The Impact of Variable Precipitation on the Performance of Wetland and Grassland Plants

by

Teresa Julia Didiano

A thesis submitted in conformity with the requirements for the degree of Master of Science
Department of Geography and Planning
University of Toronto

© Copyright by Teresa Julia Didiano 2015
The Impact of Variable Precipitation on the Performance of Wetland and Grassland Plants

Teresa Julia Didiano

Master of Science

Department of Geography and Planning
University of Toronto

2015

Abstract

Climate change is causing increased precipitation variability, leading to large, infrequent precipitation events. This will have ecological consequences for plants because hydrological inputs will become transient. Despite this, there is a lack of evidence explaining the impact of variable precipitation on plant performance. To address this, this thesis asked three questions: (i) How does variable precipitation impact wetland and grassland plant performance? (ii) Does variable precipitation impact plant functional groups differently? And, (iii) do wetland and grassland plants respond similarly or differently to variable precipitation? A greenhouse and field experiment was conducted with wetland and grassland monocot and eudicot species, the amount and frequency of precipitation was manipulated, and plant performance was measured. This thesis reveals that large, infrequent precipitation events negatively impacted plant performance with larger effects on eudicots than monocots. This suggests that variable precipitation could have implications for the diversity and abundance of species in plant communities.
Acknowledgments

Thank you to my supervisors, Tim Duval and Marc Johnson, for their support and guidance throughout my studies. To the SWEHR Group and EvoEco Lab, I appreciate the opportunity to work with an academically diverse group of graduate students. My thesis would not be possible without numerous field assistants - Sarah Ariano, Vedrana Banjavcic, Tammy Duong, Bryn Fraser, Michael Harris, Jay Hong, Sydney Homewood, Adrian Lue, Alexus Maglalang, Matthew Malone, Danielle Radu, Courtney Soden, Axel Thomas, Prexa Vora, Heather Wilson, Victoria Wisniewski, Vivian Yip, and Samantha Zamora. Their enthusiasm, positivity, and camaraderie made fieldwork fun and enjoyable. Also, I am appreciative to Marianne Kalich, Brenda Pitton, and Phil Rudz for their assistance with various aspects of my research.

Thank you to my friends, both near and afar, for expressing curiosity in my research, as well as meaningful, positive, and fun companionship. Finally, to my family, despite joking I went to graduate school to learn how to ‘count grass’, their kind support and encouragement means a lot.
# Table of Contents

Acknowledgments........................................................................................................... iii

Table of Contents.............................................................................................................. iv

List of Tables ..................................................................................................................... vi

List of Figures .................................................................................................................... vii

List of Appendices ............................................................................................................ viii

1 Chapter One Introduction.............................................................................................. 1

1.1 Global and Regional Forecasted Climate Change ..................................................... 1

1.2 Ecological Implications of Variable Precipitation .................................................... 2

1.3 Experimental Systems ............................................................................................... 4

1.4 Thesis Objective and Research Questions ............................................................... 8

1.5 Figures ....................................................................................................................... 9

2 Chapter Two Precipitation magnitude and frequency affect plant performance but have limited effects on C and N concentration of wetland plants .................................................. 14

2.1 Introduction............................................................................................................... 14

2.2 Materials and Methods ............................................................................................ 17

2.3 Results..................................................................................................................... 20

2.4 Discussion ............................................................................................................... 21

2.5 Tables ..................................................................................................................... 26

2.6 Figures .................................................................................................................... 27

3 Chapter Three How the amount and frequency of precipitation affects the performance of 14 grassland species .................................................................................. 31

3.1 Introduction............................................................................................................... 31

3.2 Materials and Methods ............................................................................................ 34

3.3 Results..................................................................................................................... 37

3.4 Discussion ............................................................................................................... 40
3.5 Tables ................................................................................................................. 45
3.6 Figures ................................................................................................................. 47

4 Chapter Four Conclusions .................................................................................. 54

References ............................................................................................................. 57

Appendices ............................................................................................................. 64

Appendix A ............................................................................................................. 64
Appendix B ............................................................................................................. 65
Appendix C ............................................................................................................. 66
List of Tables

Table 2.1 Effect of precipitation treatments on plant performance and C and N concentration .. 26

Table 3.1 Summary of precipitation regimes................................................................. 45

Table 3.2 Effect of the amount and frequency of precipitation on plant performance ............ 46
List of Figures

Figure 1.1 Soil moisture during the growing season under small frequent vs large infrequent precipitation events. ................................................................. 9

Figure 1.2 Response of plant performance to changes in soil moisture ........................................ 10

Figure 1.3 Conceptual model of the interaction among the driver, ecosystem response, and mechanism .............................................................................. 11

Figure 1.4 Study species for Chapter 2. .................................................................................. 12

Figure 1.5 Study species for Chapter 3. .................................................................................. 13

Figure 2.1 Image of Chapter 2’s experimental design. ................................................................. 27

Figure 2.2 Average soil moisture and precipitation throughout the experiment ..................... 28

Figure 2.3 Above- and below-ground biomass for the three precipitation treatments .......... 29

Figure 2.4 C and N concentration for the three precipitation treatments ................................. 30

Figure 3.1 Experimental layout at the field site. ...................................................................... 47

Figure 3.2 Rainout shelter at the field site ................................................................................. 48

Figure 3.3 Soil volumetric moisture content for the amount and frequency of precipitation .... 49

Figure 3.4 Above-ground biomass for the amount and frequency of precipitation ............... 50

Figure 3.5 Below-ground biomass for the amount and frequency of precipitation .............. 51

Figure 3.6 Plant height for the amount and frequency of precipitation ................................. 52

Figure 3.7 Cohen’s d effect sizes for metrics of plant performance for the amount and frequency of precipitation .......................................................... 53
List of Appendices

TABLES

Table A.1 Locality information for seed sources, Chapter 3………………………………………………..64
Table C.1 Impact of precipitation regimes on stomatal conductance, Chapter 3………………..66

FIGURES

Figure B.1 Temporal changes in soil volumetric moisture content and precipitation regimes, Chapter 3…………………………………………………………………………………………..65
Chapter One
Introduction

Anthropogenic activity and natural phenomena are driving unprecedented changes in the abiotic and biotic environment. Temperature is rising, precipitation is becoming more variable, atmospheric CO\textsubscript{2} concentration is growing, droughts have increased in length and severity, and glaciers and snow cover are disappearing (Easterling \textit{et al.} 2000; Diffenbaugh & Field 2013; IPCC 2013). These climatic factors are subjecting ecosystems to novel environmental conditions that are modifying the structure and function of natural systems by threatening the health of biological organisms, driving species extinction, and increasing the invasion of exotic species (Walther \textit{et al.} 2002; Parmesan 2006; Wu \textit{et al.} 2011; Blois \textit{et al.} 2013; Reyer \textit{et al.} 2013; Zeppel, Wilks & Lewis 2014). Ecologists are confident that climatic factors will alter ecosystems but the magnitude and direction of these alterations is poorly understood and remains a key challenge to address in the 21\textsuperscript{st} century. For this reason, it is imperative that future research focuses on asking “how will these climatic factors alter ecosystems” and “what does this mean for the preservation and stability of ecosystems”. This thesis will tackle these questions by examining the ecological consequences of variable precipitation on the performance of wetland and grassland plants.

In this chapter, I will outline the forecasted changes in climate with an emphasis on precipitation variability in Canada. This will be followed by a discussion of the ecological implications of variable precipitation for plants. This chapter will conclude by introducing the experimental system and thesis objective and research questions.

1.1 Global and Regional Forecasted Climate Change

Over the last century, climate change has altered temperature and precipitation patterns which are key drivers of plant processes and patterns. Global mean surface temperature rose by 0.85°C between 1880 and 2012, and will continue to increase by 0.3-0.7°C within the next century. Similarly, global average precipitation increased between 1901 and 2008, and is projected to grow by 1% to 3% per degree Celsius increase in temperature (IPCC 2013). In concert with these changes, the number, magnitude, and frequency of weather extremes will increase (Easterling \textit{et al.} 2000; Diffenbaugh & Field 2013; IPCC 2013). Warm temperatures, large precipitation
events, droughts, and floods will become more frequent (Jentsch & Beierkuhnlein 2008; Orlowsky & Seneviratne 2012). Notable examples of these occurrences include the 2003 European heat wave (Ciais et al. 2005), and the 1930s Dust Bowl (Schubert et al. 2004), as well as more recent examples such as the ongoing drought in California, the 2015 Indian heat wave, and the 2013 Toronto flood when 126 mm of precipitation fell in a few hours. Weather extremes can have different and potentially greater ecological consequences for plants than changes in average climatic conditions (Robinson et al. 2013; Thompson et al. 2013). Thus, it is crucial to elucidate the response of plants to weather extremes to grasp the current and future dynamics of plant communities.

Regionally, simultaneous changes in temperature and precipitation will drive an increase in weather extremes in Canada, in particular variable precipitation (Qian et al. 2010; IPCC 2013). Across Canada, temperature increased by 1.4°C between 1950 and 2007 (Zhang et al. 2011), and precipitation increased by 5-35% between 1900 and 1998 (Zhang et al. 2000). Within the next century, temperature will increase and precipitation will change; however, the direction of this change is uncertain with some models projecting an increase and others a decrease (IPCC 2013). Although models lack quantitative agreement for precipitation, all models agree that precipitation will become more variable, characterized by large, infrequent precipitation events. This variability will occur because increased temperatures will cause higher evaporation rates that will alter the water-holding capacity of the atmosphere (Huntington 2006). For example, Cao & Ma (2009) assessed southern Ontario summer precipitation records (June to August) from 1979 to 2000 and found an increase in the number of large, infrequent precipitation events by eleven events per decade. Despite evidence that variable precipitation is reflective of precipitation regimes in Canada, there is relatively little knowledge as to the repercussions of this variability on plant communities.

1.2 Ecological Implications of Variable Precipitation

Climate change will intensify the hydrologic cycle causing precipitation regimes to be reflective of large, infrequent precipitation events (Easterling et al. 2000; Diffenbaugh & Field 2013; IPCC 2013). This will alter plant-soil-water dynamics which could affect plant growth and survival. There is considerable observational and experimental research on how changes in the annual and seasonal amount of precipitation as well as drought impact plants (Charles & Dukes 2009; Evans
et al. 2011; Jentsch et al. 2011; Hoeppner & Dukes 2012; Kuiper et al. 2014; Churchill et al. 2015; Knapp et al. 2015). Comparatively, fewer studies examine the effects of large, infrequent precipitation events on plants (Jentsch, Kreyling & Beierkuhnlein 2007; Beier et al. 2012; Zeppel, Wilks & Lewis 2014). This represents a gap in our knowledge and therefore it is important to understand if and how forecasted precipitation variability will impact plant communities.

Variable precipitation will alter the availability of water in soil for plant uptake (Knapp et al. 2008; Sala, Gherardi & Peters 2015). Large, infrequent precipitation will cause greater fluctuations in soil moisture in comparison to small, frequent precipitation events (Fig. 1.1; Knapp et al. 2008). During large, infrequent precipitation events plants will have ample access to water and, in extreme cases, too much water if the precipitation event causes flooding. In contrast, plants will be water stressed during extended dry periods that intersperse these precipitation events. This will cause plant performance to decrease and plant mortality to increase once plants surpass their permanent wilting point (Fig. 1.2; Rodriguez-Iturbe 2000).

Some important metrics of plant performance include biomass, stomatal conductance, and nitrogen concentration. Biomass defines the productivity and growth rate of plants. Stomatal conductance provides insight into if and how plants are using water (e.g., carbon uptake and water loss). Nitrogen is an essential nutrient for plants because it contributes to plant processes such as chlorophyll production for photosynthesis and nitrogen-based plant defences. Thus, it is important to assess the performance of plants to gain insight into how plants will fair under variable precipitation.

Studies are beginning to examine the impact of precipitation variability on plant performance. Although it is expected that large, infrequent precipitation events will decrease plant performance, the results of these studies do not always support this prediction. For example, Wilcox et al. (2015) examined plant performance under ambient precipitation, small frequent precipitation events, and large infrequent precipitation events between May to August over two years in a shortgrass, northern mixed, and tallgrass prairie. They found no difference in above- and below-ground primary productivity between the small frequent and large infrequent precipitation events. In contrast, Fry et al. (2014) examined a mesic grassland’s response to ambient precipitation, drought, and large infrequent precipitation events. Relative to ambient precipitation, they found a decrease in species richness and cover in the large infrequent
precipitation event treatment, an increase in ecosystem respiration in the drought and large infrequent precipitation event treatments, and no change in net ecosystem exchange and photosynthetic rate. These studies reveal that the impact of variable precipitation on plants can be complex.

To unravel this complexity, it is important to investigate a mechanistic explanation for what drives the response of plant communities to variable precipitation (Fig. 1.3). These mechanisms encompass a suite of biotic and abiotic variables including soil type, plant functional type, and plant traits. Schneider et al. (2014) addressed this idea by examining the individual response of two mesic grassland species (*Agropyron repens* and *Lupinus perennis*) to changes in the amount and frequency of precipitation. They found that decreasing the frequency of precipitation reduced biomass in both species; while changes in the amount of precipitation or the interaction between the amount and frequency of precipitation produced distinctive species’ responses. Thus, to provide a predictive understanding of the response of plants to climate change, it is important to understand what factors drive the response of plant communities to environmental change.

Climate-driven changes in precipitation will have large impacts on ecosystems. Changes in water resources can drive shifts in the diversity, abundance, and composition of plant communities, which will have cascading effects on other components of natural systems. Observational and experimental manipulations of climate parameters can provide insight into plant performance that will be crucial for ecosystem management. If we know what species will persist or die in response to variable precipitation, we can conserve and restore plant communities to be resistant and resilient to these changes.

### 1.3 Experimental Systems

To investigate the impacts of variable precipitation on plant performance, we chose two experimental systems – wetlands and grasslands. These systems were chosen because: (i) they have been the subject of numerous studies that explore the influence of changes in water resources on plant processes and patterns. However, the majority of studies focus on alterations in the amount of precipitation, drought conditions, and fluctuations in the water table. By contrast, few studies examine the effects of precipitation variability (Jentsch, Kreyling & Beierkuhnlein 2007; Beier *et al.* 2012; Zeppel, Wilks & Lewis 2014), and this information is
crucial to comprehend the ecological consequences of climate change for ecosystems. (ii) The performance of grassland and wetland plants is tightly linked to soil water availability making plants in these systems susceptible to changes in water resources. Wetland plants are adapted to environments that are temporally saturated whereas grassland plants are adapted to environments that undergo recurrent dry periods. (iii) Wetlands and grasslands are composed of diverse communities of monocots (e.g., grass, sedge, rush) and eudicots. This presents an opportunity to examine if and how different plant functional groups respond to variable precipitation and potentially provide a mechanistic explanation for changes observed at the community-level. (iv) A significant proportion of experimental evidence for plant responses to variable precipitation is limited geographically to Midwestern USA and Europe. We have limited knowledge if and how the results of those studies will translate to other regions of the world such as southern Ontario where our study species were collected and which precipitation records are based on.

WETLANDS

Wetlands are biologically diverse and productive ecosystems that support multiple plant and animal species. Defined by the permanent or temporary presence of static or flowing water, the water table of wetlands is found at, near, or ≤ 6 m above the surface over the course of the growing season (Ramsar Convention Secretariat 2013). Based on this definition, there are five wetlands groupings: marine (e.g., coastal lagoons, coral reefs), estuarine (e.g., tidal marshes, mangroves), lacustrine (e.g., wetlands along lakes), riverine (e.g., riparian areas), and palustrine (e.g., marshes, swamps, bogs) (Millenium Ecosystem Assessment 2005). This ecosystem is intrinsically and economically valuable providing ecosystem services and functions including carbon storage, water purification, climate regulation, genetic material, refugia for plants and animals, recreational opportunities, and educational benefits (Millenium Ecosystem Assessment 2005; Mitsch & Gosselink 2007).

Covering 5.7 million km² of land (Millenium Ecosystem Assessment 2005), or 5-8% of Earth’s surface (Mitsch & Gosselink 2007), wetlands have a wide geographic distribution spanning from mangroves in the tropics, to fens and marshes in temperate and boreal regions, to peatlands in the arctic. Despite this, wetlands are degraded due to human and natural disturbance (e.g., agriculture and infrastructure development, invasive species, over-draining) and the expectation is that climate change will worsen this loss (Millenium Ecosystem Assessment 2005). During the
20th century, 41% of wetlands in Canada were lost and much of this loss, ca. 70-90%, occurred in southern Ontario (Erwin 2009). Thus, it is important to understand how to conserve and restore wetland systems to preserve their unique plant and animal biodiversity.

Wetlands represent a transitional zone between terrestrial and aquatic systems. The presence of water during the growing season creates conditions where plants temporarily or permanently have access to water and this alters physical processes such as sediment transport, soil chemistry, and water chemistry (Mitsch & Gosselink 2007). This creates a unique hydrological environment that cannot be found in other ecosystems and is conducive for water-adapted species. However, if climate change projects that precipitation regimes will be reflective of large, infrequent precipitation events this could influence the hydrological regimes of wetlands. Although there are multiple components such as evaporation and groundwater that contribute to the hydrological regime of a wetland (Labaugh 1986; Mitsch & Gosselink 2007), large, infrequent precipitation events could cause the water table to drop. Consequently, to strengthen our knowledge of the impacts of climate-driven changes in precipitation on wetlands it is important to consider scenarios where the water table is low and the plants receive transient precipitation inputs.

For Chapter 2, we selected eight perennial, herbaceous species that represent different plant functional types (Fig. 1.4). We included three eudicots: *Eupatorium perfoliatum* L. (Asteraceae), *Mimulus ringens* L. (Scrophulariaceae), and *Solidago ohioensis* Riddell (Asteraceae). We also included five monocots: *Acorus americanus* Raf. (Acoraceae), *Carex aquatilis* Wahlenb. (Cyperaceae), *Glyceria striata* (Lam.) Hitchc. (Poaceae), *Juncus effusus* L. (Juncaceae), and *Schoenoplectus tabernaemontani* K.C. Gmel. (Cyperaceae). These species are native to Ontario and found in diverse wetland habitats including fens, marshes, and wet meadows. These systems are periodically or continually flooded offering a habitat for water-tolerant species. Other studies have illustrated that species such as wetland adapted species of *Carex* and *J. effusus* are more tolerant to a wider range of hydrologic conditions than *M. ringens* (Kercher & Zedler 2004; Fraser & Karnezis 2005; Touchette et al. 2010) and *A. americanus* (Romanello et al. 2008). If and how these species will respond to alterations in other components of the water budget remains unanswered.

GRASSLANDS
Grasslands are the most productive terrestrial ecosystem and they support diverse communities of plant and animal species. This system produces high levels of plant primary productivity, contributes habitat and forage for animals, and promotes physical processes such as water regulation, nutrient cycling, soil formation, and erosion control (Samson, Knopf & Ostlie 1988; White, Murray & Rohweder 2000). Distinguished by plant communities that are dominated by grass and herbaceous species with few shrubs and trees (White, Murray & Rohweder 2000), the species composition is maintained by undergoing disturbances such as fire, grazing, periodic drought, and erosion (Samson, Knopf & Ostlie 1988).

Grasslands cover 41-56 million km$^2$ or 31-43% of Earth’s surface (White, Murray & Rohweder 2000). In North America, grasslands span from New Mexico to Ontario and throughout the western provinces of Canada (Weaver 1954). Across this geographic distribution there is a large climatic gradient with average annual precipitation and average daily temperature ranging from 200-800 mm and 8-14$^\circ$C, respectively. This creates conditions that support arid, semi-arid, and mesotrophic grasslands with diverse community assemblages such as shortgrass, mixed, and tallgrass prairies (Samson, Knopf & Ostlie 1988). In southern Ontario, less than 3% of grasslands that existed before human settlement remain, with Ojibway Prairie in Windsor being the largest remnant left (Bakowsky & Riley 1994).

For Chapter 3, we selected 14 herbaceous species that are found in southern Ontario (Fig. 1.5). We included four monocots: *Andropogon gerardii* Vitman (Poaceae), *Elymus canadensis* L. (Poaceae), *Elymus riparius* Wiegand (Poaceae), and *Panicum virgatum* L. (Poaceae). We included 10 eudicots: *Asclepias tuberosa* L. (Asclepiadaceae), *Desmodium canadense* (L.) DC (Fabaceae), *Eupatorium perfoliatum* L. (Asteraceae), *Euphorbia corollata* L. (Euphorbiaceae), *Geum triflorum* Pursh (Rosaceae), *Oenothera biennis* L. (Onagraceae), *Penstemon hirsutus* (L.) Willd (Scrophulariaceae), *Solidago nemoralis* Aiton (Asteraceae), *Verbena stricta* Vent. (Verbenaceae), and *Zizia aurea* (L.) W.D.J. Koch (Apiaceae). Other studies have examined these species (e.g., *Andropogon gerardii*, *Panicum virgatum*, and *Solidago* spp.) at the community level (Fay et al. 2008; Heisler-White et al. 2009); however, we have little evidence for the individual response of species. This is important for providing a mechanistic explanation for changes at the community- and ecosystem-level.
1.4 Thesis Objective and Research Questions

The objective of this thesis is to explore the impacts of variable precipitation on plant performance of wetland and grassland species. Specifically, this thesis addresses the following questions:

(i) How does variable precipitation impact plant performance?

(ii) Does variable precipitation impact plant functional groups differently?

(iii) Do wetland and grassland plants respond similarly or differently to variable precipitation?

By addressing these questions, this research will improve our understanding of the response of contemporary and future plant communities to climate change. This information will be influential for ecosystem managers as they develop tools and resources to mitigate the effects of environmental disturbance.
1.5 Figures

Figure 1.1 Soil moisture throughout the growing season under small frequent vs large infrequent precipitation events. The top grey box within each figure represents anoxic conditions, the middle blue box represents optimal conditions that do not limit plant performance, and the bottom box represents drought conditions. The black thick line shows temporal fluctuations in soil moisture. Under small frequent precipitation events soil moisture fluctuates within optimal conditions throughout the growing season. Under large infrequent precipitation events soil moisture fluctuates outside the bounds of optimal conditions on numerous occasions throughout the growing season. Source: Modified from Knapp et al. (2008).
Figure 1.2 The response of plant performance (e.g., plant biomass, transpiration, and photosynthesis) to changes in soil moisture. $S^*$ represents transition points in soil moisture where plants are under water stress (e.g., too much or too little water), and $S_w$ represents soil moisture where plants are limited by a lack of water to maintain osmotic turgor or too much water that leads to low soil oxygen availability and anoxia. Between these two extremes are optimal conditions for plant performance. Source: Modified from Rodriguez-Iturbe (2000).
**Figure 1.3** Conceptual model of the interaction among the driver, ecosystem response, and mechanism. Precipitation can drive changes in, for example, soil moisture, plant primary productivity, or plant physiological responses leading to alterations in community composition.
Figure 1.4 Study species for Chapter 2.
Figure 1.5 Study species for Chapter 3.
Chapter Two
Precipitation magnitude and frequency affect plant performance but have limited effects on C and N concentration of wetland plants

ABSTRACT

The structure and function of wetland plant communities is sensitive to patterns of precipitation. In some regions, climate change is expected to result in greater magnitude but infrequent precipitation events, which could lead to deeper water tables. Typically, the response of wetland plants to changing precipitation regimes is documented under alterations in the amount of precipitation or drought rather than the magnitude and frequency of precipitation events. Thus, we lack a clear understanding of the response of wetland plants to forecasted precipitation regimes and the factors governing these responses. Here, we seek to understand how changes in the magnitude and frequency of precipitation affect: i) soil moisture; ii) plant performance; and iii) plant C and N concentration. We established a greenhouse study using eight species (three eudicots, five monocots) to which we applied three precipitation treatments. Our results show a decrease in soil moisture by ca. 53% for plants grown under the 80 mm/month, 15 d (infrequent) and 110 mm/month, 15 d (greater magnitude-infrequent) treatments in comparison to the 80 mm/month, 3 d (control) treatment. This contributed to a decrease in biomass production for all eudicots by 10-49% and two of five monocots by 25-54% when subjected to the infrequent treatment in comparison to the control. In contrast, changes in C and N concentration were limited to two forb species and these species showed changes under the infrequent treatment. Our results show that some wetland species will be impacted by changes in precipitation frequency, and infrequent precipitation needs to be accompanied by greater magnitude precipitation events if drought-susceptible species are to maintain their performance.

2.1 Introduction

A consequence of climate change will be the intensification of the hydrologic cycle (Huntington 2006), including changes in the amount of seasonal and annual precipitation as well as the magnitude and frequency of precipitation events (Easterling et al. 2000; Diffenbaugh & Field 2013; IPCC 2013). In some regions, precipitation is expected to become more intense, involving large, infrequent precipitation episodes separated by prolonged dry periods (Smith 2011; IPCC
Climate driven changes in the magnitude and frequency of precipitation in concert with rising temperatures could have negative impacts on wetlands. These ecosystems house biologically diverse plant and animal communities, improve water quality, and store and sequester carbon (Millenium Ecosystem Assessment 2005; Mitsch & Gosselink 2007), all of which are dependent on the hydrological regime. Although wetland hydrology is controlled by multiple components besides precipitation (e.g., evapotranspiration, surface water, and groundwater) (Labaugh 1986; Mitsch & Gosselink 2007), the redistribution of precipitation into fewer, larger events will have cascading effects on a wetland’s watershed. This will alter surface water and groundwater as well as cause water table drawdown that forces the system to rely on transient precipitation inputs. Thus, changes to precipitation regimes have the potential to affect plant community and ecosystem dynamics.

There is mounting evidence that climate driven alterations in the amount of seasonal and annual precipitation, as well as drought will impact wetland plants (Charles & Dukes 2009; Kuiper et al. 2014; Churchill et al. 2015). Comparatively, fewer studies examine the response of wetland plants to changes in the magnitude and frequency of precipitation events (Miao, Zou & Breshears 2009; Nijp et al. 2014). Typically, observations for the impact of precipitation variability on plants typically focus on terrestrial ecosystems. Thus, we lack a comprehensive understanding of the impacts of variable precipitation events on plants, the factors governing these impacts, and how these impacts vary across different ecosystem types (Beier et al. 2012; Zeppel, Wilks & Lewis 2014). Here, we seek to address this gap in our knowledge by assessing the effects of changing magnitude and frequency of precipitation events on wetland plant performance and C and N dynamics.

Changes in precipitation variability can have different impacts on ecological patterns and processes than changes in mean precipitation (Heisler & Weltzin 2006; Reyer et al. 2013). Redistributing precipitation into fewer, larger events with longer intervening dry periods will affect the infiltration depth of water and duration of soil saturation causing changes in soil moisture (Schwinning & Sala 2004; Knapp et al. 2008; Wu et al. 2012). Shifting soil moisture regimes could cross a species’ threshold of tolerance simulating changes in, for example, physiological responses (Fay et al. 2008; Jentsch et al. 2011), plant productivity (Zhang et al. 2013a; Schneider et al. 2014), and nutrient cycling (Dijkstra et al. 2012; Evans & Burke 2013). These changes could affect the vitality of wetlands by triggering alterations in species’
abundance, distributions, and interactions which will lead to shifts in community composition and, in extreme cases, species’ extinctions (Parmesan 2006; Bellard et al. 2012). Studies are beginning to assess the impacts of variable precipitation on wetland plants. For example, Nijp et al. (2014) examined carbon exchange of various Sphagnum spp. grown under different precipitation frequencies during water table drawdown. For certain species, they found that during water table drawdown precipitation maintained carbon exchange at similar levels as plants grown under a high water table. This study provides evidence that greater magnitude, infrequent precipitation events impact wetland plants; however, more research is needed to disentangle the precipitation-water table relationship under different climate change scenarios and the ability of wetland plants to exploit transient precipitation inputs of various magnitudes and frequencies.

Understanding how plant communities will respond to variable precipitation is challenging because precipitation is region-specific and different ecosystems consist of varying abiotic and biotic factors. Species-specific responses to changing water regimes have been observed in wetlands. For example, shifts from a diverse plant wetland community consisting of mosses, grasses, and forbs to a shrub dominated community have been caused by changing climatic factors such as precipitation (Murphy, Laiho & Moore 2009; Kettridge et al. 2015), the interaction between precipitation and temperature (Weltzin et al. 2000; Weltzin et al. 2003a), and the interaction among precipitation, temperature, and carbon dioxide (Dieleman et al. 2015). Although this observation has been studied under drought, similar transitions might occur as precipitation becomes more variable. In particular, considering how different plant functional types perform under changing precipitation regimes could provide insight into the mechanistic basis of changing plant communities due to increased variability in precipitation. For example, plant functional types like monocots and eudicots frequently differ in morphological (e.g., root depth) and physiological (e.g., transpiration) features that make them more or less able to survive under changing water regimes (Jackson et al. 1996; Nippert & Knapp 2007). Thus, examining how different plant functional types will respond to variable precipitation regimes will be important for understanding plant communities under future climate scenarios.

Here, we examined the response of multiple herbaceous wetland plant species to variation in the magnitude and frequency of precipitation events during water table drawdown over an entire growing season. Specifically, we addressed the following questions: (i) How does variation in
the frequency of precipitation events affect soil moisture when there is no change in the amount of precipitation as well as an increase in the amount of precipitation? We predict that soil moisture, quantified as soil volumetric moisture content, will become more variable under large, infrequent precipitation events in comparison to small, frequent precipitation events (Knapp et al. 2008). (ii) Do wetland species exhibit decreased plant performance induced by large, infrequent precipitation events? We predict that large, infrequent precipitation events will cause extended dry periods that will reduce plant performance in comparison to small, frequent precipitation events (Knapp et al. 2008; Garssen, Verhoeven & Soons 2014). (iii) Do wetland species exhibit decreased plant N concentration induced by large, infrequent precipitation events? We predict that pulses of water from large, infrequent precipitation events will cause extended dry periods that could reduce nitrogen concentration in comparison to small, frequent precipitation events (Anderson & Mitsch 2005; Knapp et al. 2008).

2.2 Materials and Methods

STUDY SPECIES

We selected eight perennial, herbaceous species that represent different plant functional types. We included three eudicots: *Eupatorium perfoliatum* L. (Asteraceae), *Mimulus ringens* L. (Scrophulariaceae), and *Solidago ohioensis* Riddell (Asteraceae). We also included five monocots: *Acorus americanus* Raf. (Acoraceae), *Carex aquatilis* Wahlenb. (Cyperaceae), *Glyceria striata* (Lam.) Hitchc. (Poaceae), *Juncus effusus* L. (Juncaceae), and *Schoenoplectus tabernaemontani* K.C. Gmel. (Cyperaceae) (Crow & Hellquist 2000). These species are native to Ontario and found in diverse wetland habitats including fens, marshes, and wet meadows. All species can reproduce vegetatively through rhizomes and sexually via seeds (Gleason & Cronquist 1991; Crow & Hellquist 2000).

COMMON GARDEN STUDY

In summer 2013, we conducted a greenhouse study to examine the effects of precipitation variability under water table drawdown on eight wetland species (Fig. 2.1). For each species described above, seeds were collected from multiple individuals in a wetland in Walsingham, Ontario, Canada (42°40’N, 80°31’W). In June 2012, seeds were germinated on soil in ~0.05 L pots and grown in a greenhouse during the summer and overwintered outside in Walsingham. In
May 2013, we transferred the plants from Walsingham to a rooftop greenhouse at the University of Toronto Mississauga. We removed plants from their original pots, loosened the soil and roots, and transplanted the plants into 1.67 L pots with soil (Pro-mix BX Mycorrhizae, Quakertown, PA, USA), and approximately 0.38 g of slow-release fertilizer (Smartcote, N:P:K, 14:14:14, Brantford, ON, Canada). We randomized 240 plants into fifteen 81 L polyethylene pools, with 16 plants (two replicates per species) per pool. We maintained the water table at the soil surface between 23 May and 3 June 2013. From 4 June to 18 August 2013, we allowed the water table to drop, and plants only received water from the precipitation treatments (see below). This watering regime was chosen to simulate a typical summer hydroperiod of a wetland where the plants remain saturated early in the growing season following snowmelt and experience drawdown during the summer due to high rates of evapotranspiration and reduced inputs from surrounding watersheds that will both be exacerbated by climate change.

PRECIPITATION TREATMENTS

Our precipitation treatments simulated forecasted precipitation regimes that are reflective of large, infrequent precipitation events with intervening, long dry periods. We applied three treatments that manipulate the magnitude and frequency of precipitation and assigned five pools to one of the three precipitation treatments in a fully-randomized design. Treatment 1, hereafter called ‘control’, was 80 mm per month distributed as 8 mm every 3 days. On 3 August 2013 an additional 12 mm was added to an 8 mm event to prevent plant mortality during a heat wave (see Discussion). Treatment 2, hereafter called ‘infrequent’, was 80 mm per month distributed as 40 mm every 15 days. The control and infrequent treatment are based on a precipitation value of 75 mm per month which represents the 30-year average summer monthly precipitation in Mississauga, Ontario, Canada (data: Government of Canada; http://climate.weather.gc.ca/climate_normals). Treatment 3, hereafter called ‘greater magnitude-infrequent’, was 110 mm per month distributed as 55 mm every 15 days. Similar to the infrequent treatment, the greater magnitude-infrequent treatment simulated a large, infrequent precipitation event, but of large size which is in line with climate change projections for Ontario (Cao & Ma 2009; IPCC 2013).

SOIL MOISTURE AND PLANT TRAIT MEASUREMENTS
To quantify soil moisture, we measured soil volumetric moisture content within the top 12 cm using a HydroSense Soil Water Measurement System (Campbell Science Canada Corp., Edmonton, AB, Canada). We took measurements on a bi-weekly basis at the beginning of the experiment and increased the frequency to a weekly basis near the end of the experiment as the water table was drawing down.

To assess plant performance, we measured leaf number, plant height, and biomass production. On August 17, 2013, we counted the number of fully expanded leaves (eudicots and grass species) or stems (sedge and rush species) and measured plant height as the height of the tallest leaf or stem. We then harvested above- and below-ground biomass, and weighed the mass of plants following drying for 72 hours at 60°C.

We analyzed subsamples of plant leaf and below-ground tissue for C and N concentration. We randomly selected 5 replicates for each species per treatment. We ground dry samples in a Wiley mill using a 60-mesh (Arthur H. Thomas Co., Philadelphia, PA, USA), weighed samples on a microbalance, and determined the concentration of C and N (w/w) with an elemental analyzer (ECS 4010 Elemental Combustion System, Costech Analytical Technologies Inc., Valencia, CA, USA).

**STATISTICAL ANALYSIS**

We tested the effects of precipitation treatment on soil moisture, plant performance, and C and N concentration using linear mixed-effect models with the ‘lme’ function in the ‘nlme’ package (Pinheiro et al. 2014), of R 3.1.2 (R Core Team 2014). Our response variables were soil moisture, total biomass, above-ground biomass, below-ground biomass, above:below-ground biomass ratio, leaf number, plant height, C:N ratio, or C and N concentration. The fixed factor was treatment and the random factor was pool nested within treatment. We log- or square root-transformed C:N ratio and C and N concentration data for certain species to improve normality and homogeneity of variance, while we ranked-transformed leaf number and plant height data because assumptions of normality were not met. To test for statistical significance in mean responses between precipitation treatments, we conducted Tukey tests with the ‘glht’ function using the ‘multcomp’ package (Hothorn, Bretz & Westfall 2008).
2.3 Results

SOIL MOISTURE

We found that soil moisture decreased and became more variable when precipitation events decreased in frequency. We observed a decrease in soil volumetric moisture content in the infrequent (80 mm, 15 d) and greater magnitude-infrequent (110 mm, 15 d) treatments by 56% and 51%, respectively, relative to the control (80 mm, 3 d) (P < 0.0001; Fig. 2.2). We also found that the coefficient of variation in soil volumetric moisture content was greatest in the greater magnitude-infrequent treatment (49%), followed by the infrequent treatment (40%) and control (34%), respectively.

PLANT PERFORMANCE

Our precipitation treatments caused a change in plant performance for all eudicots but only some monocots. We observed a decrease in below-ground biomass for all eudicots and a decrease in above-ground biomass for only one eudicot in the large, infrequent precipitation events (Table 2.1). Specifically, *E. perfoliatum* decreased in below-ground biomass by 49% for the infrequent treatment relative to the control (Fig. 2.3a). *Solidago ohioensis* decreased in below-ground biomass for the infrequent treatment and greater magnitude-infrequent treatment by 47% and 29%, respectively, relative to the control (Fig. 2.3b). However, *M. ringens* decreased in above- and below-ground biomass for the infrequent treatment by 36% and 39%, respectively, relative to the control (Fig. 2.3c). In addition to the eudicots, we observed a decrease in biomass for two monocots. *Schoenoplectus tabernaemontani* decreased in below-ground biomass for the infrequent treatment by 54% and greater magnitude-infrequent treatment by 36% relative to the control (Fig. 2.3d). We also found a decrease in above-ground biomass of *S. tabernaemontani* by 48% for the infrequent treatment relative to the control, while the greater magnitude-infrequent treatment did not significantly differ from the control. *Acorus americanus* decreased in below-ground biomass for the infrequent treatment by 25% relative to the control (Fig. 2.3e). We did not find any significant changes in biomass across our treatments for *C. aquatilis*, *G. striata*, and *J. effusus* (Fig. 2.3f-h). Thus, our results show that for all species that experienced a change in biomass, this change was primarily driven by below-ground biomass with just two species also showing a change in above-ground biomass.
We found changes in the relative allocation to above- and below-ground biomass for two species (Table 2.1). Under the infrequent treatment, *A. americanus* (control: 0.77; infrequent: 0.87; greater magnitude-infrequent: 0.71) and *E. perfoliatum* (control: 1.57; infrequent: 2.42; greater magnitude-infrequent: 2.10) allocated relatively less to below-ground biomass than it did in the other two treatments, which increased the above:below-ground ratio. Therefore, these species allocated fewer resources to below-ground biomass production than above-ground biomass production. 

Lastly, we found a change in leaf number and/or plant height for two species (Table 2.1). *Mimulus ringens* decreased in leaf number by 58% and plant height by 23% in the infrequent treatment in comparison to the control. In contrast, *S. tabernaemontani* decreased in leaf number in the infrequent treatment by 40% in comparison to the control. 

**PLANT C AND N CONCENTRATION**

Contrary to the widespread changes in plant performance, changes in N concentration and C:N ratio were limited to *E. perfoliatum* and *M. ringens* (Table 2.1). We observed no change in C concentration in leaf and below-ground tissue of *E. perfoliatum* (Fig. 2.4a). However, N concentration increased by 33% in leaf tissue of *E. perfoliatum* in the infrequent treatment (Fig. 2.4b), which led to a decrease in C:N ratio by 22% (Fig. 2.4c). Similarly, we observed no change in C concentration in leaf and below-ground tissue of *M. ringens* (Fig. 2.4d), while the N concentration was higher in leaf tissue by 73% and below-ground tissue by 20% in the infrequent treatment in comparison to the control (Fig. 2.4e). This led to a decrease in C:N ratio in the infrequent treatment for leaf tissue by 38% and below-ground tissue by 17% (Fig. 2.4f). Thus, changes in C:N ratio of *E. perfoliatum* and *M. ringens* were driven by N concentration.

2.4 Discussion

Our study reveals three key findings on the impacts of variable precipitation on wetland plant performance and C and N dynamics. First, variable precipitation caused a decrease in average soil moisture and increase in soil moisture variability (Fig. 2.2). Second, we found that a decrease in the frequency of precipitation but not a change in the amount of precipitation caused a decrease in biomass production for all eudicots but only some monocots (Fig. 2.3). Third, we observed a decrease in N concentration and increase in C:N ratio in only two eudicots (Fig. 2.4).
Taken together, our results show that variable precipitation can impact wetland plant performance and these effects vary according to the frequency of precipitation and plant functional type.

**RESPONSE OF PLANT PERFORMANCE TO PRECIPITATION TREATMENTS**

It is predicted that large, infrequent precipitation events will decrease plant performance compared to small, frequent precipitation events (Knapp et al. 2008; Garssen, Verhoeven & Soons 2014). Our results show that plant performance decreased under large, infrequent precipitation events, but increasing the amount of precipitation by 27% compensated for the negative effects of variable precipitation. Specifically, we found 3 eudicot and 2 monocot species exhibited decreased biomass production under the infrequent treatment. The greater magnitude-infrequent treatment typically performed similarly to the small, frequent precipitation events of the control (Table 2.1; Fig. 2.3).

Our results show that the frequency of a precipitation event rather than the total amount of precipitation negatively impacts plant performance. Frequent precipitation events maintain plant performance because they provide constant access to water that reduces soil moisture variability and evapotranspiration (Sala & Lauenroth 1982; Schwinning & Sala 2004). In contrast, infrequent precipitation events decrease plant performance because they are intervened by dry periods that reduce water access and increase soil moisture variability (Knapp et al. 2008; Garssen, Verhoeven & Soons 2014). However, increasing the amount of precipitation so infrequent precipitation events are of larger magnitude compensates for the negative effects of dry periods between large precipitation events. Our results show that species in the greater magnitude-infrequent treatment were more successful than species in the infrequent treatment at maintaining plant performance. Precipitation events of greater magnitude permitted longer periods of soil recharge that provided more access for plant water uptake. Therefore, wetland plants need constant access to (at least) a small amount of water or precipitation events must be large enough to elevate soil moisture levels in order to maintain plant performance under future climate scenarios. Other studies that manipulate precipitation variability have also shown that the frequency of precipitation events is an important predictor of plant response (Miao, Zou & Breshears 2009; Nijp et al. 2014), but the response of plants to the amount and frequency of precipitation is complex and needs further assessment.
Our results also reveal that above- and below-ground biomass produced different responses to variable precipitation. We found that below-ground decreased for all species; however, *M. ringens* and *S. tabernaemontani* showed a change in both above- and below-ground biomass (Table 2.1; Fig. 2.3). During times of water deficits, we expect above-ground biomass to decrease thereby limiting photosynthesis and transpiration to minimize water loss and prevent desiccation (Knapp & Smith 2001; Wu et al. 2011). A lack of change in above-ground biomass suggests that plant response could have been physiological and these physiological changes did not translate into morphological changes over the short study period of 12 weeks. Other studies have also found that over short time scales precipitation variability can produce larger effects in physiological responses rather than above-ground biomass (Fay et al. 2002; Jentsch et al. 2011). In contrast, during times of water deficits we expect below-ground biomass to increase allowing plants to access water deep in the soil profile. However, typically wetland plants are not limited by a lack of water but a lack of oxygen due to anoxic soil conditions (Mitsch & Gosselink 2007). Therefore the observed decrease in below-ground biomass suggest that wetland plants, which are not use to finding water for plant growth, did not respond by root elongation. Our results show that below-ground structures of plants are integral for understanding plant response.

**RESPONSE OF C AND N CONCENTRATION TO PRECIPITATION TREATMENTS**

Large, infrequent precipitation events are expected to reduce N concentration in comparison to small, frequent precipitation events (Anderson & Mitsch 2005; Knapp et al. 2008). A reduction in soil moisture resulting from extended dry periods intervening large, infrequent precipitation events will cause less available N because mineralization will decrease due to lower rates of microbial activity (Fierer & Schimel 2002; Borken & Matzner 2009). We found that two species, *E. perfoliatum* and *M. ringens*, experienced an increase in N concentration in their plant tissue which led to a decrease in C:N ratio under the infrequent treatment rather than the greater magnitude-infrequent treatment (Table 2.1; Fig. 2.4).

The response of N concentration reveals two important findings. First, N concentration was higher in the frequent treatment rather than the greater-magnitude infrequent treatment. The large size of the greater-magnitude infrequent treatment could have limited oxygen availability which minimized the conversion of inorganic N to organic N for plant uptake (Gusewell et al. 2003; Borken & Matzner 2009; Nielsen & Ball 2015). There is limited knowledge on plant acquisition
of N in wetland systems in response to large, infrequent precipitation events; however, studies for other systems show that both large, infrequent precipitation events (Dijkstra et al. 2012) and small, frequent precipitation events (Yahdjian & Sala 2010) are important for maintaining plant uptake of N. Second, there is a trade-off between biomass production and N concentration. Under large, infrequent precipitation events biomass production decreased and N concentration increased. This suggests that more resources were allocated to increasing N concentration in plant tissue than biomass production. The former is important for plant processes such as photosynthesis or defences against herbivores. Moving forward, there is a need for more experimental evidence that addresses the effects of precipitation variability on nutrient dynamics and the potential trade-offs between plant traits.

RESPONSE OF PLANT FUNCTIONAL TYPES TO PRECIPITATION TREATMENTS

Plant functional types had disparate responses to precipitation variability with the performance of all eudicots being negatively affected while only some monocots were affected. In wetlands, a decrease in water availability causes graminoid monocots to outperform eudicots (Weltzin et al. 2000; Weltzin et al. 2003a; Murphy, Laiho & Moore 2009; Dieleman et al. 2015; Kettridge et al. 2015). Typically, monocots have lower leaf surface area with thin, compressed leaves, lateral root systems, and low above:below-ground biomass ratios, while eudicots have higher leaf surface area with wide, expanded leaves, deep, vertical root systems, and high above:below-ground biomass ratios (Boutin & Keddy 1993; Jackson et al. 1996; Bouchard et al. 2007). These features confer advantages and disadvantages during periods of water stress. We found that the above:below-ground ratio of E. perfoliatum and M. ringens was > 1, S. ohioensis and J. effusus was ca. 1, and A. americanus, C. aquatilis, G. striata, and S. tabernaemontani was < 1 (Fig. 2.3). Therefore, most of the eudicots produced more above- than below-ground biomass, while most of the monocots produced more below- than above-ground biomass. More below-ground than above-ground biomass, as exhibited in the monocots, suggests that these species were more effective than eudicots at exploiting pulses of water during large, infrequent precipitation events and using oxygen during dry periods. Other studies have shown that typical wetland species such as Carex spp. and J. effusus are more tolerant to a wider range of hydrologic conditions than M. ringens (Kercher & Zedler 2004; Fraser & Karnezis 2005; Touchette et al. 2010) and A. americanus (Romanello et al. 2008).
CAVEATS

We added a small volume of water to plants in the control treatment to prevent mortality during a heat wave. We added an extra 12 mm of water to the control which comprised 6% of the total precipitation received by plants in this treatment. This water was not added to the other two treatments because these plants received a large pulse event at the same time and were temporarily saturated. This additional water is unlikely to have biased our results. Consistent with this interpretation, leaf number and plant height measurements made immediately before the addition of the supplemental water showed early evidence of treatment effects in some species. For example, S. tabernaemontani showed a decrease in leaf number in the infrequent treatment relative to the control (P =0.07), while M. ringens’ height was lower in the infrequent treatment relative to the control (P = 0.006). Therefore it is unlikely this extra 6% of water significantly affected the above trends.

CONCLUSIONS

Our study provides evidence that variable precipitation patterns can have negative ecological consequences on wetland plants. We find changing the magnitude and frequency of precipitation can negatively impact plant performance and C and N concentration. Furthermore, these changes depend on the frequency of a precipitation event and plant functional type. This suggest that to understand the response of plant communities to changing precipitation regimes, we need to assess precipitation event size, precipitation event timing, and the amount of precipitation, as well as species identity and traits. To address this, more studies, especially for wetlands, are needed that tease apart the relationship among these factors from the individual- to ecosystem-level. These studies will be important for informing ecosystem management and polices that conserve and restore healthy plant communities.
### 2.5 Tables

**Table 2.1** Linear mixed-effect model results (numerator and denominator degrees of freedom, F-values, and P-values) showing the effect of three precipitation treatments on plant performance and C and N concentration for the eight species. A significant relationship (P < 0.05 shown in bold) provides evidence that a plant trait changed as a result of the precipitation treatments.

<table>
<thead>
<tr>
<th></th>
<th>E. perfoliatum</th>
<th>S. ohiensis</th>
<th>M. ringens</th>
<th>S. taberaemontani</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ndf</td>
<td>ddf</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Leaf number</td>
<td>2 11</td>
<td>1.685</td>
<td>0.230</td>
<td>2 12</td>
</tr>
<tr>
<td>Plant height</td>
<td>2 11</td>
<td>1.260</td>
<td>0.322</td>
<td>2 12</td>
</tr>
<tr>
<td>Above-ground biomass</td>
<td>2 11</td>
<td>2.329</td>
<td>0.143</td>
<td>2 12</td>
</tr>
<tr>
<td>Below-ground biomass</td>
<td>2 11</td>
<td>6.245</td>
<td>0.015</td>
<td>2 12</td>
</tr>
<tr>
<td>Above:below-ground biomass</td>
<td>2 11</td>
<td>6.009</td>
<td>0.017</td>
<td>2 12</td>
</tr>
<tr>
<td>C:N - above-ground biomass</td>
<td>2 10</td>
<td>13.462</td>
<td>0.002</td>
<td>sqrt 2</td>
</tr>
<tr>
<td>C:N - below-ground biomass</td>
<td>2 10</td>
<td>1.002</td>
<td>0.401</td>
<td>2 12</td>
</tr>
<tr>
<td>C - above-ground biomass</td>
<td>2 10</td>
<td>3.599</td>
<td>0.067</td>
<td>2 12</td>
</tr>
<tr>
<td>C - below-ground biomass</td>
<td>2 10</td>
<td>2.831</td>
<td>0.106</td>
<td>2 9</td>
</tr>
<tr>
<td>N - above-ground biomass</td>
<td>2 10</td>
<td>2.066</td>
<td>0.177</td>
<td>2 9</td>
</tr>
<tr>
<td>N - below-ground biomass</td>
<td>2 10</td>
<td>2.066</td>
<td>0.177</td>
<td>2 9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>A. ameriancus</th>
<th>C. aquatilis</th>
<th>G. striata</th>
<th>J. effusus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ndf</td>
<td>ddf</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Leaf number</td>
<td>2 12</td>
<td>0.693</td>
<td>0.519</td>
<td>2 12</td>
</tr>
<tr>
<td>Plant height</td>
<td>2 12</td>
<td>0.697</td>
<td>0.517</td>
<td>2 12</td>
</tr>
<tr>
<td>Above-ground biomass</td>
<td>2 12</td>
<td>1.608</td>
<td>0.241</td>
<td>2 12</td>
</tr>
<tr>
<td>Below-ground biomass</td>
<td>2 12</td>
<td>4.886</td>
<td>0.028</td>
<td>2 12</td>
</tr>
<tr>
<td>Above:below-ground biomass</td>
<td>log 2</td>
<td>8</td>
<td>0.018</td>
<td>0.982</td>
</tr>
<tr>
<td>C:N - above-ground biomass</td>
<td>2 12</td>
<td>4.450</td>
<td>0.036</td>
<td>2 12</td>
</tr>
<tr>
<td>C:N - below-ground biomass</td>
<td>log 2</td>
<td>8</td>
<td>1.277</td>
<td>0.330</td>
</tr>
<tr>
<td>C - above-ground biomass</td>
<td>2 12</td>
<td>1.056</td>
<td>0.241</td>
<td>2 12</td>
</tr>
<tr>
<td>C - below-ground biomass</td>
<td>2 12</td>
<td>1.056</td>
<td>0.241</td>
<td>2 12</td>
</tr>
<tr>
<td>N - above-ground biomass</td>
<td>2 12</td>
<td>0.056</td>
<td>0.946</td>
<td>2 12</td>
</tr>
<tr>
<td>N - below-ground biomass</td>
<td>2 12</td>
<td>0.056</td>
<td>0.946</td>
<td>2 12</td>
</tr>
</tbody>
</table>
2.6 Figures

Figure 2.1 Greenhouse study at the University of Toronto Mississauga in summer 2013. A pool with 16 plants (2 of each species per pool) where each pool received one treatment.
Figure 2.2 Change in soil moisture and precipitation throughout the experiment. A) Precipitation amounts for the three treatments across the entire growing season. B) Mean volumetric soil moisture content for the three precipitation treatments (±1 SE from the mean). Letters denote significance where different letters represent a significant (P < 0.05) difference between treatment means, while similar letters represent a non-significant (P > 0.05) difference between treatments means.
Figure 2.3 Above- and below-ground biomass for the three precipitation treatments for the eight species. Bars show mean above- or below-ground biomass and whiskers denote ±1 SE. Letters denote statistical significance where different letters represent a significant (P < 0.05) difference between treatment means, while similar letters represent a non-significant (P > 0.05) difference between treatment means. Letters ‘a’ and ‘b’ refer to above-ground biomass, while letters ‘c’ and ‘d’ refer to below-ground biomass.
Figure 2.4 C and N dynamics for the three precipitation treatments for *Eupatorium perfoliatum* and *Mimulus ringens*. A) and D) C concentration. B) and E) N concentration. C) and F) C:N ratio. Bars show mean above- or below-ground biomass and whiskers denote ±1 SE. Letters denote significance where different letters represent a significant (P < 0.05) difference between treatment means, while similar letters represent a non-significant (P > 0.05) difference between treatment means. Letters ‘a’, ‘b’, and ‘c’ refer to above-ground biomass, while letters ‘d’ and ‘e’ refer to below-ground biomass.
Chapter Three
How the amount and frequency of precipitation affects the performance of 14 grassland species

ABSTRACT

Climate change is causing shifts in the amount and frequency of precipitation in many regions, which could have implications for plant performance. Most research on the ecological effects of precipitation only examines the impact of the amount of precipitation on plants. Few studies have considered the effect of both the amount and frequency of precipitation on plants. To understand how climate-driven changes in precipitation can affect 14 grassland monocot and eudicot species, we asked: (i) How does the amount and frequency of precipitation affect plant performance? (ii) Do plant functional types (i.e. graminoid monocots vs. eudicots) vary in their response to variable precipitation? To answer these questions, we conducted a factorial manipulation of the amount (70 vs 90mm/month) and frequency (3, 15, or 30 days with little events) of precipitation. Our results show that both factors impact plants but these effects vary between metrics of plant performance with larger effects on eudicots than monocots. Above- and below-ground biomass were affected by the amount of precipitation and/or the interaction between the amount and frequency of precipitation. Above-ground biomass increased by 21-30% when the amount of precipitation was increased. Below-ground biomass generally decreased by 18-34% in the 70 mm treatment and increased by 33-40% in the 90 mm treatment when event frequency was decreased from 3 to 15 or 30 days. In contrast, changes in plant physiological traits were largely driven by changes in event frequency. Our results reveal that it is important to consider changes in both the amount and frequency of precipitation when predicting how plant communities will respond to variable precipitation, and eudicots are more likely to be affected than monocots.

3.1 Introduction

Within the next century, climate change will cause alterations in annual and seasonal precipitation, as well as increased precipitation variability (Easterling et al. 2000; Diffenbaugh & Field 2013; IPCC 2013). In some regions, summer precipitation regimes will be characterized by large, infrequent precipitation events with longer intervening dry periods (Smith 2011; IPCC
2013; Westra et al. 2014). Changing the size and timing of precipitation events will introduce novel hydrological conditions that could influence plant community dynamics (Craine et al. 2012; Zhang et al. 2013b; Shi et al. 2014), which remains a key challenge to understanding the ecosystem-level consequences of climate change (Jentsch, Kreyling & Beierkuhnlein 2007; Beier et al. 2012; Zeppel, Wilks & Lewis 2014). Here, we address this challenge by examining the individual and interactive effects of the amount and frequency of precipitation on plant performance of grassland monocot and eudicot species.

Plant performance can be limited by changes in water availability (Weltzin et al. 2003b; D’Odorico et al. 2010). Precipitation regimes that are characterized by large, infrequent events separated by dry periods can transition a grassland system from a state of low water stress (precipitation > evapotranspiration) to periodic, high water stress (precipitation < evapotranspiration) (Knapp et al. 2008). Under this precipitation regime, soil moisture is recharged in pulses with transient resources available for plants (Schwinning & Sala 2004). Interspersed between these pulses of precipitation will be periods of low water availability with limited resources for plants. As these periods of high water stress become more pronounced, alterations in plant community structure and function can occur. For example, research has demonstrated precipitation-related changes in above- and below-ground plant productivity (Heisler-White, Knapp & Kelly 2008; Wilcox et al. 2015), plant physiology (Jentsch et al. 2011; Thomey et al. 2011), nutrient cycling (Dijkstra et al. 2012; Evans & Burke 2013), and community composition (Evans et al. 2011; Fry, Manning & Power 2014). However, the impacts of changing precipitation regimes on plants are typically documented by manipulating the amount of seasonal or annual precipitation, while few studies manipulate both the amount and frequency of precipitation to tease apart the relative importance and interactive effects of these factors (Jentsch, Kreyling & Beierkuhnlein 2007; Beier et al. 2012; Zeppel, Wilks & Lewis 2014). Variability in precipitation regimes can have different and potentially greater ecological consequences on plants than changes in the amount of precipitation (Reyer et al. 2013; Robinson et al. 2013).

Experimental manipulations of the amount and/or frequency of precipitation reveal that large, infrequent precipitation events can have immense effects on plant performance. For example, Heisler-White et al. (2009) added the average seasonal precipitation to three types of plant communities (mesic, semi-arid, and prairie) in 4, 6, and 12 events per month. They found that
less frequent but larger precipitation events caused an 18% decrease in above-ground net primary productivity in the mesic grassland and a 30% and 70% increase in the semi-arid and prairie grassland, respectively. Similarly, Fay et al. (2008) examined the response of plant communities to variation in the amount and frequency of precipitation. They found that above-ground net primary productivity and photosynthesis were positively affected, while soil CO$_2$ efflux was negatively affected by precipitation variability but which factor (e.g., frequency) drove the response varied according to the metric measured. Schneider et al. (2014) examined the individual response of two mesic grassland species (Agropyron repens and Lupinus perennis) to changes in the amount and frequency of precipitation. They found that decreasing the frequency of precipitation reduced biomass in both species; however, changes in the amount of precipitation and interaction between the amount and frequency of precipitation produced distinctive species and trait responses. The variation in results among these studies illustrates that we need better insights into the magnitude and direction of plant responses to projected climate change to grasp community- and ecosystem-level changes.

Distinguishing between plant functional types could provide a mechanistic and predictive explanation for variation in plant responses to precipitation variability. The composition of grasslands is dominated by grasses and eudicots, which represent two plant functional types that differ in morphology and physiology. Typically, grasses have a small leaf area, vertical leaf orientation, and, in comparison to eudicots, their root systems are shallower. In contrast, most eudicots have a larger leaf area, horizontal leaf orientation, and more diversity in rooting depth (Jackson et al. 1996; Sun, Coffin & Lauenroth 1997). Also, groups of species have distinct photosynthetic pathways and transpiration rates that enable different water use efficiencies (Pearcy & Ehleringer 1984; Turner, Kneisler & Knapp 1995). These characteristics could predict a species’ response because certain traits could make types of plants preferentially vulnerable to environments with periodic, high water stress. Therefore, classifying species by plant functional type could help comprehend how the composition of a plant community will change in response to projected precipitation variability.

The objective of this study was to experimentally examine the impacts of variable precipitation on plant performance of 14 common native grassland plant species. These species encompassed both graminoid monocots and eudicots and were grown individually under rainout shelters in southern Ontario. Forecasted precipitation regimes that are characterized by large, infrequent
precipitation events are reflective of this area (Cao & Ma 2009; IPCC 2013). After assessing how the amount and frequency of precipitation affected soil moisture variability, we addressed two specific research questions: (i) How does the amount and frequency of precipitation affect plant performance? (ii) Do plant functional types vary in their response to precipitation variability? Addressing these questions will provide empirical evidence into how plant performance will change under climate-driven precipitation regimes. This will be important for forecasting changes in plant community composition and, ultimately, the maintenance and persistence of grassland biodiversity.

3.2 Materials and Methods

STUDY SITE AND SPECIES

We conducted the experiment in an old field meadow at University of Toronto Mississauga in Mississauga, Ontario, Canada (43°32' N, 79°39' W). The field site is dominated by Dactylis glomerata, Plantago lanceolata, Phleum pratensis, Solidago spp., Taraxacum officinale, and Trifolium pratense. The soils consist of a sandy loam topsoil with a sandy loam, clay subsoil horizon. During the experimental period of May to August 2014 at the field site, total precipitation was 254 mm (63.5 mm/month) and average temperature was 18.0°C (range = 1.8°C – 32.0°C) (data: University of Toronto Mississauga Meteorological Station; http://www.utm.utoronto.ca/geography/resources/meteorological-station).

At the field site, we planted 15 grassland species that represent a wide diversity of monocots and eudicots found in grasslands of southern Ontario. We included five monocots: Andropogon gerardii Vitman (Poaceae), Elymus canadensis L. (Poaceae), Elymus riparius Wiegand (Poaceae), Panicum virgatum L. (Poaceae), and Sorghastrum nutans (L.) Nash (Poaceae), but S. nutans failed to grow. We included 10 eudicots: Asclepias tuberosa L. (Asclepiadaceae), Desmodium canadense (L.) DC (Fabaceae), Eupatorium perfoliatum L. (Asteraceae), Euphorbia corollata L. (Euphorbiaceae), Geum triflorum Pursh (Rosaceae), Oenothera biennis L. (Onagraceae), Penstemon hirsutus (L.) Willd (Scrophulariaceae), Solidago nemoralis Aiton (Asteraceae), Verbena stricta Vent. (Verbenaceae), and Zizia aurea (L.) W.D.J. Koch (Apiaceae). Plant material was obtained from fields in southern Ontario (see Appendix A -Table A.1).
EXPERIMENTAL DESIGN

We employed a fully factorial, randomized, complete block design to examine the effects of changing the amount and frequency of precipitation on plant performance. In May 2014, we established 10 rainout shelters that were 6 m$^2$ with ca. 1 m spacing between shelters (Fig. 3.1). The frame of the rainout shelter was constructed of four wooden corner posts anchored 0.6 m into the ground (Fig. 3.2a). Two adjacent posts stood at 1.8 m above the soil surface, while the other two adjacent posts stood at 1.2 m above the soil surface. To construct the rainout shelter’s roof, we secured a wooden square frame with a middle support beam to the four corner posts that allowed the roof to sit on an angle of 45°. Overtop of the rainout shelter’s roof, we attached 6 mil clear polysheeting (Uline, Brampton, Ontario, Canada) that transmitted ca. 90% of photosynthetic active radiation. To the lowest side of the rainout shelter, we connected an eavestrough to collect ambient rainfall into a bucket that added an additional 0.3 m to the shelter. The rainout shelters and eavestroughs covered 2.4 m × 2.7 m, while our plants were planted within a 1.6 m × 1.9 m area which gave them a 0.4 m buffer on all sides reducing exposure to ambient precipitation. A buffer of at least 0.2 m has been shown to limit lateral water flow (Yahdjian & Sala 2002). Each rainout shelter contained 6 plots that were 0.38 m × 0.76 m (w × l) with 0.3 m spacing between plots (Fig. 3.2b). We randomly assigned one of the six precipitation regimes (see below) to each plot. To control for lateral water flow between blocks and plots, each plot contained 18 - 5 L pots (0.127 m × 0.127 m × 0.305 m; w × l × d) that were sunken into the ground at a depth of 0.25 m and arranged in a 3 × 6 rectangular grid. To these 18 pots, we randomly assigned the 15 study species and left three pots without plants for soil volumetric moisture content (VMC) measurements (see below). In May 2014, we transferred plants grown from seed in 0.06 L pots since May 2013 in St. Williams, Ontario, Canada (42°41'N, 80°26'W) to the field site. We removed plants from their original pots, loosened the soil and roots, and transplanted the plants into the pots using the soil at the site and 0.5 g of slow-release fertilizer (Smartcote, N:P:K, 14:14:14, Brantford, Ontario, Canada).

Our experiment consisted of 10 replicates per precipitation regime of each study species, with one replicate of each species in a plot and six replicates of each species in a block (Fig. 3.1). Our experiment also consisted of 30 replicates per treatment of soil VMC, with three replicates per plot and 18 unplanted pots (three per plot × six plots) per block. In total, the experiment included 900 plants and 180 unplanted pots for soil VMC.
PRECIPITATION REGIMES

To all plants we imposed a factorial manipulation of the amount and frequency of precipitation (Table 3.1). The amount of precipitation was manipulated by giving plants either 70 mm/month or 90 mm/month. The frequency was manipulated for precipitation events to occur at 3 days, 15 days, and 30 days. The latter frequency also had smaller precipitation events of 5 mm every 7 days for 70 mm/month or 5 days for 90 mm/month. We ran precipitation regimes between 30 May 2014 and 27 August 2014 by manually adding tap water to the soil surface. Large events were applied over a 2 to 3 h period so that plants never received more than 20 mm of precipitation per hour to minimize run-off and evaporation from water ponding at the surface.

Our six regimes were based on precipitation records from 1938 to 2012 (data: Government of Canada; http://climate.weather.gc.ca), with 70 mm/month representing the average monthly precipitation value received in Mississauga, Ontario, Canada for the last 75 years. In this region, the number of large, infrequent summer precipitation events has and is likely to continue to increase (Cao & Ma 2009; IPCC 2013). Our precipitation regimes reflect realistic scenarios that have low recurrence in our historical precipitation data.

SOIL AND PLANT PERFORMANCE MEASUREMENTS

Throughout the experiment, we measured soil VMC to quantify soil moisture variability. Using a soil core that was 6 cm deep and 5 cm in diameter, we removed soil from unplanted pots, immediately weighed the sample in the lab, oven dried the sample for 24 h at 100°C, and then weighed the dry sample. We randomly collected three cores on a weekly basis and at the start of a month for all precipitation regimes, as well as three cores before/after precipitation regimes with event frequencies of 15 and 30 days. Throughout the duration of the experiment, we returned to unplanted pots at most three times to collect soil VMC measurements.

To assess plant performance, we measured plant morphological and physiological traits. Near the beginning (26 May 2014) and end (22 August 2014) of the experiment we measured leaf number and plant height. We do not show leaf number results because they reveal few changes. On four occasions (2 and 4 July, 9 and 11 July, 23 and 25 July, 31 July and 1 August 2014), we used a Delta-T AP4 Porometer (Delta-T Devices, Burwell, Cambridge, UK) to measure stomatal conductance on a fully expanded, top leaf for six of the 10 replicates for all treatments and
species. On a few occasions a middle or bottom leaf was used because leaves were too small or exhibited herbivore damage. We harvested above-ground biomass on 29 August 2014 and below-ground biomass from 2-4 September 2014. We dried plant tissue in an oven for 72 h at 60°C.

STATISTICAL ANALYSIS

We tested for the effects of the precipitation regimes on metrics of plant performance using R 3.1.2 (R Core Team 2014). Our predictor variables were amount (70 vs 90 mm), frequency (3, 15 or 30 days), and amount × frequency, which were all treated as fixed effects in the models. Plot nested within rainout shelter was included as a random effect in the model with a fixed intercept. Our response variables were soil moisture and plant performance including above- and below-ground biomass, above:below-ground biomass ratio, plant height, and stomatal conductance. All analyses were performed using the ‘lmer’ function in the ‘lme4’ package (Bates et al. 2014). We tested for the significance of our predictor variables using the ‘anova’ function in the ‘lmerTest’ package (Kuznetsova, Brockhoff & Christensen 2014), with Kenward-Roger denominator degrees of freedom calculated with the ‘pbkrtest’ package (Halekoh & Højsgaard 2014). To improve normality and homogeneity of variance, we log- or square root-transformed our data where necessary.

3.3 Results

SOIL MOISTURE

Changes in soil moisture were driven by the frequency of precipitation events rather than the amount of precipitation (Fig. 3.3; see Appendix B Fig. B.1). Decreasing event frequency from 3 to 15 days and from 3 to 30 days caused a decrease in soil VMC by 7% and 12%, respectively (F=3.45, P=0.04, ndf=2, ddf=47.92). The amount of precipitation did not affect soil VMC (F=2.38, P=0.13, ndf=1, ddf=47.97), and the amount and frequency of precipitation did not interact (F=0.96, P=0.39, ndf=2, ddf=45.17). As expected, the coefficient of variation in soil VMC was lowest for the 3 day frequency precipitation treatment (70 mm: 18%, 90 mm: 21%), and greatest for the 15 day (70 mm: 37%, 90 mm: 35%) and 30 day (70 mm: 34%, 90 mm: 29%) frequency treatments. The latter two treatments show similar coefficients of variation suggesting
that soil moisture variability was muted by the small precipitation events that intervened the large 30 day events.

PLANT PERFORMANCE

Manipulating the amount and frequency of precipitation affected multiple components of plant performance, with larger effects on eudicots than monocots (Table 3.2). Our precipitation regimes affected at least one measure of plant performance in 50% of the monocots (two of the four species) and 60% of the eudicots (six of the 10 species). Of the 14 significant effects detected on morphological traits, four of these were caused by changes in the amount of precipitation, four of these were due to the effect of frequency, and six of these resulted from an interaction between the amount and frequency of precipitation. Of the 19 significant effects on physiological traits, two of these were caused by changes in the amount of precipitation, 15 of these were due to the effect of frequency, and two of these resulted from an interaction between the amount and frequency of precipitation.

Plant above- and below-ground biomass was affected by the amount of precipitation or the interaction between the amount and frequency of precipitation. We found increasing the amount of precipitation from 70 to 90 mm drove an increase in above-ground biomass in three of the 10 eudicot species (Table 3.2; Fig. 3.4): Desmodium canadense (Fig. 3.4a), G. triflorum (Fig. 3.4b), and Z. aurea (Fig. 3.4c), by 21%, 30%, and 30%, respectively. We also found four of the 10 eudicot species showed a change in below-ground biomass (Table 3.2; Fig. 3.5). Zizia aurea exhibited a 37% increase in below-ground biomass when precipitation amount was increased from 70 to 90 mm (Fig. 3.5a). In contrast, P. hirsutus, E. perfoliatum, and V. stricta experienced a change in below-ground biomass due to the interaction between the amount and frequency of precipitation. Penstemon hirsutus’ below-ground biomass decreased by 28-34% in the 70 mm treatment and increased by 34-36% in the 90 mm treatment when event frequency decreased from 3 to 15 or 30 days (Fig. 3.5b). Eupatorium perfoliatum’s below-ground biomass decreased by 18% in the 70 mm-15 day regime and increased by 40% in the 90 mm-15 day regime relative to the 3 day treatment (Fig. 3.5c). Verbena stricta’s below-ground biomass decreased by 20% in the 70 mm-30 day regime and increased by 33% in the 90 mm-30 day regime relative to the 3 day treatment (Fig. 3.5d). Thus, increasing the amount of precipitation, even when the frequency...
of precipitation events is reduced, generally leads to higher plant performance than average conditions.

Our results show that the relative allocation of above- and below-ground biomass for *E. perfoliatum* and *E. riparius* was strongly affected by the interaction between the amount and frequency of precipitation (Table 3.2). With decreasing event frequency, the above:below-ground biomass ratio of *E. perfoliatum* increased by 16-19% in the 70 mm treatment (3 day=2.07, 15 day=2.47, 30 day=2.40) and decreased by 23-37% in the 90 mm (3 day=2.73, 15 day=1.73, 30 day=2.09) relative to the 3 day treatment. The above:below-ground biomass ratio of *E. riparius* increased by 33% in the 70 mm-30 day regime relative to the 3 day treatment (3 day=0.86, 15 day=0.81, 30 day=1.14), and decreased by 22-55% in the 90 mm treatment with decreasing event frequency (3 day=1.33, 15 day=1.04, 30 day=0.59). These changes were driven by an increase in above-ground biomass in the 70 mm treatment and decrease in above-ground biomass in the 90 mm treatment with decreasing event frequency.

Plant height was influenced by the frequency and the interaction between the amount and frequency of precipitation (Table 3.2; Fig. 3.6). *Geum triflorum* did not show a change in plant height under the 70 mm treatment, but an increase by 31% in the 90mm-15 day treatment and decrease by 26% in the 90 mm-30 day treatment relative to the 3 day treatment (Fig. 3.6a). Plant height of *E. perfoliatum* was similar under the 70 and 90 mm treatment, except the 90 mm-3 day treatment showed an increase by 12-18% relative to the 15 and 30 day treatment (Fig. 3.6b). *Asclepias tuberosa* showed a decrease in plant height by 37% as event frequency decreased in the 70 mm treatment but no change in the 90 mm treatment (Fig. 3.6c). Lastly, *O. biennis*’ plant height increased by 38% in the 70 mm-15 day regime and decreased by 28% in the 90 mm-15 day regime relative to the 3 day treatment (Fig. 3.6d).

Stomatal conductance was largely impacted by the frequency of precipitation events for nine species (seven eudicots and two monocots) (see Appendix C - Table C1). On nine occasions, typically ca. 1.5 weeks after the 15 day or ca. 1.5-3.5 weeks after a 30 day treatment, we found a decrease in stomatal conductance when event frequency was reduced. We also observed an increase in stomatal conductance on three occasions for both the 15 day treatment (when measurements were made 2 or 8 days, but not 11 days, after the application of a precipitation regime) and 30 day treatment relative to the 3 day treatment.
3.4 Discussion

Our experimental test of the effects of the amount and frequency of precipitation on 14 monocot and eudicot grassland plant species reveals four key findings. First, increasing the total amount of precipitation resulted in increased plant biomass (Fig. 3.4). Second, decreasing the frequency of precipitation often led to decreased plant performance, especially in physiological traits (Fig. 3.6; Appendix C - Table C1). Third, the interaction between the amount and frequency of precipitation typically caused a decrease in plant performance in the 70 mm treatment and an increase in plant performance in the 90 mm treatment with decreasing event frequency (Fig. 3.5-6). Lastly, our precipitation regimes impacted both monocots and eudicots, with the latter group experiencing larger changes (Table 3.2). As a whole, our results reveal that to understand the impact of precipitation variability on plant communities, it is not only important to consider the individual and interactive effects of the amount and frequency of precipitation, but the species and trait composition of the community.

PRECIPI TATION AMOUNT

Increasing the monthly amount of precipitation caused an increase in above-ground biomass in three of the 10 eudicot species but no monocot species (Table 3.2; Fig. 3.4). This observation supports the well-defined relationship between vegetation response and precipitation, quantified as rain-use efficiency, which shows that increasing the amount of precipitation stimulates above-ground biomass production (Knapp & Smith 2001; Huxman et al. 2004a; Campos et al. 2013). Larger amounts of precipitation could lead to greater water infiltration and an increase in the duration of high soil moisture, especially at deeper soil depths where evaporation is low (Sala, Lauenroth & Parton 1992; Schwinning & Sala 2004). Therefore plants, especially those with deep roots, have more access to water resources that promote growth.

PRECIPI TATION FREQUENCY

Infrequent precipitation events are predicted to decrease plant performance because increasing the length of dry periods between precipitation events lowers soil moisture (Knapp et al. 2008). Frequent precipitation events are more effective at reducing temporal variability in soil moisture by providing plants with a constant supply of water (Sala & Lauenroth 1982; Schwinning & Sala 2004). Although our study confirms that decreasing precipitation frequency results in a reduction
in soil moisture (Fig. 3.3), we did not always find a reduction in plant performance (Table 3.2; Fig. 3.6; Appendix C - Table C1). With decreasing event frequency, plant height was reduced in three eudicots and increased in one eudicot (Fig. 3.6). Stomatal conductance was affected in three monocot and seven eudicot species (Appendix C - Table C1), with the majority of changes reflecting a reduction in conductance as precipitation frequency decreased. Thus, the frequency of precipitation events had larger effects on physiological traits than allocation to plant biomass because physiological traits respond more quickly to changes in water resources (Huxman et al. 2004b; Schwinning & Sala 2004). Other studies that manipulate the amount and frequency of precipitation typically find plant morphological responses largely affected by the amount rather than the frequency of precipitation (Fay et al. 2008; Jankju 2008; Zhang et al. 2013a).

Our results reveal that the timing and size of precipitation events are important predictors of plant physiological responses. For some species, we observed an increase in stomatal conductance in the 15 day treatment when measurements were made 2 or 8 days, but not 11 days, after the application of a precipitation regime. Therefore a longer duration between precipitation events will decrease soil moisture which will lead to an increase in stomatal closure to maintain leaf water potential. The trade-off to this water stress avoidance could be a decrease in carbon assimilation leading to less biomass production as observed in *E. perfoliatum, D. canadense, G. triflorum*, and *Z. aurea*. Similarly, the 30 day treatment with small, intervening precipitation events reveals that for some species precipitation events as small as 5 mm can compensate for infrequent precipitation by increasing stomatal conductance. Thus, if large, infrequent precipitation events are intervened by small precipitation events, plant physiological responses can maintain a non-stressed state and conductance similar to small, frequent precipitation events but for only so long. This has also been observed in arid and semi-arid grasslands (Sala & Lauenroth 1982; Heisler-White, Knapp & Kelly 2008), suggesting that mesic grasslands (where our experiment was based) also requires small, frequent precipitation events to maintain plant physiology. Consequently, there are scenarios where the physiological response of plants to changes in the frequency of precipitation is beneficial and thus requires further exploration to comprehend the impact of this precipitation factor.

INTERACTIONS BETWEEN THE AMOUNT AND FREQUENCY OF PRECIPITATION
Large, infrequent precipitation events are expected to decrease plant performance (Knapp et al. 2008), but our study reveals that the relationship between the amount and frequency of precipitation can be more complex. Four of the 10 eudicot species showed a decrease in belowground biomass under the 70 mm treatment and an increase in plant performance in the 90 mm treatment with decreasing event frequency (Table 3.2; Fig. 3.6). Thus, larger amounts of precipitation can compensate for high, periodic water stress caused by infrequent precipitation by increasing the level of soil moisture and the infiltration depth of water where evaporative losses are low. An increase in root structures, as exhibited in the 90 mm treatment, will aid water uptake and further contribute to an increase in plant performance. This result is important because some climate models for this region forecast that precipitation will increase by 10-20% (IPCC 2013), and thus plants could maintain their performance under future climate scenarios.

The interaction between precipitation amount and frequency drove disparate responses in plant height among four species (Table 3.2; Fig. 3.7). Plant height both increased and decreased with decreasing event frequency and this variation depended on the amount of precipitation. This discrepancy among species could be influenced by competition from neighbouring individuals late in the growing season when species reached full growth. Therefore, it is important to consider other biotic and abiotic factors beyond the amount and frequency of precipitation (e.g., other climatic factors, plant-plant interactions, soil type) in order to understand the impacts of precipitation variability. In particular, elucidating how the individualistic responses of species might change when in competition with another species would be an important next step to explore.

Overall, the results for the interaction between the amount and frequency of precipitation show that large, infrequent precipitation events can stimulate, reduce, or have no effect on plant performance depending on the amount of precipitation. Other studies that have manipulated the amount and frequency of precipitation have found similar effects. For example, Fry et al. (2014) applied an ambient precipitation, drought, and large infrequent precipitation event treatment to a mesic grassland. In the large infrequent treatment they found a decrease in species richness and cover, and in both the drought and large infrequent precipitation event treatments they found an increase in ecosystem respiration and no change in net ecosystem exchange and photosynthetic rate relative to ambient precipitation. In contrast, Heisler-White et al. (2008) added the average seasonal precipitation to a semi-arid plant community in 4, 6, and 12 events per month. They
found that above-ground net primary productivity and photosynthetic rate was greatest in the 4 and 6 events per month and lowest in the 12 events per month, but leaf water potential was only affected by the 12 event per month treatment. Our results for the interaction between the amount and frequency of precipitation, as well as the examples above, show that the impact of large, infrequent precipitation events on plant performance is complex and requires more manipulative experiments to understand how water in the soil is made available for plant uptake.

OVERALL IMPACT OF VARIABLE PRECIPITATION ON PLANT PERFORMANCE

Across all species and traits, our study reveals two important patterns (Fig. 3.7). First, increasing the amount of precipitation from 70 to 90 mm stimulated an increase in all metrics of plant performance. Second, decreasing the frequency of precipitation events from 3 to 15 or 30 days led to a reduction in plant performance for most metrics. For some metrics, precipitation events every 15 days led to higher performance than precipitation events every 30 days, and vice versa. In cases where plants performed better with water every 30 days, as opposed to 15 days, indicates that the little precipitation events (5 mm) in the 30 day treatment compensated for high water stress. Despite these findings, we did not observe an individual change in all species for all metrics of plant performance. Conducting the experiment beyond our 13 week timeline over multiple growing seasons might allow all study species to exhibit more significant changes in plant performance. However, as a whole these results show that across all species and traits, plant performance increases when the amount of precipitation increases and decreases with decreasing event frequency.

PLANT FUNCTIONAL TYPES

Changes in the amount and frequency of precipitation had larger and more consistent effects on eudicots than monocots. There are two potential explanations for these changes. First, monocot species exhibited no or minimal changes because of their fibrous root system that allows them to capture water before it infiltrates deep into the soil profile. Second, we observed larger changes in C3 (e.g. *E. canadensis* and eudicots) than C4 (*A. Gerardii* and *P. virgatum*) species. C4 species are more tolerant to changes in water resources because they have higher photosynthetic and lower transpiration rates than C3 species making them more efficient at using water (Knapp 1984; Knapp 1993; Fay *et al.* 2002). This suggests that there is a possibility of an increase in C4 plants with less frequent rain and a decrease in species diversity.
CONCLUSIONS

Here, we provide an experimental test of the impacts of variable precipitation on plant performance of temperate grassland species and show that changing precipitation regimes can have both positive and negative effects on plant performance. Our study is unique as it teases a part the relative importance and interactive effects of the amount and frequency of precipitation, while providing a mechanistic explanation for changes in individual species. We found an increase in the amount of precipitation stimulated plant biomass, a decrease in the frequency of precipitation typically reduced plant performance, and the interaction between the amount and frequency of precipitation showed that increasing the amount of precipitation by 22% compensated for the negative effects of the dry period between precipitation events. However, not all species were affected by the precipitation regimes which suggest that some species (e.g., A. geradii, P. virgatum, P. hirsutus, and V. stricta) will be resistant to variable precipitation. This information is particularly important for conserving and restoring plant communities under environmental stress. Taken together, our study shows that it important to consider both the amount and frequency of precipitation as well as the species and trait composition of communities to predict the response of plant communities to forecasted precipitation regimes.
3.5 Tables

Table 3.1 Summary table of the factorial manipulation of precipitation amount (70 vs 90 mm) and frequency (3, 15 or 30 days) used in the experiment. Precipitation amount was manipulated as either 70 mm/month or 90 mm/month. Precipitation frequency was manipulated as 3 days, 15 days, and 30 days. The latter treatment had smaller precipitation events of 5 mm every 7 days for 70 mm/month or 5 days for 90 mm/month. Precipitation regimes were implemented between 30 May 2014 and 27 August 2014. The average precipitation conditions between May and August for Mississauga, Ontario, Canada from 1938 to 2012 are provided at the bottom of the table (Government of Canada; http://climate.weather.gc.ca).

<table>
<thead>
<tr>
<th>Precipitation Amount (mm/month)</th>
<th># of Events (per month)</th>
<th>Event Magnitude (mm)</th>
<th>Event Frequency (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>10</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>70</td>
<td>2</td>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td>70</td>
<td>1 &amp; 4</td>
<td>50 &amp; 5</td>
<td>30 &amp; 7</td>
</tr>
<tr>
<td>90</td>
<td>10</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>90</td>
<td>2</td>
<td>45</td>
<td>15</td>
</tr>
<tr>
<td>90</td>
<td>1 &amp; 6</td>
<td>60 &amp; 5</td>
<td>30 &amp; 5</td>
</tr>
</tbody>
</table>

Southern Ontario May-August Average Precipitation Conditions 1938-2012

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>72.57</td>
<td>10.27</td>
<td>7.07</td>
<td>2.99</td>
</tr>
</tbody>
</table>
Table 3.2 Linear mixed-effect model results for metrics of plant performance (numerator and denominator degrees of freedom, F-values, and P-values) showing the effect of amount (70 vs 90 mm), frequency (3, 15 or 30 days), and the interaction between amount and frequency for 14 species. A significant relationship (P < 0.05 shown in bold) provides evidence that plant performance changed as a result of the precipitation regimes.

<table>
<thead>
<tr>
<th>Species</th>
<th>Plant height</th>
<th>Amount</th>
<th>Frequency</th>
<th>Amount X Frequency</th>
<th>Belowground biomass</th>
<th>Amount</th>
<th>Frequency</th>
<th>Amount X Frequency</th>
<th>Above/Below-ground biomass</th>
<th>Amount</th>
<th>Frequency</th>
<th>Amount X Frequency</th>
<th>E. coreanum</th>
<th>G. triflorum</th>
<th>A. gerardii</th>
<th>P. virginatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. canadense</td>
<td>log 44.15</td>
<td>0.796</td>
<td>0.377</td>
<td>log 44.17</td>
<td>1.960</td>
<td>0.153</td>
<td>log 44.17</td>
<td>0.542</td>
<td>0.596</td>
<td>log 41.59</td>
<td>1.791</td>
<td>0.180</td>
<td>log 41.55</td>
<td>1.583</td>
<td>0.215</td>
<td>log 41.59</td>
</tr>
<tr>
<td>A. gerardii</td>
<td>log 44.17</td>
<td>1.611</td>
<td>0.211</td>
<td>log 44.17</td>
<td>1.152</td>
<td>0.325</td>
<td>log 44.17</td>
<td>1.344</td>
<td>0.208</td>
<td>log 44.29</td>
<td>0.691</td>
<td>0.506</td>
<td>log 44.29</td>
<td>0.376</td>
<td>0.031</td>
<td>log 44.29</td>
</tr>
<tr>
<td>P. virginatum</td>
<td>log 44.29</td>
<td>0.665</td>
<td>0.352</td>
<td>log 44.29</td>
<td>2.194</td>
<td>0.123</td>
<td>log 44.29</td>
<td>2.436</td>
<td>0.069</td>
<td>log 44.29</td>
<td>0.373</td>
<td>0.691</td>
<td>log 44.29</td>
<td>0.373</td>
<td>0.691</td>
<td>log 44.29</td>
</tr>
</tbody>
</table>

The table continues with similar entries for other species and metrics.
3.6 Figures

Figure 3.1 The experimental layout at the field site showing the placement of the amount (70 vs 90 mm) and frequency (3, 15, or 30 days) of precipitation. There were 10 rainout shelters with six plots in a rainout shelter and 18 pots in a plot. Precipitation regimes, study species, and volumetric soil moisture content were randomly assigned to plots and pots, respectively. Dimensions not to scale.
Figure 3.2 A) A rainout shelter at the field site at University of Toronto Mississauga, Mississauga, Ontario, CAN. B) Experimental layout underneath the rainout shelter consisting of six plots (one for each combination of precipitation amount (70 vs 90 mm) and frequency (3, 15, or 30 days)) with 18 pots per plot (15 pots for plants and three pots for soil volumetric moisture content measurements).
Figure 3.3 Mean soil volumetric moisture content (± 1 SE from the mean) for the amount (70 vs 90 mm) and frequency (3, 15, or 30 days) of precipitation from 30 June to 30 August 2014. There was a significant effect of frequency on soil moisture ($F=3.45$, $P=0.04$, ndf=2, ddf=47.92), while the effects of amount ($F=2.38$, $P=0.13$, ndf=1, ddf=47.97) and the interaction between amount and frequency were non-significant ($F=0.96$, $P=0.39$, ndf=2, ddf=45.17). A significant factor is denoted by an abbreviation: A, amount; F, frequency; and/or A × F, interaction between amount and frequency with $P < 0.07$†, $P < 0.05$* or $P < 0.01$** in the corner of the graph.
Figure 3.4 Above-ground biomass for the amount (70 vs 90 mm) and frequency (3, 15, or 30 days) of precipitation for three species. Points show least square means and whiskers denote ± 1 SE from the least square mean. There was a significant effect of amount, while the effects of frequency and the interaction between amount and frequency were non-significant and not shown. A significant factor is denoted by an abbreviation: A, amount; F, frequency; and/or A × F, interaction between amount and frequency with P < 0.07†, P < 0.05* or P < 0.01** in the corner of the graph.
Figure 3.5 Below-ground biomass for the amount (70 vs 90 mm) and frequency (3, 15, or 30 days) of precipitation for four species. Points show least square means and whiskers denote ± 1 SE from the least square mean. A significant or marginally significant factor is denoted by an abbreviation: A, amount; F, frequency; and/or A × F, interaction between amount and frequency with P < 0.07†, P < 0.05* or P < 0.01** in the corner of the graph.
Figure 3.6 Plant height for the amount (70 vs 90 mm) and frequency (3, 15, or 30 days) of precipitation for four species. Points show least square means and whiskers denote ±1 SE from the least square mean. A significant or marginally significant factor is denoted by an abbreviation: A, amount; F, frequency; and/or A × F, interaction between amount and frequency with $P < 0.07\dagger$, $P < 0.05\ast$ or $P < 0.01\ast\ast$ in the corner of the graph.
Cohen’s d effect sizes for metrics of plant performance for the amount (70 vs 90 mm) and frequency (3, 15 or 30 days) of precipitation. For amount, the ‘control’ was 70 mm and the ‘treatment’ was 90 mm. For frequency, the ‘control’ was 3 days and the ‘treatment’ was 15 or 30 days. Cohen’s d was calculated for each species and then all species were averaged. For stomatal conductance, the four time points were grouped together and then a value of Cohen’s d was calculated using the same method as above. Points show mean values of Cohen’s d and whiskers denote 95% confidence intervals.
Chapter Four
Conclusions

The objective of my thesis was to examine the impact of variable precipitation on the performance of wetland and grassland plants. It is predicted that large, infrequent precipitation events will decrease plant performance because these events are interspersed by dry periods that negatively impact plants. My thesis generally supports the prediction above and reveals three conclusions: (i) Large, infrequent precipitation events usually affected plant performance but this effect varied among precipitation regimes and was inconsistent among species. (ii) Variable precipitation had larger effects on eudicots than monocots. And, (iii) wetland and grassland species were both affected by variable precipitation with more consistent changes among wetland species.

First, the major conclusion of my thesis is that both the performance of wetland and grassland species was affected by variable precipitation. A decrease in plant performance typically occurred when the historical amount of precipitation for southern Ontario was redistributed into large, infrequent events. Increasing the amount of precipitation by 22-27% compensated for the negative impacts of variable precipitation. It is also important to note that some species were unaffected by the precipitation regimes suggesting that plant communities could be resistant and/or resilient to future precipitation scenarios.

In Chapter 2, infrequent precipitation events decreased wetland plant performance, but increasing the magnitude of the infrequent precipitation event allowed species to maintain their performance similar to plants experiencing small, frequent precipitation events. This means that larger magnitude precipitation events permit access to adequate water during dry periods that intervene infrequent events. This also means that to maintain drought intolerant species under variable precipitation regimes, infrequent precipitation needs to be accompanied by larger magnitude events. This implies that future studies that manipulate the water budget of wetlands, should focus on precipitation because it is an integral component that controls plant performance.

In Chapter 3, the factorial manipulation of the amount and frequency of precipitation allowed for an understanding of the individual and interactive effects of these precipitation factors on plants. An increase in the amount of precipitation stimulated plant performance, a decrease in the
frequency of precipitation typically reduced plant performance, and the interaction between the amount and frequency of precipitation showed that increasing the amount of precipitation by 22\% compensated for the negative effects of large, infrequent precipitation events. However, these results were not consistent among species and metrics of plant performance because some species and metrics were unaffected. This suggests that the relationship between the amount and frequency of precipitation is complex. It is imperative that future studies assess the interaction between the timing and size of precipitation events to tease apart their relative effects and importance.

Overall, my thesis shows that variable precipitation, depending on the amount and/or frequency, have the potential to impact plant performance. Although the precipitation regimes used in Chapter 2 and 3 reflect current and future precipitation regimes, if and how these controlled experimental results will translate to natural systems depends on the direction and magnitude of change in precipitation. Out of all climatic factors, precipitation is the most difficult to forecast due to its spatial and temporal nature. Climate models are confident that precipitation will become more variable, but there is disagreement among models as to whether precipitation will increase or decrease (IPCC 2013). Scientists must devote time to producing consistent precipitation forecasts among climate models. This will allow ecologists to grasp if and how climate-driven changes in precipitation will impact plant community dynamics.

The second conclusion is that variable precipitation had larger effects on eudicots than monocots. This suggests over the long-term, large, infrequent precipitation events will lead to greater losses in the richness and diversity of eudicots, causing shifts from diverse to monocot-dominated plant communities. Eudicots promote processes such as insect and animal pollination, have high aesthetic value, and produce fruits, among other things. Therefore, the loss of this plant functional type will have cascading impacts on plant communities and ecosystems.

The differential response of plant functional types has practical applications for the conservation and restoration of plant communities. Variable precipitation, as illustrated by the results of my thesis, will cause a decrease in plant performance of some species. Therefore, traditional practices of protecting plant communities from environmental change will not be conducive (e.g., planting local plants because they are adapted to local environment conditions). Instead it is
important to acknowledge that species have disparate response to variable precipitation, and selectively maintain or add species that have optimal performance across a range of environmental conditions. This can be accomplished by adding plugs or seeds to the seed bank of species that will performance well under variable precipitation, increasing genetic variation by adding species from across a latitudinal gradient, or including both water-tolerant and drought-tolerant species. Although these are plausible solutions for the maintenance of plant communities, this should not undermine the fact that all species are integral to ecosystems. It is imperative that humans make every effort to reduce their impact on the environment to preserve the integrity of the world’s natural systems.

The third conclusion is that both the performance of wetland and grassland plant species was negatively affected by variable precipitation. However, the impacts of large, infrequent precipitation events were more consistent across species in wetlands than grasslands. This finding illustrates that water-tolerant species are less resilient and resistant to large, infrequent precipitation events than species that are adapted to environments with low water availability. Therefore, ecosystems that have ample access to water will also experience the same or potentially greater ecological consequences than terrestrial ecosystems if they dry out. This conveys that future research should evaluate the impact of variable precipitation on diverse ecosystems types to gain a comprehensive understanding of contemporary and future plant communities.

Taken together, this thesis reveals important questions that need to be addressed to provide a better perspective of the impacts of variable precipitation on plants. First, studies that manipulate variable precipitation in concert with other climatic factors (e.g., temperature, CO₂) will be extremely important for creating a holistic understanding of the impact of climate change on plants. Second, more studies that evaluate the mechanisms driving species’ responses to variable precipitation will provide an explanation as to what causes a species to thrive or not thrive under variable precipitation. For example, future studies should assess the species used in this thesis under controlled competition to evaluate whether inter- and intra-specific interactions alter an individual species’ response. Addressing these questions will help solve the complexity of climate change impacts on plant communities, and provide critical knowledge that will be important for maintaining the biological diversity of plant communities and ecosystems.
References


Appendices

Appendix A

Locality information for seed sources, Chapter 3

Table A.1 Locations of seed sources in Ontario, Canada for the 15 study species of Chapter 3. The species were germinated in St. Williams, ON, Canada in pots in May 2013 and then transferred to an old field meadow at the University of Toronto Mississauga, Mississauga, ON, Canada in May 2014.

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asclepias tuberosa</td>
<td>St. Williams</td>
</tr>
<tr>
<td>Andropogon gerardii</td>
<td>St. Williams</td>
</tr>
<tr>
<td>Desmodium canadense</td>
<td>Lynde Shores Conservation Area, Whitby</td>
</tr>
<tr>
<td>Elymus canadensis</td>
<td>Walsingham</td>
</tr>
<tr>
<td>Elymus riparius</td>
<td>Haldimand County</td>
</tr>
<tr>
<td>Eupatorium perfoliatum</td>
<td>Greenwood Conservation Area, Ajax</td>
</tr>
<tr>
<td>Euphorbia corollata</td>
<td>St. Williams</td>
</tr>
<tr>
<td>Geum triflorum</td>
<td>Walsingham</td>
</tr>
<tr>
<td>Oenothera biennis</td>
<td>Clarington</td>
</tr>
<tr>
<td>Panicum virgatum</td>
<td>St. Williams</td>
</tr>
<tr>
<td>Penstemon hirsutus</td>
<td>Walsingham</td>
</tr>
<tr>
<td>Solidago nemoralis</td>
<td>Whitby</td>
</tr>
<tr>
<td>Sorghastrum nutans</td>
<td>St. Williams</td>
</tr>
<tr>
<td>Verbena stricta</td>
<td>Greenwood Conservation Area, Ajax</td>
</tr>
<tr>
<td>Zizia aurea</td>
<td>Haldimand County</td>
</tr>
</tbody>
</table>
Appendix B

Temporal changes in soil volumetric moisture content and precipitation regimes, Chapter 3

Figure B.1 Changes in soil volumetric moisture content and precipitation from 30 June to 30 August 2014. A-C) Soil volumetric moisture content (%) for two amounts (70 and 90 mm) and three frequencies of precipitation (3, 15, or 30 days). Mean soil volumetric moisture contents (± 1 SE from the mean) are shown on the far right of each graph. D-F) Precipitation regimes for two amounts (70 and 90 mm) and three frequencies of precipitation (3, 15, or 30 days).
Appendix C

**Impact of precipitation regimes on stomatal conductance for 14 species, Chapter 3**

**Table C.1** Linear mixed-effect model results for stomatal conductance (numerator and denominator degrees of freedom, F-values, and P-values) showing the effect of amount (70 vs 90 mm), frequency (3, 15 or 30 days), and the interaction between amount and frequency for 14 species. A significant relationship (P < 0.05 shown in bold) provides evidence that stomatal conductance changed as a result of the precipitation regimes. Sampling dates 2 and 4 July & 31 July and 1 August occurred 2-3 days following a 3, 15, and 30 day event, 9 and 11 July occurred 1.5 weeks following a 15 or 30 day event, and 23 and 25 July occurred ca. 1.5 weeks after a 15 day event and 3.5 weeks after a 30 day event. We did not sample a sufficient number of replicates for *Asclepias tuberosa* on 2 and 4 July because the leaves were too small.
<table>
<thead>
<tr>
<th>E. riparius</th>
<th>E. canadensis</th>
<th>A. gerardii</th>
<th>P. virgatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount</td>
<td>Frequency</td>
<td>Amount</td>
<td>Frequency</td>
</tr>
<tr>
<td>2 &amp; 4 July 2014</td>
<td>25</td>
<td>0.010</td>
<td>0.921</td>
</tr>
<tr>
<td>9 &amp; 11 July 2014</td>
<td>25</td>
<td>0.788</td>
<td>0.466</td>
</tr>
<tr>
<td>25 &amp; 31 July 2014</td>
<td>25</td>
<td>0.012</td>
<td>0.867</td>
</tr>
<tr>
<td>31 July &amp; 1 August 2014</td>
<td>25</td>
<td>0.045</td>
<td>0.030</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D. canadensis</th>
<th>G. triflorum</th>
<th>Z. aurea</th>
<th>P. hirsutum</th>
<th>E. perfoliatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount</td>
<td>Frequency</td>
<td>Amount</td>
<td>Frequency</td>
<td>Amount</td>
</tr>
<tr>
<td>2 &amp; 4 July 2014</td>
<td>25</td>
<td>0.000</td>
<td>0.987</td>
<td>25</td>
</tr>
<tr>
<td>9 &amp; 11 July 2014</td>
<td>25</td>
<td>0.012</td>
<td>0.867</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>V. stricta</th>
<th>A. tuberosa</th>
<th>O. biennis</th>
<th>E. corollata</th>
<th>S. nemoralis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount</td>
<td>Frequency</td>
<td>Amount</td>
<td>Frequency</td>
<td>Amount</td>
</tr>
<tr>
<td>2 &amp; 4 July 2014</td>
<td>25</td>
<td>0.057</td>
<td>0.813</td>
<td>25</td>
</tr>
</tbody>
</table>

31 July & 1 August 2014

| Amount | Frequency | Amount | Frequency | Amount | Frequency | Amount | Frequency |
| 2 & 4 July 2014 | 25 | 0.000 | 0.987 | 25 | 1.820 | 0.183 | 24.278 | 1.539 | 0.235 |