the leaching with increased precipitation, as Albrecht described because one would expect sodium levels to be higher in east Texas, yet I found the highest concentrations in samples of central Texas. One reason for this may be the leaching of sodium cations with higher amounts of precipitation.

One surprising result was the lack of significant change in available nitrogen with an increase in precipitation. It is important to note that phosphorus is necessary for bacteria in the soil to convert atmospheric nitrogen (N_2) into usable forms for plants, like ammonium (NH_4^+; Divito and Sadras 2014). I expected soils in east Texas would have higher levels of nitrogen, due to the increased precipitation leading to an increase in decomposition in the nitrogen cycle. However, there was no significant change of nitrogen with precipitation ($F_{1,77}$=3.132; p=0.792) or with longitude ($F_{1,77}$=0.579; p=0.449). While not significant, the slight decrease in phosphorus in the soils of east Texas could potentially affect nitrogen available in the soil. Nitrogen in the samples in east Texas could also be occurring deeper than the top 20 cm sampled.

The increase in the effect of climate change may be detrimental to both our agriculture and our natural ecosystems. One phenomenon we see as the climate changes is the extreme weather patterns increasing in frequency: areas of drier conditions have had increased drought events, and areas with more rainfall have had increased flooding events. Plants living in wetter regions with increased flooding may experience further leaching of important cations and soil erosion, the increase in concentration of those cations that are less mobile, the reduction in soil water absorption, and increased fungal infection as flood events increase in frequency (Rosenzweig *et al.* 2001). I predict that as climate change continues and intensifies, plants in areas of higher precipitation, like east Texas, will continue to see a decrease in nutrient quality for herbivores. While leaf and plant quantity may increase, herbivory will also increase as the nutrient quality of the leaf diminishes. Water use will also suffer as soils become waterlogged and soil nutrients and oxygen become unavailable to plant roots, leading to plant death. I also predict the increase in drought in dry areas will cause plants to store water, refrain from putting leaves or fruits out during a drier growing season, or not have enough water for cation exchange or survival, resulting in eventual plant death. Understanding the changes in plant nutritional quality and leaf size and quantity is important in further understanding what may happen to our ecosystems as our climate changes.

Conclusions and Future Directions

Overall, support for my hypotheses regarding leaf characteristics were supported, but may have suffered from low sample size in some cases. This lends support to the idea that changes in soil chemistry with broad climate factors can be indirectly examined by sampling broadly distributed perennial species. On the other hand, Albrecht's generalization about the effect of precipitation on nutrient concentrations were generally not supported, and more strongly governed by differences in parent materials.

The interactions of precipitation with plant nutrient availability is relevant to natural ecosystems but also agriculture, as shift to warmer, more extreme rainfall conditions are bound to affect plant and soil chemical compositions. Thus, Albrecht's curve (Figure 2) maintains relevance as we try to better understand the relationships between nutrient content and plant productivity, and between precipitation and soil quality.

Plants living in wetter regions with increased flooding may experience further leaching of important cations and soil erosion, the increase in concentration of those cations that are less mobile, the reduction in soil water absorption, and increased fungal infection as flood events increase in frequency (Rosenzweig *et al.* 2001). This may cause further decrease in nutrient quality for herbivores. While leaf and plant quantity may increase, herbivory will also increase as the nutrient quality of the leaf diminishes. Water use may suffer as soils become waterlogged and soil nutrients and oxygen become unavailable to plant roots, possibly leading to increased plant mortality. Intensifying drought in dry areas may cause diminished leaf and fruit growth or death if pants do not have enough water for cation exchange or survival. Understanding the changes in plant nutritional quality and leaf size and quantity is important in further understanding what may happen to our ecosystems as our climate changes.

Celtis reticulata was the best species for this study, as it was the most widely distributed and is found more often in different habitats with less specific growth requirements, from the deserts of west Texas through central and east-central Texas. Assessing the change in C/N across a gradient showed a lot of variation for each sampled plant. Repeating the study using only *C. reticulata* and increasing the number of samples collected could create a better, less varied, understanding of the relationship between C/N, leaf size (area and SLA, especially), precipitation, and soil quality analysis. This will also allow for comparative studies on how a large sample in west Texas compares to a large sample in central Texas and how the soil mineral nutrients may more directly affect plant nutritional quality. *Celtis ehrenbergiana* has two main habitat locations, the drier deserts of west Texas and the central Texas hill country. Finding more samples of *C.*

ehrenbergiana and running comparative studies on C/N and leaf size may also provide interesting results on how a desert adapted plant reacts to changes in precipitation.

The Mehlich-3 soil test and the C/N analyzer only determine the total minerals within a sample, but do not assess the form of those minerals and if they are usable to the plant. Since nitrogen is important for plant growth, running an inorganic nitrogen colorimetric test would allow me to determine what forms of nitrogen exist in the soil and what is available to the plant. Depending on the direction of the study, the same can be done to detect usable forms of aluminum, iron, calcium, or phosphorus. Assessing changes in aluminum and iron may also be important, as both are required in tiny amounts and are often present (e.g., east Texas) in high concentrations that may be toxic and reduce the availability of essential nutrients. Determining amounts of these mineral nutrients within the leaf would also be interesting in determining how the plants change growth strategies with different available nutrients and water resources. Analyzing changes in pH would also provide more information on the changes in soil conditions across a gradient. Some minerals can only be broken down into forms usable to the plant under acidic conditions. The pH of soils usually decreases as rainfall increases, so assessing if changes in pH is present along this large precipitation gradient and to what extent it may play in nutrient availability and cation exchange would also aid in understanding soil nutrient change along a precipitation gradient.

Phosphorus is often a limiting nutrient within an ecosystem, as phosphorus amounts tend to be limited in the soil and a vital nutrient to plants and herbivores (Brady and Weil 2002). A future study focusing on phosphorus changes and the C:N:P ratio in soils and within the leaf may also provide new understanding to nutrient change along precipitation gradients as well as net primary productivity in relation to phosphorus (for similar studies, see Wang *et al.* 2015). Finally, the results of this study may aid in the predictions and executions of future studies encompassing a wider geographic range. Texas mirrors the precipitation gradient throughout the United States, so the results of this study allow us to hypothesize what may happen to plant nutrient change in our ecosystems and agriculture as well as the possible changes of food web health across this much wider gradient. With these predictions, we can then run future studies along this larger range, which may be key in assessing nutrient health as the climate changes.

APPENDIX SECTION

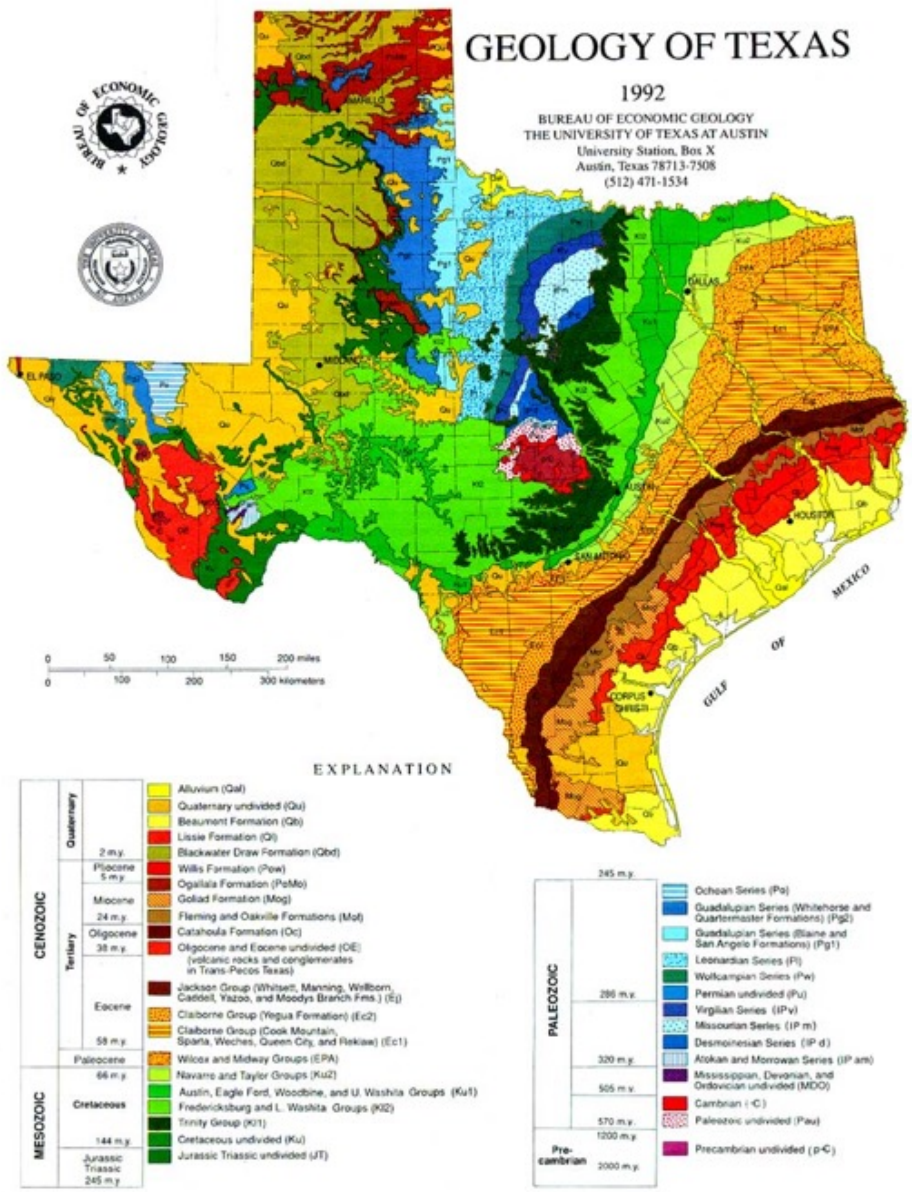

Figure 17: Geology of Texas. Map created by the Bureau of Economic Geology (1992) depicting the diverse surface geology of Texas.

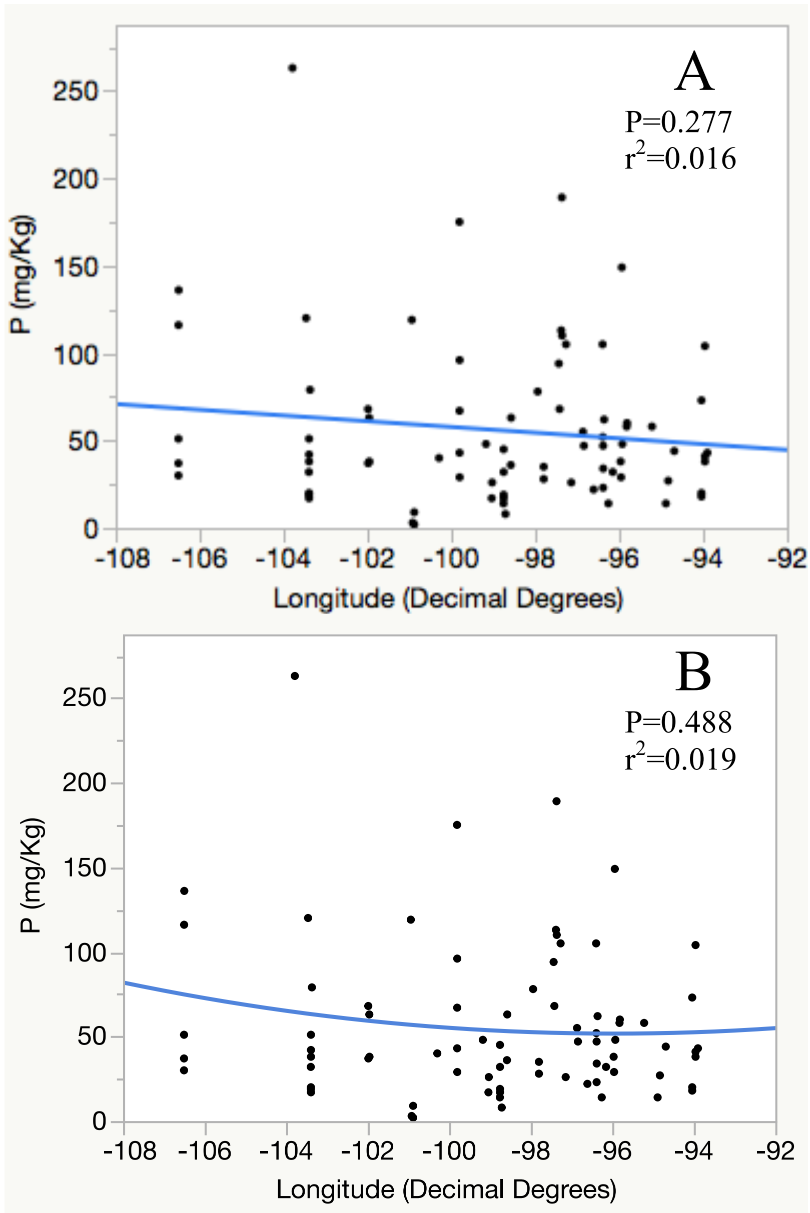

Figure 18: Analysis of phosphorus amounts in all soil samples over the longitudinal gradient. Linear regression (A) and quadratic regression (B) were both non-significant. Linear regression showed that, in general, phosphorus decreased (mg/Kg) as sampling continued from west to east.

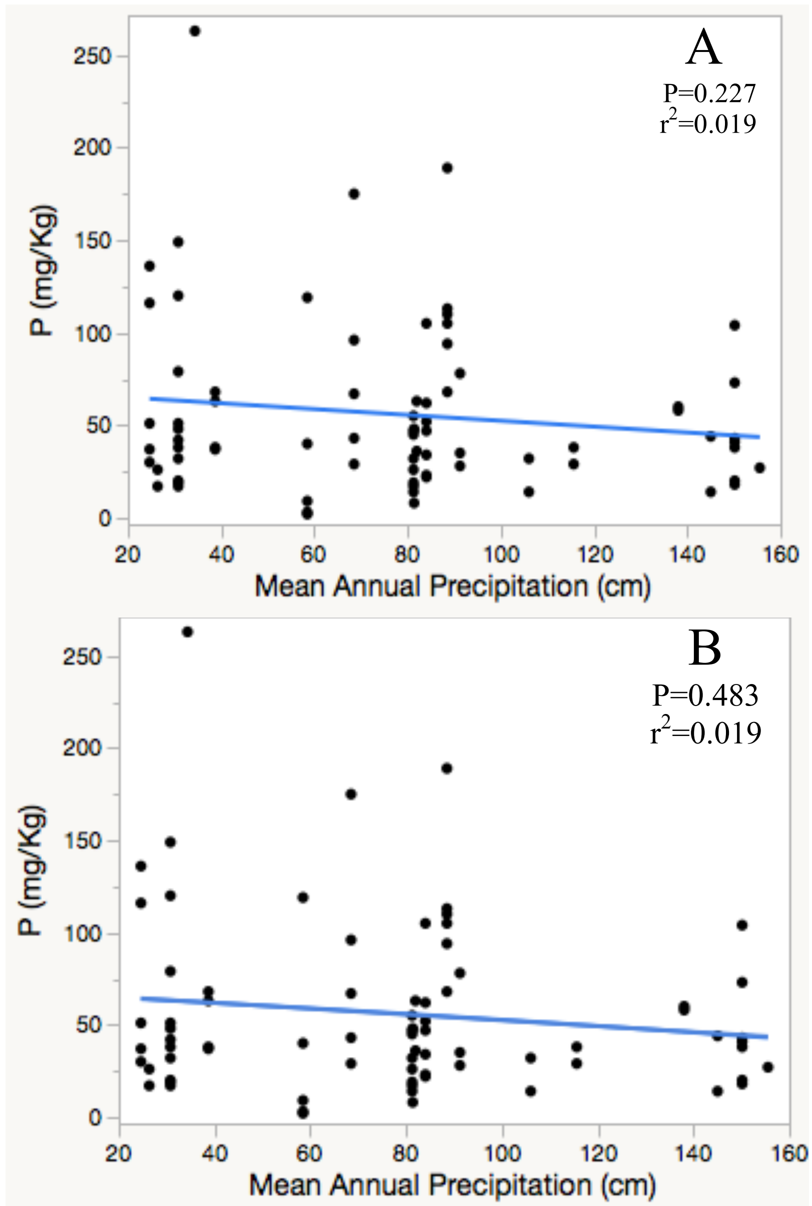

Figure 19: Analysis of phosphorus amounts in all soil samples over a precipitation gradient. Mean annual precipitation was the average annual rainfall received at each sampling location from 1981-2010. Linear regression (A) and quadratic regression (B) were both non-significant, but both showed that, in general, phosphorus decrease (mg/Kg) as precipitation increased.

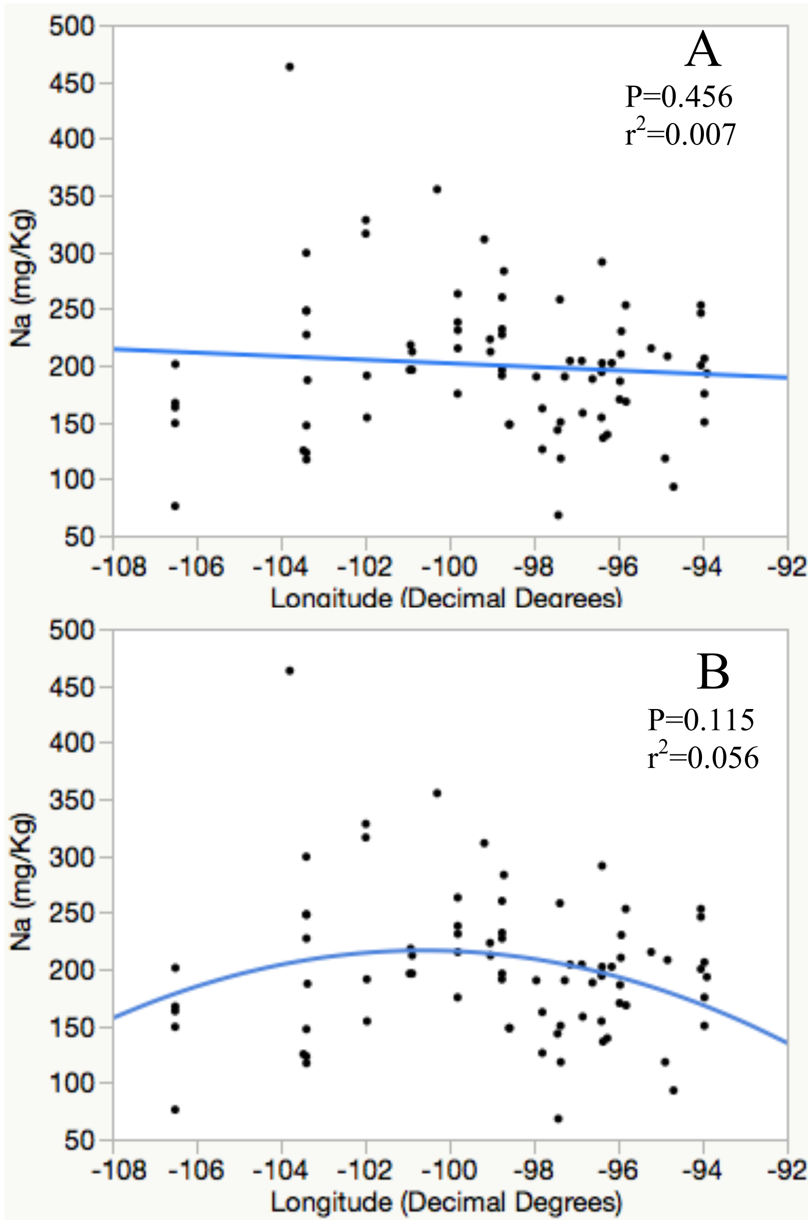

Figure 20: Analysis of sodium amounts in all soil samples over the longitudinal gradient. Linear regression (A) and quadratic regression (B) were both non-significant, however in general our quadratic regression shows a pattern similar to Albrecht's Curve with higher amounts of sodium (mg/Kg) found in central Texas.

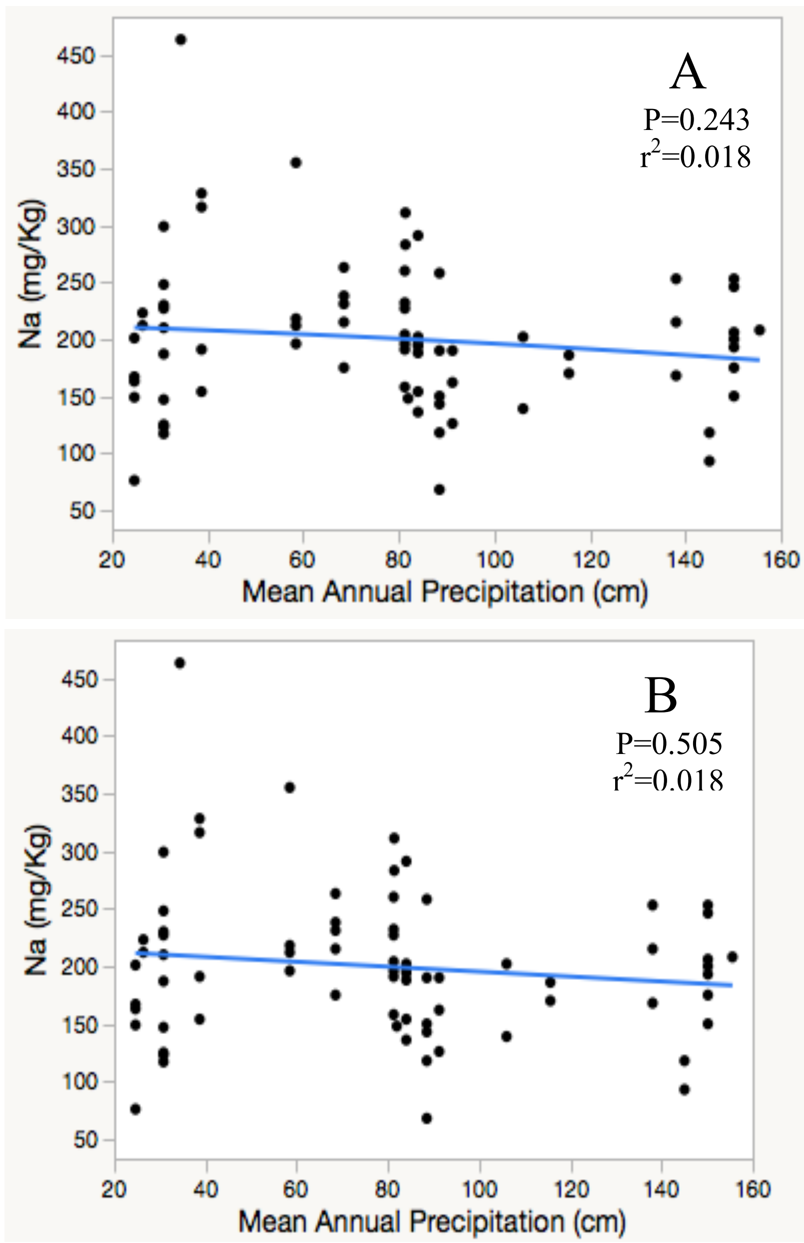

Figure 21: Analysis of sodium amounts in all soil samples over a precipitation gradient. Mean annual precipitation was the average annual rainfall received at each sampling location from 1981-2010. While both the linear regression (A) and the quadratic regression (B) were not significant, the general trend shows a slight decrease in sodium (mg/Kg) as precipitation increases.

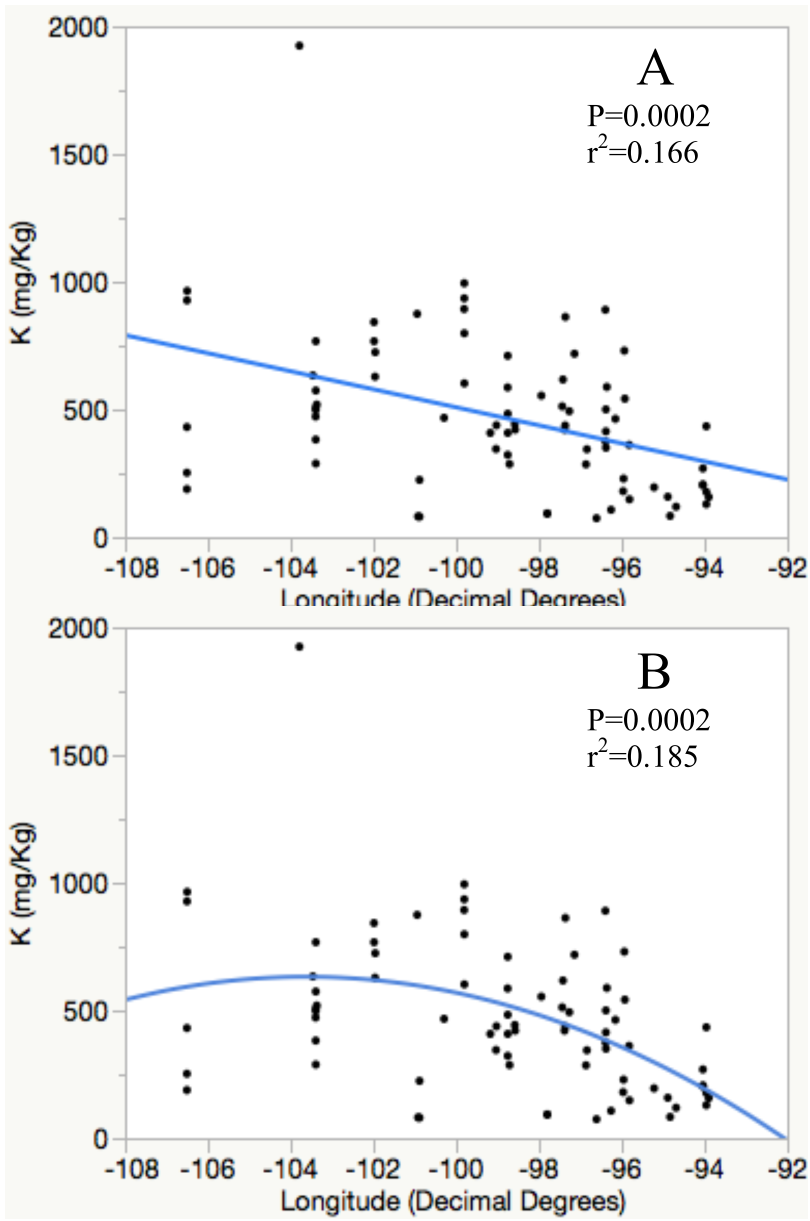

Figure 22: Analysis of potassium amounts in all soil samples over the longitudinal gradient. Both the linear regression (A) and quadratic regression (B) are statistically significant. The linear regression had high variability (r^2=0.166) and showed potassium (mg/Kg) decreased as sampling occurred from west to east. Parameter estimates for the quadratic regression were not significant, however in general we see a pattern similar to Albrecht's Curve.

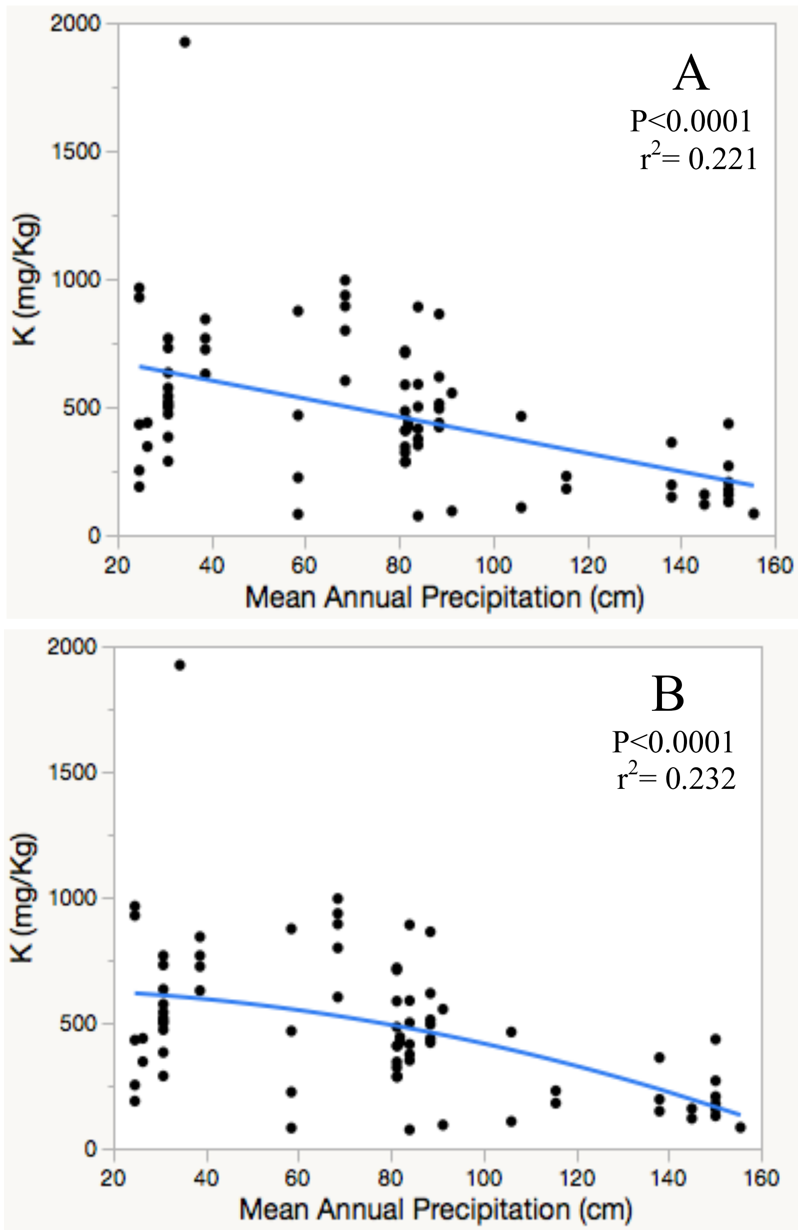

Figure 23: Analysis of potassium amounts in all soil samples over a precipitation gradient. Mean annual precipitation was the average annual rainfall received at each sampling location from 1981-2010. Linear regression (A) and quadratic regression (B) are shown, with a statistically significant linear regression with high variation (r^2=0.221). This shows potassium (mg/Kg) decreased as precipitation increased. Parameter estimates for the quadratic regression were not significant, however in general a pattern similar to Albrecht's Curve is seen.

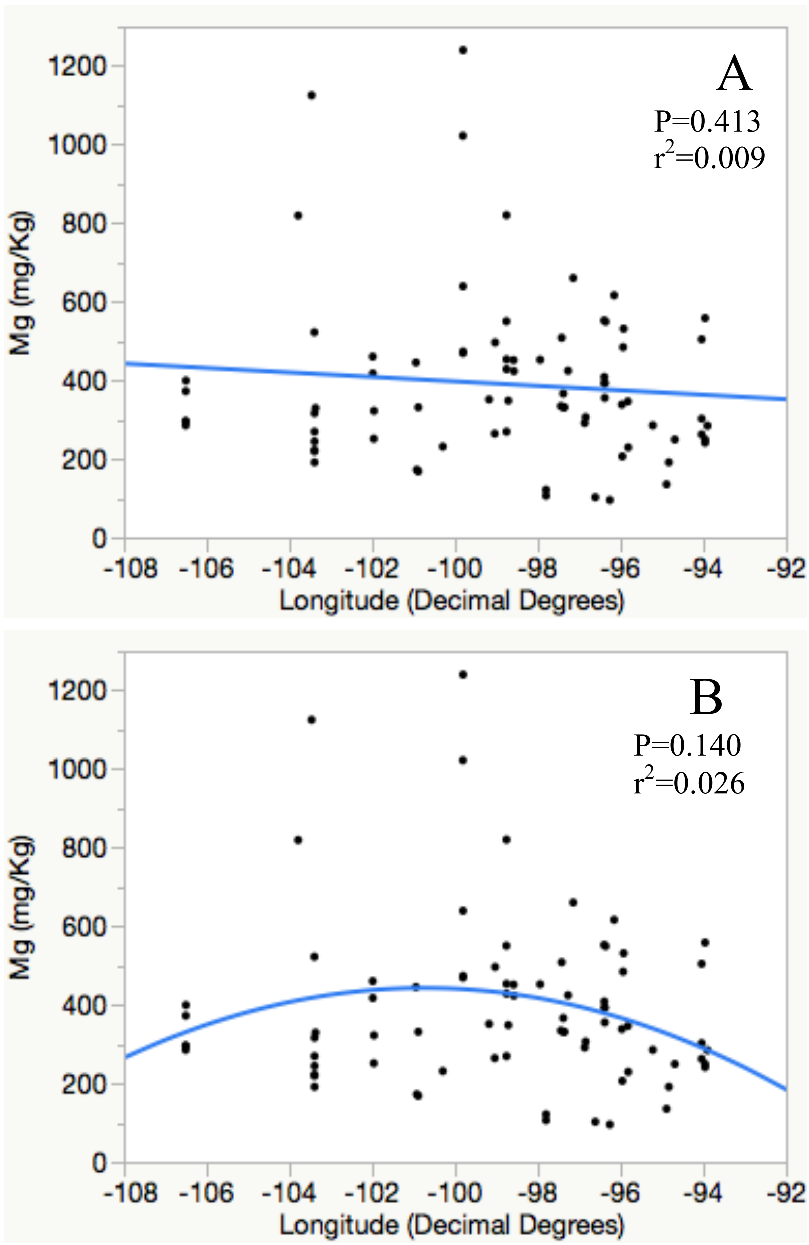

Figure 24: Analysis of magnesium amounts in all soil samples over the longitudinal gradient. While the linear regression (A) was not statistically significant, the general trend shows a slight decrease in the amount of magnesium (mg/Kg) as sampling occurred east. The quadratic regression (B) was also not significant, however in general a pattern similar to Albrecht's Curve is seen.

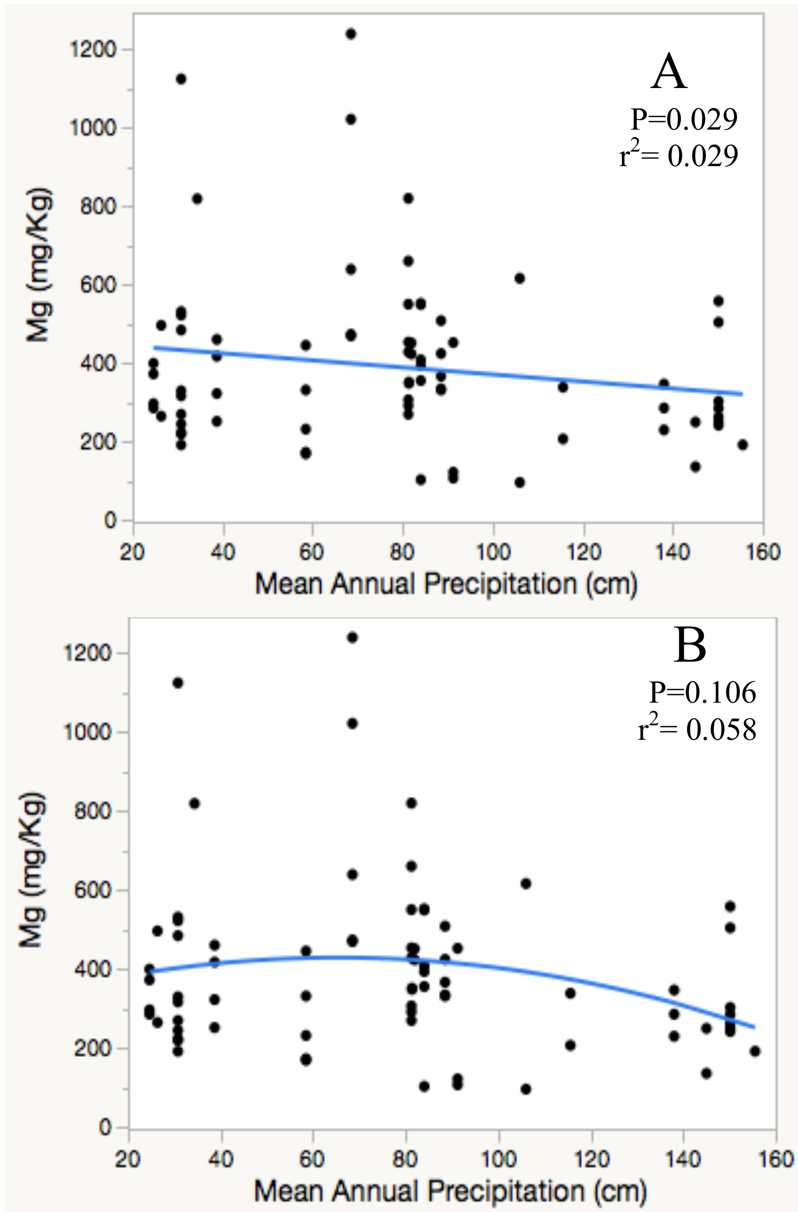

Figure 25: Analysis of magnesium amounts in all soil samples over a precipitation gradient. Mean annual precipitation was the average annual rainfall received at each sampling location from 1981-2010. While both linear regression (A) and quadratic regression (B) showed no significance, in general we see a decrease in magnesium (mg/Kg) as the 12-month antecedent precipitation increases.

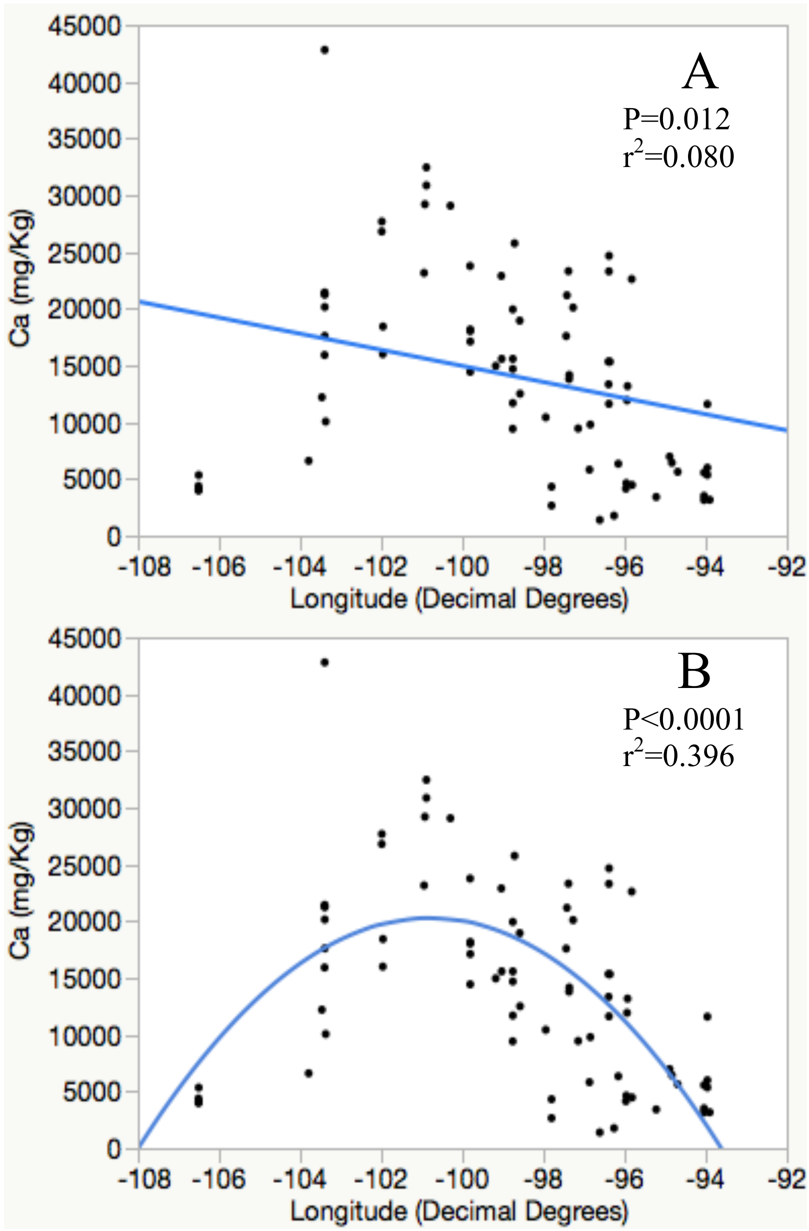

Figure 26: Analysis of calcium amounts in all soil samples over the longitudinal gradient. Both the linear regression (A) and the quadratic regression (B) were statistically significant, but showed high variability (r^2=0.080 and r^2=0.396, respectively). The linear regression showed calcium (mg/Kg) decrease as sampling continued from west to east. The quadratic regression showed a relationship similar to Albrecht's Curve.

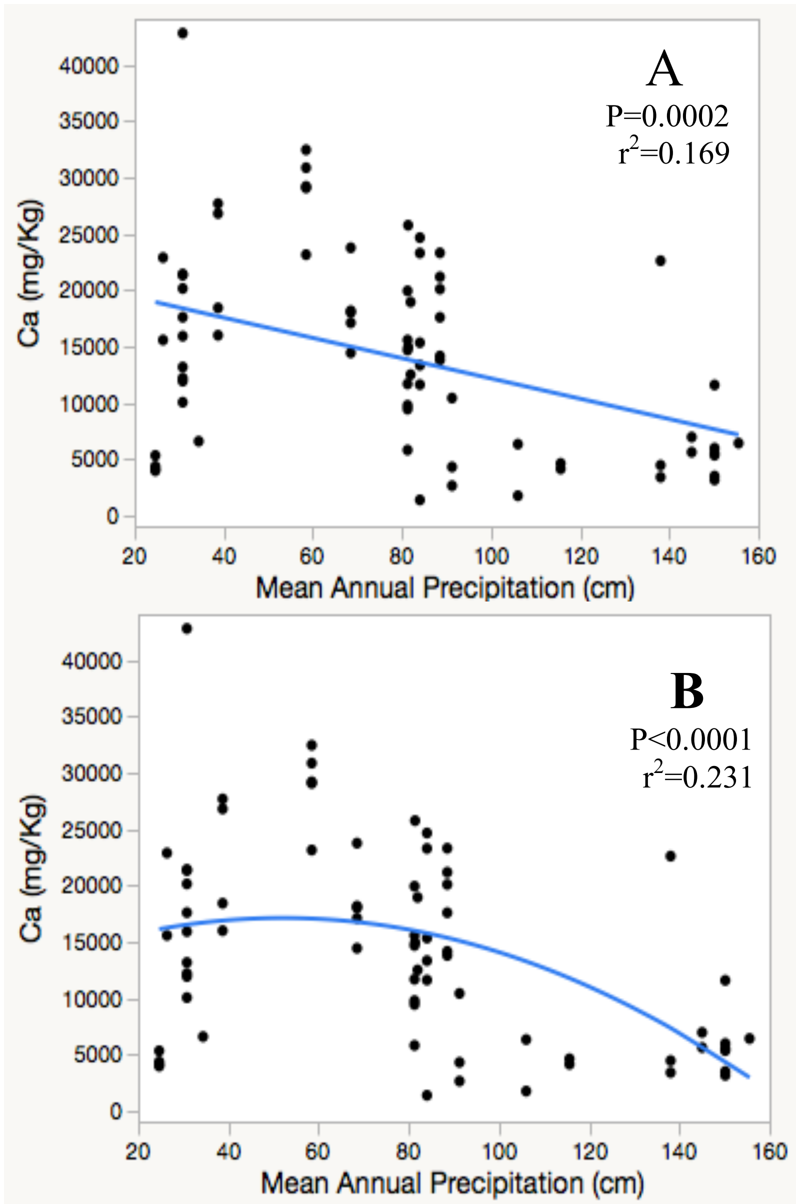

Figure 27: Analysis of calcium amounts in all soil samples over a precipitation gradient. Mean annual precipitation was the average annual rainfall received at each sampling location from 1981-2010. Both the linear regression (A) and quadratic regression (B) showed significance, and in general we see a decrease in calcium (mg/Kg) as precipitation increases.

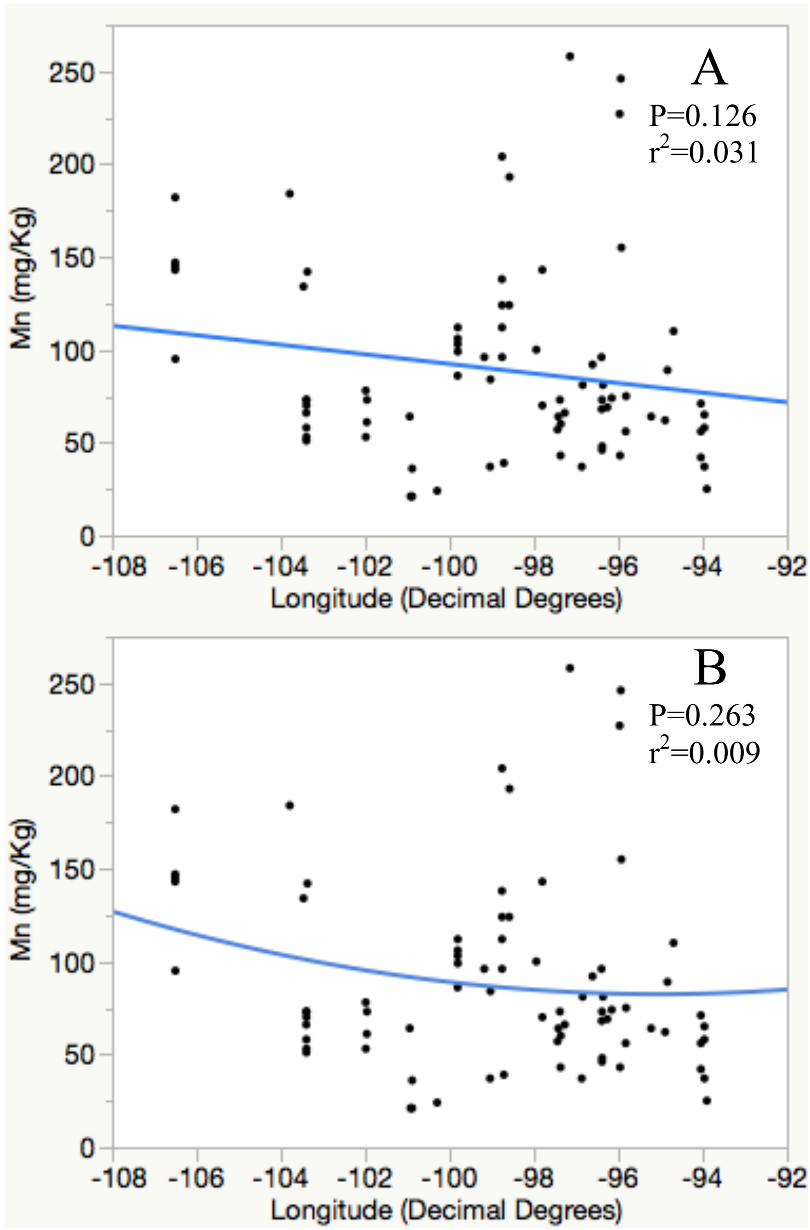

Figure 28: Analysis of manganese amounts in all soil samples over the longitudinal gradient. While not statistically significant, the linear regression (A) showed a decrease in manganese (mg/Kg) as sampling continued from west to east. Quadratic regression (B) was also not statistically significant.

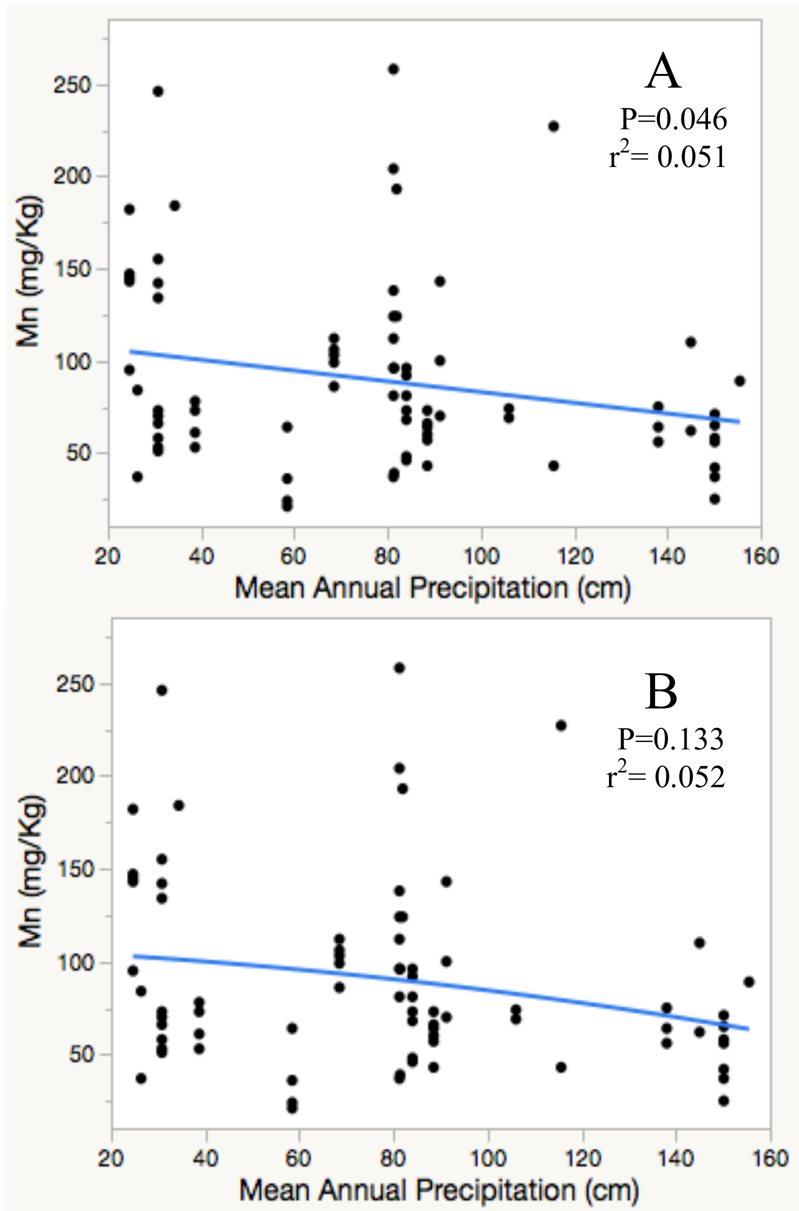

Figure 29: Analysis of manganese amounts in all soil samples over a precipitation gradient. Mean annual precipitation was the average annual rainfall received at each sampling location from 1981-2010. Linear regression (A) showed significance with high variability (r^2=0.051), and in general we see a decrease in total manganese (mg/Kg) as precipitation increases. The quadratic regression (B) was not significant, but did show a similar decrease with precipitation.

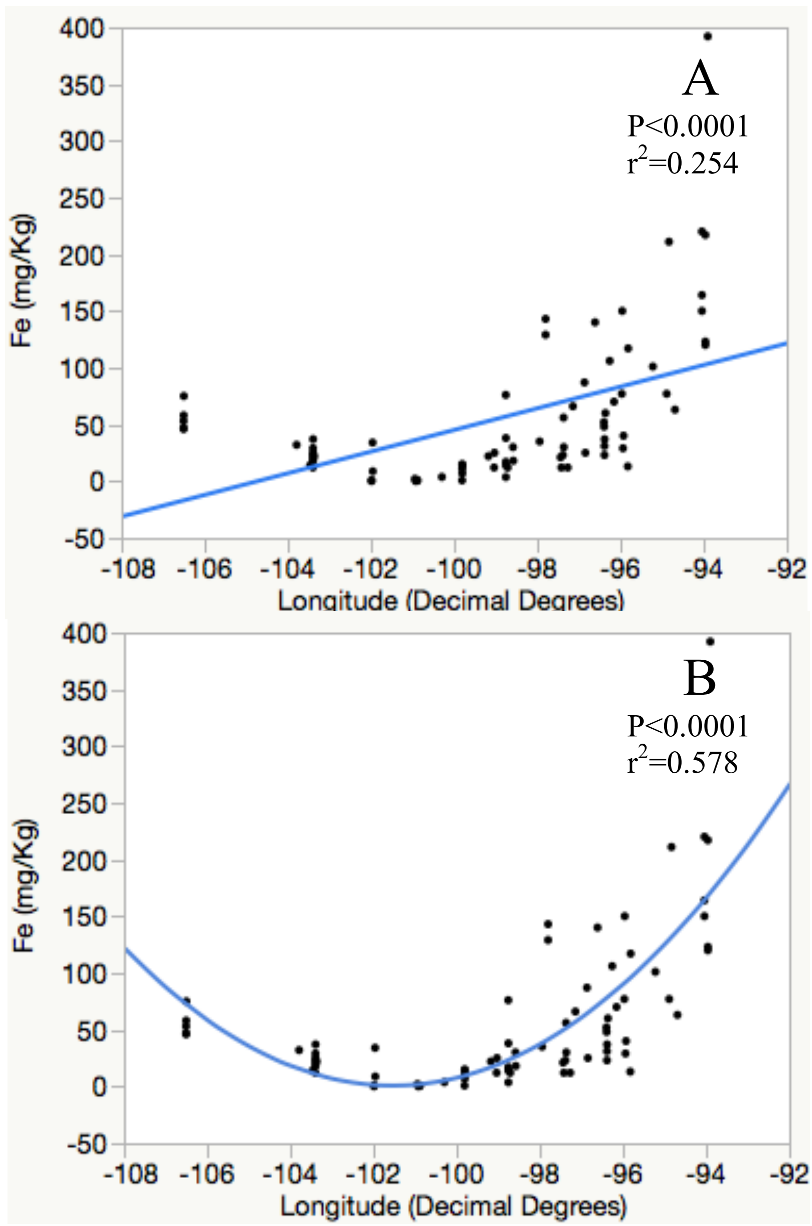

Figure 30: Analysis of iron amounts in all soil samples over the longitudinal gradient. Both the linear regression (A) and quadratic regression (B) were statistically significant. The quadratic regression had lower variability (r^2=0.567) and explained more of the data than the linear regression (r^2=0.254). Both relationships showed iron increasing (mg/Kg) as sampling moved from west to east.

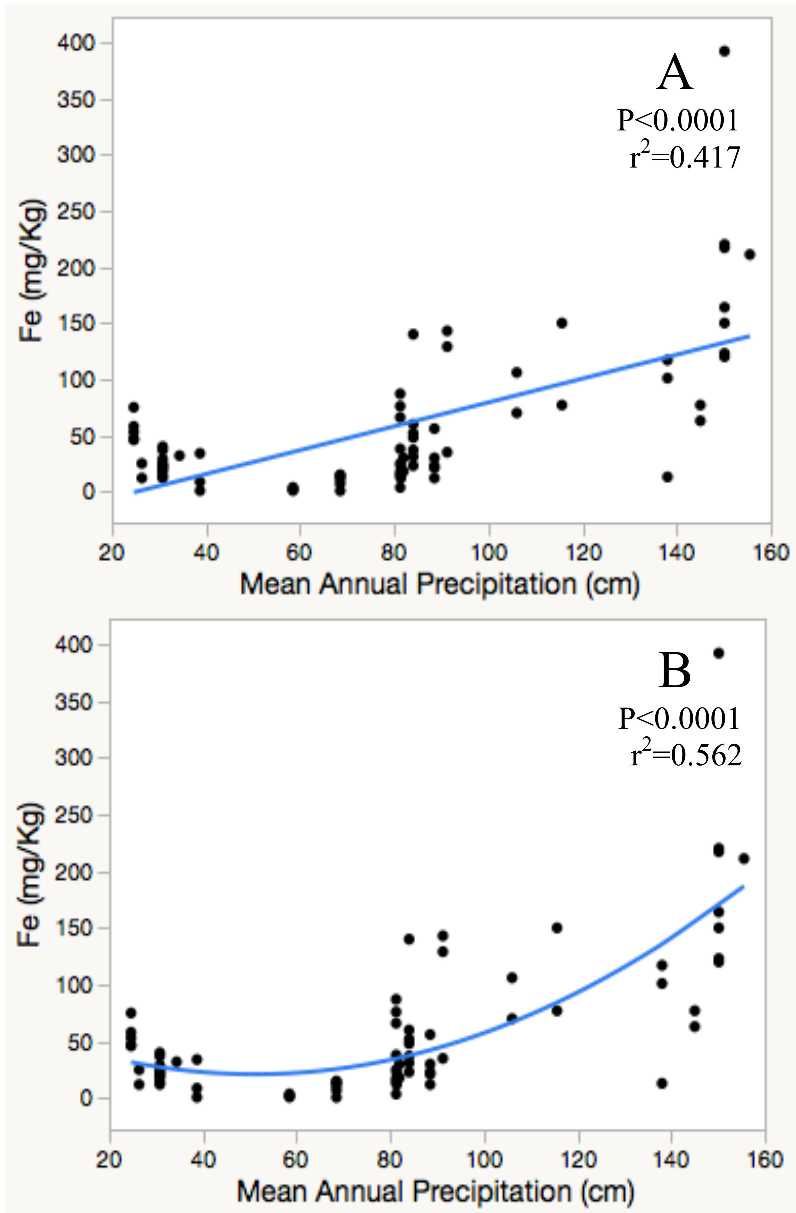

Figure 31: Analysis of iron amounts in all soil samples over a precipitation gradient. Mean annual precipitation was the average annual rainfall received at each sampling location from 1981-2010. Linear regression (A) and quadratic regression (B) both showed significance, with an increase in iron (mg/Kg) as precipitation increased.

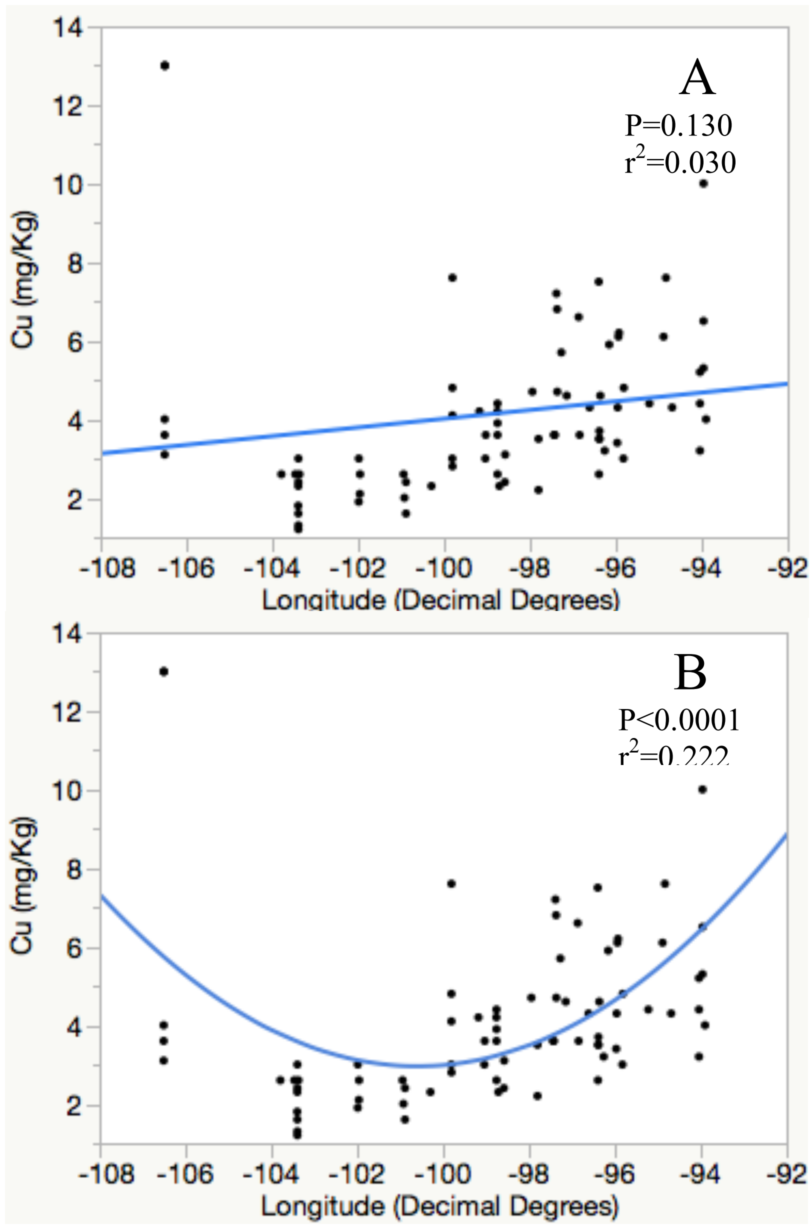

Figure 32: Analysis of copper amounts in all soil samples over the longitudinal gradient. Linear regression (A) was non-significant, but showed a general increase in copper (mg/Kg) as samples continued west to east. The quadratic regression (B) and showed highest amounts at the westernmost and easternmost locations.

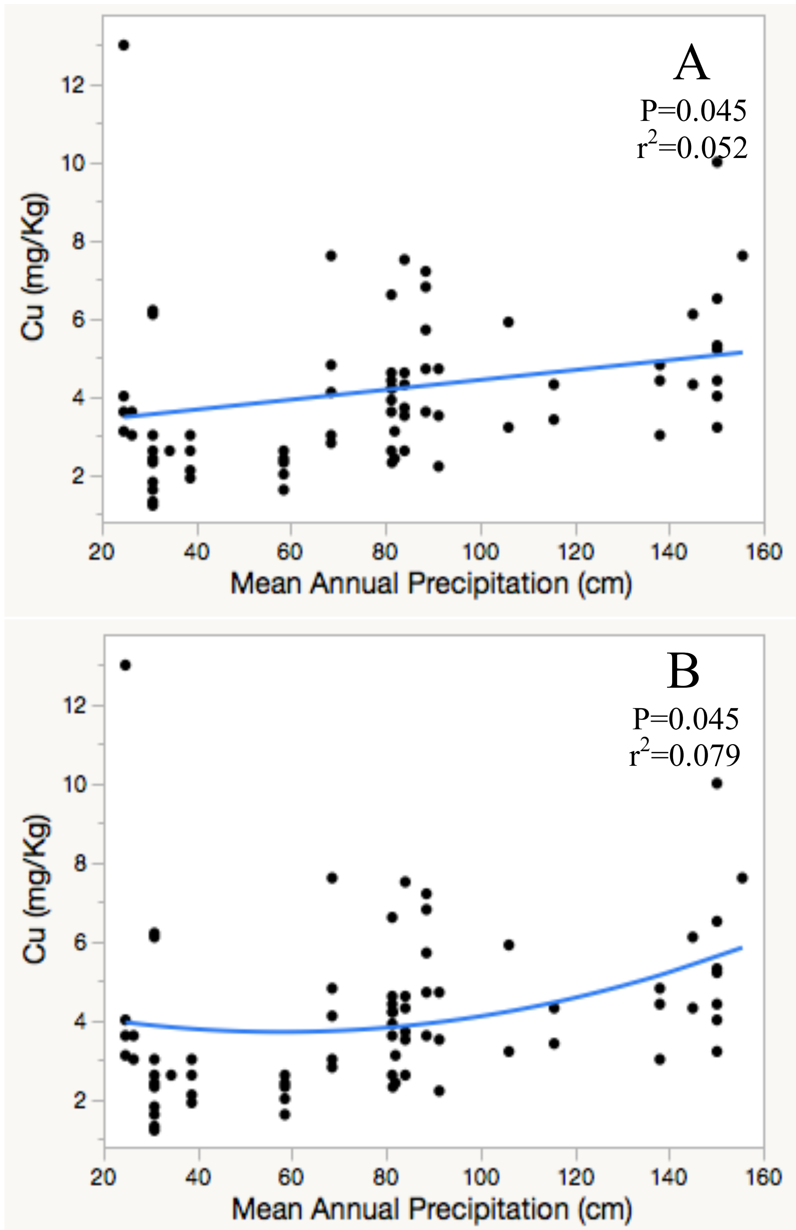

Figure 33: Analysis of copper amounts in all soil samples over a precipitation gradient. Mean annual precipitation was the average annual rainfall received at each sampling location from 1981-2010.While the linear regression (A) was not significant, a general increase in copper (mg/Kg) was seen as precipitation increased. The quadratic regression (B) was significant and showed an increase in copper (mg/Kg) as precipitation increased.

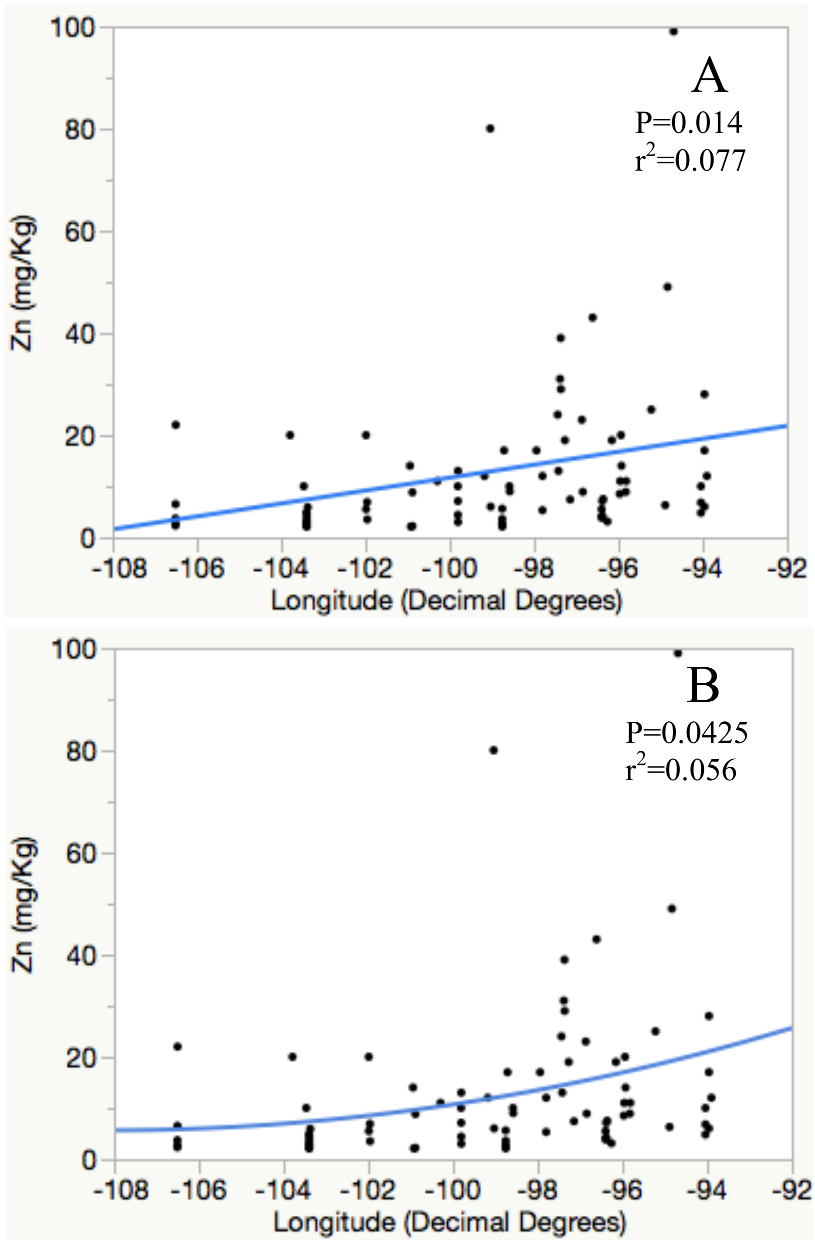

Figure 34: Analysis of zinc amounts in all soil samples over the longitudinal gradient. Linear regression (A) showed statistical significance, with zinc increasing (mg/Kg) as sampling occurred from west to east. Quadratic regression (B) increases in general, but parameter estimates made the relationship non-significant.

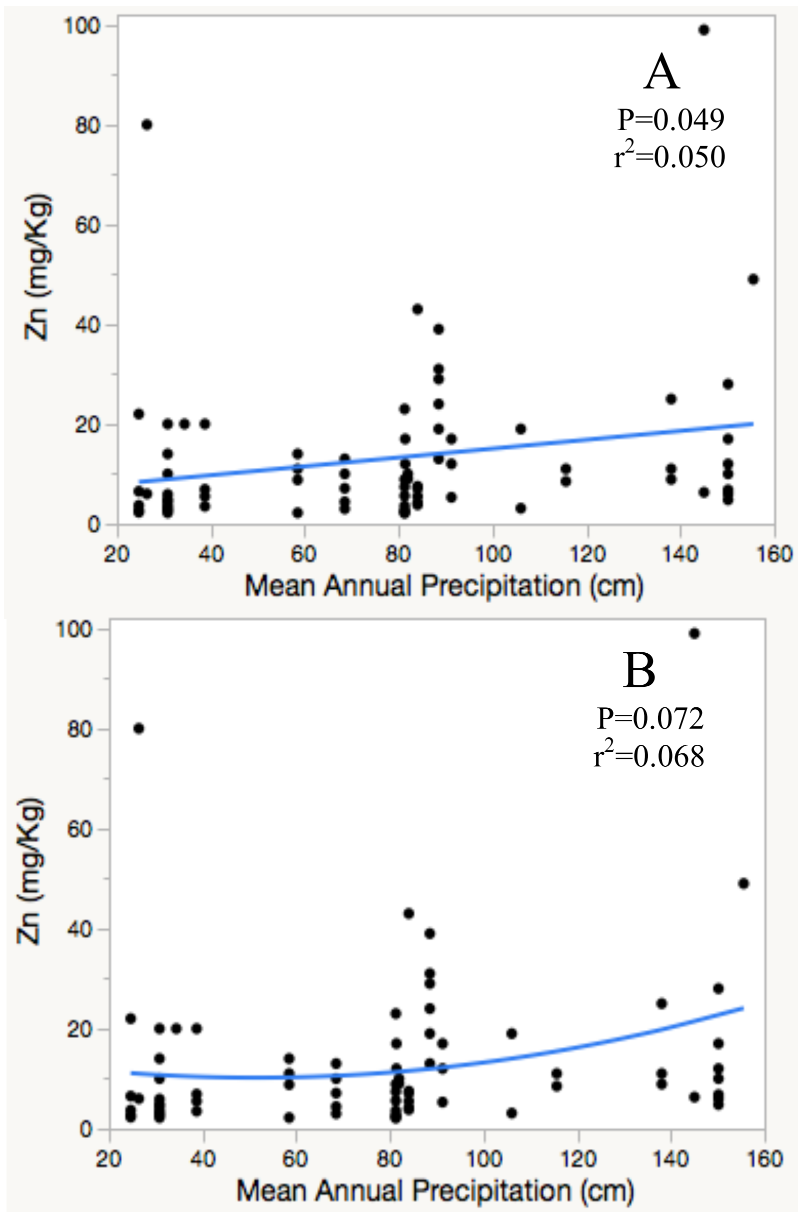

Figure 35: Analysis of zinc amounts in all soil samples over a precipitation gradient. While both the linear regression (A) and the quadratic regression (B) were non-significant, they both showed a general increase in zinc (mg/Kg) as precipitation increased.

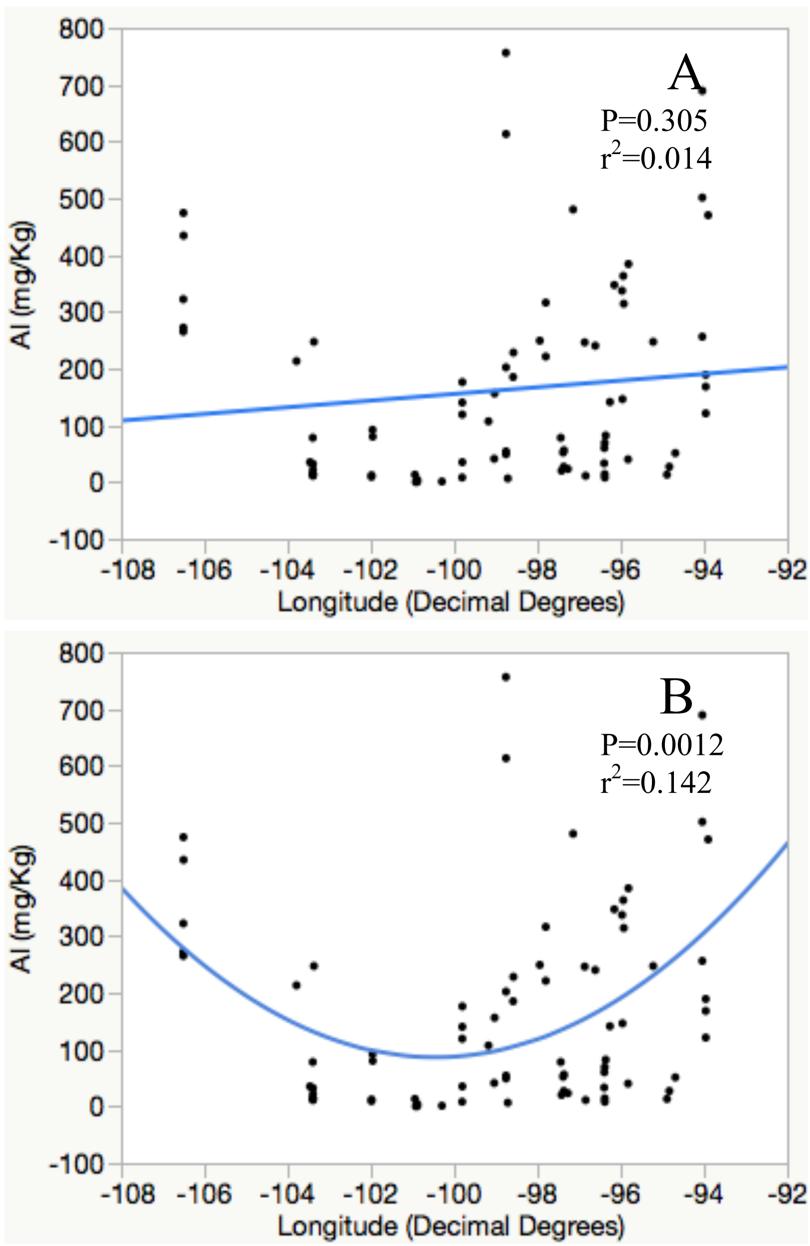

Figure 36: Analysis of aluminum amounts in all soil samples over the longitudinal gradient. The linear regression (A) was non-significant, but showed aluminum increase (mg/Kg) in general as sampling moved from west to east. The quadratic regression (B) was significant, and showed highest amounts of aluminum at the westernmost and easternmost locations.

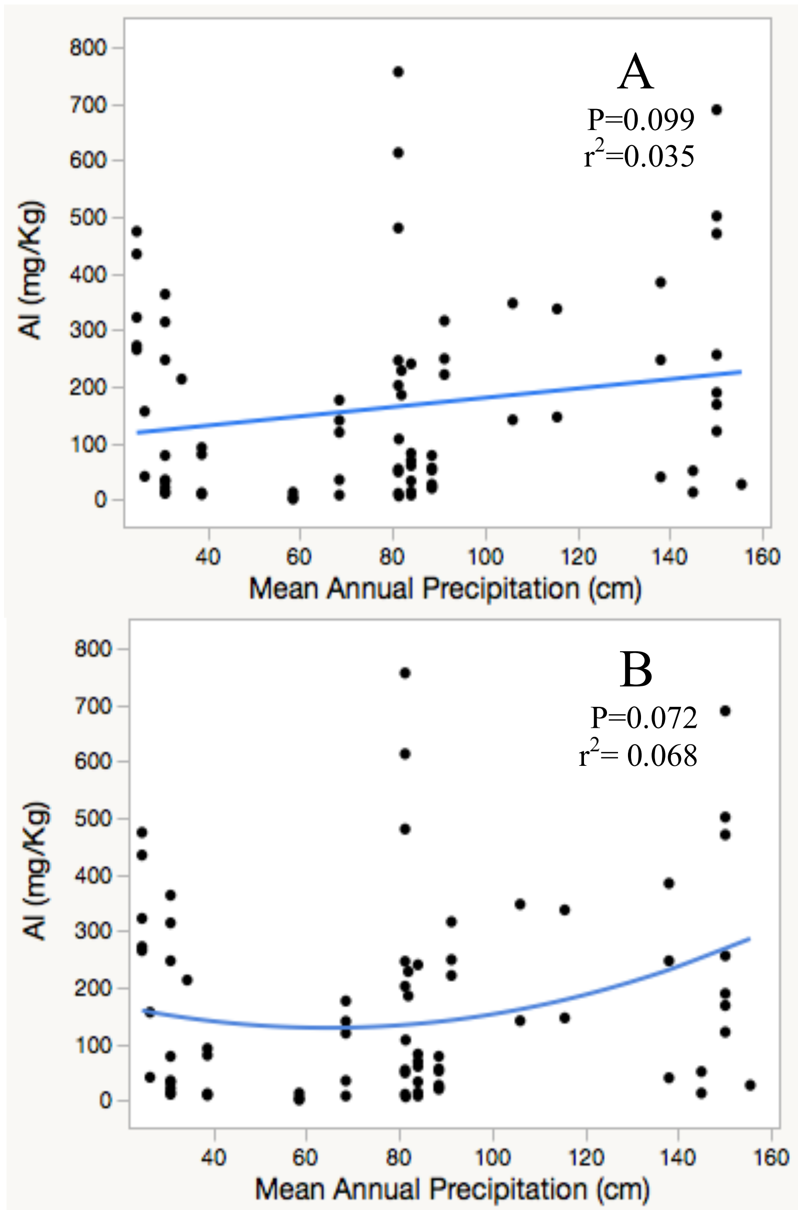

Figure 37: Analysis of aluminum amounts in all soil samples over a precipitation gradient. Mean annual precipitation was the average annual rainfall received at each sampling location from 1981-2010. While both the linear regression (A) and the quadratic regression (B) were non-significant, they both showed a general increase in aluminum (mg/Kg) as precipitation increased.

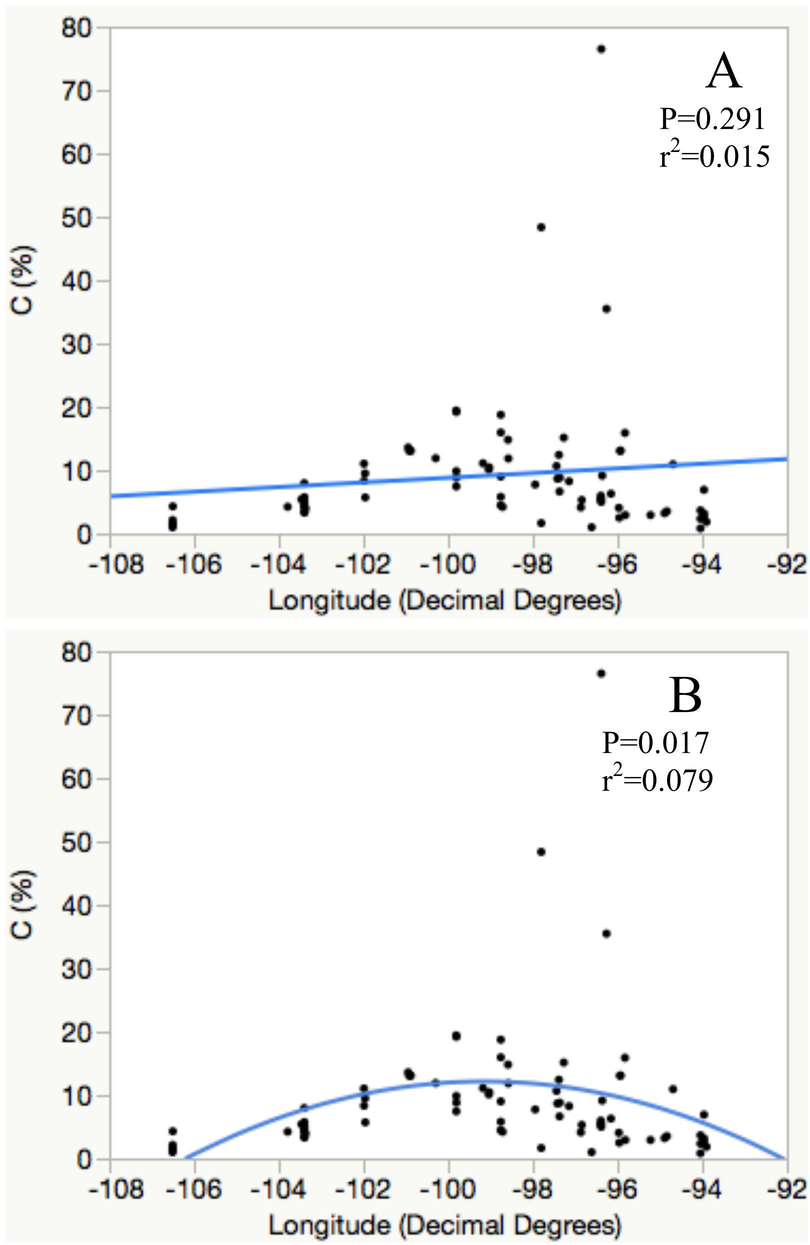

Figure 38: Analysis of carbon amounts in all soil samples over the longitudinal gradient. The linear regression (A) was non-significant, but showed a general increase in soil carbon (%) as samples were taken from west to east. Quadratic regression (B) had non-significant parameters, but resembled Albrecht's curve.

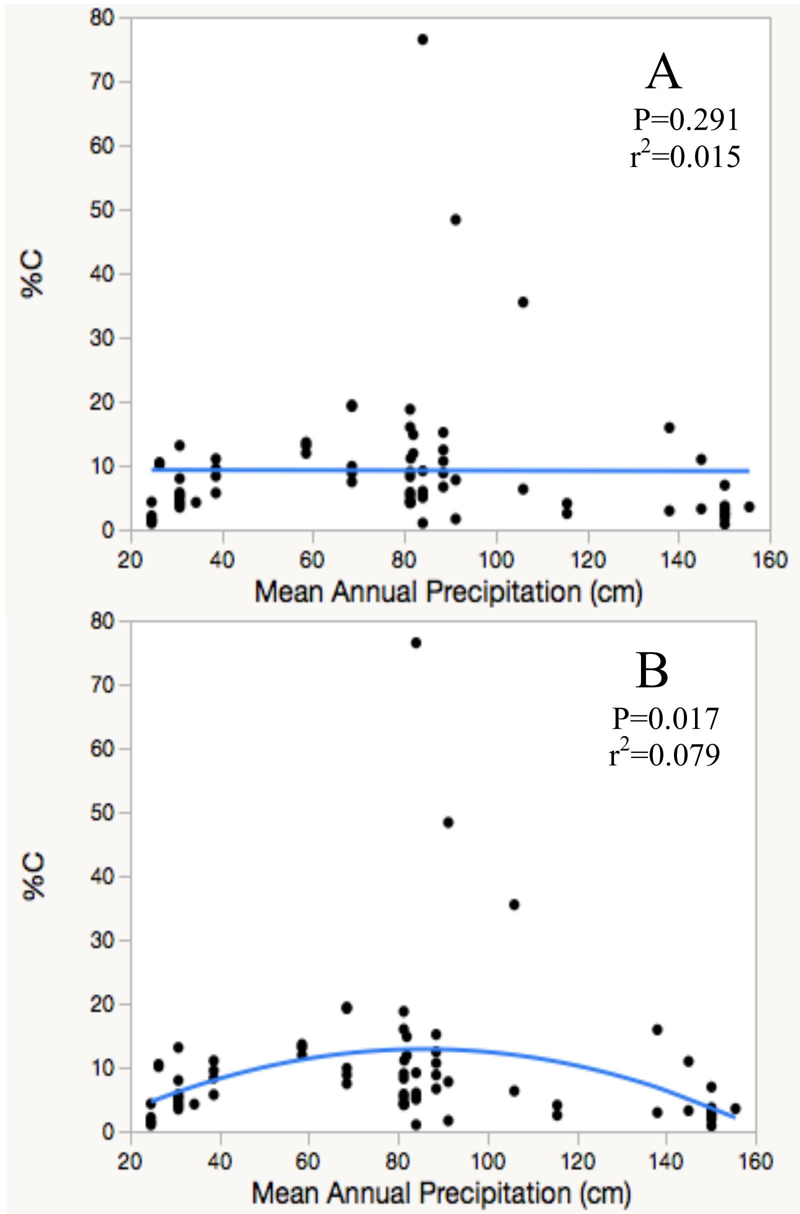

Figure 39: Analysis of carbon amounts in all soil samples over a precipitation gradient. Mean annual precipitation was the average annual rainfall received at each sampling location from 1981-2010. The linear regression (A) was not significant, and showed very little change in %C along the precipitation gradient. The quadratic regression (B) was also not significant due to non-significant parameter estimates, but showed a unimodal relationship in soil carbon (%) as precipitation increased.

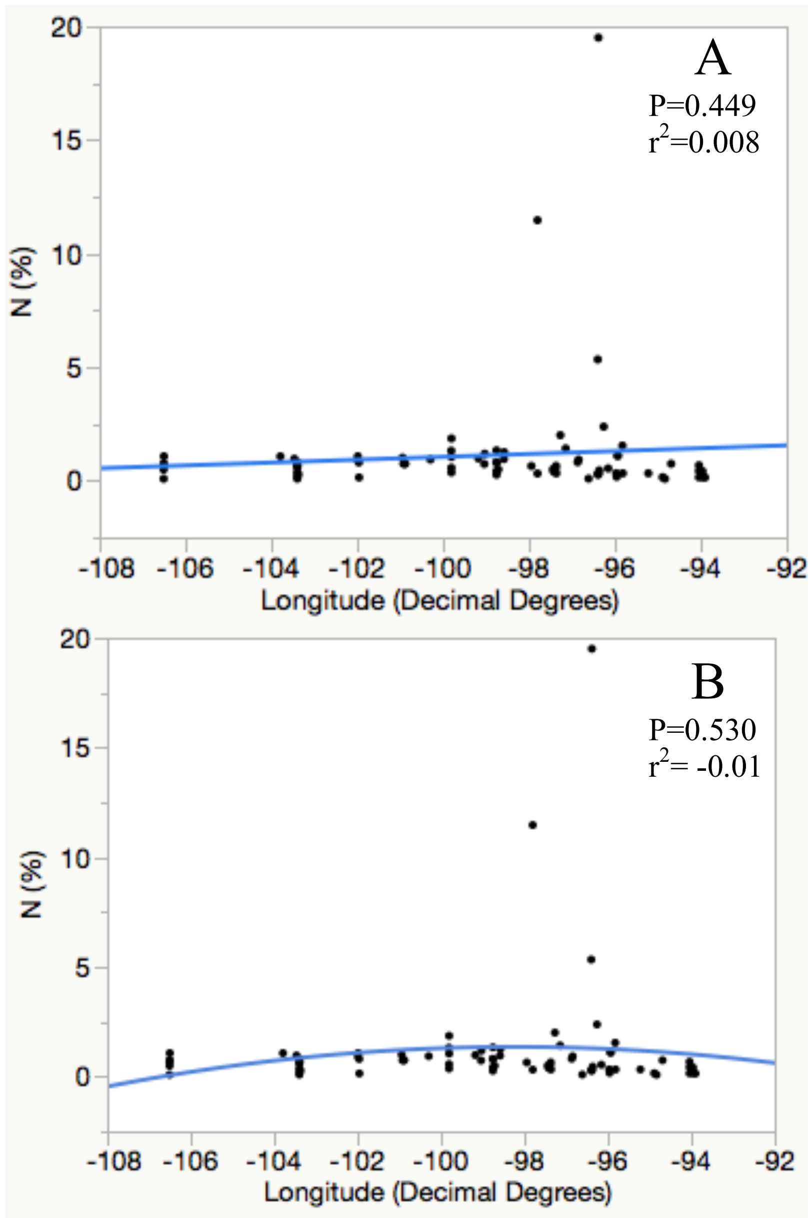

Figure 40: Analysis of nitrogen amounts in all soil samples over the longitudinal gradient. The linear regression (A) was non-significant, but showed a general increase in soil nitrogen (%) as samples were taken from west to east. The quadratic regression (B) had non-significant parameters, but slightly resembled Albrecht's curve.

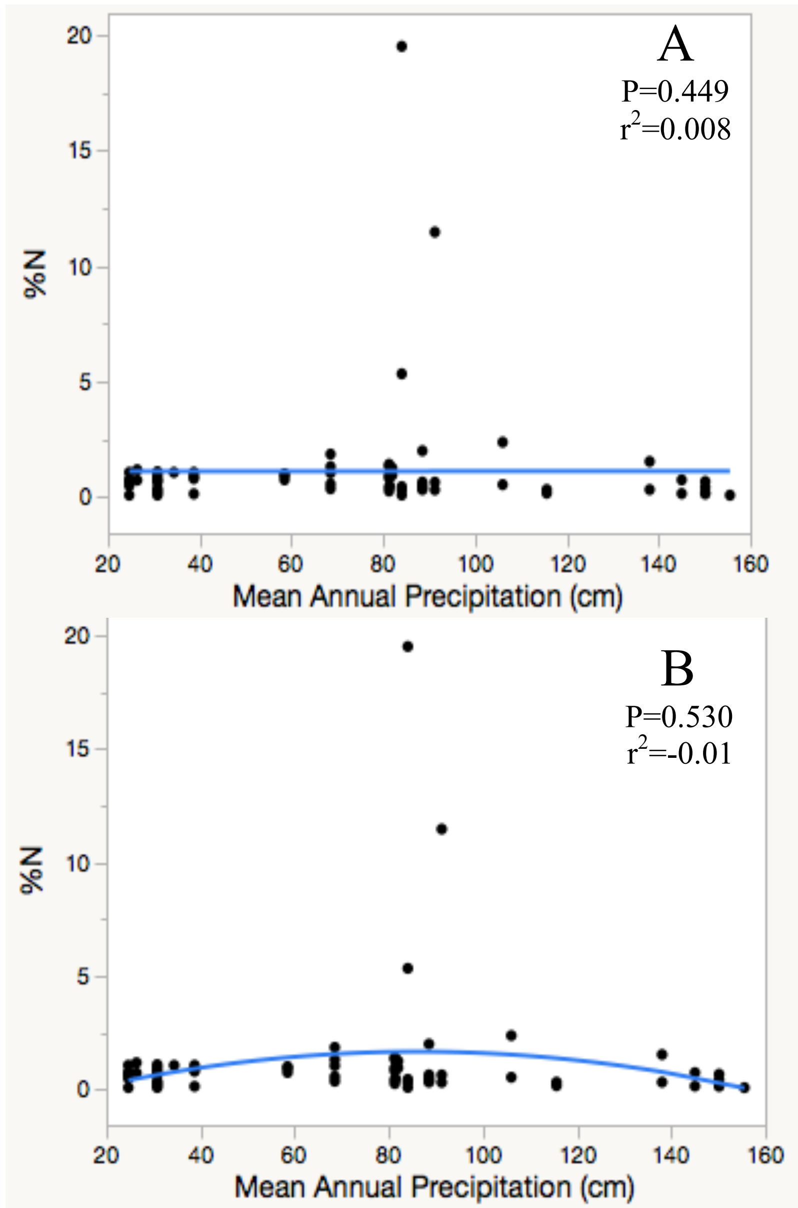

Figure 41: Analysis of nitrogen amounts in all soil samples over a precipitation gradient. Mean annual precipitation was the average annual rainfall received at each sampling location from 1981-2010.Both the linear regression (A) and the quadratic regression (B) were non-significant and showed very little change along the precipitation gradient.

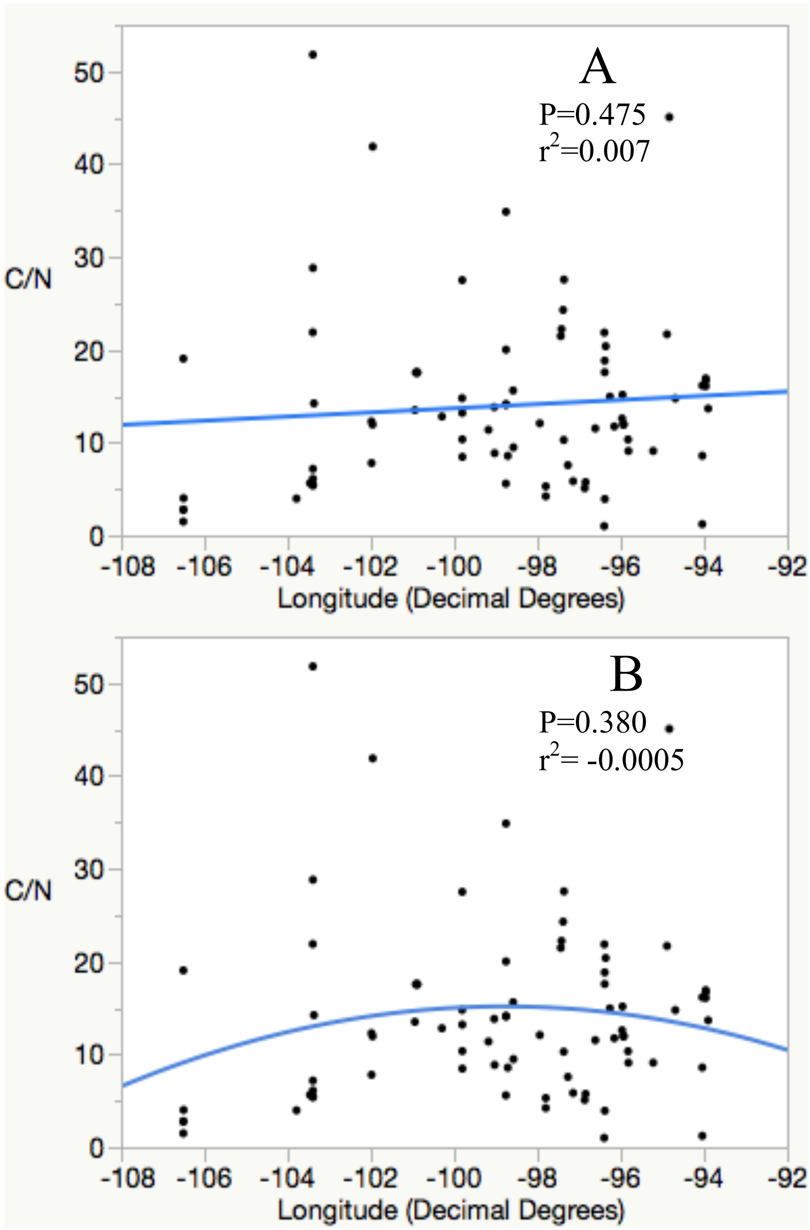

Figure 42: Analysis of the soil C/N ratio in all soil samples over the longitudinal gradient. Linear regression (A) was not significant, but increased as sampling moved west to east. The quadratic regression was not significant, but had a similar, unimodal relationship to Albrecht's Curve.

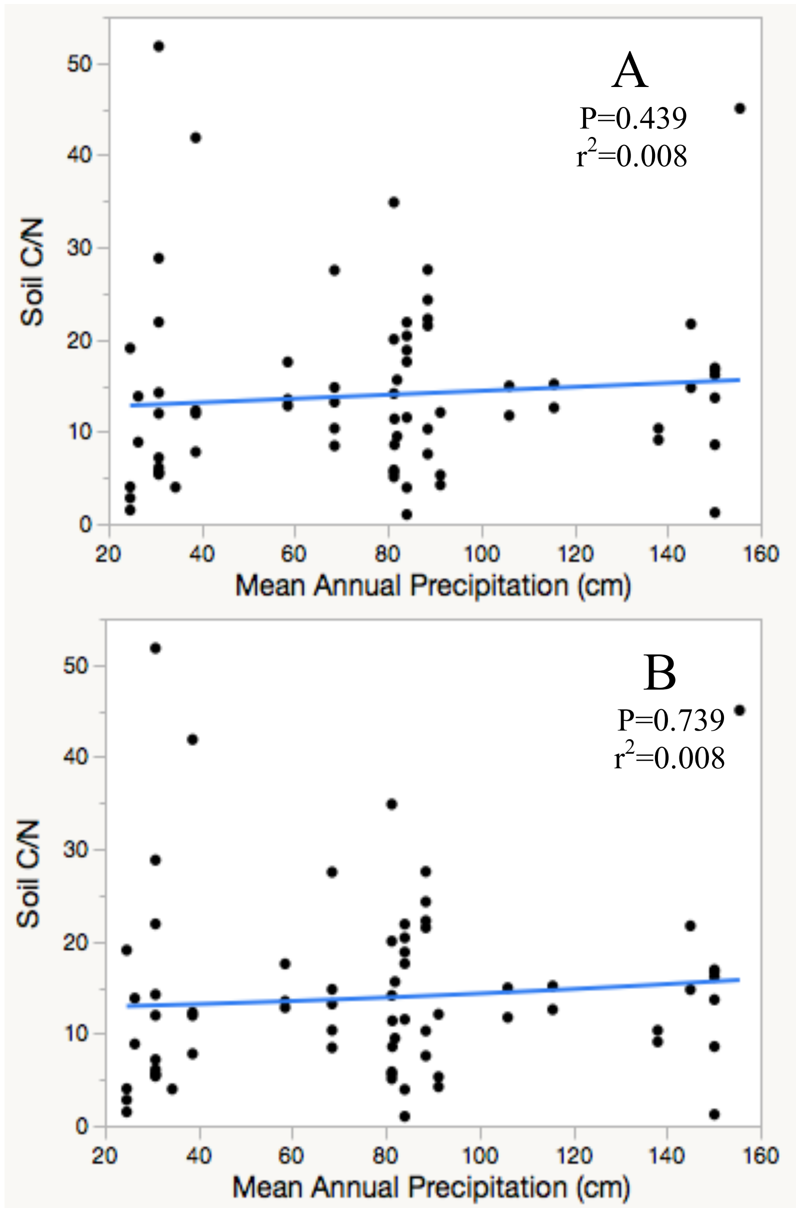

Figure 43: Analysis of the soil C/N ratio in all soil samples over a precipitation gradient. Mean annual precipitation was the average annual rainfall received at each sampling location from 1981-2010. While both the linear regression (A) and the quadratic regression (B) were non-significant, they both showed a very slight increase in soil C/N as precipitation increased.

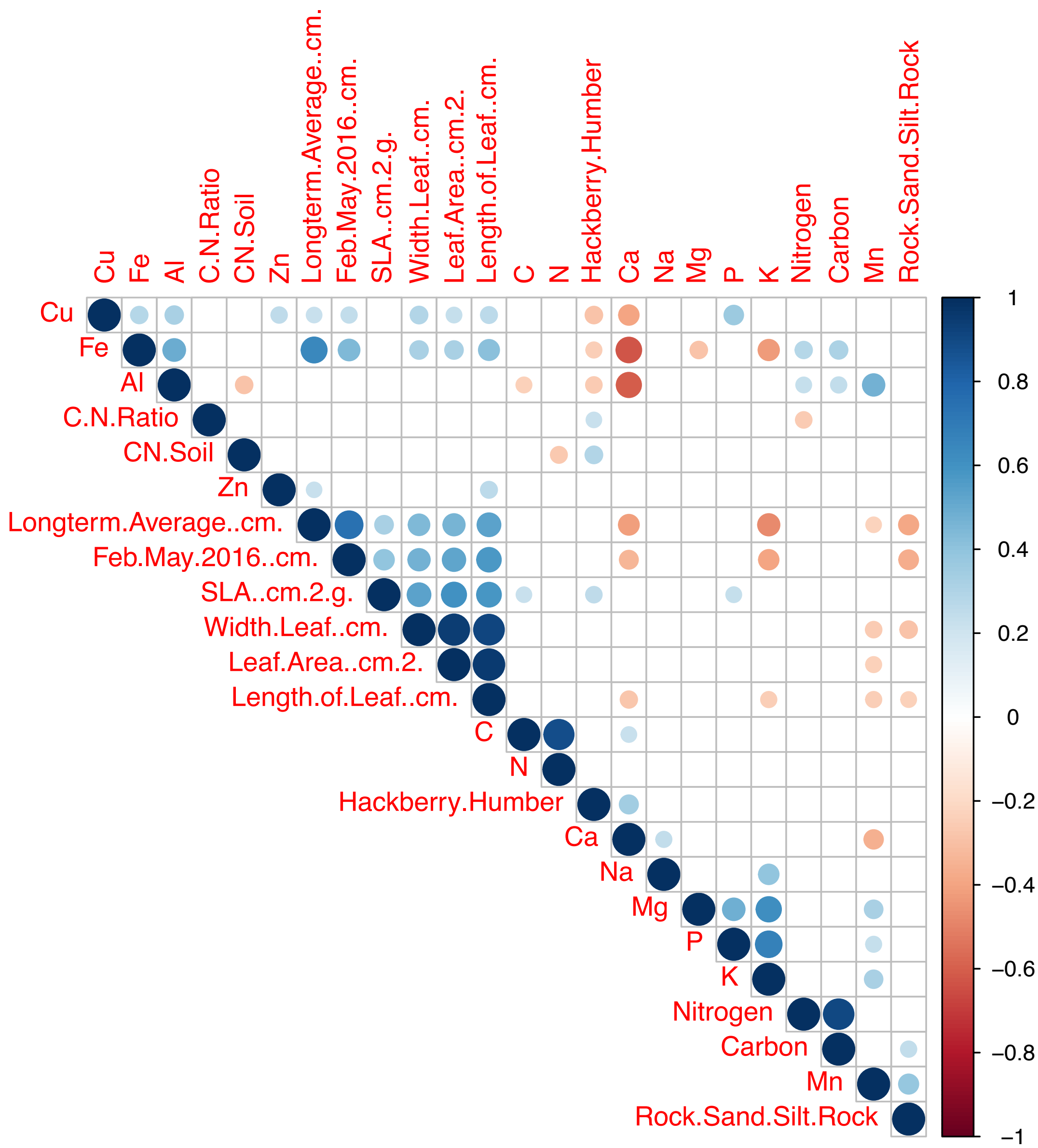

Figure 44: Correlation matrix for all precipitation measurements, soil mineral nutrients, leaf analysis. High negative correlations were found between calcium, iron and aluminum. High positive correlations where found between leaf area, leaf length, leaf width, and leaf carbon and nitrogen.

Table 6: Statistical analysis results for changes in soil mineral nutrients in response to mean annual precipitation. Mean annual precipitation was calculated as the average precipitation from 1981-2010. Calcium and iron were significant for both the linear and quadratic regressions, and potassium was significant for the linear regression.

Mineral	F Ratio	p-value	R^2	Intercept Estimate	Slope Estimate	Regression
P (mg/Kg)	$F_{1,77}$=1.485	0.227	0.019	68.299; p<0.0001	-0.159; p=0.227	Linear
	$F_{2,76}$=0.734	0.483	0.019	68.346; p<0.0001	X= -0.156; p=0.274 x^2= -0.0002; p=0.95	Quadratic
K (mg/Kg)	$F_{1,77}$=21.572	<0.0001	0.221	745.369; p<0.0001	-3.553; p<0.0001	Linear
	$F_{2,76}$=11.310	<0.0001	0.232	750.499; p<0.0001	X=-3.236; p=0.0002 x^2= -0.019; p=0.312	Quadratic
Na (mg/Kg)	$F_{1,77}$=1.383	0.243	0.018	216.69; p<0.0001	-0.214; p=0.243	Linear
	$F_{2,76}$=0.690	0.505	0.018	216.84; p<0.0001	X= -0.205; p=0.304 x^2=-0.0005; p=0.903	Quadratic
Mg (mg/Kg)	$F_{1,77}$=2.286	0.135	0.029	461.003; p<0.0001	-0.903; p=0.135	Linear
	$F_{2,76}$=0.2.316	0.106	0.058	466.93; p<0.0001	X= -0.537; p=0.403 x^2= -0.022; p=0.133	Quadratic
Ca (mg/Kg)	$F_{1,77}$= 15.449	0.0002	0.169	21161.6; p<0.0001	-89.967; p=0.0002	Linear
	$F_{2,76}$=11.251	<0.0001	0.231	21520.8; p<0.0001	X= -67.85; p=0.006 x^2= -1.320; p=0.016	Quadratic
Mn (mg/Kg)	$F_{1,77}$=4.120	0.646	0.051	112.13; p<0.0001	-0.291; p=0.046	Linear
	$F_{2,76}$=2.077	0.133	0.052	112.41; p<0.0001	X= -0.274; p=0.082 x^2= -0.001; p=0.774	Quadratic
Fe (mg/Kg)	$F_{1,77}$=54.342	<0.0001	0.417	-26.92; p=0.036	1.063; p<0.0001	Linear
	$F_{2,76}$=48.022	<0.0001	0.562	-31.042; p=0.006	X=0.809; p<0.0001 x^2= 0.015; p<0.0001	Quadratic
Cu (mg/Kg)	$F_{1,77}$=4.151	0.045	0.052	3.150; p<0.0001	0.013; p=0.045	Linear
	$F_{2,76}$=3.227	0.045	0.079	3.089; p<0.0001	X=0.009; p=0.184 x^2=0.0002; p=0.139	Quadratic
Zn (mg/Kg)	$F_{1,77}$=4.013	0.049	0.050	6.176; p=0.115	0.089; p=0.049	Linear
	$F_{2,76}$=2.727	0.072	0.068	5.829; p=0.137	X=0.068; p=0.162 x^2= 0.001; p=0.237	Quadratic
Al (mg/Kg)	$F_{1,77}$=2.782	0.099	0.035	98.154; p=0.025	6.819; p=0.099	Linear
	$F_{2,76}$=2.731	0.072	0.068	92.974; p=0.032	X=0.499; p=0.344 x^2=0.019; p=0.110	Quadratic
C (%)	$F_{1,77}$=1.129	0.291	0.15	9.328; p=0.0007	-0.001; p=0.963	Linear
	$F_{2,76}$=5.006	0.009	0.118	9.927; p=0.0002	X=0.036; p=0.256 x^2= -0.002; p=0.002	Quadratic
N (%)	$F_{1,77}$=0	0.995	4.4e-7	1.11; p=0.084	4.207; p=0.995	Linear
	$F_{2,76}$=1.849	0.1645	0.047	1.204; p=0.058	X=0.006; p=0.468 x^2= -0.0003; p=0.058	Quadratic
Soil C/N	$F_{1,77}$= 0.604	0.440	0.008	12.305; p<0.0001	0.021; p=0.440	Linear
	$F_{2,76}$=0.303	0.740	0.008	12.288; p=0.499	X=0.020; p=0.499 x^2=6.33e-5; p=0.925	Quadratic

Table 9: Statistical analysis results for changes in soil mineral nutrients in response to geographic location (longitudinal decimal degrees W). Analysis showed statistically significant relationships for important mineral nutrients (K, Ca) as well as significant relationships with the heavier, more toxic minerals (Fe, Cu, Zn, Al). Parameter estimates for Al was not significant, making the relationship not significant.

Mineral	F Ratio	p-value	R^2	Intercept Estimate	Slope Estimate	Regression
P (mg/Kg)	$F_{1,77}$=1.201	0.277	0.016	-106.4; p=0.48	-1.64; p=0.28	Linear
	$F_{2,77}$=0.725	0.488	0.019	-67.63; p=0.687	X=-1.22; p=0.69 x^2= 0.21; p=0.61	Quadratic
K (mg/Kg)	$F_{1,77}$=15.07	0.0002	0.166	-3025.5; p=0.001	-35.3; p=0.0002	Linear
	$F_{2,77}$=9.733	0.0002	0.185	-3910.4; p=0.0002	X=-44.9 p=0.0002 x^2=-4.73; p=0.054	Quadratic
Na (mg/Kg)	$F_{1,77}$=0.561	0.456	0.007	44.556; p=0.831	-1.573; p=0.456	Linear
	$F_{2,77}$=2.229	0.115	0.056	-160.81; p=0.485	X=-3.782; p=0.11 x^2=-1.098; p=0.053	Quadratic
Mg (mg/Kg)	$F_{1,77}$=0.676	0.413	0.009	-173.39; p=0.801	-5.705; p=0.413	Linear
	$F_{2,77}$=2.022	0.140	0.026	-805.65; p=0.292	X=-12.51; p=0.292 x^2=-3.38; p=0.07	Quadratic
Ca (mg/Kg)	$F_{1,77}$=6.579	0.0123	0.080	-56067.6; p=0.04	-710.225; p=0.0123	Linear
	$F_{2,77}$=26.277	0.0001	0.396	-129454.1; p<0.0001	X=-1499.6; p<0.001 x^2=-392.3; p<0.0001	Quadratic
Mn (mg/Kg)	$F_{1,77}$=2.394	0.126	0.031	-165.698; p=0.32	-2.580; p=0.126	Linear
	$F_{2,77}$=1.358	0.263	0.009	-116.043; p=0.53	X=-2.046; p=0.287 x^2=0.265; p=0.560	Quadratic
Fe (mg/Kg)	$F_{1,77}$=25.934	<0.0001	0.254	999.78; p<0.0001	9.546; p<0.0001	Linear
	$F_{2,77}$=51.406	<0.0001	0.578	1544.6; p<0.0001	X=15.41; p<0.0001 x^2=2.913; p<0.0001	Quadratic
Cu (mg/Kg)	$F_{1,77}$=2.345	0.129	0.030	15.096; p=0.0383	0.111; p=0.129	Linear
	$F_{2,77}$=11.964	0.0001	0.222	30.016; p<0.0001	X=0.271; p=0.0004 x^2=080; p<0.0001	Quadratic
Zn (mg/Kg)	$F_{1,77}$=6.329	0.014	0.077	138.34; p=0.0069	1.267; p=0.014	Linear
	$F_{2,77}$=3.295	0.0425	0.056	152.782; p=0.008	X=1.422; p=0.0.016 x^2=0.077; p=0.574	Quadratic
Al (mg/Kg)	$F_{1,77}$=1.065	0.3054	0.014	744.24; p=0.1915	5.890; p=0.3054	Linear
	$F_{2,77}$=7.379	0.0012	0.142	1727.79; p=0.0044	X=16.47; p=0.0078 x^2=5.258; p=0.0004	Quadratic
C (%)	$F_{1,77}$=1.129	0.291	0.015	45.692; p=0.187	0.369; p=0.2913	Linear
	$F_{2,77}$=4.289	0.017	0.079	-0.048; p=0.99	X=-0.123; p=0.747 x^2=-0.244; p=0.008	Quadratic
N (%)	$F_{1,77}$=0.579	0.449	0.008	7.384; p=0.373	0.063; p=0.449	Linear
	$F_{2,77}$=0.640	0.530	-0.01	3.846; p=0.680	X=0.025; p=0.791 x^2=-0.02; p=0.405	Quadratic
Soil C/N	$F_{1,77}$=0.515	0.475	0.007	36.18; p=0.247	0.225; p=0.475	Linear
	$F_{2,77}$=0.981	0.380	-0.0005	17.185; p=0.622	X=0.020; p=0.954 x^2=-0.102; p=0.233	Quadratic

LITERATURE CITED

1. Abrams, M.D., Kubiske, M.E., Mostoller, S.A. 1994. Relating wet and dry year ecophysiology to leaf structure in contrasting temperate tree species. *Ecology,* 75, 123-133.
2. Albrecht, W.A. 1957. Soil fertility and biotic geography. *Geographical Review*, 47(1), 86-105.
3. Aranibar, J.N., Otter, L., Macko, S.A., Feral, C. J. W., Epstein, H. E., Dowty, P. R., Eckardt, F., Shugart, H. H., Swap, R. J. 2004. Nitrogen cycling in the soil-plant system along a precipitation gradient in the Kalahari sands. *Global Change Biology*, 10, 359-373.
4. Austin, A.T., Vitousek, P.M. 1998. Nutrient dynamics on a precipitation gradient in Hawai'i. *Oecologia,* 113, 519-529.
5. Barbosa, E.R.M., Tomlinson, K.W., Carvalheiro, L.G., Kirkman, K., de Bie, S., Prins, H.H.T., van Langeveld, F., 2014. Short-term effect of nutrient availability and rainfall distribution on biomass production and leaf nutrient content of savanna tree species. *Plos One*, 9, 1-9.
6. Bigham, J.M., Fitzpatrick, R.W., Schulze, D.G. 2002. Iron Oxides. In: *Soil Mineralogy with Environmental Applications* (Dixon, J.B. and Schulz, D.G., eds.). Soil Science Society of America, Inc., Madison, Wisconsin, USA. Pp. 323-366.
7. Brady, N.C., R.R. Weil. 2002. The nature and properties of soils. 13th Edition. Prentice-Hall, Engelwood Cliffs, New Jersey, USA.
8. Bureau of Economic Geology. 2015. Texas GeoSciences.
9. Burns, R.M., Honkala, B.H. 1990. Silvics of North America Volume 2: Hardwoods. United States Department of Agriculture. Washington, D.C.
10. Castro-Díez, P., Villar-Salvador, P., Pérez-Rontomé, C., Maestro-Martínez, M., Montserrat-Martí, G. 1997. Leaf morphology and leaf chemical composition in three *Quercus* (Fagaceae) species along a rainfall gradient in NE Spain. *Trees*, 11, 127-134.
11. Coley, P.D., Bryant, J.P., Chapin, F.S. 1985. Resource availability and plant antiherbivore defense. *Science*, 230, 895-899.
12. Cunningham, S.A., Summerhayes, B., Westoby, M. 1999. Evolutionary divergences in leaf structure and chemistry comparing rainfall and soil nutrient gradients. *Ecology*, 69(4), 569-588.
13. Deng, Y., Dixon, J.B., 2002. Soil Organic Matter and Soil-Mineral Interactions. pg 69-108 *in* Dixon, J.B. and Schulz, D.G., editors. Soil Mineralogy with Environmental Applications, Soil Science Society of America, Inc. Madison, Wisconsin, USA.
14. Diamond, D.D., Riskind, D.H., Orzell, S.L. 1987. A framework for plant community classification and conservation in Texas. *Texas Journal of Science*, 39(3), 203-221
15. Dimmit, M. A. 1985. An ecological overview of the Sonoran Desert. *Bios*, 56(2) 66-74.

16. Divito, G.A., Sadras, V.O. 2014. How do phosphorus, potassium and sulfur affect plant growth and biological nitrogen fixation in crop and pasture legumes? A meta-analysis. *Field Crops Research*, 156, 161-171.
17. Dixon, J.B. and Schulz, D.G., editors. 2002. Soil Mineralogy with Environmental Applications, Soil Science Society of America, Inc. Madison, Wisconsin, USA.
18. Dixon, J.B., White G.N. 2002. Manganese Oxides. In: *Soil Mineralogy with Environmental Applications* (Dixon, J.B. and Schulz, D.G., eds.). Soil Science Society of America, Inc., Madison, Wisconsin, USA. Pp. 367-388.
19. Doner, H.E., Grossl, P.R. 2002. Carbonates and Evaporites. In: *Soil Mineralogy with Environmental Applications* (Dixon, J.B. and Schulz, D.G., eds.). Soil Science Society of America, Inc., Madison, Wisconsin, USA. Pp. 199-228.
20. Eskelinen, A., Harrison, S.P. 2015. Resource colimitation governs plant community responses to altered precipitation. *Proceedings of the National Academy of Sciences of the United States,* 112(42).
21. Evert, R. F, Eichhorn, S. E. 2013. Biology of Plants. 8th Edition. W. H. Freeman and Company Publishers, New York, NY, USA.
22. Ferris, F.G. 1989. Metallic ion interactions with the outer membrane of Gram-negative bacteria. In: Metal ions and bacteria (Beveridge, T.J. and Doyle, R.J., eds.). John Wiley, New York, NY. Pp. 295-323.
23. Frank, K. Beegle, D. Denning, J. 1998. Phosphorus. In: Recommended Chemical Soil Test Procedures for the North Central Region (Nathan, M. and Gelderman, R., eds.). Missouri Agricultural Experiment Station, USA. Pp 6.1-6.9.
24. Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Vörösmarty, C.J. 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry*, 70, 153-226.
25. Garten, C.T., M.A. Huston, and C. Thoms. 1994. Topographic variation of soil nitrogen dynamics at Walker Branch Watershed, Tennessee. *Forest Science* 40:497-513.
26. Gillman, L.N., Wright, S.D., Cusens, J., McBride, P.D., Malhi, Y., Whittaker, R.J. 2015. Latitude, productivity and species richness. *Global Ecology and Biogeography*, 24, 107-117.
27. Girard, R.M. 1964. *Texas Rocks and Minerals: An Amateur's Guide*. The University of Texas, Austin, Texas, USA.
28. Girardin, C.A.J., Malhi, Y., Aragão, L.E.O.C., Mamani, M., Huaraca Huasco, W., Durand, L., Feeley, K.J., Rapp, J., Silva-Espejo, J.E., Silman, M., Salinas, N., Whittaker, R.J. 2010. Net primary productivity allocation and cycling of carbon along a tropical forest elevational transect in the Peruvian Andes. *Global Change Biology,* 16, 3176-3192.

29. Grace, J.B., Anderson, T.M., Seabloom, E.W., Borer, E.T., Adler, P.B., Harpole, W.S., Hautier, Y., Hillebrand, H., Lind, E.M., Pärtel, M., Bakker, J.D., Buckley, Y.M., Crawley, M.J., Damschen, E.I., Davies, K.F., Fay, P.A., Firn, J., Gruner, D.S., Hector, A., Knops, J.M.H., MacDougall, A.S., Melbourne, B.A., Morgan, J.W., Orrock, J.L., Prober, S.M., Smith, M.D. 2016. Integrative modelling reveals mechanisms linking productivity and plant species richness. *Nature,* 529, 390-399.
30. Harris, W.G. 2002. Phosphate Minerals. In: *Soil Mineralogy with Environmental Applications* (Dixon, J.B. and Schulz, D.G., eds.). Soil Science Society of America, Inc., Madison, Wisconsin, USA. Pp. 637-665.
31. Hoffmann, W.A., Franco, A.C., Moreira, M. Z., Haridasan, H. 2005. Specific leaf area explains differences in leaf traits between congeneric savanna and forest trees. *Functional Ecology*, 19(6), 932-940.
32. Huang, P.M., Wang, M.K., Kämpf, N., Schulze, D.G. 2002. Aluminum Hydroxides. In: *Soil Mineralogy with Environmental Applications* (Dixon, J.B. and Schulz, D.G., eds.). Soil Science Society of America, Inc., Madison, Wisconsin, USA. Pp. 261-289.
33. Huston, M.A. 2012. Precipitation, soils, NPP, and biodiversity: resurrection of Albrecht's curve. *Ecological Monographs*, 82(3), 277-296.
34. Huston, M.A., Wolverton, S. 2009. The global distribution of net primary production: resolving the paradox. *Ecological Monographs*, 79, 343-377.
35. Jenny, H., Leonard, C.D. 1934. Functional relationships between soil properties and rainfall, *Soil Science,* 38, 363-381.
36. Marron, N. Dreyer, E. Boudouresque, E. Delay, D. Petit, J.M. Delmotte, F.M, Brignolas, F. 2003. Impact of successive drought and re-watering cycles on growth and specific leaf area of two *Populus x canadensis* (Moench) clones, "Dorskamp" and "Luisa Avanzo". *Tree Physiology*, 18: 1225-35.
37. McDonald, P.G., Fonesca, C.R., Overton, J.McC., Westoby, M. 2003. Leaf-size divergence along rainfall and soil-nutrient gradients: is the method of size reduction common along clades? *Functional Ecology*, 17, 50-57.
38. NOAA. National Climate Data Center. NESDIS NOAA, U.S. Department of Commerce. 2017.
39. Pardo, J.M., Quintero, F.J. 2002. Plants and sodium ions: keeping company with the enemy. *Genome Biol,* 3(6): 1-4
40. PRISM Climate Group, Oregon State University. http://prism.oregonstate.edu, created 25 June 2017
41. Reich, P.B., Walters, M.B., Ellsworth, D.S. 1997. From tropics to tundra: Global convergence in plant functioning. *Proceedings of the National Academy of Sciences of the United States*, 94, 13730-13734.
42. Sanchez, P.A. 1976. Properties and management of soils in the tropics. John Wiley and Sons, New York, New York, USA.

43. Schulze, D.G. 2002. An introduction to soil mineralogy. In: *Soil Mineralogy with Environmental Applications* (Dixon, J.B. and Schulz, D.G., eds.). Soil Science Society of America, Inc., Madison, Wisconsin, USA. Pp. 1-35.
44. Schuur, E.A.G., Matson, P.A. 2001. Net primary productivity and nutrient cycling across a mesic to wet precipitation gradient in Hawaiian montane forest. *Oecologia*, 128, 431-442.
45. Shao, H.B., Chu, L.Y., Jaleel, C.A., Zhao, C.X. 2008. Water-deficient stress-induced anatomical changes in higher plants. *Comptes Rendus Biologies*, 331, 215-225.
46. Silver, W., Lugo A.E., Keller, M. 1999. Soil oxygen availability and biogeochemistry along rainfall and topographical gradients in upland wet tropical forest soils. *Biogeochemistry* 44: 301-328.
47. Sterrett, D.B. 1923. *Mica Deposits of the United States*. Government Printing Office, Washington, USA. 1-342.
48. Stiling, P., Cornelissen, T. 2007. How does elevated carbon dioxide (CO_2) affect plant-herbivore interactions? A field experiment and meta-analysis of CO_2-mediated changes on plant chemistry and herbivore performance. *Global Change Biology*, 13, 1823-1842.
49. Thompson, M.L., Ukrainczyk, L. 2002 Micas. In: *Soil Mineralogy with Environmental Applications* (Dixon, J.B. and Schulz, D.G., eds.). Soil Science Society of America, Inc., Madison, Wisconsin, USA. Pp. 431-466.
50. Veteli, T.O., Kuokkanen, K., Julkunen-Tiitto, R., Roininen, H. Tahvanainen, J. 2002. Effects of elevated CO_2 and temperature on plant growth and herbivore defensive chemistry. *Global Change Biology*, 8, 1240-1252.
51. Vinton, M.A., Burke, I.C. 1997. Contingent effects of plant species on soils along a regional moisture gradient in the Great Plains. *Oecologia,* 110, 393-402.
52. Viola, R. E., Morrison, J.F., Cleland, W.W. 1980. Interactions of metal (III)-adenosine 5'-triphosphate complexes with yeast hexokinase. *Biochemistry*, 19, 3131-3137.
53. Wang, L. Zhao, G., Li, M. Zhang, M. Zhang, L. Zhang, X., An, L., Xu, S. 2015. C:N:P stoichiometry and leaf traits of halophytes in an arid saline environment, Northwest China. Plos One, 10(3).
54. White T.C. 1993. *The Inadequate Environment: Nitrogen and the Abundance of Animals.* Springer-Verlag, New York, New York, USA.
55. Wright, I.J., Reich, P.B., Westoby, M. 2001. Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. *Functional Ecology*, 15, 423-434.
56. Xu, F., Guo, W., Xu, W., Wei, Y., Wang, R. 2009. Leaf morphology correlates with water and light availability: What consequences for simple and compound leaves? *Progress in Natural Sciences*, 19:12, 1789-98.