# ABOVE- AND BELOWGROUND EFFECTS OF NUTRIENT APPLICATIONS AND MOWING TREATMENTS ON RESTORED NORTH DAKOTA GRASSLANDS

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**Title**

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

**DOCTOR OF PHILOSOPHY**

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## ABSTRACT

Although more than half of the annual production within North American grasslands occurs beneath the soil surface, this portion is oftentimes overlooked in ecological studies. In this study, we investigated the above- and belowground responses in restored grassland plots that were subjected to different nutrient and mowing treatments. This study was conducted at two locations in North Dakota: the Dickinson Research Extension Center (DREC); and the Albert K. Ekre Grassland Preserve (Ekre). We evaluated the above- and belowground responses using visual surveys, biomass harvesting, and minirhizotrons.

At the DREC site, we found the belowground variables to be relatively unresponsive to the nutrient and mowing treatments – there were no significant differences in root length, surface area, number of tips, or branches. The aboveground variables did exhibit responses to repeated nitrogen applications (200 kg/ha·yr). Nitrogen applications were found to increase aboveground biomass over the control (660 g/m$^2$ vs. 265 g/m$^2$, respectively) and decrease the species richness in comparison to the control (3.25 vs. 7.29, respectively). There was a decrease in the ratio of root volume to average aboveground biomass in the DREC nitrogen plots that resulted from the increase in aboveground biomass despite no apparent changes in the root systems, indicating that the root systems in the nitrogen plots were able to support significantly more aboveground biomass than similarly sized root systems in plots that did not receive nitrogen.

At the Ekre site, there were no significant differences in the belowground variables attributed to mowing. However, aboveground biomass was higher in the mowed sub-plots ($\overline{x}_M$=530 g/m$^2$) than in the control sub-plots ($\overline{x}_C$=485 g/m$^2$). Species richness was lower in the nitrogen ($\overline{x}_N$=4.46) than in the phosphorus plots ($\overline{x}_P$=5.66). Species richness was also lower in the plots that received the high application rates (200 kg/ha·yr nitrogen or 40 kg/ha·yr

phosphorus) ($\overline{x}_H$=4.30) than the low application rates (20 kg/ha·yr nitrogen or 4 kg/ha·yr phosphorus) ($\overline{x}_L$=5.90). Root growth was highest in the low phosphorus and the high nitrogen plots. There were different responses in the root variables throughout the growing season as a function of nutrient type and application rate.

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# CHAPTER 1. GENERAL INTRODUCTION

## Introduction

Some of the earliest and best known research on root systems was conducted in the Great Plains Region of the United States. In 1919, John Weaver published *The Ecological Relations of Roots*, an extensive text that provided detailed descriptions of the root systems and ecological interactions of 140 species and their corresponding communities. In the introduction to his volume, Weaver acknowledged the progress that had been made towards an ecological understanding of root systems but indicated that this body of knowledge is far from complete. Nearly a century later, contemporary researchers make similar statements about the dearth of ecological studies focused on belowground patterns and processes (Jackson et al. 1996; Sun et al. 1997; Milchunas et al. 2005; Frank et al. 2010). This study was intended to add to the growing body of research on the ecological relations of roots.

Root systems are extremely important to terrestrial plants; they perform many essential functions including anchorage, uptake of resources, storage of materials, and the sensing and modification of their surrounding environment (Gregory 2006; Jackson et al. 2007). Although the significance of root systems has long been recognized (Weaver 1919; Weaver and Clements 1938), relatively few ecological studies have focused on belowground biomass production or root dynamics due in part to the difficulty of observing roots *in situ*. Complicating matters further, research on root systems has yielded varied and often conflicting results for root production, responses to stress, longevity, and turnover (Eissenstat and Yanai 2002; Reich 2002).

Traditionally, studies of root systems employed various techniques such as soil coring, excavation, and trenching to observe roots growing in field settings (Box et al. 1989; Johnson et al. 2001; Polomski and Kuhn 2002; Hendricks et al. 2006). These techniques have yielded

invaluable information, but were somewhat limited in that they were rather destructive and did not allow for repeated observations of the same root system (Box et al. 1989; Johnson et al. 2001). In recent years, research on root systems has advanced greatly through the use of minirhizotrons and highly specialized imaging technology (Johnson et al. 2001).

When properly installed, minirhizotrons are able to provide researchers with a valuable method for making multiple observations of root systems *in situ* with relatively little disturbance. To date, minirhizotrons have been used to address many different research objectives including measurements and/or estimates of belowground production, nutrient cycling, turnover rates, root longevity, carbon allocation, and root responses to inputs or stimuli (Box et al. 1989; Fitter et al. 1998; Johnson et al. 2001; Polomski and Kuhn 2002; Hendricks et al. 2006; Fischer et al. 2007; Volder et al. 2007). This study utilized minirhizotrons to determine the effects of nutrient additions and mowing treatments on the root characteristics of restored grasslands in the Northern Great Plains of North Dakota.

## Roots: Structure and Function

### Root Production and Distribution

Belowground production has been determined to exceed aboveground production in several ecosystems (Fitter 1987; Eissenstat and Yanai 1997; Coleman et al. 2004). For instance, in grasslands, estimates of belowground production range from 60-90% of annual net primary production (Stanton 1988; Hui and Jackson 2006). Hui and Jackson (2006) determined the mean fraction of belowground net primary production to be 70-74% for humid temperate grasslands. Estimates of root biomass have been determined for several biomes and range from 0.15 $kg/m^2$ for cropland biomes to 4.90 $kg/m^2$ for tropical evergreen forest biomes (Jackson et al. 1996;

Gregory 2006). For temperate grasslands, estimates of root biomass range from 1.40 kg/$m^2$ to 1.51 kg/$m^2$ (Jackson et al. 1996; Jackson et al. 1997).

There is widespread interest in measuring the longevity and turnover of individual roots and entire root systems. Unfortunately, estimates and/or measurements of root longevity and turnover have yielded results that are often contradictory. For example, Dahlman and Kucera (1965) estimated an annual turnover rate of 25% of the entire root system while Gill and Jackson (2000) estimated annual turnover to be nearer to 50%. Tjoelker et al. (2005) reported estimates of average root longevity ranging from 32 to 1,409 days, with pronounced differences among species and species groups. Tjoelker et al. (2005) estimated mean root longevity to be 504 days for selected cool-season grasses, 791 days for selected warm-season grasses, and 182 days for selected forb species. Estimates of root longevity may be influenced by the techniques employed, e.g., isotope residence time studies versus minirhizotron sampling (Strand et al. 2008). Estimates of root growth and mortality may also vary widely for a single sampling technique. For example, Stewart and Frank (2008) found a wide discrepancy in estimates of root growth and mortality related to the length of the interval between samples.

Over the past century, many researchers have examined root distribution by depth in various biomes. In a review of approximately 80 references, Jackson et al. (1996) found 83% of roots to occur within 30 cm of the soil surface in temperate grasslands. In a more recent study of root biomass distribution within a single study area, Mueller et al. (2013) found 51%-100% of root biomass occurring within the top 30 cm of the soil profile in restored grassland plots located near Bethel, MN. Although the exact percentage may not be agreed upon, there is general consensus that the vast majority of root production occurs nearest the soil surface in grassland

systems (Dahlman and Kucera 1965; Jackson et al. 1996; Sun et al. 1997; Gregory 2006; Mueller et al. 2013).

Many factors are known to influence the distribution of roots in a soil profile including: the genetic characteristics of an individual plant, environmental conditions, soil conditions, and interactions with other species (Weaver and Clements 1938; Sun et al. 1997; Cahill 1999; Gregory 2006). Several researchers have examined root distribution patterns in relation to these factors. One pattern that has been detailed is a localized proliferation of roots in an area of higher nutrient content with a corresponding decrease in root growth in a separate region of the root system (Robinson 1996; Johnson and Biondini 2001; Waisel and Eshel 2002; Berendse et al. 2007; Jackson et al. 2007). A similar pattern has been observed for water availability in certain instances (Robinson 1996; Jackson et al. 2007).

Results from studies evaluating the responses of roots to various aboveground factors and/or experimental treatments have not always led to consistent conclusions. For instance, several studies have supported contrasting conclusions about the effects of grazing/mowing on belowground plant production. Pucheta et al. (2004) determined belowground production to increase dramatically following grazing, while Gao et al. (2008) documented a decrease in belowground production following grazing. Balogianni et al. (2014) found no significant effect of mowing on the belowground portion of their grassland plots.

Biological interactions, such as competition, also play important roles in the spatial distribution of roots (Schenk 2006; Frank et al. 2010). A common theme in Weaver's extensive studies of the morphology and dynamics of root systems throughout the Great Plains of North America was the grouping (or partitioning) of roots into "absorbing layers" (Weaver 1919, 1958; Weaver and Clements 1938). Weaver consistently observed that the more deeply rooted species

had relatively few absorbing roots near the soil surface. Weaver hypothesized that this separation may have reduced competition among species growing in close proximity to each other. In addition, Weaver (1958) observed that forb species (depths of 20-38 cm at maturity) were generally more deeply-rooted than grass species (depths generally less than 15 cm) in Great Plains plant communities. However, Weaver (1958) noted several forb species with roots in the upper layers of the soil, in direct competition with the grasses. Decades later, ecologists are still working to discern the effects of belowground competition on root systems and link these potential impacts to the structure and function of plant communities (Schenk 2006; Frank et al. 2010).

**Root Morphology and Architecture**

Individual roots show relatively little morphological variation, especially when compared to individual shoots. The aboveground portions of plants are remarkably variable in both form and function. Conversely, roots, which also perform a vast array of vital functions, appear to be fairly uniform at first glance (Fitter 1987). However, though individual roots may resemble each other morphologically, these roots may possess many distinguishing anatomical, physiological, or functional features that aren't easily observed by the naked eye (Fitter 1987; Fitter 2002).

Since individual roots tend to look alike, it is extremely difficult to identify plants or to make ecological inferences based on the morphological appearance of individual roots (Fitter 2002). Root architecture, the spatial arrangement of the root system in soil is perhaps more useful (Fitter 1987; Gregory 2006). There are two important features of root architecture – topology (connections and branching patterns) and distribution (the spatial arrangement in the soil profile) (Gregory 2006). The architecture of a root system has important implications for the plant in an ecological context, e.g., the uptake of resources and the costs of construction (Fitter

1987; Gregory 2006). Root systems are extremely plastic; a feature that has proven adaptive in heterogeneous environmental conditions (Fitter 2002). Root architecture may respond to soil heterogeneity by concentrating growth in a relatively nutrient-rich area (Robinson 1996), but may also induce soil heterogeneity through the depletion or accumulation of nutrients (Gregory 2006).

Root architecture varies widely within and among species (Weaver 1919, 1958; Weaver and Clements 1938; Fitter 1987; Gregory 2006). Nonetheless, many researchers have attempted to describe, classify, and model root systems. Some of the earliest and most influential descriptions of root systems were conducted by J.E. Weaver and his colleagues at the University of Nebraska (i.e. *The Ecological Relations of Roots,* 1919). Throughout his career, Weaver compiled detailed drawings of thousands of plants in order to produce generalized descriptions for dozens of species (Weaver 1919; 1958), many of which are familiar to contemporary ecologists. In more recent decades, ecologists have made extensive progress modeling root systems using the technologies that they have at hand (Fitter 1987, 2002; Tilman 1988; Biondini 2001, 2008). Although extensive progress has been made, these models and classification systems are often complicated by the tremendous degree of plasticity inherent to root systems within and among species (Fitter 1987, 2002).

**Root System Functions**

Many significant functions are accredited to root systems, including the uptake of resources, storage of materials, anchorage, propagation, dispersal, and sensing and/or modification of the surrounding environment (Fitter 2002; Gregory 2006; Jackson et al. 2007). Of the many functions performed by root systems, two are generally considered to be the primary functions in terrestrial plants – the uptake of soil-based resources and anchorage (Fitter

2002). The remaining functions, while undeniably important to individual plants and their corresponding ecosystems, are commonly considered to be secondary (Fitter 2002). However, it is important to bear in mind that multiple functions may be performed simultaneously within a root system or even within a single root (Gregory 2006).

Both shoots and roots participate in the uptake of resources in terrestrial plants. Shoots are responsible for the uptake of carbon and energy, while roots are responsible for the uptake of water and nutrients (Jackson et al. 2007). The uptake processes occurring in roots are inextricably linked to the uptake processes occurring in shoots, and vice versa. In the interest of brevity, this review will remain focused on the belowground processes.

Three simultaneous processes come into play as soil resources are taken up by roots – mass flow, diffusion, and interception (Gardiner and Miller 2004; Jackson et al. 2007). These three processes operate constantly for as long as the plant continues to grow (Gardiner and Miller 2004). Mass flow is driven by transpiration (Jackson et al. 2007) and is defined as the movement of dissolved nutrients in water flowing towards the root (Gardiner and Miller 2004). As such, mass flow is dependent on water movement and the concentration of dissolved nutrients in the soil solution (Jackson et al. 2007). Diffusion is simply the movement down a concentration gradient from a region of higher concentration to one of lower concentration (Campbell and Reece 2002). Diffusion occurs when root uptake exceeds mass flow and a depletion zone is created in the area surrounding the root (Jackson et al. 2007). Interception occurs as a root grows through the soil and encounters water and nutrients (Gardiner and Miller 2004; Jackson et al. 2007).

The uptake of water by roots is a passive process; it does not require energy expenditure by the plant (Gurevitch et al. 2006; Jackson et al. 2007). In contrast, the uptake of nutrients can

also have an active component that requires energy expenditure on behalf of the plant (Campbell and Reece 2002; Jackson et al. 2007). Researchers have shown that plants may selectively forage by proliferating roots in regions enriched in soil water and/or nutrients (Gregory 2006; Berendse et al. 2007; Jackson et al. 2007). In addition, the belowground competitive ability of a plant is often directly proportional to the size of its root system (Jackson et al. 2007).

Less is understood about anchorage than about the uptake of water and nutrients although anchorage has been recognized as one of the primary functions of root systems (Gregory 2006). Roots must anchor plants against multiple forces simultaneously, e.g., vertical and horizontal forces, in order to withstand herbivores, trampling, and wind (Fitter 2002; Gregory 2006; Jackson et al. 2007). Root quantity, distribution, strengthening, and branching pattern are all known to play a role in the anchorage of plants (Fitter 2002; Gregory 2006). Root systems are known to respond to certain environmental stressors. For instance, researchers have documented the asymmetrical development of structural roots in woody species in response to prevailing wind conditions (Jackson et al. 2007). In addition, some species produce aerial roots – specialized structures that originate aboveground and help to brace the plant (Gregory 2006). Found in certain grasses and in familiar crop species (i.e. maize), these aerial roots can branch into laterals and aid in the absorption of soil-based resources (Gregory 2006).

Many plant species store nutrients, carbohydrates, and/or water in belowground tissue to be used at a later date (Gregory 2006; Jackson et al. 2007). Interestingly, this belowground storage can occur in both shoots and roots. Belowground storage often occurs as modified shoots within bulbs, rhizomes, tubers, corms, etc. (Campbell and Reece 2002; Jackson et al. 2007). These specialized organs function similarly to roots specialized for storage (Jackson et al. 2007). Specialized roots that are large in diameter (i.e. root tubers and taproots) are often the

most important roots involved in storage although storage can occur in roots of all size classes (Gregory 2006; Jackson et al. 2007). In contrast, uptake is known to occur mainly in small diameter roots and root hairs (Gregory 2006; Evert and Eichhorn 2013).

## Researching Roots

Many different techniques have been employed for researching root systems. These techniques are commonly divided into two broad categories – destructive methods and nondestructive methods (Johnson et al. 2001). The earliest methods utilized to study root systems generally fell into the destructive category (Box et al. 1989; Johnson et al. 2001; Gregory 2006). Typical historic methods used to study root systems involved mechanically extracting roots from the soil, separating roots from other materials, and then quantifying the roots (Box et al. 1989). There are many ways to sample roots using these destructive techniques and many are still at use today in certain situations, e.g., soil coring, in-growth cores, trenching, and excavation (Weaver 1919; Johnson et al. 2001). While these techniques certainly yield invaluable data, they are somewhat limited since they do not allow for repeated measures of the same root system and they tend to disturb the soil (Box et al. 1989; Johnson et al. 2001). Thus, it may not always be desirable to use a destructive method to harvest root samples and nondestructive techniques may be preferred depending on the context of the ecological study.

Nondestructive techniques generally involve rhizotrons or minirhizotrons (Johnson et al. 2001). Rhizotrons are structures built belowground with transparent windows and/or walls that allow observations and measurements of root systems *in situ* (Gregory 2006). Minirhizotrons are essentially small-scale versions of rhizotrons. Current minirhizotron systems consist of a transparent tube installed into the ground coupled with a specialized imaging device that can be inserted into the tube to capture video or still images of the root system *in situ* (Gregory 2006).

Rhizotrons and minirhizotrons both provide valuable information about root systems. However, rhizotrons are relatively expensive to construct and the information gathered may be fairly site-specific (Gregory 2006). Minirhizotrons are less costly to install, allowing researchers to expand the scope of their research.

Minirhizotrons have found favor with researchers since they allow frequent observations of the same root system, allow for large numbers of observations, can provide measurements of production and disappearance simultaneously, minimize soil disturbance, and do not significantly impact fine root processes (Box et al. 1989; Johnson et al. 2001; Crocker et al. 2003; Gregory 2006; Hendricks et al. 2006). In addition, minirhizotrons are versatile – they can be installed in a wide variety of ecological conditions and the data yielded can be used to answer a wide variety of ecological questions (Gregory 2006). This study employed minirhizotrons to allow for repeated, nondestructive observations of root systems in restored grasslands.

Grasslands cover about one-quarter of the Earth's land surface (Hui and Jackson 2006). Prior to European settlement, the extent of native prairie throughout the Great Plains Region exceeded 160 million hectares (Samson and Knopf 1994). Following the advance of European settlement and the expansion of agriculture, the vast majority of the native prairies encountered in the Great Plains Region were converted to other uses; an estimated 87% of tallgrass prairies and 71% of mixed grass prairies have been lost (Samson et al. 2004; Murphy and Grant 2005). In the past several decades, grassland restoration has become an increasingly widespread conservation practice. In turn, an extensive body of research on grassland restoration practices and the resulting novel ecological communities has developed. However, comparatively little research has focused on the belowground patterns and processes occurring within restored grasslands. This study concentrated on the belowground aspects of restored grassland systems in

order to add to the body of knowledge related to grassland restoration by examining the impacts of repeated nutrient applications and mowing treatments on above- and belowground components of restored grassland systems (e.g., species richness, aboveground biomass, root growth, etc.).

## Organization of Dissertation

This dissertation is organized into four chapters. Chapter 1 provides a general introduction to the topics that will be discussed at length in the proceeding chapters. Chapter 1 is intended to provide the background information and the justification for the information presented in the proceeding chapters. Chapters 2 and 3 present the methods and results of this research project, carried out at two separate locations in North Dakota. Chapters 2 and 3 are organized as complete manuscripts. Thus, there is unavoidably redundancy among Chapters 2 and 3 in order to provide the information necessary to make each chapter complete on its own. Chapter 4 serves as the conclusion to this dissertation and includes future directions for research.

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# CHAPTER 2. BELOWGROUND RESPONSES TO NUTRIENT AND MOWING TREATMENTS DO NOT MIRROR ABOVEGROUND RESPONSES IN A RESTORED SEMI-ARID GRASSLAND


## Abstract

This study was conducted in the northern mixed grass prairie region of western North Dakota. Restored grassland plots were subjected to different nutrient and mowing treatments and the above- and belowground responses were measured. Experimental plots were planted with different numbers of species and received nitrogen, phosphorus, or no fertilizer additions while half of each plot was mowed annually. Estimates of aboveground biomass, species richness, root length, root surface area, number of root tips, number of root branches, and root growth were compared among nutrient and mowing treatments. In general, the belowground variables measured were found to be relatively unresponsive to the nutrient and mowing treatments while the aboveground variables exhibited a pronounced response to nitrogen applications (200 kg/ha·yr). Nitrogen applications increased aboveground biomass compared to the control/no nutrient application (660 $g/m^2$ vs. 265 $g/m^2$, respectively) while no differences were detected in the belowground variables. Nitrogen applications also reduced average species richness compared to the control (3.25 species vs. 7.29 species, respectively). A decrease in the ratio of root volume to average aboveground biomass occurred in the nitrogen plots as a result of the increase in aboveground biomass without a corresponding increase belowground. The decreased ratio of root volume to average aboveground biomass indicated that the root systems of the plots that received nitrogen additions were capable of supporting more aboveground biomass than were similarly sized root systems in the plots that received no nitrogen additions.

## Introduction

A significant portion of production occurs below the soil surface in North American grasslands. Estimates of belowground production in grasslands are as high as 60-90% of annual net primary productivity (Stanton 1988; Hui and Jackson 2006). Unfortunately, the portion of grassland systems located below the soil surface is often neglected by ecologists, but not necessarily due to a lack of recognition of its significance. In actuality, grassland ecologists have long recognized the importance of root systems – some of the most extensive studies of root systems conducted in the Great Plains Region occurred decades ago (Weaver 1919; Weaver and Clements 1938). Instead, the disparity of ecological studies incorporating the belowground portion of grassland systems is more likely due to the inherent difficulties of studying roots *in situ*. To complicate matters further, research on root systems has yielded varied and oftentimes conflicting results for root production, responses to stress, longevity, and turnover (Eissenstat and Yanai 2002; Reich 2002).

Mowing and grazing are used as management techniques throughout the Great Plains Region and have been shown to have somewhat similar effects on grassland plant communities (Van Dyke et al. 2004; Holechek et al. 2011). Studies on the impacts of grazing and/or mowing on the above- and belowground components of grassland systems have yielded conflicting results. Some studies have reported increases in aboveground production as a result of grazing, while many others have reported no significant changes or reductions in aboveground production following grazing (Biondini et al. 1998; Pucheta et al. 1998; Pucheta et al. 2004). Similarly, conclusions about the effects of grazing/mowing on belowground plant production have been varied. For example, Pucheta et al. (2004) determined belowground production to increase dramatically following grazing, while Gao et al. (2008) documented a decrease in belowground

production following grazing. To date, many studies examining the effects of nutrient applications on root production have shown no increases in belowground plant production despite pronounced aboveground increases (Cahill 1999; Son and Hwang 2003; Ladwig et al. 2011; Yavitt et al. 2011). In addition, certain authors have documented decreases in root biomass following nutrient applications and/or changes in root-to-shoot ratios (Gregory 2006; Yavitt et al. 2011).

Minirhizotrons are increasingly used by ecologists to study root systems *in situ*. Minirhizotrons typically involve transparent tubes inserted into the ground coupled with specialized imaging devices that can be inserted into the tube to capture video or still images of the root system (Gregory 2006). Minirhizotrons have found favor with researchers since they allow frequent and/or repeated observations of the same root system, allow for large numbers of observations, provide measurements of production and disappearance simultaneously, minimize soil disturbance, and do not significantly impact fine root processes (Box et al. 1989; Johnson et al. 2001; Crocker et al. 2003; Gregory 2006; Hendricks et al. 2006). In addition, minirhizotrons are versatile – they can be installed in a wide variety of ecological conditions and the data yielded can be used to answer a wide variety of ecological questions (Gregory 2006).

The specific objectives of this study included:

- Combining above- and belowground sampling methods to provide a comprehensive evaluation of restored grassland plant communities in the mixed grass prairie region of North Dakota,
- An evaluation of the above- and/or belowground responses within restored grassland plant communities to annual nitrogen (20 $g/m^2$) and phosphorus (4 $g/m^2$) additions, and

- An examination of the above- and/or belowground effects of annual fall mowing and biomass removal on restored mixed grass prairie plant communities

## Methods

### Study Site Description

This study was conducted at the Dickinson Research Extension Center (DREC) located near the city of Dickinson in Stark County, North Dakota (46° 53' 54.799"N, 102° 49' 54.799"W). The DREC lies within the northern mixed grass prairie region (Barker and Whitman 1988). The 30-year average annual temperature is 3.33 °C and the 30-year average annual precipitation is 43.2 cm (USDA 2011). The soils for the experimental plots are mapped as Reeder-Farnuf loams (Fine-loamy, mixed, superactive, frigid Typic Argiustolls) on 3-6% slopes, these soils are well drained (USDA 2015).

Prior to the establishment of the experimental plots at the DREC, the area was planted with smooth brome (*Bromus inermis* Leyss.) and crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) and had been hayed intermittently since the 1930s. In 2005, the area was treated three times with glyphosate (Roundup, Monsanto, St. Louis, MO, USA) before seeding in order to improve the establishment of the seeded species (Biondini et al. 2011). The experimental plots are surrounded by pasturelands (mixed grasses and forbs).

### Experimental Design

The original restoration experiment consisted of 210 experimentally-planted plots (Biondini et al. 2011). Each plot was 25 $m^2$ (5 m x 5 m) and surrounded by a 3 m buffer. The plots were organized as a completely randomized factorial design with two factors (Biondini et al. 2011). Factor one (species richness) had seven levels: 1) one species belonging to one functional form, 2) two species belonging to two functional forms, 3) five species belonging to

two functional forms, 4) five species belonging to three functional forms, 5) 10 species belonging to three functional forms, 6) 10 species belonging to four functional forms, and 7) 20 species belonging to five functional forms. The functional form classifications of the species were derived by Levang-Brilz and Biondini (2002) and Biondini (2007). Species and functional forms were assigned randomly to each replication. A list of the species included in the experiment is provided in Appendix A (Table A.1). Factor two (nutrient application) had three levels: 1) no fertilization (control), 2) nitrogen fertilization (200 kg/ha·yr) and 3) phosphorus fertilization (40 kg/ha·yr). Sierra© slow release fertilizer was applied annually to the treatment plots in the spring of each year.

Most of the plots were planted in the fall of 2005, with a few plots planted the following spring (Biondini et al. 2011). The seeding rate for each plot was 400 live seeds/$m^2$, divided among species. For example, if five species were to be planted within a plot, the seeding rate for each species was 80 live seeds/$m^2$. Since 2006 was an extremely dry year in western North Dakota, (29.0 cm of growing season precipitation) the plots were irrigated with about 10.0 cm to allow for reasonable establishment (Biondini et al. 2011). Without this irrigation, the plantings would have failed. Irrigation was only used in 2006.

In the fall of 2009, a harvesting experiment was superimposed on the experimental plots using a split-plot design with two additional treatments: 1) yearly fall harvest (mowed) and 2) no harvest/control (un-mowed). The mowed portions of the split-plots were harvested each fall after frost (Mulkey et al. 2006) using a sickle-bar mower at a cutting height of 10-15 cm followed by a landscape rake pulled behind an ATV. In the fall of 2010, 40 minirhizotron tubes were installed within a subset of the 210 experimental plots at the DREC. Twenty plots were selected for this study based on a gradient of species richness and biomass data (Biondini et al.

2011). Appendix A (Table A.2) contains information about the plots selected for minirhizotron tube installation. Two minirhizotron tubes were installed in each of the selected plots – one in each of the mowed sub-plot and un-mowed sub-plots.

Each minirhizotron tube was installed to a depth of approximately 1 m angled 45° from the vertical using a trailer-mounted Amity 9800E sampler (Amity Technology, Fargo, ND, USA). The 45° angle was chosen in order to observe root growth at multiple depths and to discourage roots from growing along the tube (Johnson et al. 2001). Each tube was approximately 2 m in length and consisted of clear acrylic plastic (Crown Plastics Inc., Plymouth, MN, USA). Each tube had a rubber stopper (VWR International, Radnor, PA, USA) and a vinyl cap (Caplugs, Buffalo, NY, USA) glued onto the lower end and a removable vinyl cap on the portion that remained aboveground. Each tube was insulated with removable foam pipe insulation (Armacell, Mebane, NC, USA or ITP, Brampton, ON, Canada) in an attempt to reduce the effects of the tube on local soil temperatures (Tingey et al. 2003; Phillips et al. 2006). The aboveground portion of each tube was first painted black (Rust-Oleum, Vernon Hills, IL, USA) in order to reduce light penetration to the soil at depth and then white (Rust-Oleum, Vernon Hills, IL, USA) in order to reduce the heating effects from the sun (Box et al. 1989; Johnson et al. 2001; Tingey et al. 2003; Phillips et al. 2006). Tubes were checked often throughout the subsequent growing seasons to insure good contact with the soil surface and were repainted as needed.

**Sampling Methods**

Species richness and aboveground production surveys were conducted on all 210 plots at the DREC from 2006 through 2011 (Biondini et al 2011; DiAllesandro et al. 2013). Since this study was intended to examine the impacts of mowing and nutrient applications on roots and

shoots, only the data obtained in 2010 and 2011 for the 20 plots in which minirhizotron tubes were installed were included in the current analysis. Aboveground biomass was estimated by clipping two 0.25 $m^2$ quadrats in each sub-plot in 2010 and 2011. Quadrats were randomly placed in an area that was not clipped in the previous growing season. Clipping was timed to coincide with peak aboveground biomass and completed in July of each year. Biomass was separated by species, oven-dried, and weighed (Biondini et al. 2011). In addition, species richness was determined annually by surveying the entire area of each sub-plot.

In order to allow plenty of time for the establishment of roots in the soil adjacent to the minirhizotron tubes, belowground observations did not begin until the spring of 2012. Minirhizotron images were collected four times during the growing season of 2012, timed in a manner intended to provide a thorough examination of the root systems present. The first minirhizotron sampling session occurred in mid-May in order to coincide with early season growth. The second occurred in late June and was intended to capture early growth of warm-season species. The third occurred in mid-July, timed to coincide with peak aboveground biomass. The fourth occurred in late September/early October, coinciding with the end of the growing season.

Images were obtained using the CI-600 In-Situ Root Imager (CID Bio-Science, Camas, WA, USA) which rotated along the length of the tube to provide a $360^o$ image (400 dpi). Images were collected from the soil surface to the maximum depth allowed by the equipment. For the current study, analysis was conducted on the 0-0.45 m depth interval since the vast majority of root growth occurs within this interval (Dahlman and Kucera 1965; Jackson et al. 1996; Sun et al. 1997; Gregory 2006; Mueller et al. 2013).

Images collected in the 0-0.45 m depth interval were processed using WinRhizo Tron MF software (2014 Regent Instruments, Quebec, Canada), a program specifically designed for use with images obtained from minirhizotrons. A trained user identified roots within the image space and selected a diameter class for each root segment (Figure 2.1). The software then performed additional measurements and calculations; thereby producing estimates for several features (e.g., root length, surface area, and volume). In addition, the software tracked features such as branching and number of root tips.

**Statistical Analysis**

Estimates of root length, surface area, number of root tips, and number of branches for the 2012 growing season were obtained from WinRhizo Tron MF. Estimates of aboveground biomass and species richness were obtained from clipping and visual surveys conducted in 2010 and 2011. Biomass and species richness estimates were averaged in order to account for some of the variation among years. Data were transformed prior to analysis in order to help satisfy the distributional assumptions of linear statistical methods (McCune and Grace 2002; Quinn and Keough 2002).

Root length, root surface area, number of root tips, and number of branches were analyzed using a split-plot design with repeated measures for the entire 0-0.45 m depth interval. A log transformation was applied to root length and surface area estimates. A fourth root transformation was applied to the number of root tips and number of branches. Statistical analyses were conducted in SAS 9.4 (SAS Institute, Cary, NC). Nutrient type (nitrogen, phosphorus, or control) and mowing treatment (mowed or un-mowed) were specified as fixed effects and interaction terms were included. Time (i.e. sampling session) was the repeated factor (interaction terms included). The covariance structure was specified as first-order autoregressive

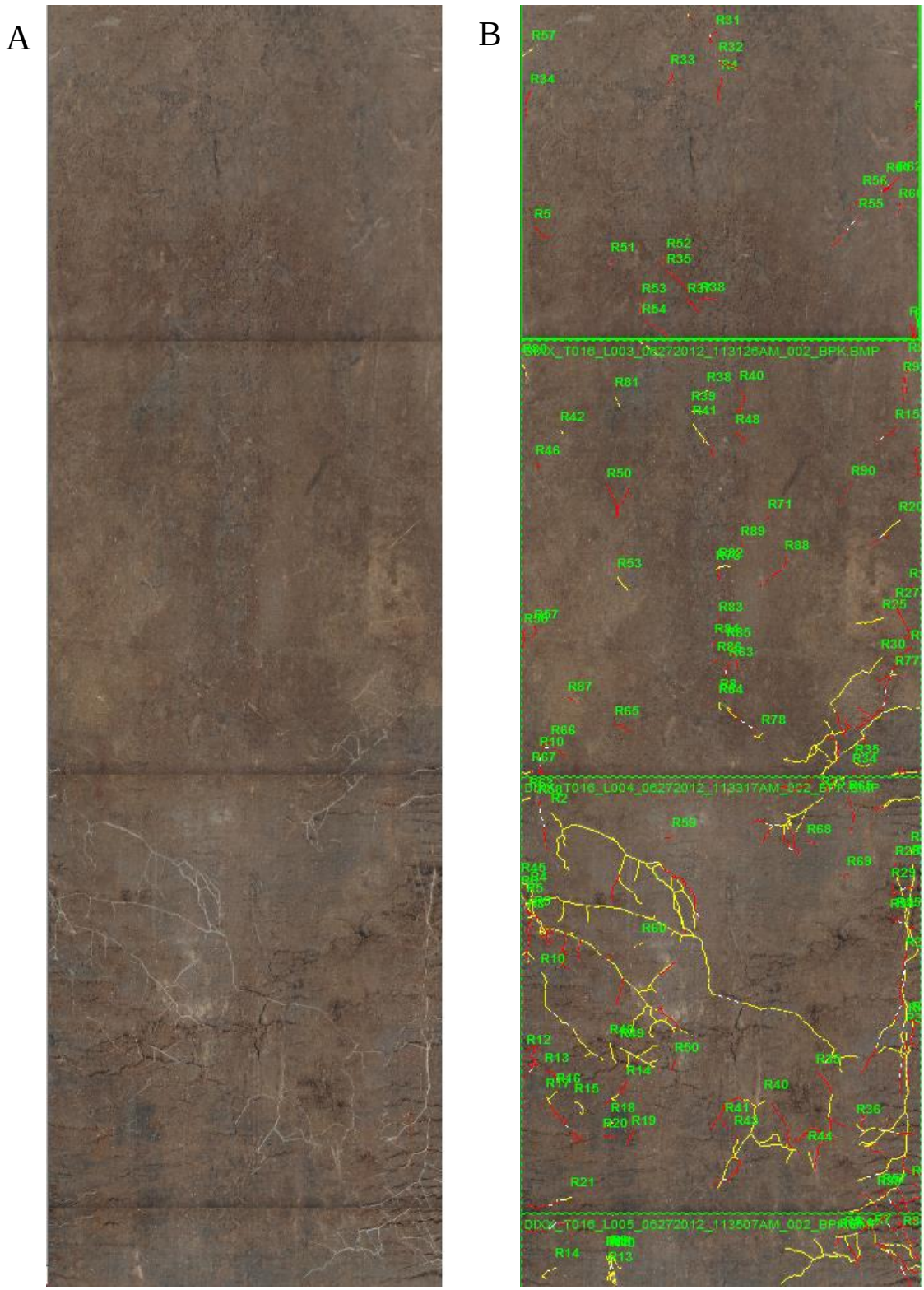


Figure 2.1. Example of image processing using WinRhizo Tron MF software (DREC, control plot). A) Unprocessed images obtained for the 0-0.45 m depth interval. B) Processed images wherein a trained user identified the individual roots.

in recognition that observations collected during adjacent sampling sessions were expected to be more highly correlated than observations collected at longer time intervals (Littell et al. 2006). Degrees of freedom calculations employed the Kenward-Rogers (first-order) procedure since it was appropriate for use with both the repeated measures and correlated errors (Littell et al. 2006). A residual (restricted) maximum likelihood estimation method was specified. Least squares estimates of population means were calculated for statistically significant fixed effects and specific pair-wise comparisons among treatment populations were made. P values were corrected for multiple comparisons using the Studentized maximum modulus-based/Hochberg's GT2 method (SAS Institute Inc. 2008).

In addition to the overall split-plot design with repeated measures used to look across the entire growing season, each sampling session was analyzed separately using a split-plot design in order to incorporate estimates of average aboveground biomass, species richness, root growth, and the ratio of root volume to average aboveground biomass. Root length and volume estimates obtained from WinRhizo were used to calculate root growth and the ratio of root volume to average aboveground biomass. Root growth was estimated by subtracting the initial root length from the maximum root length of each plot, then dividing by the initial root length.

$$G = (l_m - l_i) \div l_i$$

Where $G$ is root growth, $l_m$ is the maximum root length detected for all sampling sessions, and $l_i$ is the initial root length (measured in mid-May). The ratio of root volume to average aboveground biomass was calculated using root volume (obtained at peak aboveground biomass) and peak aboveground biomass estimates (root volume ÷ aboveground biomass).

Nutrient type and mowing treatments were specified as fixed effects (interaction term included) for all split-plot models. A log transformation was applied to root length, root surface

area, aboveground biomass estimates, and the ratio of root volume to average aboveground biomass. A square root transformation was applied to the number of root tips, number of branches, average species richness, and root growth estimates. (A square root transformation was selected for these variables rather than the fourth root transformation used above since these data were not as skewed.) A residual (restricted) maximum likelihood estimation method was specified. Degrees of freedom calculations employed the Satterthwaite approximation since it was appropriate for use with split-plot designs (Schabenberger and Pierce 2002; Littell et al. 2006). P values were corrected for multiple comparisons using the Studentized maximum modulus-based/Hochberg's GT2 method (SAS Institute Inc. 2008).

Regression analysis was used to examine the relationships between above- and belowground variables. Maximum root length, maximum root surface area, and root growth were regressed against average species richness, average forb richness, and average grass richness in order to determine if significant relationships existed ($p < 0.05$). Maximum root length and maximum root surface area estimates were log transformed. Average species richness, average forb richness, average grass richness, and root growth estimates were square root transformed. Regression coefficients were compared among nutrient treatments in order to determine if there were any significant differences ($p < 0.05$) in the slope parameters.

## Results

Neither nutrient type nor mowing treatment affected the average root length, average surface area, average number of root tips, or average number of branches for the 0-0.45 m depth interval (Tables 2.1-2.4). However, time (sampling session) had an effect ($p < 0.05$) on average root length, average surface area, average number of root tips, and the average number of

branches for the 0-0.45 m depth interval. No significant interaction among nutrient treatments, mowing treatments, or time was found (Tables 2.1-2.4).

Table 2.1. Repeated measures ANOVA for average root length by treatment and interaction at the Dickinson Research Extension Center (Type III estimation method).

| **Effect** | **Num. DF[1]** | **Den. DF[2]** | **F Value** | **p value** |
|---|---|---|---|---|
| Nutrient | 2 | 34.0 | 0.11 | 0.89 |
| Mow | 1 | 34.0 | 0.03 | 0.86 |
| Session | 3 | 72.1 | 18.2 | <0.01 |
| Nutrient*Mow | 2 | 34.0 | 0.47 | 0.63 |
| Nutrient*Session | 6 | 79.4 | 1.09 | 0.38 |
| Mow*Session | 3 | 72.1 | 1.39 | 0.25 |
| Nutrient*Mow*Session | 6 | 79.4 | 0.72 | 0.64 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Kenward-Rogers first-order method)

Since time (sampling session) affected average root length, surface area, number of root tips, and number of branches, specific pair-wise comparisons were conducted among least squares means for the four sampling sessions. The p values resulting from these pair-wise comparisons are presented in Tables 2.5-2.8. Average root length, surface area, number of root tips, and number of branches were found to follow a similar pattern throughout the growing season. Each of these four variables increased from mid-May through June, peaked in mid-July, and declined in the fall (Figures 2.2-2.5).

For all four sampling sessions, nutrient types and mowing treatments had no effect on the average root length, surface area, number of root tips, number of root branches, or root growth for the 0-0.45 m depth interval (Appendix B, Tables B.1-B.17). The interaction term (nutrient*mowing) was also not significant for any of the sampling sessions. However, nutrient type impacted average aboveground biomass, species richness, and the ratio of root volume to average peak aboveground biomass (Tables 2.9-2.14). Mowing treatments had no effect on

average aboveground biomass, species richness, or the ratio of root volume to average peak aboveground biomass.

Table 2.2. Repeated measures ANOVA for average root surface by treatment and interaction at the Dickinson Research Extension Center (Type III estimation method).

| **Effect** | **Num. DF[1]** | **Den. DF[2]** | **F Value** | **p value** |
|---|---|---|---|---|
| Nutrient | 2 | 34.0 | 0.08 | 0.92 |
| Mow | 1 | 34.0 | 0.00 | 0.95 |
| Session | 3 | 72.0 | 27.2 | <0.01 |
| Nutrient*Mow | 2 | 34.0 | 0.33 | 0.72 |
| Nutrient*Session | 6 | 79.4 | 1.97 | 0.08 |
| Mow*Session | 3 | 72.0 | 1.19 | 0.32 |
| Nutrient*Mow*Session | 6 | 79.4 | 1.21 | 0.31 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Kenward-Rogers first-order method)

Table 2.3. Repeated measures ANOVA for average number of root tips by treatment and interaction at the Dickinson Research Extension Center (Type III estimation method).

| **Effect** | **Num. DF[1]** | **Den. DF[2]** | **F Value** | **p value** |
|---|---|---|---|---|
| Nutrient | 2 | 34.0 | 0.17 | 0.85 |
| Mow | 1 | 34.0 | 0.05 | 0.82 |
| Session | 3 | 72.1 | 79.6 | <0.01 |
| Nutrient*Mow | 2 | 34.0 | 0.44 | 0.65 |
| Nutrient*Session | 6 | 79.5 | 1.57 | 0.17 |
| Mow*Session | 3 | 72.1 | 0.76 | 0.52 |
| Nutrient*Mow*Session | 6 | 79.4 | 0.37 | 0.90 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Kenward-Rogers first-order method)

Table 2.4. Repeated measures ANOVA for average number of branches by treatment and interaction at the Dickinson Research Extension Center (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 34.8 | 0.79 | 0.46 |
| Mow | 1 | 34.8 | 0.18 | 0.68 |
| Session | 3 | 73.4 | 34.5 | <0.01 |
| Nutrient*Mow | 2 | 34.8 | 0.39 | 0.68 |
| Nutrient*Session | 6 | 80.5 | 1.17 | 0.33 |
| Mow*Session | 3 | 73.4 | 2.08 | 0.11 |
| Nutrient*Mow*Session | 6 | 80.5 | 0.50 | 0.81 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Kenward-Rogers first-order method)

Table 2.5. P values from pair-wise comparisons of least squares means estimates for root length by sampling session at the Dickinson Research Extension Center (corrected for multiple comparisons).

| | Session 1 | Session 2 | Session 3 | Session 4 |
|---|---|---|---|---|
| **Session 1** | | 0.06 | <0.01 | 0.12 |
| **Session 2** | 0.06 | | <0.01 | 1.00 |
| **Session 3** | <0.01 | <0.01 | | <0.01 |
| **Session 4** | 0.12 | 1.00 | <0.01 | |

Table 2.6. P values from pair-wise comparisons of least squares means estimates for root surface area by sampling session at the Dickinson Research Extension Center (corrected for multiple comparisons).

| | Session 1 | Session 2 | Session 3 | Session 4 |
|---|---|---|---|---|
| **Session 1** | | 0.28 | 0.001 | <0.01 |
| **Session 2** | 0.28 | | 0.19 | <0.01 |
| **Session 3** | 0.001 | 0.19 | | <0.01 |
| **Session 4** | <0.01 | <0.01 | <0.01 | |

Table 2.7. P values from pair-wise comparisons of least squares means estimates for number of root tips by sampling session at the Dickinson Research Extension Center (corrected for multiple comparisons).

| | Session 1 | Session 2 | Session 3 | Session 4 |
|---|---|---|---|---|
| **Session 1** | | 0.41 | <0.01 | <0.01 |
| **Session 2** | 0.41 | | <0.01 | <0.01 |
| **Session 3** | <0.01 | <0.01 | | <0.01 |
| **Session 4** | <0.01 | <0.01 | <0.01 | |

Table 2.8. P values from pair-wise comparisons of least squares means estimates for number of root branches by sampling session at the Dickinson Research Extension Center (corrected for multiple comparisons).

| | Session 1 | Session 2 | Session 3 | Session 4 |
|---|---|---|---|---|
| **Session 1** | | <0.01 | <0.01 | 0.01 |
| **Session 2** | <0.01 | | <0.01 | 0.80 |
| **Session 3** | <0.01 | <0.01 | | <0.01 |
| **Session 4** | 0.01 | 0.80 | <0.01 | |

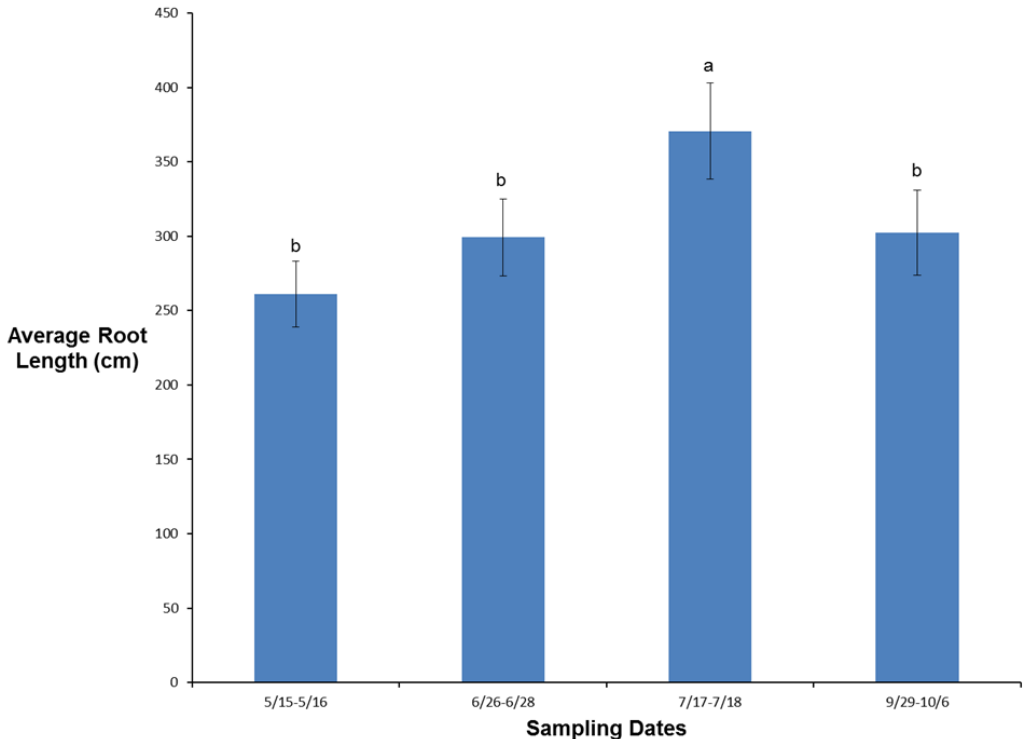


Figure 2.2. Average root length by sampling session for the Dickinson Research Extension Center ± SE. Significant differences indicated by different letters ($p < 0.05$).

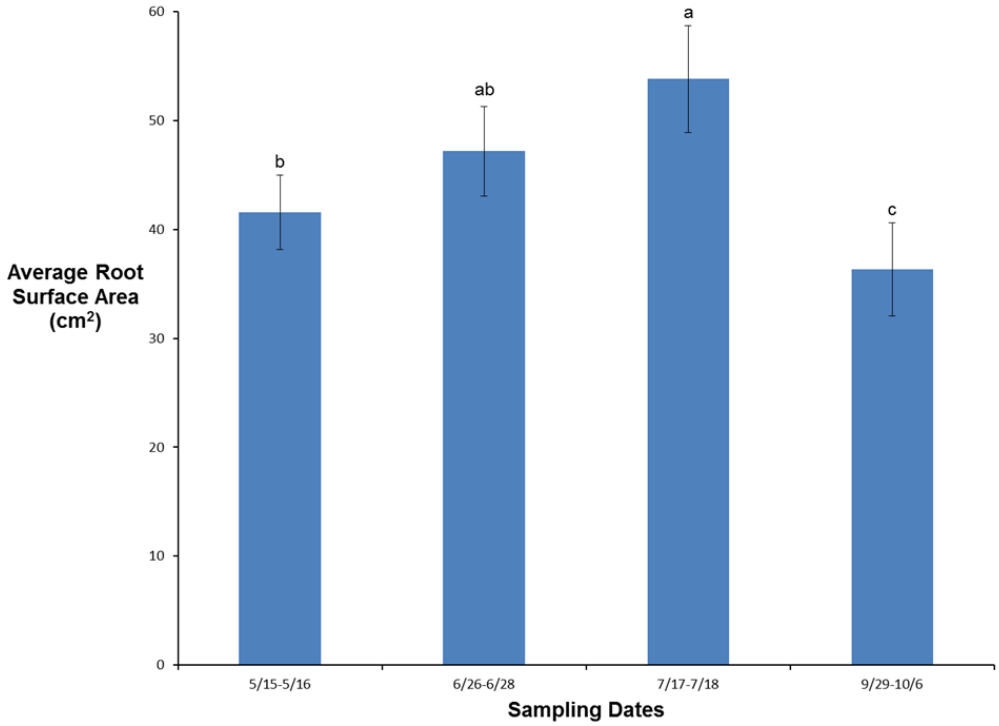


Figure 2.3. Average root surface area by sampling session for the Dickinson Research Extension Center ± SE. Significant differences indicated by different letters ($p < 0.05$).

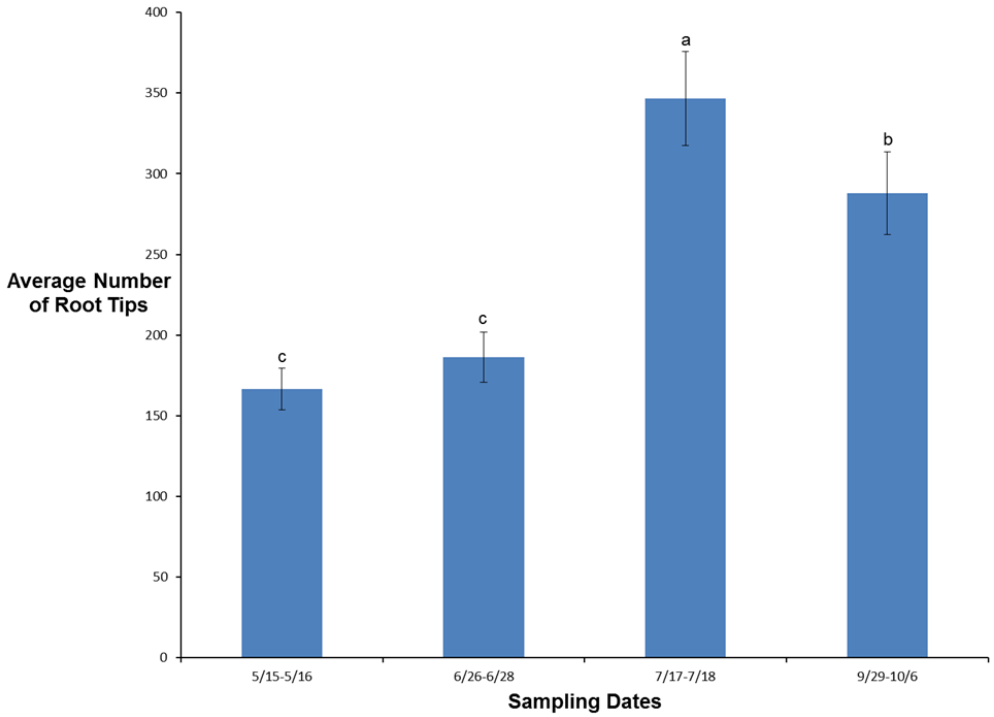


Figure 2.4. Average number of root tips by sampling session for the Dickinson Research Extension Center ± SE. Significant differences indicated by different letters ($p < 0.05$).

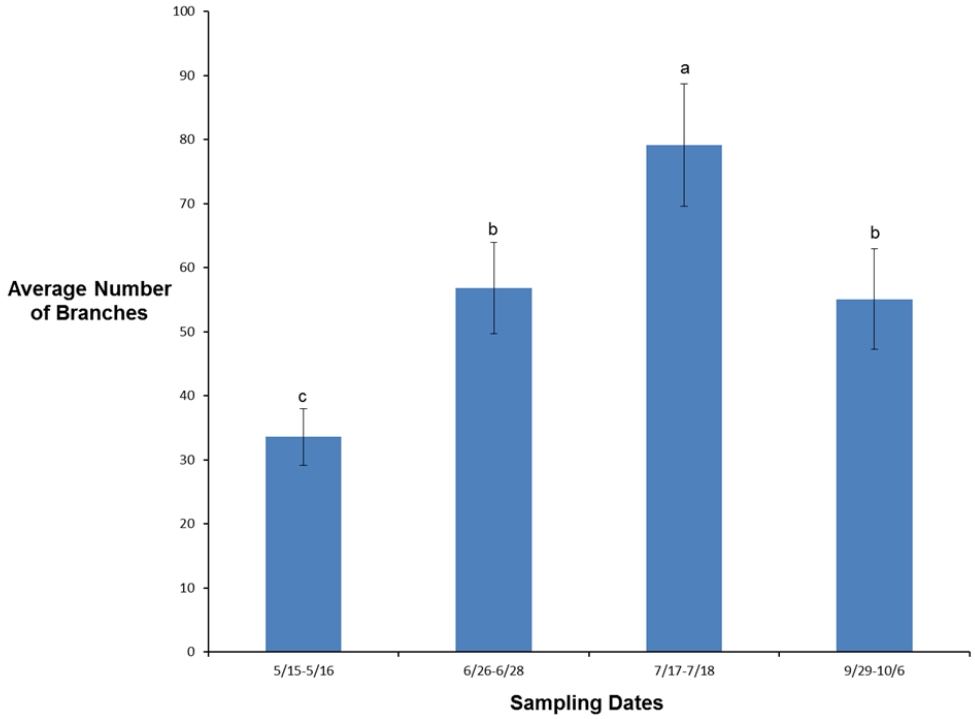


Figure 2.5. Average number of branches by sampling session for the Dickinson Research Extension Center ± SE. Significant differences indicated by different letters ($p < 0.05$).

Nitrogen application increased average aboveground biomass ($\overline{x}_N$ = 660 g/m$^2$, SE = 28.1) compared to the control ($\overline{x}_C$ = 265 g/m$^2$, SE = 17.7), while there was no difference in average aboveground biomass following phosphorus application ($\overline{x}_P$ = 307 g/m$^2$, SE = 21.7; Figure 2.6). Nitrogen application reduced the average species richness ($\overline{x}_N$ = 3.25, SE = 0.33) compared to the control ($\overline{x}_C$ = 7.29, SE = 0.49) while phosphorus application had no change ($\overline{x}_P$ = 6.28, SE = 0.36) (Figure 2.7). Nitrogen application decreased the ratio of root volume to average peak aboveground biomass ($\overline{x}_N$ = 1.08 x10$^{-3}$, SE = 2.2 x 10$^{-4}$) compared to the control ($\overline{x}_C$ = 3.07 x 10$^{-3}$, SE = 7.30 x 10$^{-4}$). Again, there was no difference in the ratio of root volume to average peak aboveground biomass following phosphorus application ($\overline{x}_P$ = 2.61 x 10$^{-3}$, SE = 4.70 x 10$^{-4}$) (Figure 2.8).

Regression analysis showed no linear relationships or differences in the slopes between maximum root length, maximum root surface area, and root growth with: 1) average species

richness, 2) average forb richness, or 3) average grass richness (see Appendix C Figs. C.1-9 and Tables C.1-3 for details).

Table 2.9. Analysis of Variance for average aboveground biomass by treatment and interaction at the Dickinson Research Extension Center (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 30.9 | <0.01 |
| Mow | 1 | 17 | 0.26 | 0.62 |
| Nutrient*Mow | 2 | 17 | 0.37 | 0.69 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Table 2.10. P values from pair-wise comparisons of least squares means estimates for average aboveground biomass by nutrient treatment at the Dickinson Research Extension Center (corrected for multiple comparisons).

| | Nitrogen | Control | Phosphorus |
|---|---|---|---|
| **Nitrogen** | | <0.01 | <0.01 |
| **Control** | <0.01 | | 0.58 |
| **Phosphorus** | <0.01 | 0.58 | |

Table 2.11. Analysis of Variance for average species richness by treatment and interaction at the Dickinson Research Extension Center (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 15.6 | <0.01 |
| Mow | 1 | 17 | 0.35 | 0.56 |
| Nutrient*Mow | 2 | 17 | 0.45 | 0.65 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Table 2.12. P values from pair-wise comparisons of least squares means estimates for average species richness by nutrient treatment at the Dickinson Research Extension Center (corrected for multiple comparisons).

| | Nitrogen | Control | Phosphorus |
|---|---|---|---|
| **Nitrogen** | | <0.01 | 0.001 |
| **Control** | <0.01 | | 0.50 |
| **Phosphorus** | <0.01 | 0.50 | |

Table 2.13. Analysis of Variance for the ratio of root volume (at peak aboveground biomass) to average peak aboveground biomass by treatment and interaction at the Dickinson Research Extension Center (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 34 | 5.47 | <0.01 |
| Mow | 1 | 34 | 0.00 | 0.95 |
| Nutrient*Mow | 2 | 34 | 0.51 | 0.60 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Table 2.14. P values from pair-wise comparisons of least squares means estimates for the ratio of root volume (at peak aboveground biomass) to average peak aboveground biomass by nutrient treatment at the Dickinson Research Extension Center (corrected for multiple comparisons).

| | Nitrogen | Control | Phosphorus |
|---|---|---|---|
| **Nitrogen** | | 0.01 | 0.02 |
| **Control** | 0.01 | | 0.99 |
| **Phosphorus** | 0.02 | 0.99 | |

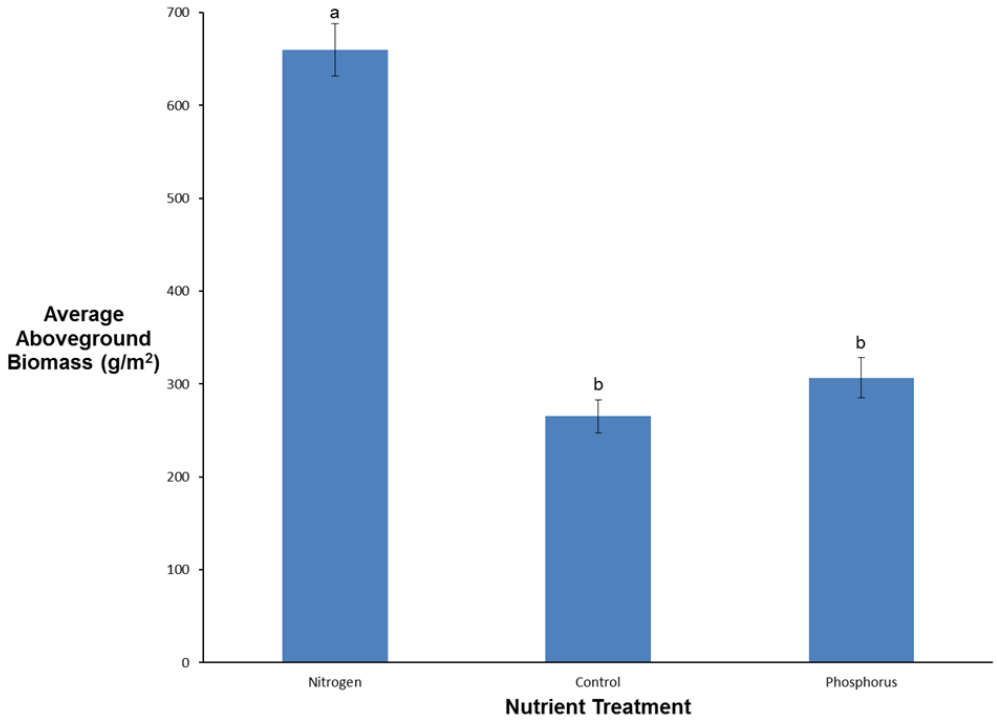


Figure 2.6. Average aboveground biomass by nutrient treatment for the Dickinson Research Extension Center ± SE. Significant differences indicated by different letters ($p < 0.05$).

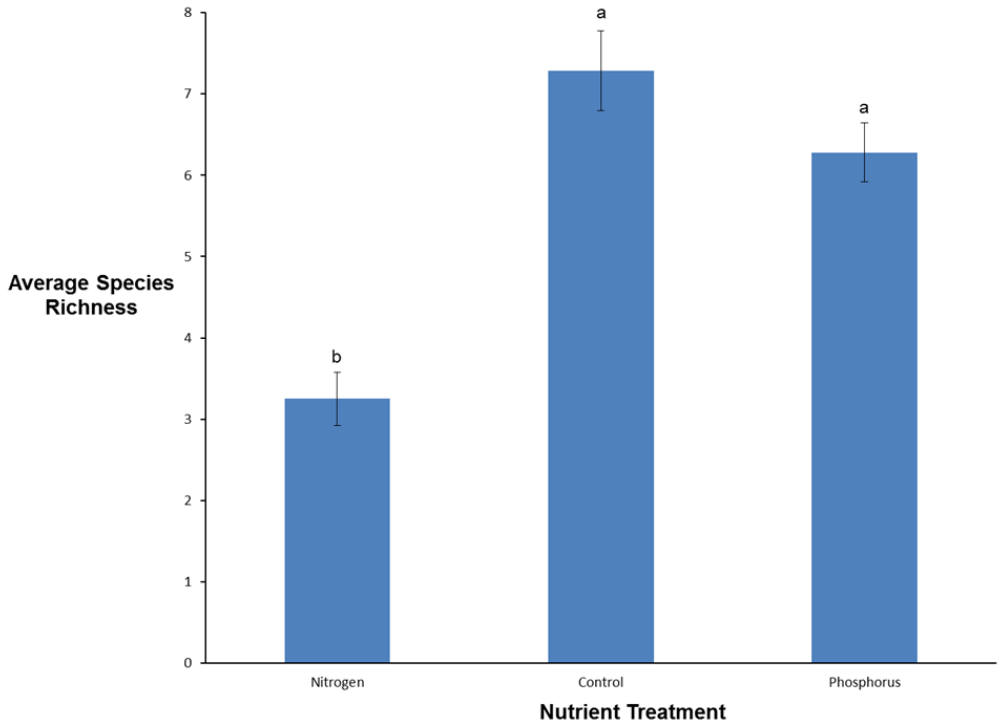


Figure 2.7. Average species richness by nutrient treatment for the Dickinson Research Extension Center ± SE. Significant differences indicated by different letters ($p < 0.05$).

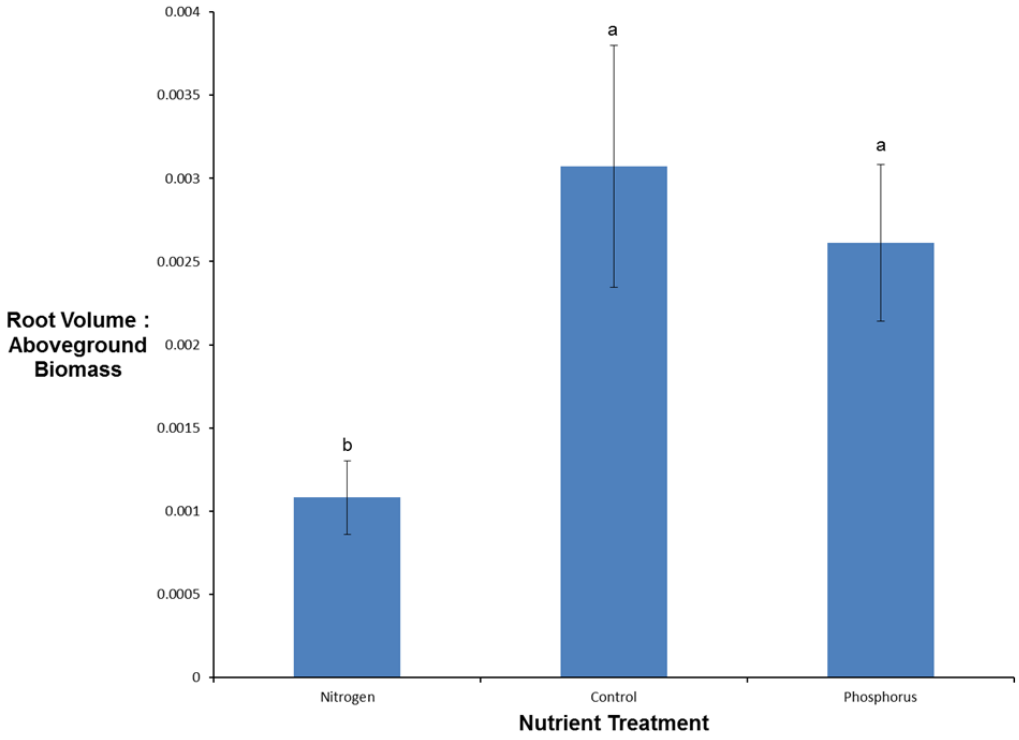


Figure 2.8. Ratio of root volume (obtained at peak biomass) to average peak aboveground biomass for the Dickinson Research Extension Center ± SE. Significant differences indicated by different letters ($p < 0.05$).

## Discussion

In general, the root systems within the restored grassland plots included in this study were found to be relatively insensitive to nutrient applications and mowing treatments. When the entire 0-0.45 m depth interval was analyzed for the 2012 growing season, there were no differences in average root length, surface area, number of root tips, number of root branches, or root growth estimates among nutrient applications or mowing treatments. Regression analysis of above- and belowground variables did not detect significant linear relationships among root estimates and species richness factors. Furthermore, there were no differences in the responses of the nutrient treatments.

Nutrient applications impacted aboveground biomass, species richness and the ratio of root volume to average peak aboveground biomass, while mowing treatments did not. Average aboveground biomass increased substantially following nitrogen applications, while species richness and the ratio of root volume to average aboveground biomass declined. There was no difference between the control (no nutrient application) and the phosphorus treatment. Previous studies involving the experimental plots at the DREC have detected similar responses in the aboveground plant community following nitrogen applications (Biondini et al. 2011; DiAllesandro et al. 2013). For example, Biondini et al (2011) documented increases in non-seeded species biomass following nitrogen additions that were not apparent following phosphorus additions. Similarly, DiAllesandro et al. (2013) showed an increase in smooth brome and crested wheatgrass biomass in plots that received nitrogen applications while no increase was shown in the control plots or those that received phosphorus.

The apparent disconnect in the above- and belowground response to nutrient treatments was reflected in the decline in the ratio of root volume to average peak aboveground biomass

following nitrogen applications. This ratio links the above- and belowground responses, thus, it follows that a difference was detected after repeated nitrogen applications since there were pronounced effects observed in the aboveground variables even though no belowground effects were observed. The decline in the ratio of root volume to average aboveground biomass suggests that similar root volumes were able to support increased aboveground biomass when additional nitrogen was provided. A similar effect was not observed in the plots that received additional phosphorus. Gusewell (2004) noted that although biomass allocation to roots is expected to increase when there is a deficiency of either nitrogen or phosphorus, the effect of nitrogen deficiency is often more pronounced. Following this line of evidence, the root-to-shoot ratios would be expected to decrease when nitrogen and phosphorus are readily available. This effect was observed for the nitrogen treatment, but not the phosphorus treatment in the current study.

As in the current study, many researchers have documented different above- and belowground responses to nutrient additions (Cahill 1999; Gregory 2006; Balogianni 2014). In a study of the annual productivity of a semi-arid grassland, Ladwig et al. (2011) found that nitrogen fertilization did not result in increased root production although there was a significant aboveground response. Similarly, Balogianni et al. (2014) determined that nitrogen additions did not have a significant effect on root length or root mass, despite a pronounced effect on shoot mass in native and invaded grasslands in Montana. In a study of a single weedy species (*Abutilon theophrasti* Medik.), Cahill (1999) found an increase in aboveground biomass with no corresponding increase in belowground biomass following fertilization with nitrogen, phosphorus, and potassium.

Although perhaps counterintuitive, differences in the observed responses to the above- and belowground portions of restored grassland plant communities may be explained. Plants are known to have a limited amount of resources available to carry out of all the physiological functions required throughout their lifecycle and roots, like all plant organs, have costs associated with their production and maintenance (Bloom et al 1985; Gurevitch et al. 2006; Yavitt et al. 2011). Thus, when resources are readily available, as may be the case following nutrient applications, it may be advantageous for the plant to invest more of its resources in aboveground production than in belowground production (Bloom et al. 1985; Gregory 2006; Yavitt et al. 2011). For the current study, it is possible that there were no differences detected belowground due to an investment in aboveground tissue rather than belowground tissue as a result of readily available nutrients. This also helps to explain the observed increase in aboveground biomass following nitrogen additions.

The significant differences in the ratios of root volume to average peak aboveground biomass indicated that the observed increase in aboveground biomass could be sustained in the plots receiving nitrogen applications despite no increase in the size of their root systems. This is likely an additional illustration of an increased investment in aboveground biomass following nitrogen applications due to the relative availability of soil resources. Many researchers have documented similar decreases in root-to-shoot ratios following nutrient applications (Craine et al. 2002; Levang-Brilz and Biondini 2002; Gusewell 2004; Henry et al. 2005; Gregory 2006). Gregory (2006) noted that relatively small root systems can be sufficient for plant growth under optimal conditions, i.e. readily available soil nutrients.

Grazing is widely recognized as an important component of grassland systems and its impacts have been widely studied. Mowing is also used as a management tool for North

American grasslands although it may not be as well-studied as grazing. Previous studies on the impacts of grazing and/or mowing have yielded varied and/or conflicting results. To date, researchers have reported increases, decreases, or no changes in above- and/or belowground production following grazing and/or mowing (Biondini et al. 1998; Pucheta et al. 1998; Pucheta et al. 2004; Gao et al. 2008; Balogianni et al. 2014).

For example, Balogianni et al. (2014) found that while mowing had an effect on the aboveground biomass in native and invaded grassland plots in Montana there was no effect detected belowground. This study sought to elucidate the effects of mowing treatments on the above- and belowground components of restored grasslands but found no effect attributable to mowing treatments (or to the interaction of mowing with nutrient applications), either above- or belowground. It is not completely unexpected that the mowing treatment included in this study had no effect on the restored plant communities present in the DREC plots. The plots evaluated in this study were mowed once in the fall of each year, beginning in 2009. Data for this study was collected in 2010-2012. As of 2012, there were no differences in the above- or belowground variables that were attributable to mowing. It is possible that differences may develop over time or that mowing one time in the fall of each year after a frost may not have any effect in either the above- or belowground variables included in this study.

Time was the only factor found to impact the belowground variables examined in this study. This result may not be very informative since roots are known to grow, develop, and senesce throughout the growing season. Perhaps more pertinent to the current study is the marked similarity in the patterns observed throughout the growing season for the belowground variables for the 0-0.45 m depth interval. Average root length, surface area, number of root tips, and number of branches each was shown to increase throughout the early growing season, peak

in mid-July (near the peak in aboveground biomass), and decline to the end of the growing season. While some researchers have detected multiple peaks in root biomass/production within a single growing season (Son and Hwang 2003) this study did not. Having only four samples from the single growing season, it was only possible to make inferences about relatively course-scale dynamics of the belowground aspects of the restored grassland plots. A future study with more frequent sampling intervals would be required to answer questions related to fine-scale dynamics such as daily or weekly fluctuations within the restored grassland plots.

## Concluding Remarks

- In general, the belowground aspects of the restored grasslands evaluated in this study were found to be unaffected by nitrogen and phosphorus additions. In contrast, the aboveground aspects – species richness and biomass – exhibited a response to nutrient treatments, especially to nitrogen additions.
- Repeated fertilization with nitrogen increased aboveground biomass while reducing the root to shoot ratio, indicating that smaller root systems were able to support an increase in biomass in the plots where nitrogen was readily available in this restored semi-arid grassland.
- Repeated nitrogen applications reduced species richness, thereby changing the structure and function of restored grassland plots. Repeated phosphorus applications did not alter species richness or aboveground biomass.
- None of the variables measured in this study was impacted by three years of annual fall mowing.

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# CHAPTER 3. ABOVE- AND BELOWGROUND EFFECTS OF NUTRIENT APPLICATIONS AND MOWING IN A RESTORED MESIC GRASSLAND


## Abstract

This study evaluated the effects of repeated nutrient applications and mowing on above- and belowground components of a restored tallgrass prairie community located in southeastern North Dakota. Thirty experimentally-planted plots received nitrogen or phosphorus applications at a high (200 kg/ha·yr for nitrogen or 40 kg/ha·yr for phosphorus) or low (20 kg/ha·yr for nitrogen or 4 kg/ha·yr for phosphorus) rate for more than 10 years. In addition, half of each plot was mowed annually in the fall for four years. There were no differences in root length, surface area, number of root tips, or number of root branches due to the annual fall mowing. However, aboveground biomass was higher in the mowed sub-plots ($\overline{x}_M$ = 530 g/m$^2$, SE = 39.6) than control sub-plots ($\overline{x}_C$ = 485 g/m$^2$, SE = 67.2). Average species richness was lower in the plots that received repeated nitrogen applications ($\overline{x}_N$ = 4.46, SE = 0.54) than those that received repeated phosphorus applications ($\overline{x}_P$ = 5.66, SE = 0.42). Species richness was lower in the plots that received the high application rates ($\overline{x}_H$ = 4.30, SE = 0.45) than those that received the low application rates ($\overline{x}_L$ = 5.90, SE = 0.48). Root growth was highest in the plots that received phosphorus additions at the low rate (4 kg/ha·yr) and in those that received nitrogen additions at the high rate (200 kg/ha·yr). There were different responses in the root variables throughout the growing season as a function of nutrient type and application rate.

## Introduction

Tallgrass prairies are known for their extensive belowground production (Stanton 1988; Hui and Jackson 2006). Hui and Jackson (2006) determined the mean fraction of belowground net primary production to be 70-74% for humid temperate grasslands. Stanton (1988) determined belowground production to make up 48-64% of annual net primary production in North American tallgrass prairies. Although a significant portion of the annual production within tallgrass prairie ecosystems occurs below the soil surface, most ecological research focuses on the aboveground portion. This is likely due to the inherent difficulties of studying the belowground portions of grasslands rather than a lack of recognition of the importance of the belowground portion. Further complicating matters, research on root systems has yielded varied and often conflicting results when root production and dynamics have been evaluated (Eissenstat and Yanai 2002; Reich 2002).

Mowing has a history of use as a management strategy in restored and native grasslands (Van Dyke et al. 2004; Rowe 2010; Holechek et al. 2011). In some instances, mowing is used to simulate the effects of grazing or burning, both major components of historic tallgrass prairie ecosystems. Research on the effects of grazing and/or mowing on aboveground production in grassland systems has generated mixed results – varying from no impact to increases or even decreases (Biondini et al. 1998; Pucheta et al. 1998; Pucheta et al. 2004). Similarly, studies focused belowground have reached varied conclusions about the effects of mowing or grazing in grasslands (Pucheta et al. 2004; Gao et al. 2008). In addition, studies evaluating the belowground impacts of nutrient applications in grasslands have also produced mixed results – i.e. decreases, no impact, or increases following fertilizer applications have all been reported (Cahill 1999; Son and Hwang 2003; Gregory 2006; Ladwig et al. 2011; Yavitt et al. 2011).

This study examined the above- and belowground aspects of restored grassland plots located in southeastern North Dakota using a combination of methods. Visual surveys and clipping methods were employed to evaluate the aboveground features of the restored plant community. A minirhizotron system was used to explore the belowground features.

The specific objectives of this study included:

- Combining above- and belowground sampling methods to provide a comprehensive evaluation of restored grassland plant communities in the tallgrass prairie ecoregion of North Dakota,
- An evaluation of the above- and belowground responses within restored grassland plant communities to annual nitrogen (200 kg/ha·yr or 20 kg/ha·yr) or phosphorus (40 kg/ha·yr or 4 kg/ha·yr) additions, and
- An examination of the above- and/or belowground effects of annual fall mowing with biomass removal on restored tallgrass prairie plant communities.

## Methods

### Study Site Description

This study was conducted at the Albert Ekre Grassland Preserve (Ekre) located in the northern tallgrass prairie region of southeastern North Dakota (Barker and Whitman 1989). The Ekre Preserve is located near the city of Walcott in Richland County, North Dakota (46° 33' 14.148"N, 97° 8' 0.336"W). The 30-year average annual temperature at Ekre is 3.33 °C and the 30-year average annual precipitation is 53.4 cm (USDA 2011). The soils for the experimental plots are mapped as Mantador-Delamere-Wyndmere fine sandy loams, slightly saline, 0-2% slopes (USDA 2015). Mantador soils are Coarse-loamy, mixed, surperactive, frigid Aquic Pachic Hapludolls; Delamere soils are Coarse-loamy, mixed, surperactive, frigid Typic

Endoaquolls; and Wyndmere soils are Coarse-loamy, mixed, surperactive, frigid Aeric Calciaquolls. The Mantador-Delamere-Wyndmere soil complex is somewhat poorly drained.

Prior to the establishment of the experimental plots, the area had been planted in a corn and soybean rotation (Biondini 2007). In 1997 and 1998, the area was disked and treated with glyphosate (Roundup, Monsanto St. Louis, MO, USA) in order to reduce the seedbank thereby enhancing the establishment of the seeded species. The area adjacent to the plots has been continually planted with various crops since their establishment in 1999 and was planted with soybeans or alfalfa for the years of this study.

**Experimental Design**

The original restoration experiment at Ekre consisted of 400 experimental plots (Biondini 2007). Each plot was 9 $m^2$ (3 m x 3 m) and separated by a 1 m buffer. The plots were organized as a completely randomized factorial design with three factors (Biondini 2007; Biondini et al. 2011). Factor one (nutrient type) had two levels: nitrogen or phosphorus application. Factor two (application rate) had two levels: high application rate (200 kg/ha·yr for nitrogen or 40 kg/ha·yr for phosphorus) or low application rate (20 kg/ha·yr for nitrogen or 4 kg/ha·yr for phosphorus). Sierra© slow release fertilizer prills (Pursell Technologies, Inc., Sylacauga, AL, USA) were applied annually in the spring of each year. Factor three (species richness) had five levels: one species, two species, five species, 10 species, or 20 species. Species were randomly assigned to each replication from the list of species included in Appendix D (Table D.1).

The majority of the plots were planted in the fall of 1998 while certain plots were planted in the spring of 1999. Each plot was seeded at a rate of 400 live seeds/$m^2$ divided among species. For example, if 20 species were to be planted within a plot, the seeding rate for each species was 20 live seeds/$m^2$. The plots were burned once in the fall of 2007, a common

management practice within the region (Biondini et al. 2011). In the fall of 2008, a mowing experiment was superimposed on the original restoration experiment. At that time, each plot was split into a no harvest/control (continuation of the current treatment) portion and a yearly fall harvest portion. The mowed portions of the split-plots were harvested after a killing frost (Mulkey et al. 2006) using a sickle bar mower at a cutting height of 10-15 cm and a landscape rake pulled behind an ATV.

In the fall of 2010, 60 minirhizotron tubes were installed within a subset of the experimental plots. Plots were selected based on species richness and biomass data collected within the plots. Appendix D (Table D.2) contains information about the plots selected for minirhizotron tube installation. Two minirhizotron tubes were installed in each plot in order to examine the effects of the mowing treatment – one in each of the mowed and un-mowed sub-plots.

Minirhizotron tubes were installed to a depth of approximately 1 m using a trailer-mounted Amity 9800E sampler (Amity Technology, Fargo, ND, USA). Tubes were installed at a 45° angle in order to observe root growth at multiple depths and to discourage roots from growing along the tube (Johnson et al. 2001). The tubes consisted of clear acrylic plastic (Crown Plastics Inc., Plymouth, MN, USA) approximately 2 m in length. Each tube had a rubber stopper (VWR International, Radnor, PA, USA) and vinyl cap (Caplugs, Buffalo, NY, USA) glued onto the belowground end and a removable rubber stopper and vinyl cap on the aboveground end. Each tube was insulated with removable foam pipe insulation (Armacell, Mebane, NC, USA or ITP, Brampton, ON, Canada) in order to reduce the effects of the tube on local soil temperatures (Tingey et al. 2003; Phillips et al. 2006). The aboveground portion of each tube was first painted black (Rust-Oleum, Vernon Hills, IL, USA) in order to reduce light penetration to the soil at

depth and then white (Rust-Oleum, Vernon Hills, IL, USA) in order to reduce the heating effects from the sun (Box et al. 1989; Johnson et al. 2001; Tingey et al. 2003; Phillips et al. 2006).

**Sampling Method**

Species richness and aboveground production surveys began in 2000 (Biondini 2007; Biondini et al 2011; DiAllesandro et al. 2013). Since this study was intended to examine the impacts of mowing and nutrient applications on roots and shoots, only the data obtained after the mowing treatments were initiated for the plots in which minirhizotron tubes were installed were included in the current analysis. Estimates of aboveground biomass and species richness were obtained from clipping and visual surveys conducted in 2011. Aboveground biomass was estimated by clipping two quadrats (0.4 m x 0.4 m) within each sub-plot. Quadrats were randomly located within the sub-plots in areas that were not clipped the previous growing season. Clipping was timed to coincide with peak aboveground biomass. Biomass was separated by species, oven-dried, and weighed (Biondini et al. 2011). Species richness was determined annually by surveying the entire area of each sub-plot.

Although the minirhizotron tubes were installed in the fall of 2010, belowground data collection did not begin until the spring of 2012 in order to allow plenty of time for the establishment of roots in the soil adjacent to the minirhizotron tubes. Minirhizotron images were collected four times throughout the growing season of 2012, timed in a manner intended to provide a thorough examination of the root systems present. The first minirhizotron sampling session was completed in early-May in order to coincide with early season root growth. The second occurred in late June/early July and was intended to capture early growth of warm-season species. The third occurred in late-July and was timed to coincide with peak aboveground

biomass. The fourth occurred in late October/early November, coinciding with the end of the growing season.

Images were collected using the CI-600 In-Situ Root Imager (CID Bio-Science, Camas, WA, USA) which rotated along the length of the tube to provide a $360^{o}$ image (400 dpi). Images were collected from the soil surface to the maximum depth allowed by the equipment. For the current study, only the 0-0.45 m depth interval was included since the vast majority of root growth occurs within this interval (Dahlman and Kucera 1965; Jackson et al. 1996; Sun et al. 1997; Gregory 2006; Mueller et al. 2013). Minirhizotron images were processed using WinRhizo Tron MF software (2014 Regent Instruments, Quebec, Canada), in which a trained user identified roots within the image space while the program performed measurements and calculations of several features including root length, surface area, number of root tips, and number of root branches (Figure 3.1).

**Statistical Analysis**

Root length, root surface area, number of root tips, and number of branches were analyzed using a split-plot design with repeated measures for the 0-0.45 m depth interval. These data were transformed prior to analysis in order to help satisfy the distributional assumptions of linear statistical methods (McCune and Grace 2002; Quinn and Keough 2002). A log transformation was applied to root length and surface area estimates. A square root transformation was applied to the number of root tips and number of root branches. Statistical analyses were conducted in SAS 9.4 (SAS Institute, Cary, NC, USA). Nutrient type (nitrogen or phosphorus) and nutrient application rate (high or low) were whole-plot factors while mowing treatment (mowed or un-mowed) was the split-plot factor, with all interaction terms included.


Figure 3.1. Example of image processing using WinRhizo Tron MF software (Ekre, low phosphorus plot). A) Unprocessed images obtained for the 0-0.45 m depth interval. B) Processed images wherein a trained user identified the individual roots.

Time was the repeated factor (interaction terms included). The covariance structure was specified as first-order autoregressive in recognition that observations collected during adjacent sampling sessions were expected to be more highly correlated than observations collected at longer time intervals (Littell et al. 2006). Degrees of freedom calculations employed the Kenward-Rogers (first-order) procedure since it was appropriate for use with both the repeated measures and correlated errors of the split-plot design (Littell et al. 2006). A residual (restricted) maximum likelihood estimation method was used. Least squares estimates of population means were calculated for statistically significant effects and pair-wise comparisons among treatment populations were made. P values were corrected for multiple comparisons using the Bonferroni adjustment (SAS Institute Inc. 2008).

Root length and volume estimates obtained from WinRhizo were used to calculate root growth and the ratio of root volume to average aboveground biomass. Root growth was estimated by subtracting the initial root length from the maximum root length observed for each plot, then dividing by the initial root length.

$$G = (l_m - l_i) \div l_i$$

Where $G$ is root growth, $l_m$ is the maximum root length detected for all sampling sessions, and $l_i$ is the initial root length (measured in mid-May). The ratio of root volume to aboveground biomass was calculated using root volume (obtained at peak aboveground biomass) and peak aboveground biomass estimates (root volume ÷ aboveground biomass).

Root growth, aboveground biomass, species richness, and the ratio of root volume to aboveground biomass were analyzed using a split-plot design. In these instances, nutrient type (nitrogen or phosphorus) and nutrient application rate (high or low) were treated as whole-plot factors while the mowing treatment (mowed or un-mowed) was treated as a split-plot factor, with

all interaction terms included. A log transformation was applied to the aboveground biomass estimates and the ratio of root volume to aboveground biomass, while a square root transformation was applied to the average species richness estimates. A residual (restricted) maximum likelihood estimation method was specified. Degrees of freedom calculations employed the Satterthwaite approximation since it was appropriate for use with the split-plot design (Schabenberger and Pierce 2002; Littell et al. 2006). P values were corrected for multiple comparisons using the Bonferroni adjustment (SAS Institute Inc. 2008).

Regression analysis was used to examine the relationships between above- and belowground variables. Maximum root length, maximum root surface area, and root growth estimates were regressed against species richness, forb richness, and grass richness in order to determine if linear relationships existed ($p < 0.05$). Where linear relationships existed, regression coefficients were compared among treatment groups (nutrient type x application rate) in order to determine if there were differences ($p < 0.05$) in the slope parameters.

## Results

The annual fall mowing treatment did not have an effect on the average root length, surface area, number of root tips, or number of root branches for the 0-0.45 m depth interval (Tables 3.1-3.4). Conversely, time and the three-way interaction among time, nutrient type, and nutrient application rate (time x nutrient type x application rate) were found to affect ($p < 0.05$) average root length, surface area, number of root tips, and number of branches for the 0-0.45 m depth interval. Nutrient type (nitrogen or phosphorus) affected the number of branches while the two-way interaction of nutrient type and application rate (nutrient type x application rate) affected the number of root tips and number of branches (Tables 3.3 and 3.4).

Table 3.1. Repeated measures ANOVA for average root length by treatment and interaction at the Ekre Preserve (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient Type | 1 | 52 | 1.27 | 0.26 |
| Rate of Application | 1 | 52 | 2.18 | 0.15 |
| Mow | 1 | 52 | 0.00 | 0.98 |
| Sampling Session | 3 | 110 | 204 | <0.01 |
| Nutrient Type * Rate | 1 | 52 | 1.49 | 0.23 |
| Nutrient Type * Mow | 1 | 52 | 0.02 | 0.88 |
| Nutrient Type * Session | 3 | 110 | 0.51 | 0.68 |
| Rate * Mow | 1 | 52 | 0.03 | 0.86 |
| Rate * Session | 3 | 110 | 0.49 | 0.69 |
| Mow*Session | 3 | 110 | 0.40 | 0.75 |
| Nutrient * Rate * Mow | 1 | 52 | 0.31 | 0.58 |
| Nutrient * Rate * Session | 3 | 110 | 17.3 | <0.01 |
| Nutrient * Mow * Session | 3 | 110 | 0.29 | 0.83 |
| Rate * Mow * Session | 3 | 110 | 0.56 | 0.64 |
| Nutrient * Rate * Mow * Session | 3 | 110 | 0.57 | 0.64 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Kenward-Rogers first-order method)

Table 3.2. Repeated measures ANOVA for average root surface area by treatment and interaction at the Ekre Preserve (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient Type | 1 | 52 | 1.11 | 0.30 |
| Rate of Application | 1 | 52 | 1.47 | 0.23 |
| Mow | 1 | 52 | 0.01 | 0.94 |
| Sampling Session | 3 | 111 | 80.6 | <0.01 |
| Nutrient Type * Rate | 1 | 52 | 1.10 | 0.30 |
| Nutrient Type * Mow | 1 | 52 | 0.06 | 0.80 |
| Nutrient Type * Session | 3 | 111 | 1.28 | 0.28 |
| Rate * Mow | 1 | 52 | 0.12 | 0.73 |
| Rate * Session | 3 | 111 | 1.66 | 0.18 |
| Mow*Session | 3 | 111 | 0.04 | 0.99 |
| Nutrient * Rate * Mow | 1 | 52 | 0.14 | 0.71 |
| Nutrient * Rate * Session | 3 | 111 | 15.6 | <0.01 |
| Nutrient * Mow * Session | 3 | 111 | 0.11 | 0.96 |
| Rate * Mow * Session | 3 | 111 | 0.35 | 0.79 |
| Nutrient * Rate * Mow * Session | 3 | 111 | 0.51 | 0.68 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Kenward-Rogers first-order method)

Table 3.3. Repeated measures ANOVA for average number of root tips by treatment and interaction at the Ekre Preserve (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient Type | 1 | 52 | 2.47 | 0.12 |
| Rate of Application | 1 | 52 | 0.59 | 0.45 |
| Mow | 1 | 52 | 0.31 | 0.58 |
| Sampling Session | 3 | 111 | 211 | <0.01 |
| Nutrient Type * Rate | 1 | 52 | 4.83 | 0.03 |
| Nutrient Type * Mow | 1 | 52 | 0.00 | 0.99 |
| Nutrient Type * Session | **3** | 111 | 1.66 | 0.18 |
| Rate * Mow | 1 | 52 | 0.00 | 0.96 |
| Rate * Session | 3 | 111 | 0.23 | 0.88 |
| Mow*Session | 3 | 111 | 0.67 | 0.58 |
| Nutrient * Rate * Mow | 1 | 52 | 0.32 | 0.57 |
| Nutrient * Rate * Session | 3 | 111 | 17.2 | <0.01 |
| Nutrient * Mow * Session | 3 | 111 | 0.36 | 0.78 |
| Rate * Mow * Session | 3 | 111 | 0.89 | 0.45 |
| Nutrient * Rate * Mow * Session | 3 | 111 | 0.43 | 0.73 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Kenward-Rogers first-order method)

Table 3.4. Repeated measures ANOVA for average number of root branches tips by treatment and interaction at the Ekre Preserve (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient Type | 1 | 55.6 | 5.65 | 0.02 |
| Rate of Application | 1 | 55.6 | 0.86 | 0.36 |
| Mow | 1 | 55.6 | 0.17 | 0.69 |
| Sampling Session | 3 | 155 | 115 | <0.01 |
| Nutrient Type * Rate | 1 | 55.6 | 4.44 | 0.04 |
| Nutrient Type * Mow | 1 | 55.6 | 0.09 | 0.76 |
| Nutrient Type * Session | 3 | 155 | 0.45 | 0.72 |
| Rate * Mow | 1 | 55.6 | 0.02 | 0.88 |
| Rate * Session | 3 | 155 | 0.14 | 0.93 |
| Mow*Session | 3 | 155 | 0.16 | 0.93 |
| Nutrient * Rate * Mow | 1 | 55.6 | 0.25 | 0.62 |
| Nutrient * Rate * Session | 3 | 155 | 18.2 | <0.01 |
| Nutrient * Mow * Session | 3 | 155 | 0.59 | 0.62 |
| Rate * Mow * Session | 3 | 155 | 1.31 | 0.27 |
| Nutrient * Rate * Mow * Session | 3 | 155 | 0.35 | 0.79 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Kenward-Rogers first-order method)

Pair-wise comparisons were conducted among least squares means estimates across the entire growing season for average root length, surface area, number of root tips, and number of branches. Average root length in the first sampling session ($\overline{x}_1$ = 790 cm, SE = 41.8) was lower than the second ($\overline{x}_2$ = 1371 cm, SE = 84.2, p <0.01), third ($\overline{x}_3$ = 1432 cm, SE = 76.6, p <0.01), and fourth ($\overline{x}_4$ = 1355 cm, SE = 83.6, p <0.01) sampling sessions. There were no differences in root length between sampling sessions throughout the growing season (Figure 3.2). Average root surface area in the first sampling session ($\overline{x}_1$ = 109 cm$^2$, SE = 5.95) was lower than the second ($\overline{x}_2$ = 159 cm$^2$, SE = 10.1, p <0.01), third ($\overline{x}_3$ = 164 cm$^2$, SE = 9.11, p <0.01), and fourth ($\overline{x}_4$ = 153 cm$^2$, SE = 9.37, p <0.01) sampling sessions. There were no other differences in root surface area between sampling sessions throughout the growing season (Figure 3.3).

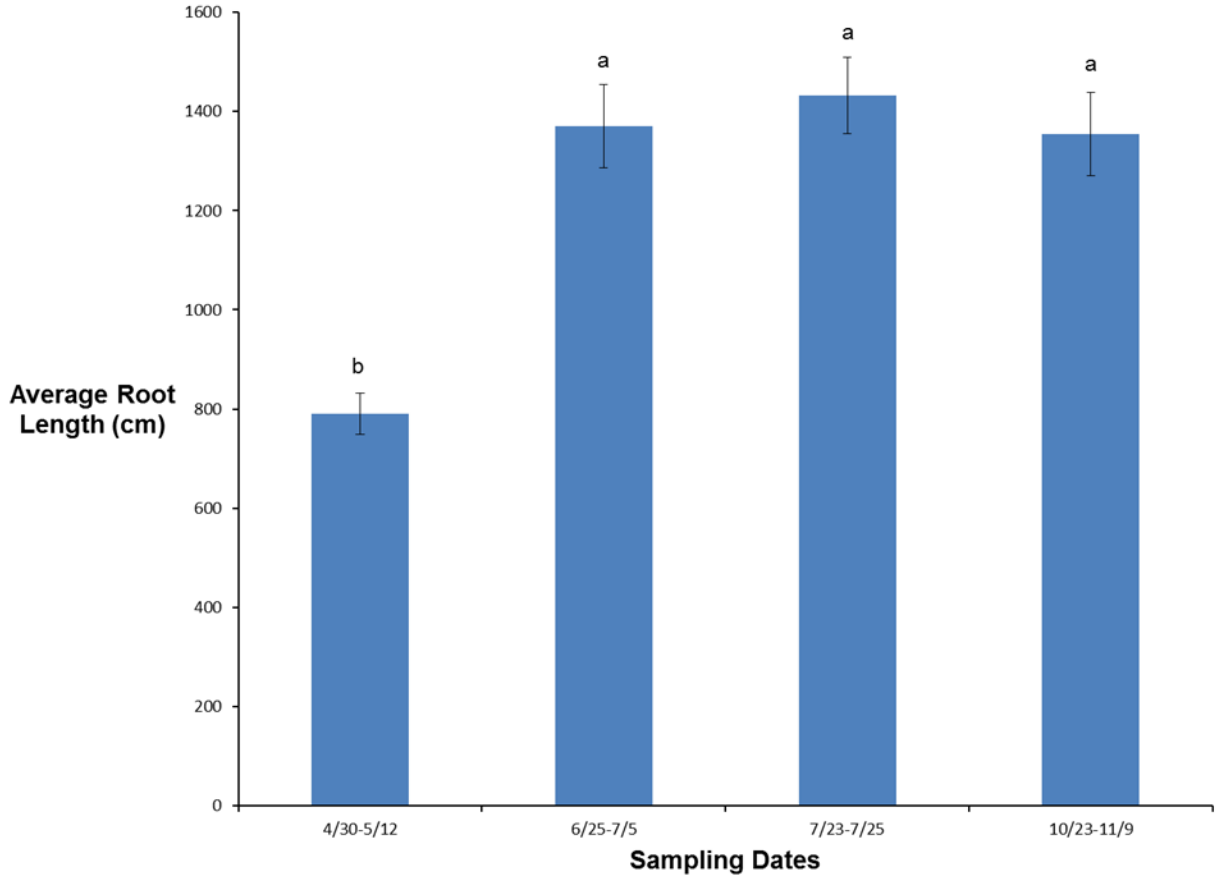


Figure 3.2. Average root length by sampling session for the Ekre Preserve ± SE. Significant differences indicated by different letters (p < 0.05).

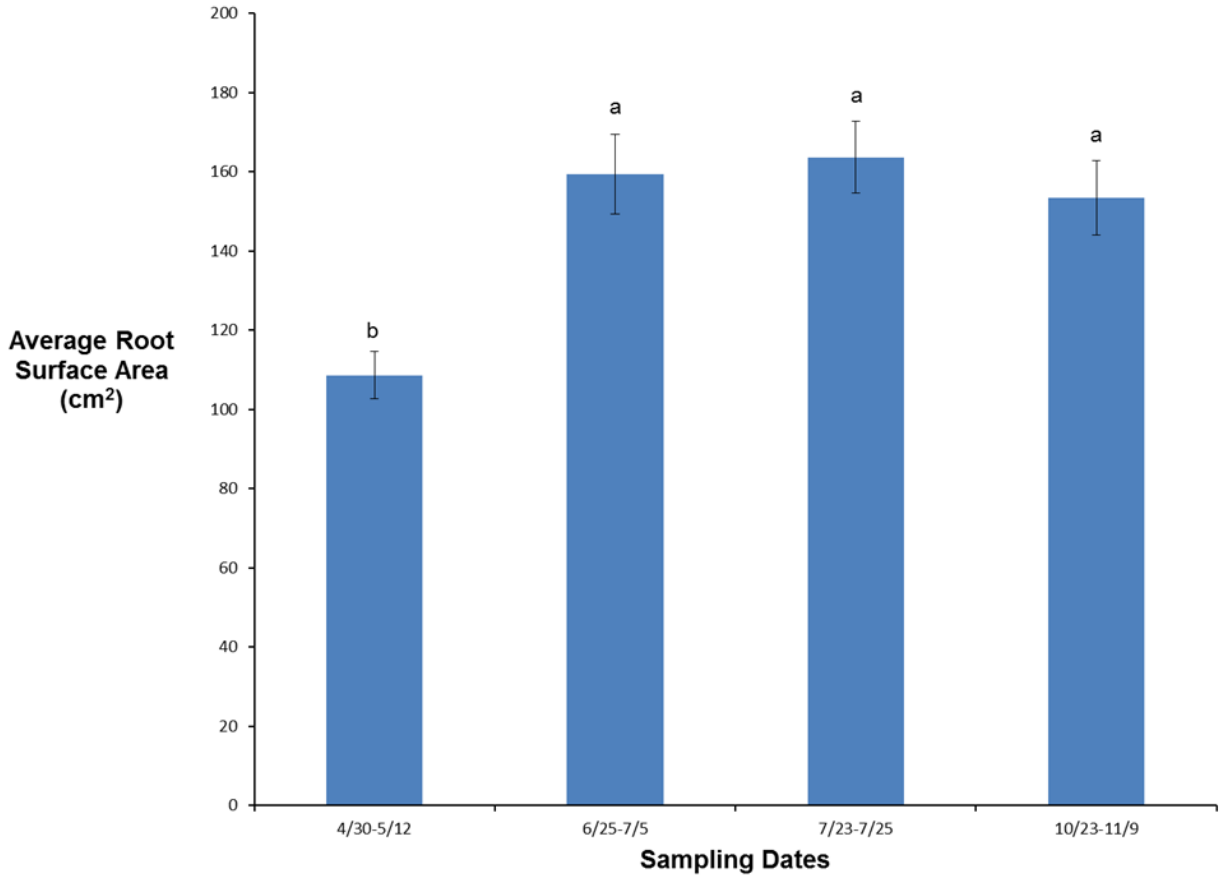


Figure 3.3. Average root surface area by sampling session for the Ekre Preserve ± SE. Significant differences indicated by different letters ($p < 0.05$).

The average number of root tips was lower in the first sampling session ($\overline{x}_1$ = 377, SE = 21.3) than the second ($\overline{x}_2$ = 873, SE = 59.6, $p < 0.01$), third ($\overline{x}_3$ = 943, SE = 52.4, $p < 0.01$), and fourth ($\overline{x}_4$ = 897, SE = 59.3, $p < 0.01$) sampling sessions. There were no differences in the average number of root tips between sampling sessions throughout the growing season (Figure 3.4). The average number of root branches followed a similar pattern, with fewer branches present in the first sampling session ($\overline{x}_1$ = 123, SE = 10.72 than the second ($\overline{x}_2$ = 360, SE = 32.4, $p < 0.01$), third ($\overline{x}_3$ = 386, SE = 30.0, $p < 0.01$), and fourth ($\overline{x}_4$ = 341, SE = 31.0, $p < 0.01$) sampling sessions (Figure 3.5). However, there were fewer branches in the fourth sampling session than in the third ($p = 0.01$).

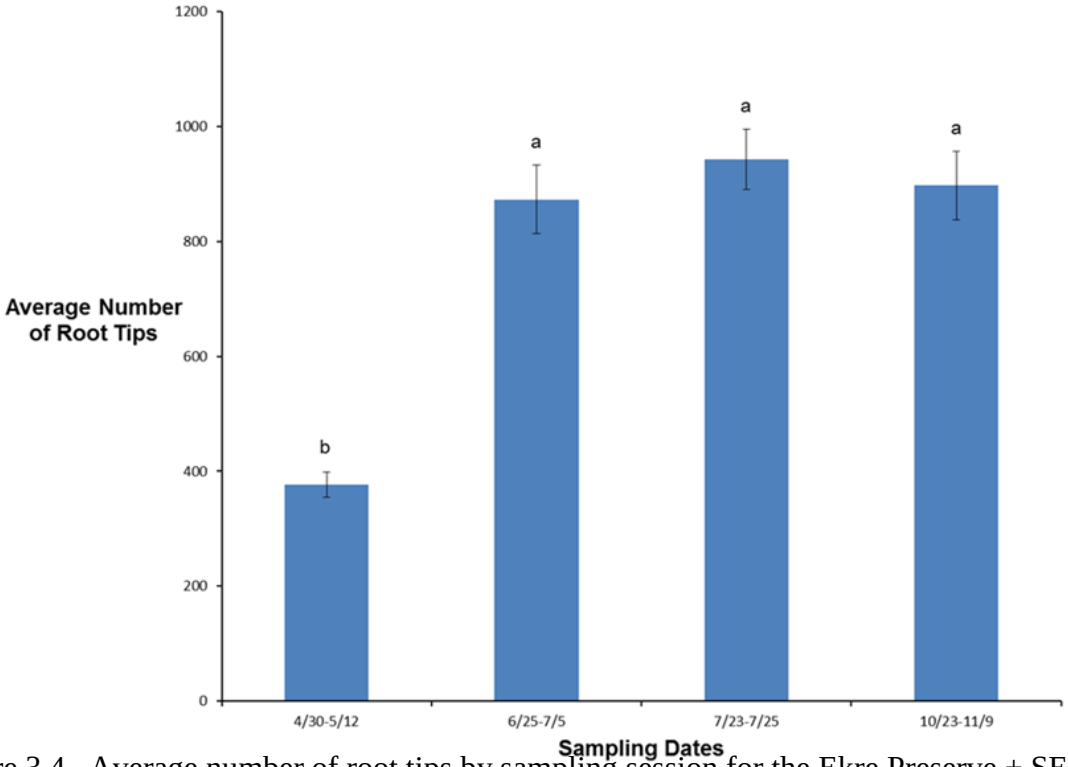


Figure 3.4. Average number of root tips by sampling session for the Ekre Preserve ± SE. Significant differences indicated by different letters ($p < 0.05$).

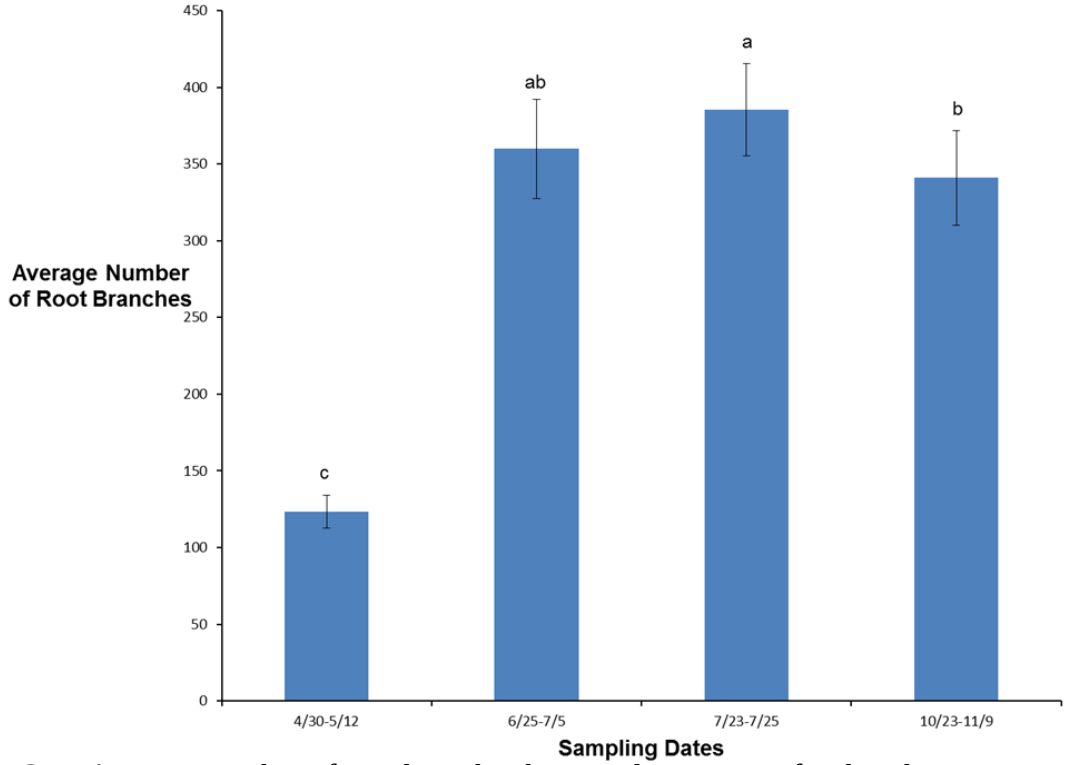


Figure 3.5. Average number of root branches by sampling session for the Ekre Preserve ± SE. Significant differences indicated by different letters ($p < 0.05$).

In addition, pair-wise comparisons were conducted among least squares means estimates for the average number of root tips and average number of branches (Figures 3.6 and 3.7). The average number of root branches in the plots repeatedly fertilized with nitrogen ($\overline{x}_N$ = 682, SE = 35.6) was lower (p = 0.02) than the number of branches present in the plots repeatedly fertilized with phosphorus ($\overline{x}_P$ = 851, SE = 44.1). There were differences in the number of branches among the groups defined by nutrient type and rate of nutrient application, with fewer (p = 0.01) branches in the plots that received repeated applications of nitrogen at the low rate ($\overline{x}_{NL}$ = 193, SE = 16.1) than the plots receiving phosphorus at the low application rates ($\overline{x}_{PL}$ = 391, SE = 39.6) (Figure 3.7).

A three-way interaction occurred among time, nutrient type, and nutrient application rate (time x nutrient type x application rate) for average root length, surface area, number of root tips, and number of branches for the 0-0.45 m depth interval (Tables 3.1-3.4, Figures 3.8-3.11). While there were differences in both the average root length and average surface area between sampling sessions, there were no differences within a single sampling session for either throughout the growing season (Figures 3.8 and 3.9). Similarly, there were no differences in the average number of root tips or branches within the first sampling session, conducted in late April/early May (Figures 3.10 and 3.11).

The average number of root tips was greater in the low phosphorus plots than in the low nitrogen plots for all sampling sessions, except the first (Figure 3.10). In late June/early July, the average number of root tips in the low nitrogen plots ($\overline{x}_{NL}$ = 639, SE = 84.1) was less (p < 0.01) than in the low phosphorus plots ($\overline{x}_{PL}$ = 1126, SE = 145). The same pattern was found in late July ($\overline{x}_{NL}$ = 723, SE = 66.4 vs. $\overline{x}_{PL}$ = 1161, SE = 145, p = 0.02), and at the end of the growing season ($\overline{x}_{NL}$ = 650, SE = 69.8 vs. $\overline{x}_{PL}$ = 1129, SE = 185, p = 0.01).

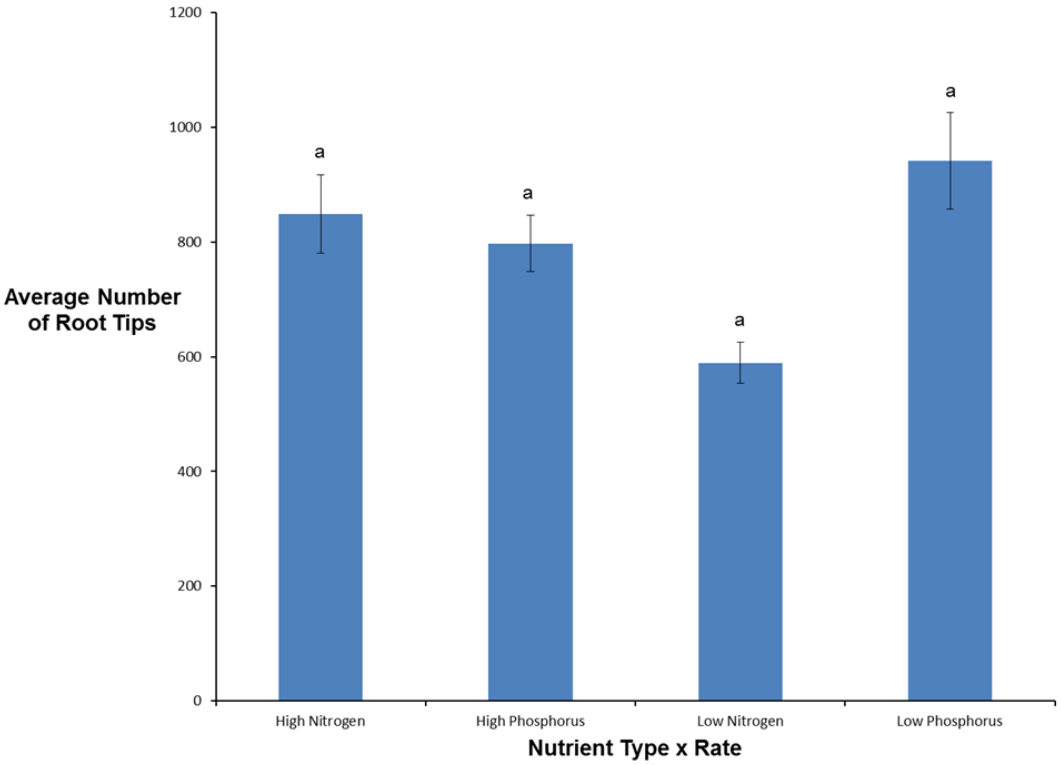


Figure 3.6. Average number of root tips by nutrient type x rate for the entire growing season at the Ekre Preserve ± SE. Significant differences indicated by different letters ($p < 0.05$).

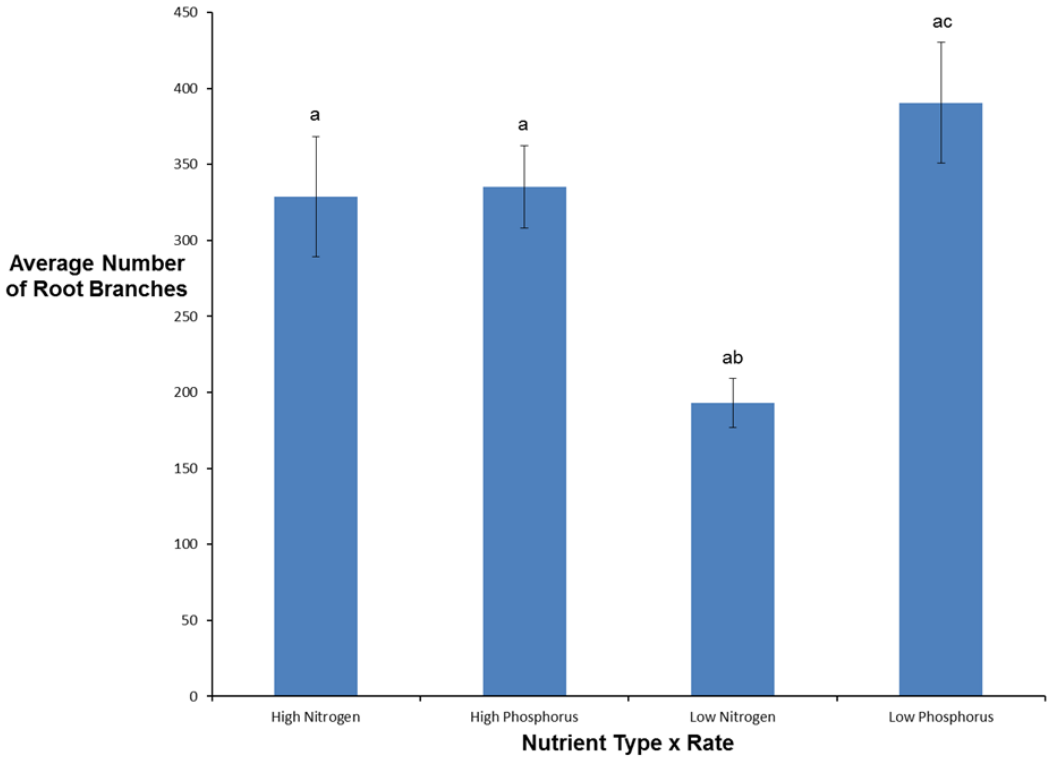


Figure 3.7. Average number of root branches by nutrient type x rate for the entire growing season at the Ekre Preserve ± SE. Significant differences indicated by different letters ($p < 0.05$).

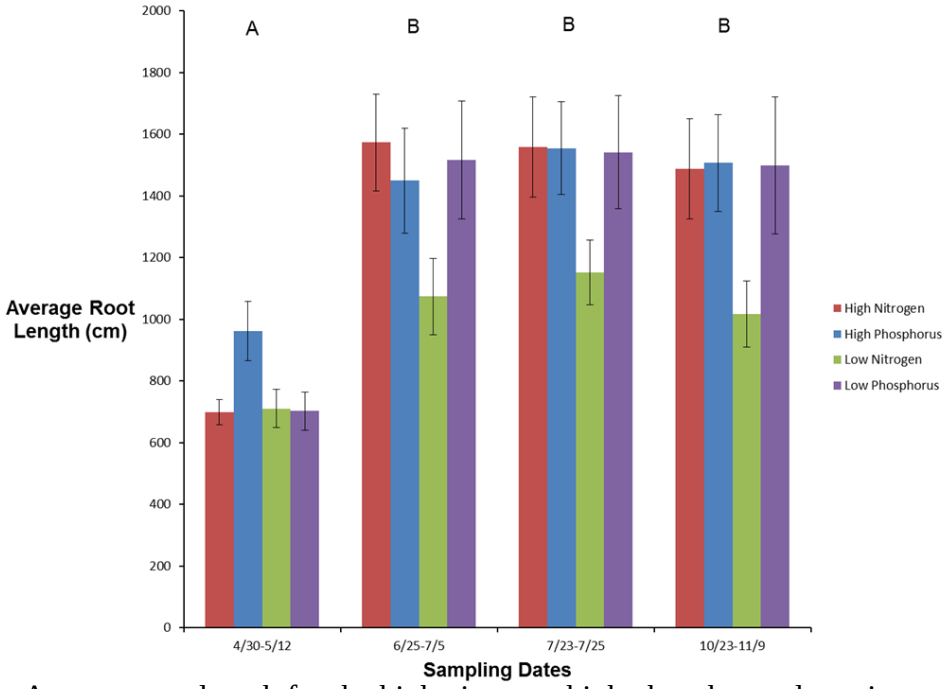


Figure 3.8. Average root length for the high nitrogen, high phosphorus, low nitrogen, and low phosphorus treatments at the Ekre Preserve ± SE. Significant differences between sampling sessions are indicated by different letters ($p < 0.05$). There were no significant differences in average root length within a sampling session.

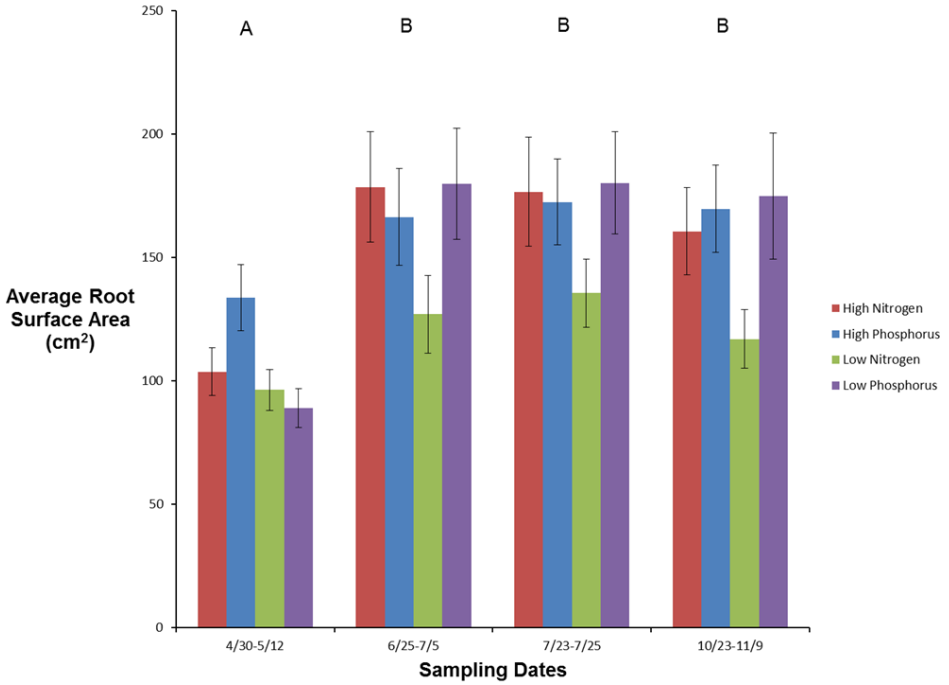


Figure 3.9. Average root surface area for the high nitrogen, high phosphorus, low nitrogen, and low phosphorus treatments at the Ekre Preserve ± SE. Significant differences between sampling sessions are indicated by different letters ($p < 0.05$). There were no significant differences in average root surface area within a sampling session.

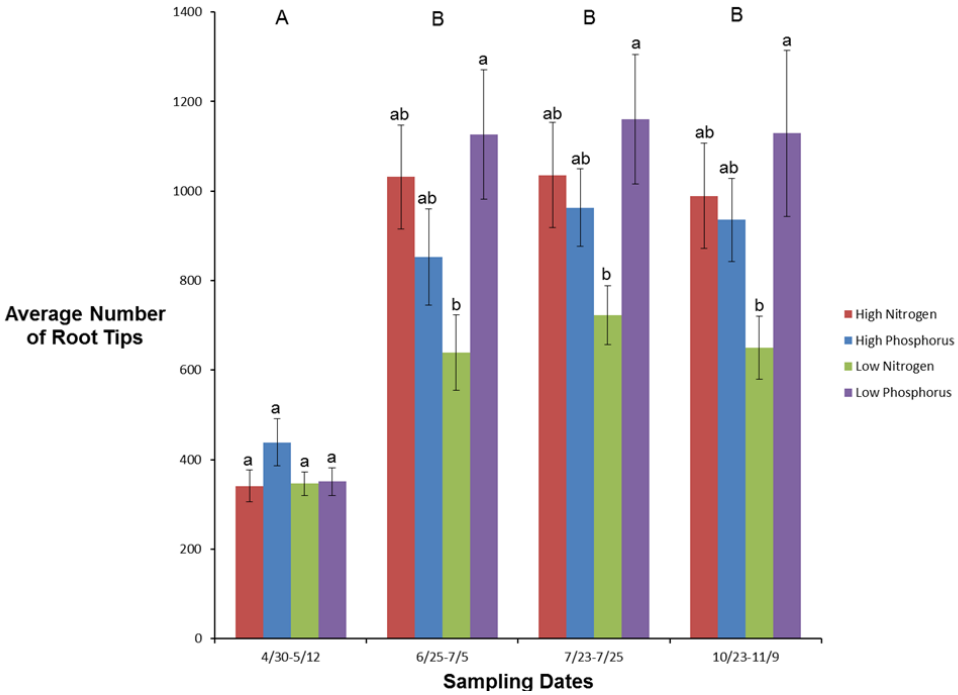


Figure 3.10. Average number of root tips for the high nitrogen, high phosphorus, low nitrogen, and low phosphorus treatments at the Ekre Preserve ± SE. Significant differences between sampling sessions are indicated by different uppercase letters ($p < 0.05$). Significant differences within a sampling session are indicated by different lowercase letters ($p < 0.05$).

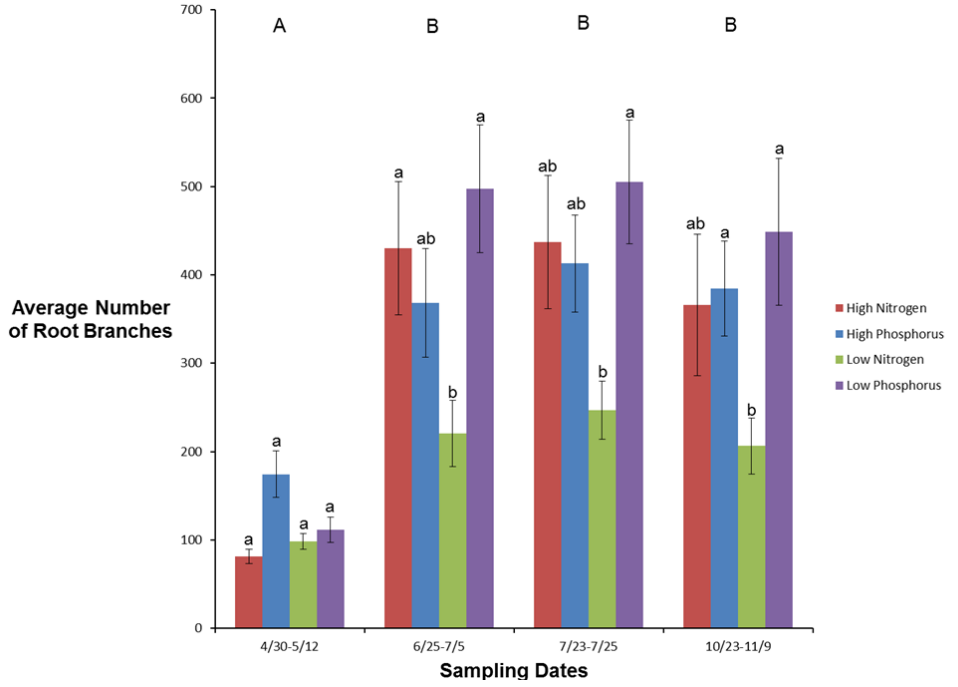


Figure 3.11. Average number of root branches for the high nitrogen, high phosphorus, low nitrogen, and low phosphorus treatments at the Ekre Preserve ± SE. Significant differences between sampling sessions are indicated by different uppercase letters ($p < 0.05$). Significant differences within a sampling session are indicated by different lowercase letters ($p < 0.05$).

The average number of root branches was greater in the low phosphorus plots than the low nitrogen plots for all sampling sessions except the first (Figure 3.11). In late June/early July, there was as average of 220 (SE = 37.5) branches in the low nitrogen plots compared to 497 (SE = 72.3) in the low phosphorus plots ($p < 0.01$). In late July, the average number of root branches in the low nitrogen plots ($\overline{x}_{NL}$ = 413, SE = 32.9) was less ($p < 0.01$) than in the low phosphorus plots ($\overline{x}_{PL}$ = 505, SE = 70.0). At the end of the growing season, the average number of root branches in the low nitrogen plots ($\overline{x}_{NL}$ = 206, SE = 31.6) was lower ($p = 0.01$) than in the low phosphorus plots ($\overline{x}_{PL}$ = 449, SE = 83.0). There were also more ($p = 0.02$) root branches in the high nitrogen ($\overline{x}_{NH}$ = 430, SE = 75.5) plots than in the low nitrogen ($\overline{x}_{NL}$ = 220, SE = 37.5) plots in the late June/early July sampling session. At the end of the growing season, there were more ($p = 0.02$) root branches in the high phosphorus ($\overline{x}_{PH}$ = 385, SE = 53.7) plots than in the low nitrogen ($\overline{x}_{NL}$ = 206, SE = 31.6) plots.

A two-way interaction occurred between nutrient type and application rate for root growth ($p < 0.01$) (Table 3.5). Root growth was greater in the plots that received repeated phosphorus applications at the low rate ($\overline{x}_{PL}$ = 1.31, SE = 0.14) than in those that received repeated phosphorus applications at the high rate ($\overline{x}_{PH}$ = 0.69, SE = 0.06, $p < 0.01$) or repeated nitrogen applications at the low rate ($\overline{x}_{NL}$ = 0.69, SE = 0.10, $p = 0.01$) (Figure 3.12). Root growth was greater in the plots that received nitrogen at the high rate ($\overline{x}_{NH}$ = 1.27, SE = 0.14) than in those that received phosphorus at the high rate ($\overline{x}_{PH}$ = 0.69, SE = 0.06, $p = 0.01$) and nitrogen at the low rate ($\overline{x}_{NL}$ = 0.69, SE = 0.10, $p = 0.02$) (Figure 3.12). No differences were detected in the ratio calculated between root volume and average aboveground biomass (Table 3.6).

Table 3.5. Analysis of Variance for root growth by treatment and interaction at the Ekre Preserve (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient Type | 1 | 26 | 0.03 | 0.87 |
| Rate of Application | 1 | 26 | 0.03 | 0.86 |
| Mow | 1 | 26 | 1.57 | 0.22 |
| Nutrient Type * Rate | 1 | 26 | 25.5 | <0.01 |
| Nutrient Type * Mow | 1 | 26 | 0.19 | 0.66 |
| Rate * Mow | 1 | 26 | 0.25 | 0.62 |
| Nutrient Type * Rate * Mow | 1 | 26 | 0.88 | 0.36 |

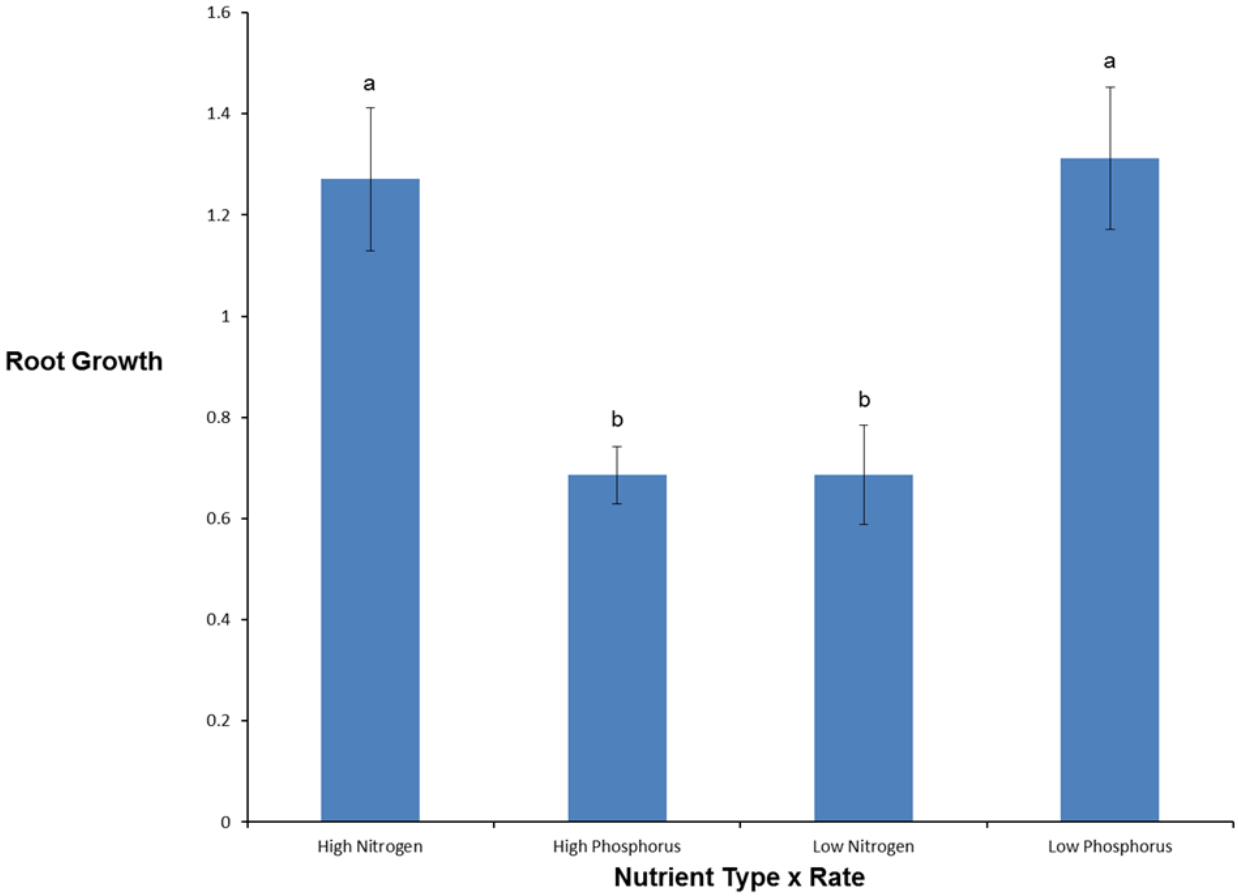


Figure 3.12. Root growth by nutrient type x rate at the Ekre Preserve ± SE. Significant differences indicated by different letters ($p < 0.05$).

Table 3.6. Analysis of Variance for the ratio of root volume (at peak aboveground biomass) to average aboveground biomass (harvested at peak biomass) by treatment and interaction at the Ekre Preserve (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient Type | 1 | 26 | 0.22 | 0.64 |
| Rate of Application | 1 | 26 | 0.08 | 0.78 |
| Mow | 1 | 26 | 1.24 | 0.28 |
| Nutrient Type * Rate | 1 | 26 | 0.31 | 0.58 |
| Nutrient Type * Mow | 1 | 26 | 0.05 | 0.83 |
| Rate * Mow | 1 | 26 | 0.06 | 0.80 |
| Nutrient Type * Rate * Mow | 1 | 26 | 0.06 | 0.81 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Average total biomass was the only above- or belowground variable affected by mowing ($p = 0.04$, Table 3.7). The average total biomass was higher in the plots that were mowed annually ($\overline{x}_M = 530$ g/m$^2$, SE = 39.6) than those that were not ($\overline{x}_C = 485$ g/m$^2$, SE = 67.2). Nutrient type, application rate, and the interaction of nutrient type and application rate all affected the average species richness (Table 3.8). The plots that received nitrogen additions at the high rate ($\overline{x}_{NH} = 2.10$, SE = 0.46) had lower average species richness than all other nutrient type and application rate combinations ($\overline{x}_{NL} = 5.78$, SE = 0.61, $p < 0.01$; $\overline{x}_{PH} = 5.40$, SE = 0.46, $p < 0.01$; $\overline{x}_{PL} = 6.08$, SE = 0.82, $p < 0.01$) (Figure 3.13).

Table 3.7. Analysis of Variance for average aboveground biomass, harvested at peak biomass by treatment and interaction at the Ekre Preserve (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient Type | 1 | 26 | 0.02 | 0.88 |
| Rate of Application | 1 | 26 | 0.76 | 0.39 |
| Mow | 1 | 26 | 4.49 | 0.04 |
| Nutrient Type * Rate | 1 | 26 | 0.29 | 0.59 |
| Nutrient Type * Mow | 1 | 26 | 1.79 | 0.19 |
| Rate * Mow | 1 | 26 | 1.69 | 0.20 |
| Nutrient Type * Rate * Mow | 1 | 26 | 0.73 | 0.40 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Table 3.8. Analysis of Variance for the average number of species by treatment and interaction at the Ekre Preserve (Type III estimation method).

| Effect | Num.DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient Type | 1 | 26 | 8.58 | 0.01 |
| Rate of Application | 1 | 26 | 11.5 | <0.01 |
| Mow | 1 | 26 | 1.49 | 0.23 |
| Nutrient Type * Rate | 1 | 26 | 6.67 | 0.02 |
| Nutrient Type * Mow | 1 | 26 | 0.71 | 0.41 |
| Rate * Mow | 1 | 26 | 0.37 | 0.55 |
| Nutrient Type * Rate * Mow | 1 | 26 | 0.26 | 0.61 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

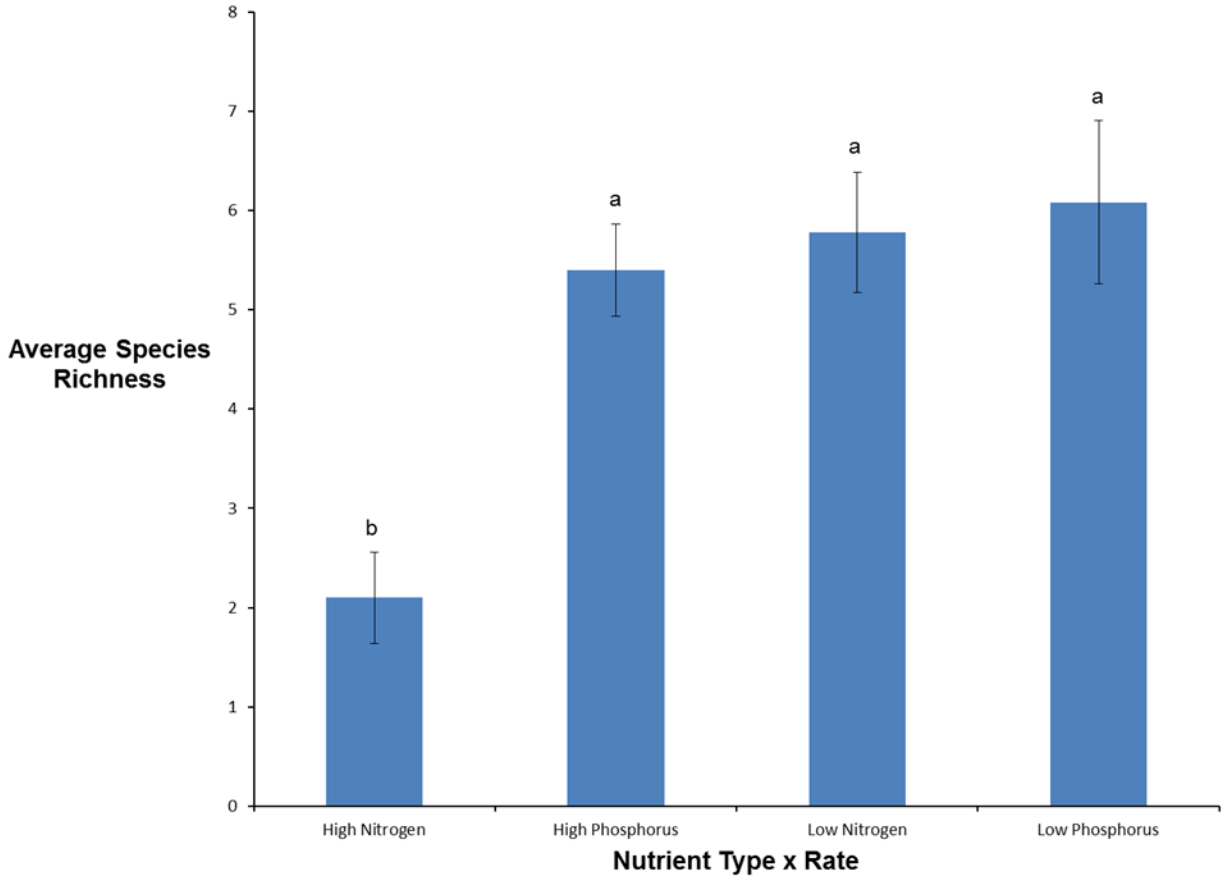


Figure 3.13. Average species richness by nutrient type x rate at the Ekre Preserve ± SE. Significant differences indicated by different letters ($p < 0.05$).

Regression analysis showed a weak inverse linear relationship between maximum root length with species richness and forb richness ($r^2 = 0.09$, $p = 0.02$; and $r^2 = 0.1$, $p = 0.03$

respectively). There was no relationship ($r^2 = 0.05$, $p = 0.09$) between maximum root length and grass richness. There were no differences in the regression coefficients for the relationships of maximum root length and species richness components among the nutrient treatments (defined by nutrient type and application rate) (Table 3.9). Maximum root surface area was weakly, inversely related to species richness ($r^2 = 0.08$, $p = 0.03$) and forb richness ($r^2 = 0.07$, $p = 0.04$) but not to grass richness ($r^2 = 0.04$, $p = 0.14$). There were no differences in the regression coefficients for the relationships of maximum root surface area and species richness components among the nutrient treatments defined by nutrient type and application rate (Table 3.10). Root growth was not related to the species richness ($r^2 = 0.05$, $p = 0.09$), forb richness ($r^2 = 0.04$, $p = 0.11$), or the grass richness ($r^2 = 0.02$, $p = 0.23$).

Table 3.9. Comparison of the regression coefficients (β) for maximum root length (cm) versus: species richness; forb richness; and grass richness at the Ekre Preserve. Upper and lower limits for the 95% confidence intervals are included.

| **Max Root Length vs.** | **Nutrient Treatment** | **Regression Coefficient** | | | **Slope comparison[3]** | |
|---|---|---|---|---|---|---|
| | | **Slope[1]** | **Lower CI[2]** | **Upper CI[2]** | **F value** | **p value** |
| **Species Richness** | High Nitrogen | 47.2 | -245 | 339 | 1.00 | 0.40 |
| | Low Nitrogen | -103 | -184 | -22.7 | | |
| | High Phosphorus | -151 | -295 | -5.75 | | |
| | Low Phosphorus | -25.7 | -205 | 154 | | |
| **Forb Richness** | High Nitrogen | 42.7 | -459 | 544 | 1.10 | 0.36 |
| | Low Nitrogen | -125 | -241 | -9.13 | | |
| | High Phosphorus | -172 | -339 | -5.39 | | |
| | Low Phosphorus | -1.27 | -205 | 203 | | |
| **Grass Richness** | High Nitrogen | 140 | -461 | 740 | 1.02 | 0.39 |
| | Low Nitrogen | -148 | -320 | 23.6 | | |
| | High Phosphorus | -341 | -988 | 305 | | |
| | Low Phosphorus | -543 | -1345 | 260 | | |

[1]Slope/Regression coefficient

[2] Upper/lower limit of 95% confidence interval

[3]Comparison of regression coefficients among nutrient treatments ($H_o$: $\beta_1 = \beta_2 = \beta_3 = \beta_4$)

Table 3.10. Comparison of the regression coefficients (β) for maximum root surface area ($cm^2$) versus: species richness; forb richness; and grass richness at the Ekre Preserve. Upper and lower limits for the 95% confidence intervals are included.

| Max Root Surface Area vs. | Nutrient Treatment | Regression Coefficient | | | Slope comparison[3] | |
|---|---|---|---|---|---|---|
| | | Slope[1] | Lower CI[2] | Upper CI[2] | F value | p value |
| **Species Richness** | High Nitrogen | -11.9 | -50.4 | 26.5 | 0.50 | 0.68 |
| | Low Nitrogen | -10.1 | -21.4 | 1.25 | | |
| | High Phosphorus | -15.8 | -33.5 | 1.90 | | |
| | Low Phosphorus | -2.37 | -22.9 | 18.2 | | |
| **Forb Richness** | High Nitrogen | -21.1 | -86.5 | 44.3 | 0.94 | 0.43 |
| | Low Nitrogen | -13.3 | -29.0 | 2.39 | | |
| | High Phosphorus | -19.3 | -39.4 | 0.79 | | |
| | Low Phosphorus | 0.70 | -22.6 | 24.0 | | |
| **Grass Richness** | High Nitrogen | -20.0 | -101 | 60.6 | 0.63 | 0.60 |
| | Low Nitrogen | -13.7 | -36.9 | 9.56 | | |
| | High Phosphorus | -20 | -98.8 | 58.8 | | |
| | Low Phosphorus | -69.8 | -159 | 19.2 | | |

[1]Slope/Regression coefficient
[2] Upper/lower limit of 95% confidence interval
[3]Comparison of regression coefficients among nutrient treatments ($H_o$: $\beta_1 = \beta_2 = \beta_3 = \beta_4$)

## Discussion

This study evaluated the effects of nitrogen and phosphorus additions at high and low rates coupled with annual fall mowing on above- and belowground aspects of a restored plant community. The belowground variables measured included root length, surface area, number of root tips, and number of branches. These four variables were selected to provide information on the amount of roots present within 0.45 m of the soil surface (e.g., length and surface area) and reveal clues about the structure and function of the root systems present in the restored plots (e.g., number of root tips and branches). Although all four root variables provided certain information about water and nutrient uptake, the number of root tips was especially revealing

since the majority of uptake is known to occur at root tips rather than along the length of a root (Campbell and Reece 2002; Gregory 2006; Evert and Eichhorn 2013).

The numbers of root tips and branches also provided clues about root architecture in the depth interval of interest. Root architecture is concerned with the spatial arrangement of root systems throughout the soil and has important ecological implications related to the uptake of soil resources and the costs of construction (Fitter 1987; Gregory 2006; Hodge et al. 2008). Root systems are known to be extremely plastic and may respond to soil heterogeneity by concentrating growth in a relatively nutrient-rich area (Robinson 1996; Fitter 2002; Gregory 2006; Hodge et al. 2008). In the current study, the number of root branches provided a proxy for lateral root formation. Lateral roots may form in response to nutrient-rich patches in a heterogeneous soil and certain species are known to form lateral roots more readily than others (Robinson 1996; Kembel and Cahill 2005; Gregory 2006; Hodge et al. 2008; Kembel et al. 2008).

When the 0-0.45 m depth interval was examined across the entire 2012 growing season, certain differences in root length, surface area, number of root tips, and number of root branches were attributable to time (Figures 3.2-3.5.). Measured values for root length, surface area, number of root tips, and number of root branches were lower at the beginning of the growing season than at any other time throughout the growing season. This is not at all surprising since roots are expected to grow, develop, and senesce within the relatively short growing season in North Dakota.

All differences in root length and surface area were between sampling sessions (Figures 3.2 and 3.3). There were no differences in root length or surface area among plots that received nitrogen or phosphorus, at the high or low rates, within a single sampling session throughout the

entire growing season (Figures 3.8 and 3.9). Similarly, there were no differences in the numbers of root tips or branches among the plots that received nitrogen or phosphorus, at the high or low rates, during the first sampling session conducted in late April/early May (Figures 3.10 and 3.11). However, there were differences in the numbers of root tips and branches within all other sampling sessions (June-November). While differences between sampling sessions may be explained by root growth, development, and senescence throughout the growing season, differences within a single sampling session indicate that the root systems were exhibiting different responses to the experimental treatments.

In general, the root systems of the plots that received repeated nitrogen additions had fewer branches than those that received repeated phosphorus additions ($\overline{x}_N$ = 682, SE = 35.6; $\overline{x}_P$ = 851, SE = 44.1) (across the entire growing season). The difference in the number of root branches present in the depth interval sampled implied that long-term nitrogen and phosphorus additions differently affected the structure of the root systems (i.e. branching pattern and lateral root formation). Researchers have long been concerned with root architecture and the integration of structure and function (Hodge et al. 2008). As in the current study, several other researchers have observed root responses to nutrient additions and/or soil heterogeneity (Robinson 1996; Johnson and Biondini 2001; Waisel and Eshel 2002; Gregory 2006; Jackson et al. 2007).

There were more root tips and branches observed in the plots that received phosphorus at the low rate than those that received nitrogen at the low rate for all sampling sessions except the first (Figures 3.10 and 3.11). The greater incidence of root branching throughout most of the growing season in the plots that received phosphorus at the low rate than in the plots that received nitrogen at the low rate indicated a response to the increased phosphorus availability in

the low phosphorus plots, similar to what was observed when the growing season was taken in its entirety (Figure 3.7).

At the end of the growing season, there was more branching in the plots that received phosphorus at the high rate than in those that received nitrogen at the low rate, an effect that was not apparent earlier in the growing season. Root proliferation in response to nutrient availability involves both physiological and morphological responses (Hodge et al. 2008). Perhaps the root systems in the plots that received phosphorus at the high rate were able to increase their phosphorus uptake via a physiological response while the type of morphological response that could be measured by this study developed later in the season (Figure 3.11).

When soil-borne resources are readily available, it is not advantageous for a plant to invest its resources in belowground production (Bloom et al 1985; Gregory 2006; Yavitt et al. 2011). Thus, the higher incidence of branched roots in late June/early July in the plots that received nitrogen at the high rate than in those that received nitrogen at the low rate is perhaps counterintuitive. However, the experimental plots that received the high nitrogen applications had the lowest species richness (Figure 3.13) and smooth brome (*Bromus inermis* Leyss.) had the highest biomass by species in 80% of these plots. Previous research conducted at this location has documented the presence of cool-season invaders in the plots, especially smooth brome (Biondini 2007; Biondini et al. 2011; DiAllesandro et al. 2013). As in the current study, DiAllesandro et al. (2013) determined that the plots with the lowest species richness had the highest smooth brome biomass. In addition, DiAllesandro et al. (2013) determined that species richness and smooth brome biomass were inversely correlated in the restored plots at this location. Furthermore, an extensive study on the root architecture of 1759 individual plants from

77 herbaceous species by Biondini (2008) showed that smooth brome has the highest branching patterns of all the species used in this study.

Research has shown smooth brome to be an excellent competitor both above- and belowground (Johnson and Biondini 2001; Levang-Brilz and Biondini 2002; Rajaniemi and Reynolds 2004; Vinton and Goergen 2006). Vinton and Goergen (2006) proposed that smooth brome has a competitive advantage over the native species of the Northern Great Plains due to its ability to respond more quickly to increased nutrient inputs. Since smooth brome has become ubiquitous in the high nitrogen plots at this location, the increased branching observed in the high nitrogen plots relatively early in the growing season is likely a measure of smooth brome's rapid response to nutrient availability.

Root growth (as calculated from estimates of root length) was higher in the plots that received nitrogen at the high rate and phosphorus at the low rate than in those that received nitrogen at the low rate and phosphorus at the high rate (Figure 3.12). In the plots that received nitrogen at the high rate, the relatively high root growth measured can again be related to smooth brome's competitive advantage over species native to the Northern Great Plains as discussed by Vinton and Goergen (2006). Since the high nitrogen plots have become invaded by smooth brome, these plots have lower species richness than any of the other nutrient treatments at this location (Figure 3.13) and smooth brome is known to exhibit belowground responses to nutrient availability and heterogeneity (Johnson and Biondini 2001; Levang-Brilz and Biondini 2002; Vinton and Goergen 2006). Thus, in the high nitrogen plots, the relatively high root growth is a result of very low species richness due to the prominence of smooth brome.

The high root growth observed in the low phosphorus plots can also be related to species richness. In contrast to the high nitrogen plots, which were typically dominated by a cool-season

grass, the low phosphorus plots had the highest species richness including both grass and forb species. Species' responses to nutrient availability are variable as forbs, in general, have been known to exhibit more pronounced root proliferation than grasses (Johnson and Biondini 2001; Kembel and Cahill 2005). The presence of more species within the low phosphorus plots likely explains some of the observed lateral root formation in these plots.

Although the high nitrogen plots had significantly lower species richness than all other nutrient type and rate combinations; there were no differences in species richness in the low nitrogen, low phosphorus, or high phosphorus plots. Thus, the lower root growth in the plots that received nitrogen at the low rate and phosphorus at the high rate is likely due to the relative availability of nutrients in these plots. As mentioned above, there is little reason to produce and maintain additional belowground tissues when nutrients are readily available to a plant (Bloom et al. 1985; Gregory 2006; Yavitt et al. 2011). Many researchers have documented no resulting increase in root production following nutrient additions that are hypothesized to be a result of the plant allocating more of its resources aboveground (Cahill 1999; Ladwig et al. 2011; Balogianni et al. 2014).

While the mowing treatment evaluated in this study had no effect on any of the belowground variables, it did affect one of the aboveground variables – average biomass. The average aboveground biomass was higher in the plots that were mowed once in the fall of each year than in the plots that were not mowed. Average species richness and the ratio of root volume to aboveground biomass were not different between the plots that were mowed and those that were not.

Although the effects of mowing on grassland systems have not been as well-studied as have the effects of grazing, mowing is widely used as a management tool following grassland

restoration (van Dyke et al. 2004; Balogianni et al. 2014). Studies on the impacts of mowing on above- and belowground components of grasslands have reached varying conclusions – certain studies have reported increases in above- and/or belowground production following mowing while others have reported decreases or no change (van Dyke et al. 2004; Kitchen et al. 2009; Balogianni et al. 2014). There are also studies that have documented effects attributable to mowing aboveground that were not detected belowground.

For instance, Balogianni et al. (2014) found mowing to have significant effects on aboveground biomass in grassland plots while no effects were detected belowground, as was the case for the current study. Balogianni et al. (2014) observed a decrease in aboveground biomass following mowing treatments (plots were mowed twice each year, in May and June, at a cutting height of 2 cm). In the current study, an increase in aboveground biomass was observed following four years of harvesting (plots were mowed once annually in the fall at a cutting height of 10-15 cm and biomass was removed using a landscape rake). The mowing treatment used in the current study may have increased shoot production through the removal of the litter and standing dead crop from the plots, thereby promoting light penetration and increasing production.

The regression analysis conducted in this study also pointed to the role species richness played in determining belowground variables. For instance, there was a weak inverse relationship between species richness and maximum root length. This was consistent with the other results in this study that point to smooth brome's abundance in the plots with the lowest species richness. In the current study, the plots with the lowest species richness were found to have the highest root growth – hypothesized to be due to smooth brome's ability to outcompete many of the native species included in the original restoration experiment (Vinton and Goergen

2006). The additional weak inverse relationships among maximum root length and forb richness and maximum surface area and species richness/forb richness provided additional evidence of the prevalence of smooth brome in the experimental plots and of smooth brome's competitive abilities, consistent with previous research conducted at this location (DiAllesandro et al. 2013).

## Concluding Remarks

- Repeated fertilization with nitrogen at the high rate reduced species richness. Species richness was lower in the plots fertilized with nitrogen at the high rate than in the plots fertilized with nitrogen at the low rate and phosphorus at both the high and low rates.
- Root growth was higher in the plots that received nitrogen at the high rate and phosphorus at the low rate than in those that received nitrogen at the low rate and phosphorus at the high rate. Both of these results reflected species richness patterns. In the high nitrogen plots, the increased growth was due to the prevalence of smooth brome, an excellent competitor for above- and belowground resources. In the low phosphorus plots, the increased growth was due to greater species richness, more competition, and increased lateral root formation.
- None of the belowground variables measured in this study was impacted by four years of annual fall mowing. Conversely, aboveground biomass increased in the plots that were mowed annually.
- There were different responses in the root variables as measured throughout the growing season among the different experimental treatments defined by nutrient type and application rates.

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# CHAPTER 4. CONCLUSIONS AND FUTURE DIRECTIONS

## Conclusions

This study sought to improve the ecological understanding of the belowground portion of restored plant communities. Various above- and belowground variables were included in the current project in order to build a more holistic picture of restored grassland plots in the Northern Great Plains. The aboveground portion of the restored plant communities was evaluated using a combination of biomass harvesting and visual survey methods while the belowground portion was assessed through the employment of a minirhizotron system. Two separate locations in North Dakota were selected for the current research project to represent different plant community types encountered throughout the Northern Great Plains Regions. Restored grassland plots in western North Dakota were chosen to represent the mixed grass prairie ecoregion while restored grassland plots in southeastern North Dakota represented the tallgrass prairie ecoregion. Plots were subjected to different nutrient and mowing treatments and the impacts to the above- and belowground portions of the plant communities were evaluated.

In western North Dakota, the belowground variables evaluated in this study were found to be relatively unresponsive to the annual additions of nitrogen and phosphorus fertilizers (200 kg/ha·yr and 40 kg/ha·yr, respectively). In contrast, the aboveground variables did exhibit a response to the repeated nitrogen and phosphorus additions. This response was especially pronounced in response to the nitrogen additions. Repeated nitrogen additions were found to increase aboveground biomass without a corresponding increase in the root system, thereby resulting in a reduced ratio of root volume to average aboveground biomass ratio. A smaller ratio of root volume to average aboveground biomass indicated that these root systems were able to support more aboveground biomass in response to the readily available nitrogen than were the

plots that did not receive repeated nitrogen additions. In addition, repeated nitrogen applications were found to reduce species richness, thus changing the structure and function of these restored grassland plots. None of the above- or belowground variables was impacted by the annual fall mowing treatment employed at this location.

In southeastern North Dakota, repeated nitrogen applications at a rate of 200 kg/ha·yr reduced species richness and increased root growth. Smooth brome (*Bromus inermis* Leyss.), which is known to be an excellent competitor for above- and belowground resources (Johnson and Biondini 2001; Levang-Brilz and Biondini 2002; Rajaniemi and Reynolds 2004; Vinton and Goergen 2006) was very prevalent in the plots that received 200 kg/ha·yr of nitrogen annually, thus, the increase in root growth observed in these plots was a reflection of smooth brome's competitiveness. Increased root growth was also observed in the plots that received 4 kg/ha·yr of phosphorus annually. However, these plots contained a more diverse plant community and the increased root growth was a reflection of more belowground competition due to greater species richness. While none of the belowground variables were significantly affected, there was a significant increase in aboveground biomass following four years of annual fall mowing at this location.

## Future Directions

Although the increased employment of minirhizotrons in the study of root systems has advanced ecological studies of the belowground portion of many ecosystems, there is still much to be discovered (Box et al. 1989; Fitter et al. 1998; Johnson et al. 2001; Polomski and Kuhn 2002; Hendricks et al. 2006; Fischer et al. 2007; Volder et al. 2007; Balogianni et al. 2014). For instance, minirhizotrons provide a method to repeatedly observe roots *in situ*, allowing researchers to examine how root systems respond to different above- and belowground factors

over time. Additionally, minirhizotrons offer a means to examine the distribution of roots in the soil profile in relation to environmental conditions, nutrient and/or water availability, or biological interactions. Now that minirhizotrons are becoming more widespread, ecologists will be more able to include the belowground portion of ecological communities in long-term ecological research studies.

Productivity estimates are central to descriptions of ecosystems. To date, many researchers have sought to quantify production for many diverse ecological systems and at a variety of scales, with or without attempts to incorporate the belowground portion (Gregory 2006; Hui and Jackson 2006). Quantifying production can be very challenging, especially when it is located below the soil surface. The use of minirhizotrons could help to improve estimates of productivity by providing a non-destructive method that allows for repeated observations to examine belowground production patterns.

There is widespread interest among ecological researchers in estimating root longevity and/or turnover in order to better estimate ecosystem production and to enhance the understanding of ecological processes, such as nutrient cycling. Root longevity and turnover are known to vary widely among plant species and communities (Jackson et al. 1996; Gregory 2006). For example, Tjoelker et al. (2005) estimated average root longevity to be 504 days for selected cool season ($C_3$) grasses; 791 days for selected warm season ($C_4$) grasses; and 182 days for selected forb species. Estimates of root longevity are also believed to be influenced by the experimental techniques employed, i.e. isotope residence time versus minirhizotron sampling methods (Strand et al. 2008). Further, estimates of root growth and mortality may also vary widely using a single sampling technique at a single site – Stewart and Frank (2008) found a wide discrepancy in estimates of root growth and mortality related to the length of the interval

between samples. In future years, there will likely be many studies that seek to improve estimates of root longevity and turnover in a wide variety of ecosystems types using minirhizotrons.

One of the most valuable applications of minirhizotrons is to provide a literal picture of the belowground portion of plant communities – which is often overlooked due to the inherent difficulties of studying root systems. Designing studies that incorporate above- and belowground sampling techniques will undoubtedly enhance the understanding of plant community structure and function through the thorough examination of roots and shoots. In addition, minirhizotrons are conducive for repeated sampling thereby allowing researchers to observe how root systems respond to changing biotic and/or abiotic conditions, which could be very useful for examining how entire plant communities respond to experimental treatments. Finally, increased use of minirhizotrons could provide a method to examine how/whether certain ecological hypotheses developed through observing the aboveground portion of plant communities play out beneath the soil surface.

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## APPENDIX A. RESTORATION EXPERIMENT DESIGN AND MINIRHIZOTRON PLOT SELECTION FOR THE DREC PLOTS

Table A.1. Species planted at the Dickinson Research Extension Center. Nomenclature follows the USDA Plants Database.

| Forbs, Shrubs, Legumes | Grasses |
|---|---|
| *Achillea millefolium* L. | *Agropyron cristatum* (L.) Gaertn. |
| *Artemisia dracunculus* L. | *Andropogon gerardii* Vitman |
| *Artemisia frigida* Willd. | *Bouteloua curtipendula* (Michx.) Torr. |
| *Asclepias verticillata* L. | *Bouteloua gracilis* (Willd. Ex Kunth) Lag. ex Griffiths |
| *Coreopsis lanceolata* L. | *Bromus inermis* Leyss. |
| *Dalea purpurea* Vent. | *Calamovilfa longifolia* (Hook) Scribn. |
| *Gaillardia aristata* Pursh. | *Elymus canadensis* L. |
| *Geum triflorum* Pursh | *Hesperostipa comata* (Trin. & Rupr.) Barkworth |
| *Grindelia squarrosa* (Pursh) Dunal | *Hordeum jubatum* L. |
| *Helianthus maximiliani* Schrad. | *Koeleria macrantha* (Ledeb.) Schult |
| *Helianthus pauciflorus* Nutt. | *Nassella viridula* (Trin.) Barkworth |
| *Heterotheca villosa* (Pursh) Shinners | *Panicum virgatum* L. |
| *Linum perenne* L. | *Poa pratensis* L. |
| *Melilotus officinalis* (L.) Lam. | *Pseudoroegneria spicata* (Pursh) A. Love |
| *Oligoneuron rigidum* (L.) Small | *Schizachyrium scoparium* (Michx.) Nash |
| *Potentilla arguta* Pursh | *Sorghastrum nutans* (L.) Nash |
| *Ratibida columnifera* (Nutt.) Woot. & Standl. | *Sporobolus cryptandrus* (Torr.) A. Gray |
| *Rosa arkansana* Porter | |
| *Rudbeckia hirta* L. | |
| *Solidago missouriensis* Nutt. | |
| *Symphyotrichum ericoides* (L.) G.L. Nesom | |
| *Taraxacum officinale* F.H. Wigg | |
| *Tragopogon dubius* Scop. | |
| *Vicia americana* Muhl. ex Willd. | |

Table A.2. Details about the plots selected for minirhizotron tube installation at the DREC including nutrient treatment, biomass at peak aboveground production, and species richness at peak aboveground production. The biomass estimates were obtained by harvesting 0.25 $m^2$ quadrats while the species richness estimates were obtained through visual surveys. The data presented were collected in 2009 – prior to the implementation of the mowing treatment.

| Nutrient Treatment | Species Richness | Biomass ($g/m^2$) |
|---|---|---|
| Nitrogen | 8 | 918 |
| Nitrogen | 8 | 903 |
| Nitrogen | 8 | 941 |
| Nitrogen | 8 | 991 |
| Nitrogen | 11 | 439 |
| Control | 10 | 303 |
| Control | 11 | 400 |
| Control | 12 | 509 |
| Control | 14 | 549 |
| Control | 15 | 583 |
| Control | 16 | 581 |
| Control | 22 | 403 |
| Phosphorus | 9 | 186 |
| Phosphorus | 9 | 424 |
| Phosphorus | 11 | 515 |
| Phosphorus | 11 | 523 |
| Phosphorus | 13 | 452 |
| Phosphorus | 13 | 600 |
| Phosphorus | 16 | 318 |
| Phosphorus | 17 | 375 |

## APPENDIX B. ANALYSIS OF VARIANCE TABLES FOR BELOWGROUND VARIABLES BY SAMPLING SESSION FOR THE DICKINSON RESEARCH EXTENSION CENTER PLOTS

### Sampling Session 1 – May 15th-16th, 2012

Table B.1. Analysis of Variance for root length by treatment and interaction at the Dickinson Research Extension Center (mid-May) (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 0.43 | 0.65 |
| Mow | 1 | 17 | 0.36 | 0.56 |
| Nutrient*Mow | 2 | 17 | 1.44 | 0.26 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Table B.2. Analysis of Variance for root surface area by treatment and interaction at the Dickinson Research Extension Center (mid-May) (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 0.49 | 0.62 |
| Mow | 1 | 17 | 0.05 | 0.83 |
| Nutrient*Mow | 2 | 17 | 1.09 | 0.36 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Table B.3. Analysis of Variance for number of root tips by treatment and interaction at the Dickinson Research Extension Center (mid-May) (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 0.02 | 0.98 |
| Mow | 1 | 17 | 0.55 | 0.47 |
| Nutrient*Mow | 2 | 17 | 1.70 | 0.21 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Table B.4. Analysis of Variance for number of root branches by treatment and interaction at the Dickinson Research Extension Center (mid-May) (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 0.53 | 0.60 |
| Mow | 1 | 17 | 1.32 | 0.27 |
| Nutrient*Mow | 2 | 17 | 0.99 | 0.39 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

## Sampling Session 2 – June 26th-28th, 2012

Table B.5. Analysis of Variance for root length by treatment and interaction at the Dickinson Research Extension Center (late June) (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 0.05 | 0.95 |
| Mow | 1 | 17 | 0.15 | 0.70 |
| Nutrient*Mow | 2 | 17 | 0.88 | 0.43 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Table B.6. Analysis of Variance for root surface area by treatment and interaction at the Dickinson Research Extension Center (late June) (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 0.16 | 0.85 |
| Mow | 1 | 17 | 0.58 | 0.46 |
| Nutrient*Mow | 2 | 17 | 0.49 | 0.62 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Table B.7. Analysis of Variance for number of root tips by treatment and interaction at the Dickinson Research Extension Center (late June) (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 0.14 | 0.87 |
| Mow | 1 | 17 | 0.21 | 0.65 |
| Nutrient*Mow | 2 | 17 | 1.60 | 0.23 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Table B.8. Analysis of Variance for number of root branches by treatment and interaction at the Dickinson Research Extension Center (late June) (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 0.49 | 0.62 |
| Mow | 1 | 17 | 0.37 | 0.55 |
| Nutrient*Mow | 2 | 17 | 0.40 | 0.68 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

## Sampling Session 3 – July 17th-18th, 2012

Table B.9. Analysis of Variance for root length by treatment and interaction at the Dickinson Research Extension Center (mid-July) (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 0.05 | 0.96 |
| Mow | 1 | 17 | 0.48 | 0.50 |
| Nutrient*Mow | 2 | 17 | 1.98 | 0.17 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Table B.10. Analysis of Variance for root surface area by treatment and interaction at the Dickinson Research Extension Center (mid-July) (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 0.04 | 0.96 |
| Mow | 1 | 17 | 0.38 | 0.55 |
| Nutrient*Mow | 2 | 17 | 2.26 | 0.13 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Table B.11. Analysis of Variance for number of root tips by treatment and interaction at the Dickinson Research Extension Center (mid-July) (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 0.12 | 0.89 |
| Mow | 1 | 17 | 1.59 | 0.22 |
| Nutrient*Mow | 2 | 17 | 2.82 | 0.09 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Table B.12. Analysis of Variance for number of root branches by treatment and interaction at the Dickinson Research Extension Center (mid-July) (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 0.05 | 0.95 |
| Mow | 1 | 17 | 0.04 | 0.85 |
| Nutrient*Mow | 2 | 17 | 0.20 | 0.82 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

## Sampling Session 4 – September 9th – October 6th, 2012

Table B.13. Analysis of Variance for root length by treatment and interaction at the Dickinson Research Extension Center (late September/early October) (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 0.05 | 0.96 |
| Mow | 1 | 17 | 0.48 | 0.50 |
| Nutrient*Mow | 2 | 17 | 1.98 | 0.17 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Table B.14. Analysis of Variance for root surface area by treatment and interaction at the Dickinson Research Extension Center (late September/early October) (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 0.21 | 0.82 |
| Mow | 1 | 17 | 0.04 | 0.84 |
| Nutrient*Mow | 2 | 17 | 0.25 | 0.78 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Table B.15. Analysis of Variance for number of root tips by treatment and interaction at the Dickinson Research Extension Center (late September/early October) (Type III estimation method).

| Effect | Num DF[1] | Den DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 0.60 | 0.56 |
| Mow | 1 | 17 | 0.13 | 0.72 |
| Nutrient*Mow | 2 | 17 | 0.16 | 0.85 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

Table B.16. Analysis of Variance for number of root branches by treatment and interaction at the Dickinson Research Extension Center (late September/early October) (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 1.01 | 0.39 |
| Mow | 1 | 17 | 0.03 | 0.87 |
| Nutrient*Mow | 2 | 17 | 0.23 | 0.80 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

## Root Growth

Table B.17. Analysis of Variance for root growth by treatment and interaction at the Dickinson Research Extension Center (Type III estimation method).

| Effect | Num. DF[1] | Den. DF[2] | F Value | p value |
|---|---|---|---|---|
| Nutrient | 2 | 17 | 0.69 | 0.52 |
| Mow | 1 | 17 | 0.20 | 0.66 |
| Nutrient*Mow | 2 | 17 | 0.08 | 0.93 |

[1]Numerator degrees of freedom
[2]Denominator degrees of freedom (Satterthwaite method)

## APPENDIX C. REGRESSION ANALYSIS OF ABOVE- AND BELOWGROUND VARIABLES FOR THE DICKINSON RESEARCH EXTENSION CENTER PLOTS

### Maximum Root Length vs. Species Richness Components

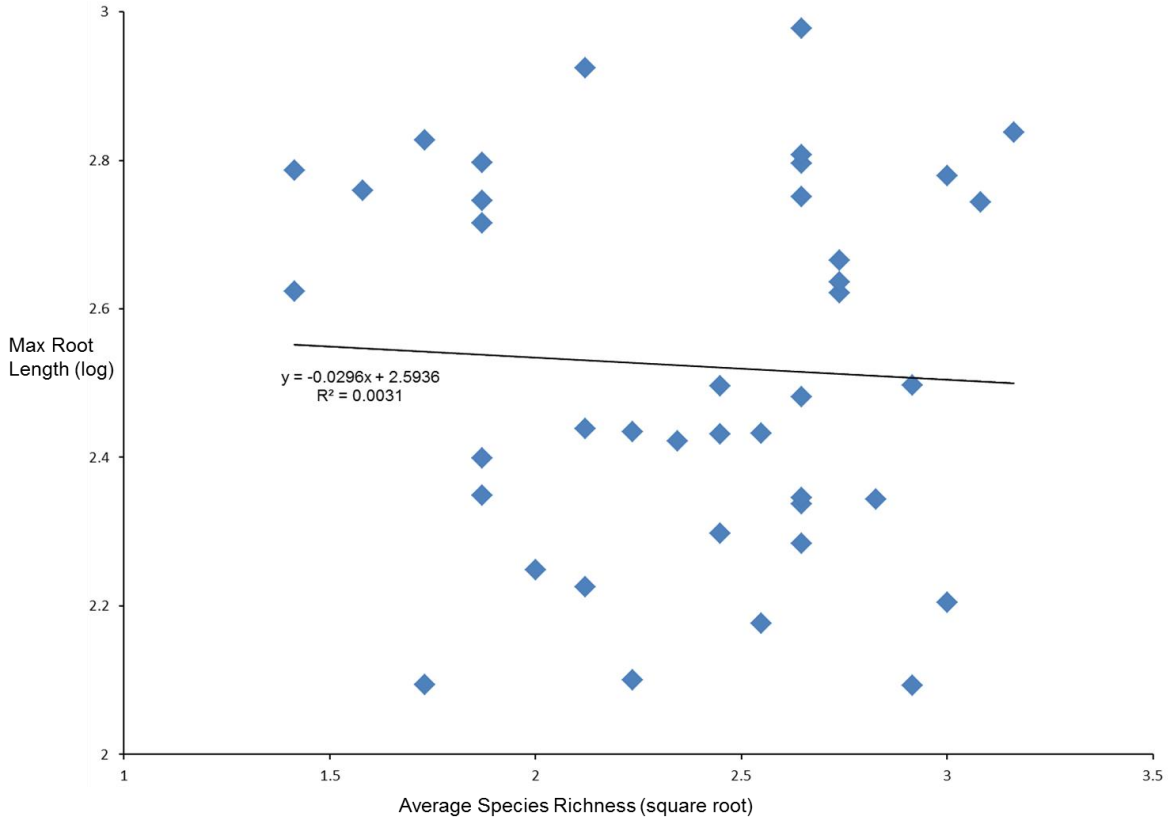


Figure C.1. Relationship between the log of maximum root length (cm) and the square root of average species richness (p = 0.73) at the Dickinson Research Extension Center.

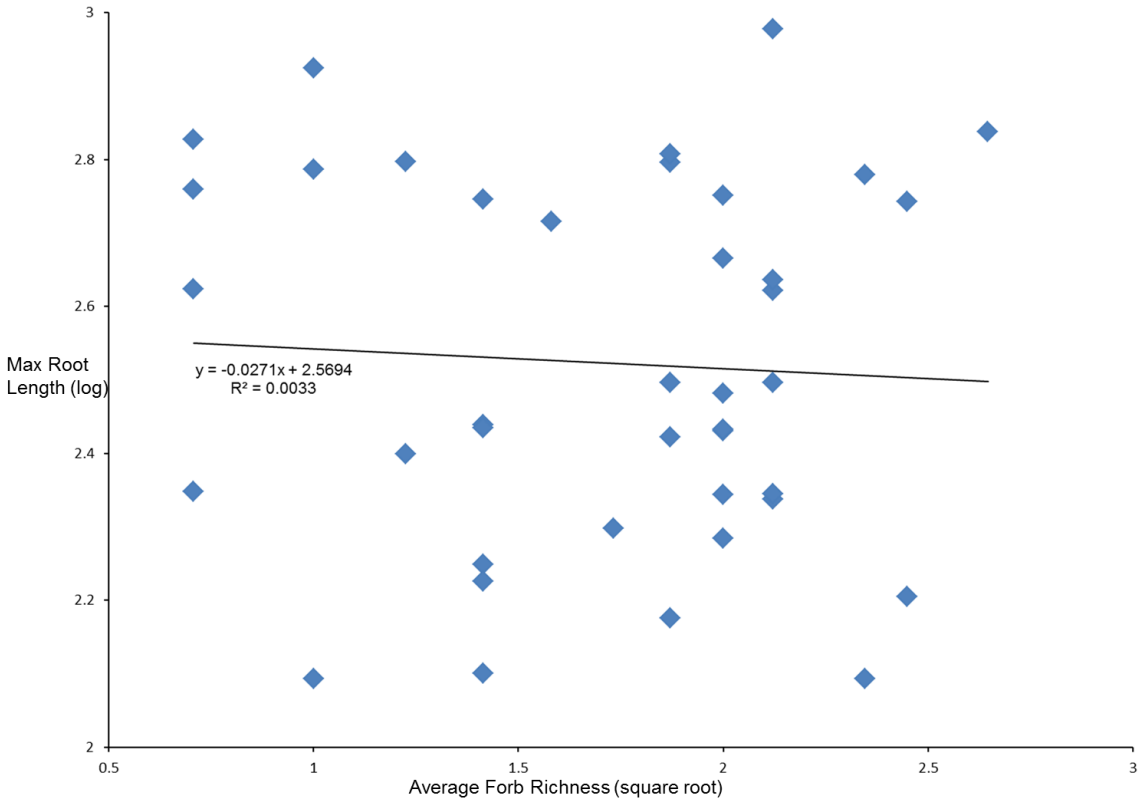


Figure C.2. Relationship between the log of maximum root length (cm) and the square root of average forb richness (p = 0.72) at the Dickinson Research Extension Center.

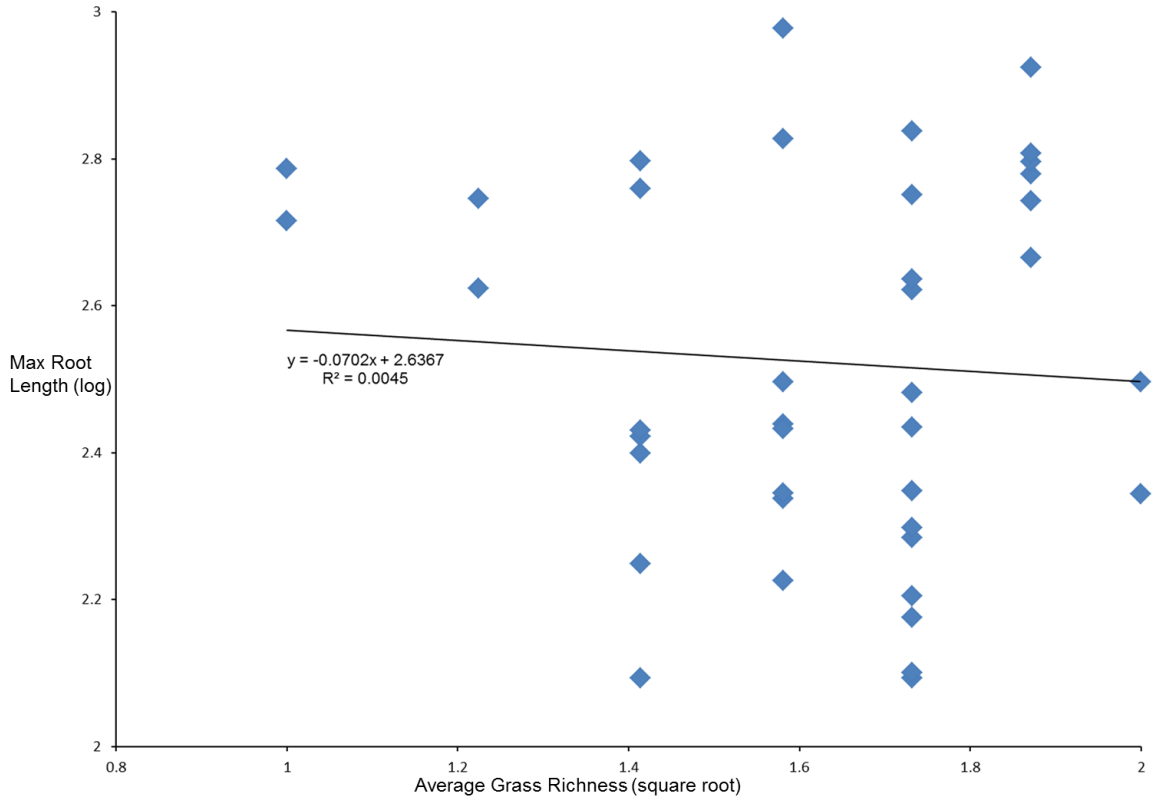


Figure C.3. Relationship between the log of maximum root length (cm) and the square root of average grass richness (p = 0.68) at the Dickinson Research Extension Center.

Table C.1. Comparison of the regression coefficients (β) for the log of maximum root length (cm) versus the square root of: average species richness; average forb richness; and average grass richness at the Dickinson Research Extension Center. Upper and lower limits for the 95% confidence intervals are included.

| **Max Root Length[1] vs.** | **Nutrient Treatment** | **Regression Coefficient** | | | **Slope comparison[5]** | |
|---|---|---|---|---|---|---|
| | | **Slope[3]** | **Lower CI[4]** | **Upper CI[4]** | **F value** | **p value** |
| **Average Species Richness[2]** | Nitrogen | -0.42 | -1.10 | 0.27 | 1.87 | 0.17 |
| | Control | 0.24 | -0.21 | 0.69 | | |
| | Phosphorus | -0.15 | -0.60 | 0.30 | | |
| **Average Forb Richness[2]** | Nitrogen | -0.23 | -0.75 | 0.29 | 1.54 | 0.23 |
| | Control | 0.21 | -0.19 | 0.60 | | |
| | Phosphorus | -0.18 | -0.61 | 0.25 | | |
| **Average Grass Richness[2]** | Nitrogen | -0.39 | -1.45 | 0.67 | 0.52 | 0.60 |
| | Control | 0.26 | -0.76 | 1.28 | | |
| | Phosphorus | -0.09 | -0.65 | 0.48 | | |

[1]Log-transformed

[2]Square root transformed

[3]Slope/Regression coefficient

[4]Upper/lower limit of 95% confidence interval

[5]Comparison of regression coefficients among nutrient treatments ($H_o$: $\beta_1 = \beta_2 = \beta_3$)

**Maximum Root Surface Area vs. Species Richness Components**

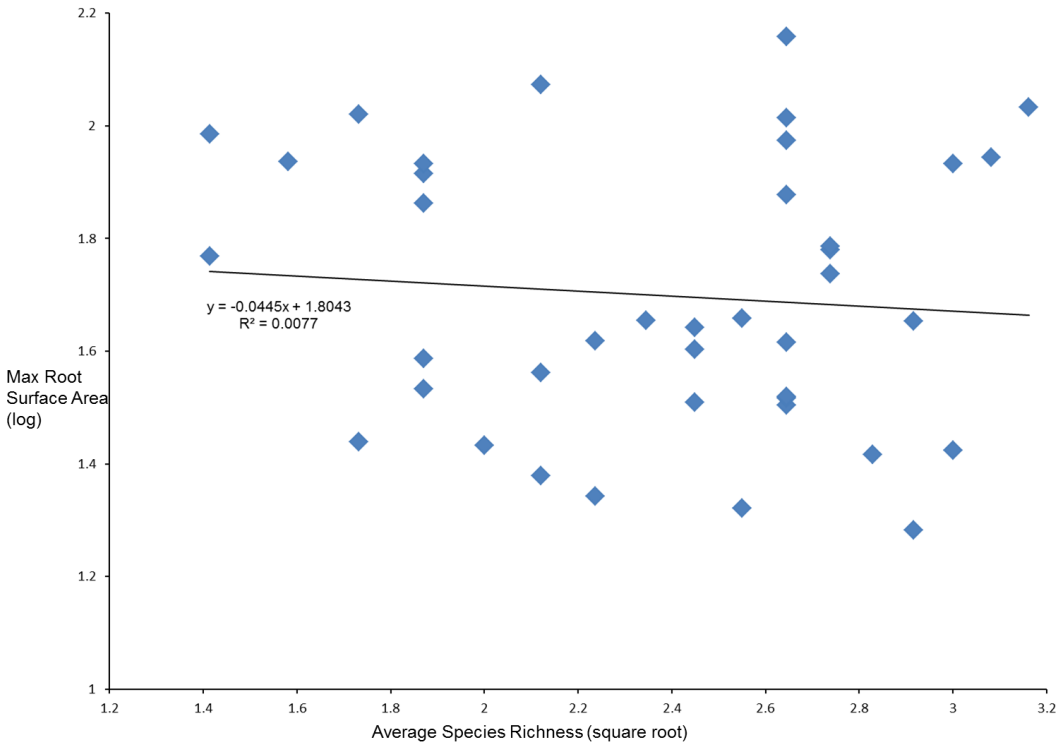


Figure C.4. Relationship between the log of maximum root surface area ($cm^2$) and the square root of average species richness (p = 0.59) at the Dickinson Research Extension Center.

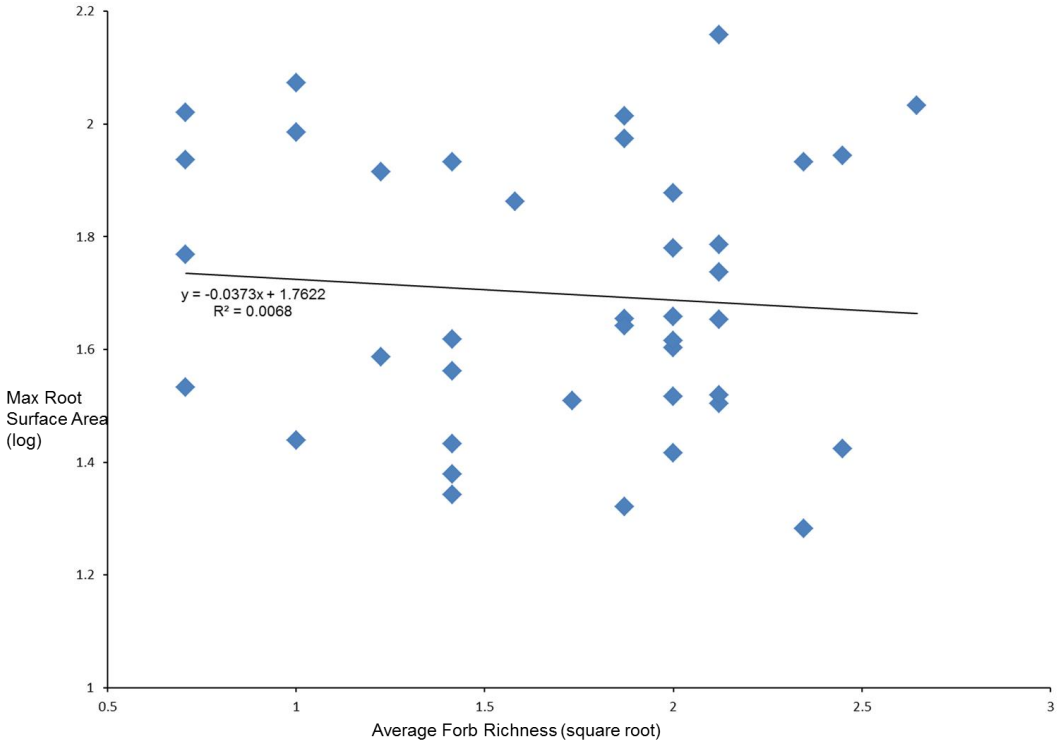


Figure C.5. Relationship between the log of maximum root surface area ($cm^2$) and the square root of average forb richness (p = 0.61) at the Dickinson Research Extension Center.

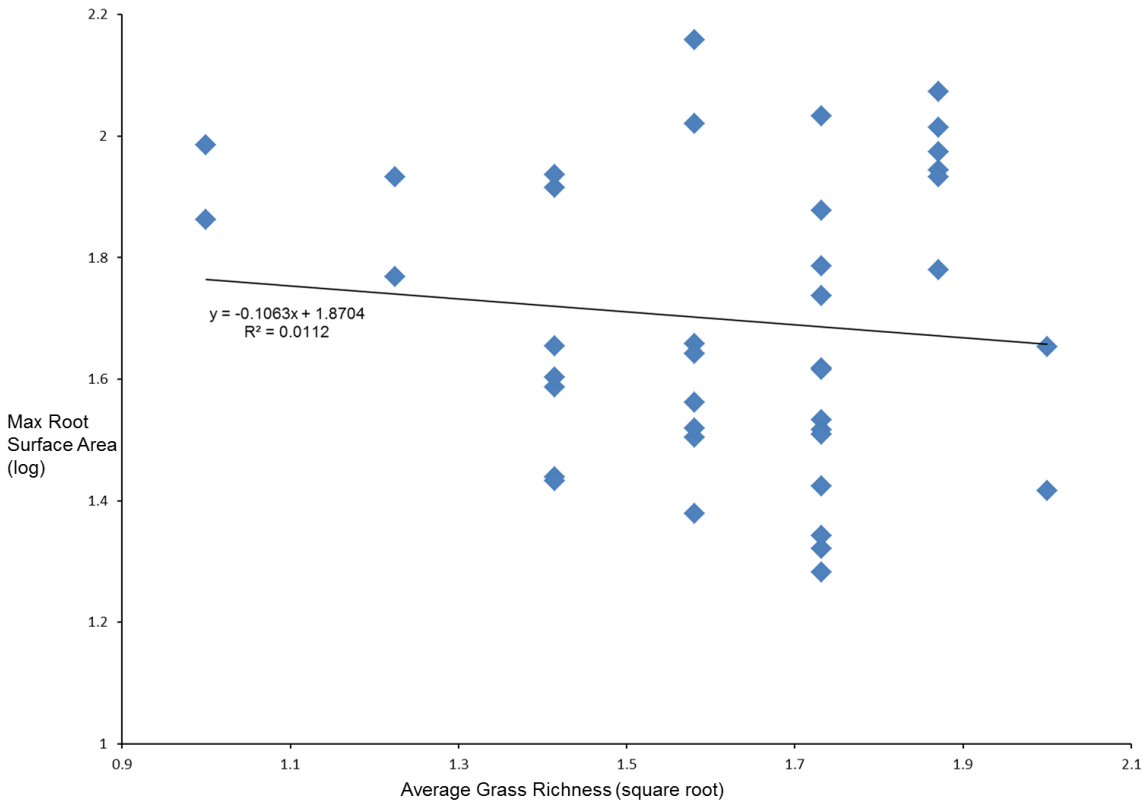


Figure C.6. Relationship between the log of maximum root surface area ($cm^2$) and the square root of average grass richness (p = 0.52) at the Dickinson Research Extension Center.

Table C.2. Comparison of the regression coefficients (β) for the log of maximum root surface area ($cm^2$) versus the square root of: average species richness; average forb richness; and average grass richness at the Dickinson Research Extension Center. Upper and lower limits for the 95% confidence intervals are included.

| **Max Root Surface Area[1] vs.** | **Nutrient Treatment** | **Regression Coefficient** | | | **Slope comparison[5]** | |
|---|---|---|---|---|---|---|
| | | **Slope[3]** | **Lower CI[4]** | **Upper CI[4]** | **F value** | **p value** |
| **Average Species Richness[2]** | Nitrogen | -0.38 | -0.97 | 0.20 | 2.22 | 0.12 |
| | Control | 0.27 | -0.19 | 0.72 | | |
| | Phosphorus | -0.17 | -0.59 | 0.25 | | |
| **Average Forb Richness[2]** | Nitrogen | -0.21 | -0.66 | 0.24 | 2.13 | 0.13 |
| | Control | 0.25 | -0.15 | 0.64 | | |
| | Phosphorus | -0.21 | -0.61 | 0.20 | | |
| **Average Grass Richness[2]** | Nitrogen | -0.38 | -1.29 | 0.53 | 0.39 | 0.68 |
| | Control | 0.16 | -0.88 | 1.21 | | |
| | Phosphorus | -0.08 | -0.61 | 0.46 | | |

[1]Log-transformed

[2]Square root transformed

[3]Slope/Regression coefficient

[4]Upper/lower limit of 95% confidence interval

[5]Comparison of regression coefficients among nutrient treatments ($H_o$: $\beta_1 = \beta_2 = \beta_3$)

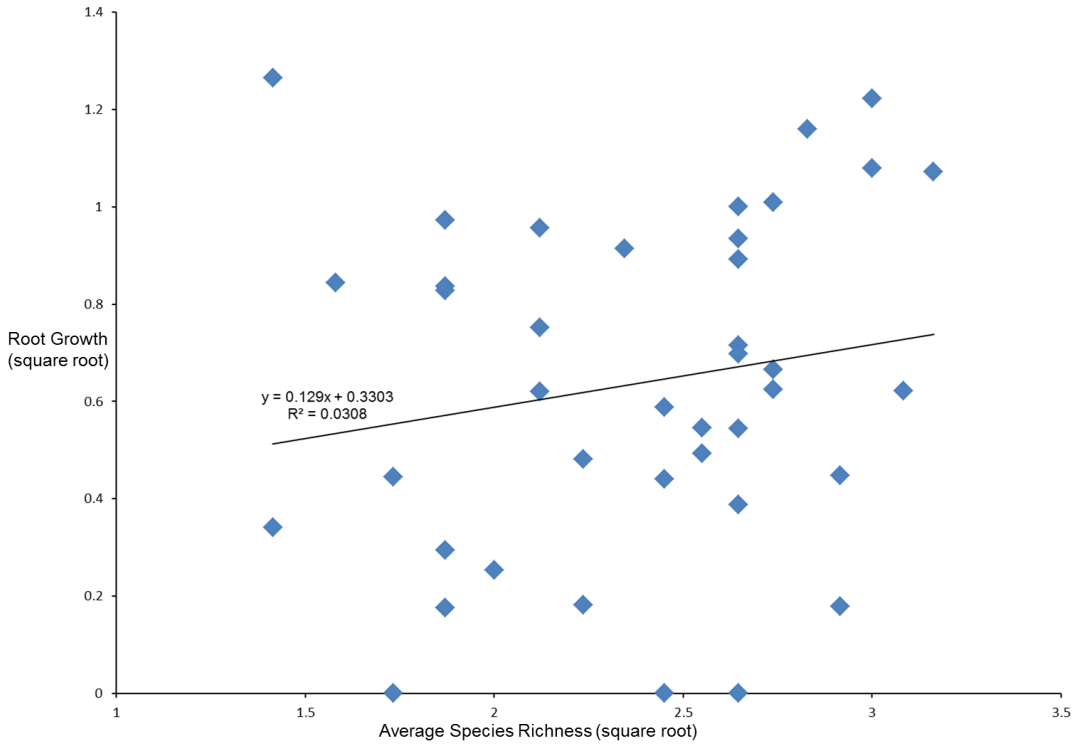


Figure C.7. Relationship between the square root of root growth and the square root of average species richness (p = 0.28) at the Dickinson Research Extension Center.

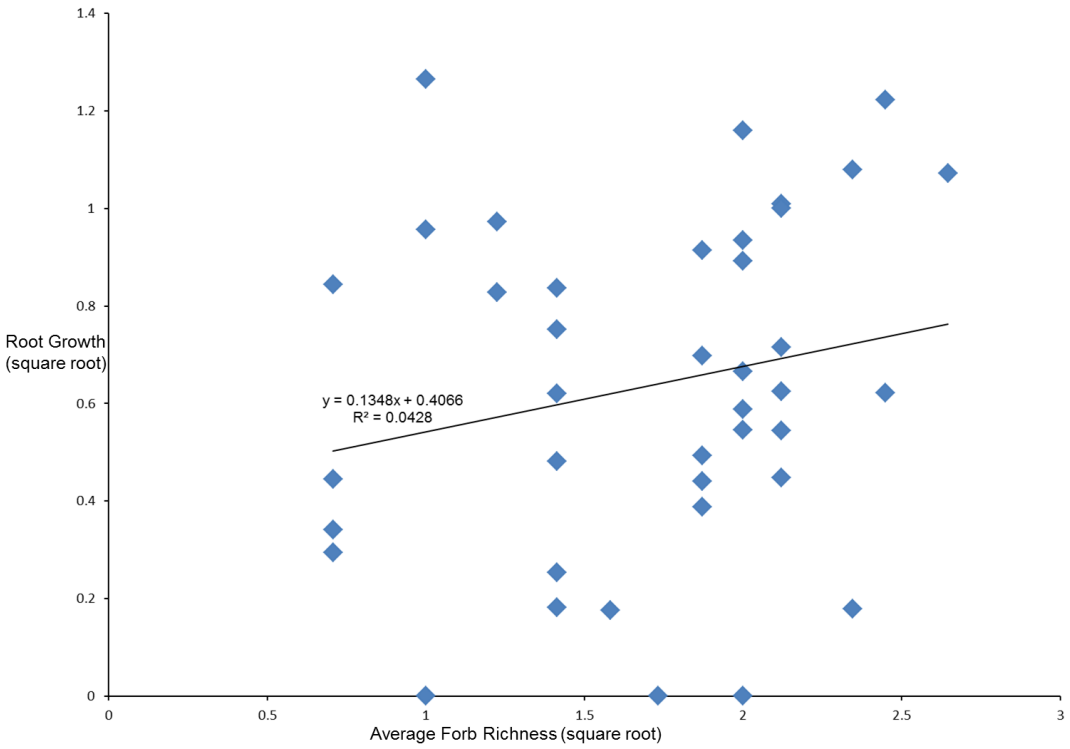


Figure C.8. Relationship between the square root of root growth and the square root of average forb richness (p = 0.20) at the Dickinson Research Extension Center.

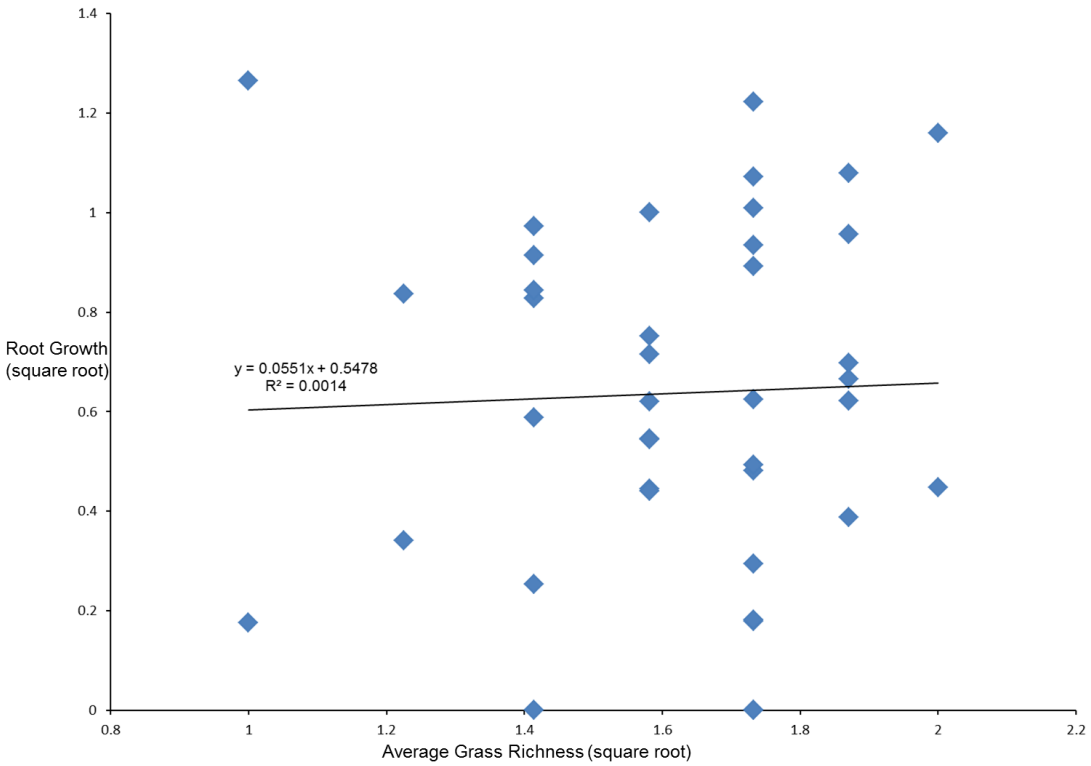


Figure C.9. Relationship between the square root of root growth and the square root of average grass richness (p = 0.82) at the Dickinson Research Extension Center.

Table C.3. Comparison of the regression coefficients (β) for the square root of root growth versus the square root of: average species richness; average forb richness; and average grass richness at the Dickinson Research Extension Center. Upper and lower limits for the 95% confidence intervals are included.

| **Root Growth [1] vs.** | **Nutrient Treatment** | **Regression Coefficient** | | | **Slope comparison[4]** | |
|---|---|---|---|---|---|---|
| | | **Slope[2]** | **Lower CI[3]** | **Upper CI[3]** | **F value** | **p value** |
| **Average Species Richness[1]** | Nitrogen | -0.05 | -1.22 | 1.12 | 0.32 | 0.73 |
| | Control | 0.35 | -0.15 | 0.84 | | |
| | Phosphorus | 0.14 | -0.51 | 0.80 | | |
| **Average Forb Richness[1]** | Nitrogen | 0.30 | -0.52 | 1.11 | 0.15 | 0.86 |
| | Control | 0.24 | -0.21 | 0.68 | | |
| | Phosphorus | 0.08 | -0.55 | 0.71 | | |
| **Average Grass Richness[1]** | Nitrogen | -1.04 | -2.51 | 0.43 | 2.49 | 0.10 |
| | Control | 0.77 | -0.29 | 1.83 | | |
| | Phosphorus | 0.13 | -0.69 | 0.94 | | |

[1] Square root transformed

[2] Slope/Regression coefficient

[3]Upper/lower limit of 95% confidence interval

[4]Comparison of regression coefficients among nutrient treatments ($H_o$: $\beta_1 = \beta_2 = \beta_3$)

## APPENDIX D. RESTORATION EXPERIMENT DESIGN AND MINIRHIZOTRON PLOT SELECTION FOR THE EKRE PLOTS

Table D.1. Species planted at the Albert Ekre Grassland Preserve. Nomenclature follows the USDA Plants Database.

| Forbs, Shrubs, Legumes | Grasses |
|---|---|
| *Achillea millefolium* L. | *Agropyron cristatum* (L.) Gaertn. |
| *Allium stellatum* Fraser ex. Ker Gawl. | *Andropogon gerardii* Vitman |
| *Artemisia dracunculus* L. | *Bouteloua curtipendula* (Michx.) Torr. |
| *Artemisia frigida* Willd. | *Bouteloua gracilis* (Willd. Ex Kunth) Lag. ex Griffiths |
| *Asclepias verticillata* L. | *Bromus inermis* Leyss. |
| *Astragalus canadensis* L. | *Calamovilfa longifolia* (Hook) Scribn. |
| *Cirsium arvense* (L.) Scop. | *Elymus canadensis* L. |
| *Coreopsis lanceolata* L. | *Hesperostipa comata* (Trin. & Rupr.) Barkworth |
| *Dalea purpurea* Vent. | *Hordeum jubatum* L. |
| *Gaillardia aristata* Pursh. | *Koeleria macrantha* (Ledeb.) Schult |
| *Galium boreale* L. | *Nassella viridula* (Trin.) Barkworth |
| *Geum triflorum* Pursh | *Panicum virgatum* L. |
| *Grindelia squarrosa* (Pursh) Dunal | *Poa pratensis* L. |
| *Helianthus maximiliani* Schrad. | *Pseudoroegneria spicata* (Pursh) A. Love |
| *Helianthus pauciflorus* Nutt. | *Schizachyrium scoparium* (Michx.) Nash |
| *Heterotheca villosa* (Pursh) Shinners | *Sorghastrum nutans* (L.) Nash |
| *Linum perenne* L. | *Sporobolus cryptandrus* (Torr.) A. Gray |
| *Lupinus perennis* L. | |
| *Melilotus officinalis* (L.) Lam. | |
| *Oenothera biennis* L. | |
| *Oligoneuron rigidum* (L.) Small | |
| *Potentilla arguta* Pursh | |
| *Ratibida columnifera* (Nutt.) Woot. & Standl. | |
| *Rosa arkansana* Porter | |
| *Rudbeckia hirta* L. | |
| *Solidago missouriensis* Nutt. | |
| *Symphyotrichum ericoides* (L.) G.L. Nesom | |
| *Taraxacum officinale* F.H. Wigg | |
| *Tragopogon dubius* Scop. | |
| *Verbena stricta* Vent. | |
| *Vicia americana* Muhl. ex Willd. | |

Table D.2. Details about the plots selected for minirhizotron tube installation at the Albert Ekre Grassland Preserve including nutrient type and application rate, species richness at peak aboveground production, and biomass at peak aboveground production. The biomass estimates were obtained by harvesting 0.25 $m^2$ quadrats while the species richness estimates were obtained through visual surveys. The data presented were collected in 2004 – prior to the implementation of the mowing treatment.

| **Nutrient Treatment** | **Species Richness** | **Biomass ($g/m^2$)** |
|---|---|---|
| High Nitrogen | 1 | 542 |
| High Nitrogen | 2 | 392 |
| High Nitrogen | 2 | 271 |
| High Nitrogen | 2 | 270 |
| High Nitrogen | 1 | 247 |
| High Nitrogen | 2 | 224 |
| High Nitrogen | 2 | 213 |
| High Nitrogen | 2 | 205 |
| High Nitrogen | 4 | 51.5 |
| High Nitrogen | 5 | 33.2 |
| Low Nitrogen | 1 | 528 |
| Low Nitrogen | 10 | 387 |
| Low Nitrogen | 1 | 303 |
| Low Nitrogen | 10 | 234 |
| Low Nitrogen | 19 | 220 |
| Low Nitrogen | 16 | 218 |
| Low Nitrogen | 10 | 210 |
| Low Nitrogen | 10 | 175 |
| Low Nitrogen | 2 | 144 |
| Low Nitrogen | 4 | 143 |
| Low Nitrogen | 4 | 133 |
| Low Nitrogen | 2 | 123 |
| Low Nitrogen | 4 | 119 |
| Low Nitrogen | 4 | 96.0 |
| Low Nitrogen | 1 | 88.1 |
| Low Nitrogen | 1 | 80.2 |
| Low Nitrogen | 2 | 34.1 |
| Low Nitrogen | 2 | 13.4 |

Table D.2. Details about the plots selected for minirhizotron tube installation at the Albert Ekre Grassland Preserve including nutrient type and application rate, species richness at peak aboveground production, and biomass at peak aboveground production (continued).

| Nutrient Treatment | Species Richness | Biomass ($g/m^2$) |
|---|---|---|
| High Phosphorus | 14 | 492 |
| High Phosphorus | 5 | 380 |
| High Phosphorus | 5 | 325 |
| High Phosphorus | 14 | 295 |
| High Phosphorus | 7 | 295 |
| High Phosphorus | 13 | 232 |
| High Phosphorus | 2 | 214 |
| High Phosphorus | 14 | 201 |
| High Phosphorus | 8 | 182 |
| High Phosphorus | 2 | 171 |
| High Phosphorus | 5 | 160 |
| High Phosphorus | 2 | 157 |
| High Phosphorus | 1 | 153 |
| High Phosphorus | 1 | 130 |
| High Phosphorus | 1 | 118 |
| High Phosphorus | 2 | 101 |
| High Phosphorus | 2 | 98.2 |
| High Phosphorus | 5 | 96.9 |
| Low Phosphorus | 3 | 575 |
| Low Phosphorus | 4 | 556 |
| Low Phosphorus | 8 | 286 |
| Low Phosphorus | 7 | 261 |
| Low Phosphorus | 15 | 178 |
| Low Phosphorus | 14 | 171 |
| Low Phosphorus | 11 | 169 |
| Low Phosphorus | 1 | 168 |
| Low Phosphorus | 12 | 131 |
| Low Phosphorus | 5 | 103 |
| Low Phosphorus | 1 | 93.6 |
| Low Phosphorus | 5 | 92.0 |