

RESPONSES OF SWITCHGRASS (*PANICUM VIRGATUM* L.) TO PRECIPITATION
AMOUNT AND TEMPERATURE

by

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Abstract

Anthropogenic climate change is likely to alter the function and composition of ecosystems worldwide through increased precipitation variability and temperatures. To predict ecosystem responses, a greater understanding of the physiological and growth responses of plants is required. Dominant species drive ecosystem responses, and it is essential to understand how they respond to understand potential ecosystem changes. Dominant species, such as switchgrass (*Panicum virgatum* L.), possess large genotypic and phenotypic variability, which will impact the degree of responses to projected climate changes. I studied the physiological and growth responses of switchgrass, a common perennial warm-season C₄ grass that is native to the tallgrass prairie, to alterations in precipitation amount and temperature. The first experiment I conducted focused on the responses of three ecotypes of *P. virgatum* to three precipitation regimes (average, 25% below, 25% above). I concluded that the physiological responses of photosynthesis, stomatal conductance, transpiration, dark-adapted fluorescence, and mid-day water potential in *P. virgatum* were explained by ecotypic differences. Robust responses to altered precipitation were seen in the water use efficiency, mid-day water potential, and aboveground biomass. Ecotypic differences were also seen in several aboveground biomass variables, and most strikingly in flowering times and rates. There were few interactions between ecotype and precipitation, suggesting precipitation is a strong driver of biomass production, whereas adaptation of ecotypes to their local environment affects physiological processes. A second experiment studied the response of local populations of *P. virgatum* to nocturnal warming. Results showed significant differences in daytime E, daytime g_s, and flowering phenology between treatments. Differences in aboveground biomass were between topographic positions. I concluded that water availability, based on topographic position, is a strong driver of *P. virgatum* aboveground biomass production, but nocturnal warming has the potential to impact flowering phenology, physiological responses, and exacerbate plant water stress. I also reviewed the literature on the ecological effects of implementing switchgrass cultivation for biofuel. From the literature review, I concluded that large-scale switchgrass cultivation will have widespread ecological impacts. If landscape heterogeneity is maintained through harvest rotations, no till farming, and mixed species composition, ecosystem services can be maintained while providing economic value.

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Chapter 1 - Introduction

Ecology of Switchgrass

Switchgrass (*Panicum virgatum* L.) is a common perennial C₄ grass that is native to the tallgrass prairie. *P. virgatum* is adapted to a broad range of environmental conditions, and naturally occurs from Central America to southern Canada and from the East coast of North America to Idaho, Nevada, and Arizona. Within the tallgrass prairie, this species is co-dominant with other grass species such as *Andropogon gerardii*, *Sorghastrum nutans*, and *Schizachyrium scoparium* (Weaver & Fitzpatrick 1932). As a dominant species within the tallgrass prairie ecosystem, it impacts both the form and the function of the ecosystem (Smith & Knapp 2003). The ability of *P. virgatum* to be broadly adapted to a wide range of environmental conditions comes from the genotypic and phenotypic variability that exists within *P. virgatum* populations (Casler et al. 2004; Das et al. 2003). Porter (1966) grouped *P. virgatum* populations into two broad forms or types, usually based on their position in the landscape. The upland type is usually found in higher, more xeric sites than the lowland type. The upland type has been found to have a smaller size, and lower water and nitrogen requirements compared to the lowland type (Porter 1966; Casler 2005). Across the natural range of switchgrass, upland types are more common in the mid to upper latitudes, while lowland types tend to be better adapted to lower latitudes. To better characterize switchgrass types, levels of ploidy have been used. To date, all lowland types have been found to be tetraploid, while upland types can be either hexaploid or octoploid (Hultquist et al. 1996; Hopkins et al. 1996). The large genotypic and phenotypic variability allows switchgrass to maintain high levels of productivity across large environmental ranges (Sanderson et al. 2006). Because *P. virgatum* is able to thrive in a variety of conditions, a high level of physiological variability occurs with adaptation to local environments (McLaughlin & Kszos 2005). For these reasons, switchgrass has been identified as a biofuel species (McLaughlin & Kszos 2005), with most of the cultivars currently available being derived from ecotypes selected from remnant prairies (Casler et al. 2004).

Biofuel Species

P. virgatum is a native to the tall-grass prairie ecosystem, and its life history has always been linked with grazing ungulates. Starting in the 1970s, dedicated research on the forage value

and yield of switchgrass became prominent (Balasko & Smith 1971; Berg 1971). Beginning in 1985, switchgrass was used in the Department of Energy (DOE) Herbaceous Energy Crops Program (HECP) to identify species that might have potential to be used as biofuel feedstock (Parrish & Fike 2005). By 1992, the DOE began to focus solely on switchgrass research for biofuels, and through genetic transformations and breeding in the Bioenergy Feedstock Development Program (BFDP), created many varieties of cultivars (Vogel & Jung 2001). The third round of research began in 1997 and was geared towards scaling up switchgrass cultivation to investigate commercial applications (Boylan et al. 2000). Large scale production of switchgrass biomass to be used as biofuel or co-fired with coal was started in southern Iowa in the late 1990's (Lemus et al. 2002; Ney & Schnoor 2002; Brown et al. 2000), and many reports have concluded that cultivation of switchgrass for energy use is ready to be deployed commercially (Tillman 2000; Boylan et al. 2000).

With the widespread implementation of switchgrass cultivation looming, the ecological effects need to be considered. There has been much research done on specific impacts of switchgrass cultivation on different aspects of ecosystem structure and function. Switchgrass cultivation can be important in soil erosion control (Lemus & Lal 2005), carbon sequestration (Al-Kaisi & Grote 2007), and increased wildlife habitat (Milder et al. 2008). However, aspects such as potential for disease and insect outbreaks (Hoffman et al. 1995; Gonzalez-Hernandez 2009), increased invasibility (Simberloff 2008), and habitat quality (Bies 2006) need to be incorporated into management and cultivation practices as well. Chapter two in this thesis aims to review the ecological impacts of switchgrass cultivation, and to condense the literature into main concepts that will minimize the negative ecological impacts of switchgrass cultivation and provide the greatest environmental and economical benefits.

Resource Limitation Responses

P. virgatum is broadly adapted across a range of growing conditions in North America. Longitudinal and latitudinal differences in ecotypes are seen, with ecotypes from a particular latitude or location displaying greatest productivity and survival when grown near the area where the ecotype came to a genetic-ecologic equilibrium (Casler & Boe 2003; Casler et al. 2004). These ecotypes display varying physiological responses to water and nitrogen limitations (Byrd & May 2000; Sanderson & Reed 2000), but compared to other co-occurring C₄ grass species like

Andropogon gerardii, *Sorghastrum nutans*, and *Schizachyrium scoparium*, *P. virgatum* is more limited by water deficits (Knapp 1985; Stout et al. 1988; Muir et al. 2001). Soil water availability is the chief regulator of the phenology and physiology of *P. virgatum* (Sanderson 1992), which makes the species potentially sensitive to predicted climate changes. Water stress can significantly reduce aboveground biomass (Berdahl et al. 2005), establishment rates (Xu et al. 2006), and affect physiological responses. *P. virgatum* shows a positive yield response to water availability (Heaton et al. 2004), and under reduced soil moisture tension (<-45 kPa) photosynthesis and xylem pressure potential decrease (Sanderson & Reed 2000). Xu et al. (2006) also showed a linear relationship between gas exchange rates and soil water content for *P. virgatum*. Variability in precipitation timing can also elicit responses in *P. virgatum* growth (Evers & Parsons 2003). Within a precipitation gradient in Nebraska (340-560mm annually), stomatal conductance was shown to decline in the first four days of a drying period, and continue to decline as the time from the precipitation event increases (Awada et al. 2002). Evers and Parsons (2003) watered a *P. virgatum* cv. Alamo in intervals ranging from 3-14 days. They found that individuals with watering intervals of longer than seven days displayed decreased establishment and seedling survival, with a 10 day interval acting as the threshold for emergence and establishment. They also found that the root to shoot ratio increased as the watering interval increased.

Soil water availability is a key driver of *P. virgatum* physiology and growth, but nitrogen availability strongly influences these responses as well (Collins et al. 1998; Baer et al. 2004). Increased water availability can increase nitrogen mineralization rates and the supply of available inorganic N in grasslands (Burke et al. 1997). *P. virgatum* yields have been shown to have greater growth responses with increasing nitrogen availability compared to other C₄ perennial grasses (Heaton et al. 2004). These yield responses originate from increases in plant tiller weight, not increases in the number of tillers per plant (Sanderson & Reed 2000). During years with an even distribution of rain, nitrogen availability is positively related with *P. virgatum* yield, and can account for about 80% of the variation in yield responses (Stout et al. 1988). Other growth variables, such as leaf appearance and lamina extension rates show positive increases with N availability (Suplick et al. 2002). The responses of *P. virgatum* to changes in precipitation and N availability, and the interaction of water availability on N availability, make *P. virgatum* an ideal species to study the effects of projected climate change conditions.

P. virgatum is an obligate mycotroph (Wilson & Hartnett 1998) which forms a mutualistic relationship with arbuscular mycorrhizal fungi that allow the species to thrive in edaphic conditions that are normally very limiting (Parrish & Fike 2005). Historically, the mycorrhizal fungi have been understood to obtain and provide nutrients such as phosphorus to the plant, and in return the mycorrhizae receive carbon from plant photosynthetic processes (Finlay 2004). However, it is now understood that networks of mycorrhizae can improve water relations, pathogen resistance, toxic soils, and increase N uptake (Allen & Allen 1984; Clark 2002; Ruiz-Lozano 2003). This relationship with mycorrhizal fungi allows *P. virgatum* to persist in ecosystems that would normally be characterized as phosphorus limited (Bredja et al. 1993). This ability to efficiently take up P has been stated as an advantage for implementing large scale switchgrass cultivation so that P would be removed from P contaminated soils (Missaoui et al. 2005).

Climate Change

Even though the climate of the central Great Plains of North America is characterized by large variability (Borchert 1950), anthropogenic climate change is expected to dramatically alter natural fluctuations in precipitation and temperature (Easterling et al. 2000; Houghton et al. 2001; Alley et al. 2003; Alley et al. 2007). Although inter-annual variability in rainfall will occur, from very wet to very dry years, the long-term mean annual precipitation is predicted to remain the same. Intra-annual variability is projected to increase, with shifts in the distribution of rainfall from 75% in the summer months and 25% in winter months, to and even 50:50 distribution (Karl et al. 2009). Variability in rain events is expected, with the time between rainfall events and the frequency of extreme events increasing (Christensen et al. 2007; Alley et al. 2007). The mean annual air temperature is projected to increase 4°C by the year 2100 (Christensen et al. 2007). Daily minimum temperatures are increasing at a greater rate than daily maximum temperatures (Karl et al. 1991; Alward et al. 1999). The changes in temperature and precipitation, and their interactions, will alter the environment and affect how ecosystems respond both in growth and physiology (Knapp et al. 2002). Because grassland systems can be highly variable in their responses, the ability to forecast responses to projected climate change is difficult (Nippert et al. 2006). Variability in precipitation and temperature has been shown to have significant effects on the physiology and growth of individual plants, to changes in

ecosystem C and N fluxes (Knapp et al. 2002; Fay et al. 2003; Fay et al. 2008; Nippert et al. 2009). In order to predict how communities and ecosystems will respond to climate change, key physiological plant processes and growth responses to simulated climate change need to be better understood (Hughes et al. 2008).

Current Research

Because climate is an important driver of how plants respond in natural environments, it is likely to affect the productivity, community composition, functioning, and distribution of grassland ecosystems (Epstein et al. 1997; Voigt et al. 2003; Jones & Donnelly 2004). Plants are able to respond to current levels of climate variability, but more needs to be known on how plants will respond to future climate change scenarios to accurately predict responses (Nippert et al. 2006). *P. virgatum* displays local adaptation (Casler et al. 2004), with ploidy appearing to be associated with habitat preference. Variation in the plastic responses of ecotypes of *P. virgatum* may have evolutionary consequences. Because we do not understand how ecotype differences affect the short-term responses to environmental variability, this thesis will discuss the responses of *P. virgatum* ecotypes to environmental variability.

This thesis investigated how *P. virgatum* leaf-level physiological and whole-plant growth responses vary when subjected to predicted climate change conditions. In chapter two I discuss the ecological impacts of implementing widespread switchgrass cultivation for biofuels. In chapter three I explore the physiological and growth responses of three ecotypes of *P. virgatum* to altered precipitation. In the fourth chapter I investigate the physiological and growth responses of local populations of *P. virgatum* to increased nighttime temperature at different topographic sites in a tallgrass prairie. Finally, in chapter five, I conclude these results and discuss future directions for *P. virgatum* research, in both biofuel cultivars and natural populations.

Literature Cited

- Allen, E.B., Allen, M.F. 1984. Competition between plants of different successional stages: mycorrhizae as regulators. *Canadian Journal of Botany* 70:1596-1602.
- Alley, R. B., Marotzke, J., Nordhaus, W.D., Overpeck, J.T., Peteet, D.M., Pielke, R.A., Pierrehumbert, R.T., Rhines, P.B., Stocker, T.F., Talley, L.D., Wallace, J.M. 2003. Abrupt climate change. *Science* 299:2005-2010.
- Alley, R.B., et al. *Climate Change 2007: The Physical Science Basis, Summary for Policy Makers*. 2007. Geneva, Switzerland, IPCC Secretariat. Working Group I Fourth Assessment Report of the Intergovernmental Panel on Climate Change. 2-2-2007.
- Al-Kaisi, M.M., Grote J.B. 2007. Cropping systems effects on improving soil carbon stocks of exposed subsoil. *Soil Science Society of America Journal* 71:1381-1388.
- Alward, R.D., Detling, J.K., Milchunas, D.G. 1999. Grassland vegetation changes and nocturnal global warming. *Science* 283:229-231.
- Awada, T., Moser, L.E., Schacht, W.H., Reece, P.E. 2002. Stomatal variability of native warm-season grasses from the Nebraska sandhills. *Canadian Journal of Plant Science* 82:349-355.
- Baer, S.G., Blair, J.M., Collins, S.L., Knapp, A.K. 2004. Soil heterogeneity effects on tallgrass prairie community heterogeneity: an application of ecological theory to restoration ecology. *Restoration Ecology* 13:413-424.
- Balaskko, J.A., Smith, D. 1971. Influence of temperature and nitrogen fertilization on the growth and composition of switchgrass (*Panicum virgatum* L.) and timothy (*Phleum pratense* L.) *Agronomy Journal* 63:853-856.

- Berdahl, J.D., Frank, A.B., Krupinsky, J.M., Carr, P.M., Hanson, J.D., Johnson, H.A. 2005. Biomass yield, phenology, and survival of diverse switchgrass cultivars and experimental strains in western North Dakota. *Agronomy Journal* 97:549-555.
- Berg, C.C. 1971. Forage yield of switchgrass (*Panicum virgatum*) in Pennsylvania. *Agronomy Journal* 63:785.
- Bies, L. 2006. The biofuels explosion: Is green energy good for wildlife? *Wildlife Society Bulletin* 34:1203-1205.
- Boylan, D., Bush, V., Bransby, D.I. 2000. Switchgrass cofiring: Pilot scale and field evaluation. *Biomass and Bioenergy* 19: 411-417.
- Borchert, J. R. 1950. The climate of the Central North American grassland. *Annals of the Association of American Geographers* 40:1-39.
- Bredja, J.J., Yocom, D.H., Moser, L.E., Waller, S.S. 1993. Dependence of 3 Nebraska Sandhills Warm- Season Grasses on Vesicular-Arbuscula. *Journal of Range Management* 46:14-20.
- Brown, R.A., Roseberg, N.J., Hay, C.J., Easterling, W.E., Mearns, L.O. 2000. Potential production and environmental effects of switchgrass and traditional crops under current and greenhouse-altered climate in the central United States: A simulation study. *Agriculture, Ecosystems and Environment* 78:31-47.
- Burke, I. C., Lauenroth, W.K., Parton, W.J. 1997. Regional and temporal variation in net primary production and nitrogen mineralization in grasslands. *Ecology* 78:1330-1340.
- Byrd, G.T., May II, P.A. 2000. Physiological comparisons of switchgrass cultivars differing in transpiration efficiency. *Crop Science* 40:1271-1277.

- Casler, M.D. 2005. Ecotypic Variation among Switchgrass Populations from the Northern USA. *Crop Science* 45:388-398.
- Casler, M.D., Boe, A.R. 2003. Cultivar X Environment Interactions in Switchgrass. *Crop Science* 43:2226–2233.
- Casler, M.D., Vogel, K.P., Taliaferro, C.M., Wynia, R.L. 2004. Latitudinal Adaptation of Switchgrass Populations. *Crop Science* 44:293–303.
- Clark, R.B. 2002. Differences among mycorrhizal fungi for mineral uptake per root length of switchgrass grown in acidic soil. *Journal of Plant Nutrition* 25:1753-1772.
- Christensen, J. H., B. Hewitson, A. Busuioc, A. Chen, X. Gao, I. Held, R. Jones, R. K. Kolli, W.-T. Kwon, R. Laprise, V. Magaña Rueda, L. Mearns, C. G. Menéndez, J. Räisänen, A. Rinke, A. Sarr and P. 2007: Regional Climate Projections. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor and H. L. Miller, eds.). Cambridge University Press, Cambridge, United Kingdom and New York, USA.
- Collins, S.L., Knapp, A.K., Briggs, J.M., Blair, J.M., Steinauer, E. 1998. Modulation of diversity by grazing and mowing in native tallgrass prairie. *Science* 280:745-747.
- Das, M.K., Fuentes, R.G., Taliaferro, C.M. 2004. Genetic Variability and Trait Relationships in Switchgrass. *Crop Science* 44:443-448.
- Easterling, D. R., Meehl, G.A., Parmesan, C., Changnon, S.A., Karl, T.R., Mearns, L.O. 2000. Climate extremes: Observations, modeling, and impacts. *Science* 289:2068-2074.
- Epstein, H.E., Lauenroth, W.K., Burke, I.C., Coffin, D.P. 1997. Productivity patterns of C₃ and C₄ functional types in the U.S. Great Plains. *Ecology* 78:722-731.

- Evers, E.W., Parsons, M.J. 2003. Soil type and moisture level influence on Alamo switchgrass emergence and seedling growth. *Crop Science* 43:288-294.
- Fay, P.A., Carlisle, J.D., Knapp, A.K., Blair, J.M., Collins, S.L. 2003. Productivity responses to altered rainfall patterns in a C₄-dominated grassland. *Oecologia* 137:245–251.
- Fay, P.A., Kaufman, D.M., Nippert, J.B., Carlisle, J.D., Harper, C.W. 2008. Changes in grassland ecosystem function due to extreme rainfall events: implications for responses to climate change. *Global Change Biology* 14:1600-1608.
- Finlay, R.D. 2004. Mycorrhizal fungi and their multifunctional roles. *Mycologist* 18:91-96.
- Gonzalez-Hernandez, J.L., Sarath, G., Stein, J.M., Owens, V., Gedye, K., Boe, A. 2009. A multiple species approach to biomass production from native herbaceous perennial feedstocks. *Vitro Cellular & Developmental Biology-Plant* 45:267-281.
- Heaton, E., Voigt, T., Long, S.P. 2004. A Quantitative review comparing the yields of two candidate C₄ perennial biomass crops in relation to nitrogen, temperature, and water. *Biomass and Bioenergy* 27:21-30.
- Hoffman W, Beyea J, and Cook JH. 1995. Ecology of agricultural monocultures: Some consequences for biodiversity in biomass energy farms. *Proceedings of the Second Biomass Conference of the Americas: Energy, Environment, Agriculture, and Industry*. Portland, Oregon. NREL/CP-200-8098. National Renewable Energy Laboratory, Golden, Colorado. 1618-1627.
- Hopkins, A.A., Taliaferro, C.M., Murphy, C.D., Christian, D. 1996. Chromosome number and nuclear DNA content of several switchgrass populations. *Crop Science* 36:1192-1195.

- Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K., Johnson, C.A., eds. 2001. Climate change 2001: the scientific basis. Contributions of working groups I to the third assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge.
- Hughes, A.R., Inouye, B.D., Johnson, M.T.J., Underwood, N., Vellend, M. 2008. Ecological consequences of genetic diversity. *Ecology Letters* 11:609-623.
- Hultquist, S.J., Vogel, K.P., Lee, D.J., Arumuganathan, K., Kaeppler, S.M. 1996. Chloroplast DNA and nuclear DNA content variations among cultivars of switchgrass, *Panicum virgatum* L. *Crop Science* 36:1049-1052.
- Jones, M.B., Donnelly, A. 2004. Carbon sequestration in temperate grassland ecosystems and the influence of management, climate and elevated CO₂. *New Phytologist* 164:423-439.
- Karl, T.R., Kukla, G., Razuvayev, V.N., Changery, M.J., Quayle, R.G., Heim Jr., R.R., Easterling, D.R., Bin Fu, C. 1991. Global warming: Evidence for asymmetrical diurnal temperature change. *Geophysical Research Letters* 18:2253-2256.
- Karl, T.R., Melillo, J.M., Peterson, T.C., (eds). 2009. Global Climate Change Impacts in the United States. Cambridge University Press.
- Knapp, A.K. 1985. Effect of fire and drought on the ecophysiology of *Andropogon gerardii* and *Panicum virgatum* in a tallgrass prairie. *Ecology* 66:1309-1320.
- Knapp, A.K., Fay, P.A., Blair, J.M., Collins, S.L., Smith, M.D., Carlisle, J.D., Harper, C.W., Danner, B.T., Lett, M.S., McCarron, J.K. 2002. Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science* 298:2202-2205.

- Lemus, R., Brummer, E.C., Moore, K.J., Molstad, M.E., Burras, C.E., Barker, M.F. 2002. Biomass yield and quality of 20 switchgrass populations in southern Iowa, USA. *Biomass and Bioenergy* 23:433-442.
- Lemus R., Lal R. 2005. Bioenergy crops and carbon sequestration. *Critical Reviews in Plant Sciences* 24:1-21.
- McLaughlin, S.B., Kszos, L.N. 2005. Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. *Biomass and Bioenergy* 28:515-535.
- Milder JC, McNeely JA, Shames SA, Scherr SJ. 2008. Biofuels and ecoagriculture: can bioenergy production enhance landscape-scale ecosystem conservation and rural livelihoods? *International Journal of Agricultural Sustainability* 6:105-121.
- Missaoui, A.M., Boerma, H.R., Bouton, J.H. 2005. Genetic variation and heritability of phosphorus uptake in Alamo switchgrass grown in high phosphorus soils. *Field Crops Research* 93:186-198.
- Muir, J. P., Sanderson, M.A., Ocumpaugh, W.R., Jones, R.M., Reed, R.L. 2001. Biomass production of 'Alamo' switchgrass in response to nitrogen, phosphorus, and row spacing. *Agronomy Journal* 93:896-901.
- Ney, R.A., Schnoor, J.L. 2002. Incremental life cycle analysis: Using uncertainty analysis to frame greenhouse gas balances from bioenergy systems for emission trading. *Biomass and Bioenergy* 22: 257-269.
- Nippert, J.B., Fay, P.A., Carlisle, J.D., Knapp, A.K., Smith, M.D. 2009. Ecophysiological responses of two dominant grasses to altered temperature and precipitation regimes. *Acta Oecologica* 35:400-408.

- Nippert, J.B., Knapp, A.K., Briggs, J.M. 2006. Intra-annual rainfall variability and grassland productivity: can the past predict the future? *Plant Ecology* 184:65-74.
- Parrish, D.J, Fike, J.H. 2005. The Biology and Agronomy of Switchgrass for Biofuels. *Critical Reviews in Plant Science* 24:423-459.
- Porter, C.L. 1966. An analysis of variation between upland and lowland switchgrass, *Panicum Virgatum* L., in Central Oklahoma. *Ecology* 47:980-992.
- Ruiz-Lozano, J. M. 2003. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. *Mycorrhiza* 13:309–317.
- Sanderson, M.A. 1992. Morphological development of switchgrass and kleingrass. *Agronomy Journal* 84:415-419
- Sanderson, M.A., Adler, P.R., Boateng, A.A., Casler, M.D., Sarath, G. 2006. Switchgrass as a biofuels feedstock in the USA. *Canadian Journal of Plant Science* 86:1315-1325.
- Sanderson, M.A., Reed, R.L. 2000. Switchgrass growth and development: Water, nitrogen, and plant density effects. *J. Range Manage.* 53:221-227.
- Simberloff, D. 2008. Invasion Biologists and the Biofuels Boom: Cassandras or Colleagues? *Weed Science* 56:867-872.
- Smith, M.D., Knapp, A.K. 2003. Dominant species maintain ecosystem function with non-random species loss. *Ecology Letters* 6:509-517.
- Stout, W. L., G. A. Jung, and J. A. Shaffer. 1988. Effects of soil and nitrogen on water use efficiency of tall fescue and switchgrass under humid conditions. *Soil Science Society of America Journal* 52:429-434.

- Suplick, M.R., Read, J.C., Matuson, M.A., Johnson, J.P. 2002. Switchgrass leaf appearance and lamina extension rates in response to fertilizer nitrogen. *Journal of Plant Nutrition* 25: 2115-2127.
- Tillman, D.A. 2000. Biomass cofiring: The technology, the experience, the combustion consequences. *Biomass and Bioenergy* 19:365-384.
- Vogel, K.P., Jung, H.J.G. 2001. Genetic modification of herbaceous plants for feed and fuel. *Critical Reviews in Plant Science* 20:15-49.
- Voigt, W., Perner, J., Davis, A.J., Eggers, T., Schumacher, J., Bährmann, R., Fabian, B., Heinrich, W., Köhler, G., Lichter, D., Marstaller, R., Sande F.W. 2003. Trophic levels are differentially sensitive to climate. *Ecology* 84:2444-2453.
- Weaver, J.E., Fitzpatrick, T.J. 1932. Ecology and relative importance of the dominants of Tall-Grass Prairie. *Botanical Gazette* 93:113-150.
- Wilson, G. W.T., Hartnett, D.C. 1998. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. *American Journal of Botany* 85:1732-1738.
- Xu, B. C., Li, F.M., Shan, L., Ma, Y.Q., Ichizen, N., Huang, J. 2006. Gas exchange, biomass partition, and water relationships of three grass seedlings under water stress. *Weed Biology and Management* 6:79-88.

Chapter 2 - Potential ecological impacts of switchgrass (*Panicum virgatum* L.) biofuel cultivation in the Central Great Plains, USA¹

Abstract

Switchgrass (*Panicum virgatum* L.) is a broadly adapted warm-season grass species native to most of central and eastern United States. Switchgrass has been identified as a potential biofuel species because it is a native species that requires minimal management, and has a large potential to sequester carbon underground. Since the 1990's, switchgrass has been bred to produce cultivars with increased biomass and feedstock quality. This review addresses potential ecological consequences of widespread switchgrass cultivation for biofuel production in the central United States. Specifically, this review address the ecological implications of changing use of marginal and CRP land, impacts on wildlife, potentials for disease and invasions, and changes in soil quality through reductions in erosion, decomposition rates, and carbon sequestrations. A central theme of the review is the utility of maintaining landscape heterogeneity during switchgrass biofuel production. This includes implementing harvest rotations, no till farming, and mixed species composition. If negative ecological consequences of switchgrass cultivation are minimized, biofuel production using this species has economical and environmental benefits.

Introduction

Panicum virgatum L. (switchgrass) is a common perennial C₄ grass that is widely distributed across North America. Ecologically, this species is a dominant plant in the central Great Plains grasslands, with impacts on both the structure and function of these ecosystems [1] [2]. Considerable genotypic and phenotypic variability exists for switchgrass [3] [4]. This variability contributes to the broad adaptation of this species across a wide geographic and environmental range [5]. For example, switchgrass has a robust distribution across North America, from 5-25°C MAT and 300-1500mm MAP (Fig. 2-1). In general, ecotypes of switchgrass are broadly divided into two types: upland and lowland [6]. Upland ecotypes have a

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smaller size, and lower water and nitrogen requirements than lowland ecotypes [6]. Additionally, upland ecotypes are typically octoploid or hexaploid, whereas lowland ecotypes are tetraploid [6] [7].

Practically, switchgrass is an important forage crop in pasture lands, and has been studied extensively over the past two decades for its potential value as an alternative energy source. In recent years, switchgrass has become a model species for biofuel production [8]. Switchgrass was chosen as a prospective biofuel for its ability to increase soil quality, sequester carbon, and its wide range of suitable habitat [9]. While the potential economic benefits of implementing switchgrass for biofuel production are enormous, the environmental consequences of cultivation must be considered [10]. Large amounts of land will be required for cultivation, and this land will be transferred from previous agricultural or conservation practices to switchgrass biofuel production [11]. The environmental impacts of changing land-use to biofuel production have yet to be adequately assessed [12]. If switchgrass cultivation for biofuel is to be successfully implemented in the Central Great Plains of the United States, the potential ecological impacts must be assessed in concert with economical impacts.

Cultivation in marginal or CRP lands

Marginal lands that are not currently used for agricultural production may be suitable for switchgrass cultivation. The use of marginal lands for biofuel production is desirable because utilization of this land minimizes competition with food crops produced on lands of higher agricultural value [13]. Switchgrass cultivation in marginal lands has great potential value because this species produces high biomass across a broad range of environments, requires low water and nutrient inputs compared to agronomic species (e.g., corn), and provides environmental benefits for degraded lands (e.g., reduced erosion, increased soil organic carbon) [14][15]. The production potential of switchgrass on marginal lands is equal to or greater than other potential herbaceous biofuel like corn [16] and switchgrass cultivation in marginal lands provides wildlife cover while promoting landscape heterogeneity and biodiversity compared to conventional corn-grain production [12][17]. However, the positive biodiversity and landscape heterogeneity benefits of switchgrass cultivation or other perennial herbaceous energy crops for biofuel are minimized when grown in monoculture [12].

The cultivation of switchgrass as a perennial energy crop has also been considered for marginal lands currently in the Conservation Reserve Program (CRP). This program, developed in 1985 as part of the Food Security Act, provides compensation for landowners to rest their land from continual agricultural production. A byproduct of removing the land from agricultural production is the establishment of permanent grass cover. As of 2008, there were 34.7 million acres enrolled in the Conservation Reserve Program [18]. The CRP program has successfully advanced conservation practices, with estimated decreases in soil erosion of 220 million tons/year, and native bird populations have increased by 2-52% [19][20]. The 2008 Farm Bill allowed for 32 million acres to be enrolled, so a large amount of land was not renewed, and is available for switchgrass cultivation [18]. While CRP lands can be cultivated, the economic value for food production is often considerably lower. Some scenarios for switchgrass cultivation on CRP lands have been estimated at 3.3 to 5.2 million hectares of CRP land being converted [21]. Within the Central Great Plains region, a large amount of agricultural land is enrolled as CRP land (Fig. 2-2). Those lands to be converted would not include CRP land that is used as buffer zones, wetlands, or critical habitats [21]. Thus, switchgrass cultivation would not be appropriate in all CRP lands, and more research is necessary to assess the biodiversity and wildlife habitat consequences of converting some CRP lands to biofuel production. Ultimately, the applicability of using marginal lands or CRP lands for switchgrass production requires effective harvesting techniques that maximize yield while minimizing land degradation and impacts on native plants and wildlife. To manage the tradeoff between productivity, long-term sustainability and habitat heterogeneity, a proportion of converted CRP land would likely need to remain unharvested in the establishment year. Schmer and colleagues estimated that switchgrass on CRP land requires 40% stand establishment of the initial switchgrass planting, for subsequent annual harvests [22]. However, these authors estimate 25% stand establishment is sufficient if the stand is harvested every few years [22].

Potential for disease, insect outbreaks, & invasive species

Historically, biofuel production has been planned and implemented similar to production agriculture, in monoculture ecosystems [23]. This technique is advantageous because monocultures are selected and cultivated for species and populations with the highest yield [23]. However, monoculture production can have negative ecological consequences. For example,

biofuel crops selected for high productivity have increased vulnerability to plant pathogens and pests due to decreases in genetic diversity and heterogeneity [23][24]. For switchgrass in particular, increased susceptibility to some strains of the yellow barley dwarf virus occurs when grown in large monocultures [5]. Monocultures accelerate the spread of pests and pathogens because the suitable host has high abundance and distribution across the landscape. For switchgrass, pests and pathogens include insects, fungi, water molds, bacteria, mollicutes, protozoa, nematodes, and viruses. In 2009, Crouch and colleagues identified a new fungal species, *C. navitas*, which is the cause of switchgrass anthracnose [25]. Previously, anthracnose had been thought to be caused by a different fungal species, *C. graminicola*. *C. navitas* displayed many characteristics of close relatives such as decreased plant vigor which led to necrotic tissue eventually covering much of the plants affected. However, *C. navitas* also displayed a few unique traits such as host association and many fixed molecular characters [25]. These pests and pathogens can negatively impact switchgrass in numerous ways, including physical and physiological damage through excessive herbivory [24]. Herbivory can result in reduced physiological functioning via toxin production which reduces cellular physiological functioning and ultimately leads to cell death of the infected tissue [24]. The fall armyworm, *Spodoptera frugiperda* has been shown capable of developing on switchgrass. In laboratory tests, the larval form showed a strong preference for feeding on the leaf tissues of young switchgrass stands [26]. These physical and physiological impacts reduce photosynthetic rates and ultimately decrease biomass production. Therefore, it is vitally important to understand the interactions between host and pathogen and minimize the potential for disease or insect outbreaks by using diverse genotypes or multi-species assemblages within the area cultivated for biofuel production.

Another negative trade off associated with monocultures is the punctuated seasonal tempo of growth and productivity, leaving large periods of time with gaps in standing biomass. Fluctuations in insect diversity and abundance mimic these fluctuations in productivity [23]. Productivity gaps affect the plant-herbivore interactions within the monoculture [23]. Changing the interaction between predators and prey has the potential to enhance the vulnerability of biofuels such as switchgrass especially if genetic diversity within the population is low. While the topic of disease potential in biofuel monocultures has been discussed initially, this is a topic requiring considerable future research, with a specific studies focused on key biofuel species,

including switchgrass [25]. Specifically, outbreaks, spread, and consequences of the pests, pathogens, and diseases on monoculture switchgrass cultivation remain to be evaluated.

Pathogens and pests have the potential to negatively impact establishment, biomass productivity, and stand survival in perennial herbaceous crops grown for biofuels [24]. The impacts of rust fungi on switchgrass have been assessed in several studies. In 1941, Cornelius and Johnston [27] examined 34 accessions of switchgrass from South Dakota, Nebraska, Oklahoma, and Texas and found that collections from South Dakota and Nebraska were more susceptible to the rust *Uromyces graminicola*, than those from Oklahoma and Texas. In 1967, Barnett and Carver [28] reported lowland ecotypes were more rust resistant than upland ecotypes due to coarser stems. Moreover, Gustafson and colleagues examined the impacts of another rust species, *Puccinia emaculata* [29]. Their results showed variation within and among populations of switchgrass at two different sites in South Dakota. These results suggest selection of cultivars for biomass production should consider populations with appropriate pest resistance as well as appropriate environmental tolerance (e.g., winter hardy) [29].

Monocultures of switchgrass and other biofuel crops increase the potential for future invasion of non-native species. Reduced landscape heterogeneity increases the susceptibility of an area to new invasive species [23] [24]. Simberloff in 2008 [30] states that many invasive species remain restricted or dormant for decades until such a time when environmental conditions change in favor of their growth and subsequent spread. The potential for the release from environmental restriction for invasive species increases as more land is allocated to monoculture biofuel production. Additionally, many of the species chosen for biofuel cultivation share similar characteristics with invasive species including phenological characteristics such as a perennial lifespan and rapid spring growth, as well as physiological characteristics such as the C₄ photosynthetic pathway and high water-use efficiency [31]. These types of potential biofuel species may be candidate species for undesirable spread from their natural or agricultural areas. For example, native species have the potential to become invasive as grazing or fire suppression is increased [30], or as climate change expands the potential habitat of the species [11]. The invasive risk from biofuel species can also increase as different genotypes are engineered and introduced across the landscape. For switchgrass, this threat is already eminent and worthy of future consideration. Barney and DiTomaso (2008) [32] relate the extensive bioengineering of switchgrass cultivars and varieties to invasion potential in introduced regions in California and

the Pacific Northwest, where switchgrass cultivation trials with engineered genotypes are currently being conducted. Although their evaluations concluded switchgrass was not likely to become an extensive invader under current climate conditions, an altered future climate could shift the invasive capability of switchgrass in these regions. This potential invasive capability under climatic changes needs to be studied experimentally in the future.

Impacts on Wildlife

Switchgrass cultivation in marginal farming lands and CRP land can provide needed habitat for bird and insect populations if landscape heterogeneity is maintained via mixed-species assemblages and rotational harvests [33]. By retaining the structural (grassy) composition of CRP land or marginal land when converted to biofuel production, native grassland wildlife species are supported by a habitat more closely resembling their native grassland communities [34]. The maintenance of vertical and horizontal habitat structure supports multiple ecological niches for insect, bird, reptile and mammal populations [35]. One way to decrease the impact on wildlife biodiversity would be through crop rotation. Milder et al. (2008) [35] suggested that short rotations with both perennial grass and fast-growing woody species would maintain biodiversity. McCoy and colleagues (2001) [36] suggested CRP land-conversion should focus on a combination of warm and cool season grasses to maximize the potential benefits to wildlife rather than single species plantings of warm-season grasses, such as switchgrass. This strategy provides wildlife populations a shifting mosaic of available habitats. Semere and Slater (2007) [37] showed that the diversity of invertebrates increase indirectly through the abundance of mixed species composition within biomass crop fields. The consequence of reduced landscape heterogeneity and viable habitat is reduced wildlife biodiversity.

Appropriate harvest rotations have the potential to increase the stability of grassland bird populations [37] [38] [39]. When switchgrass was cultivated in CRP land in Iowa, nest cover was available early in the year, reducing the impact of harvests that occur later in the fall [38]. As long as the CRP fields were a mix of harvested and non-harvested fields, stable breeding habitat would still be available for those species that breed later in the year. For example, fields not harvested in the fall provide much needed over-wintering cover and forage sites for bird species that feed on invertebrates and seeds [37]. Similarly, when CRP land is converted to cultivate switchgrass, the diversity of local grassland bird populations increases only when there

is a mixture of harvested and unharvested fields. Harvested fields showed increased diversity in shortgrass bird species, while unharvested fields increased in tallgrass bird species diversity [40]. To date, most research has been conducted over the short-term, so further monitoring of bird populations and assessments of habitat availability and suitability must be continued as more land is converted to switchgrass production [41][42][43].

Changes in soil quality

Soil type

The broad distribution of suitable habitat for switchgrass in the United States spans a range of soil types. The direct impacts of soil type on switchgrass productivity may be less than other grasses [44]. Soil type effects on distribution are likely indirectly related via rainfall patterns. Evers and Parsons (2003) [45] report that rainfall every 7 to 10 days is required for switchgrass to survive in sandy soils, but less frequent rainfall is required in clay soils. Therefore, climate is likely to exert a greater influence on switchgrass survival and productivity across suitable habitat, rather than differences in soil type. Switchgrass is tolerant of both extreme soil moisture conditions for short periods of time, from flooded soils to low levels of soil moisture [46]. This broad soil moisture tolerance is a direct contributor to the broad habitat distribution in the United States for flooded and drought conditions. Future predictions for suitable switchgrass habitat include most of the eastern and Midwestern regions of the United States, with habitat boundaries shifting northward towards the end of the century as the average air temperature increases [11].

Decomposition

Rates of decomposition affect soil quality, driven largely by changes in precipitation, temperature, soil factors, and litter quality [47]. For the Great Plains region, annual precipitation is predicted to increase slightly over the next century with a greater increase in annual temperature [48]. The impact of high temperature to increase decomposition rates is present only when precipitation is not limiting. Since precipitation is limiting grassland productivity across most of this region [49], increased temperature would decrease root decomposition, and therefore increase the carbon storage of grasslands [50]. Another component of decomposition is the litter quality, which is affected by the allocation of nutrients by the plant. Plants that allocate

large amounts of carbon to structural components, like lignin, generally have low quality litter. This low quality litter decomposes slowly which adds more carbon to the soil [51]. Litter quality has been found to be related to precipitation, in that increased precipitation leads to lower litter quality [50] [51]. Therefore, switchgrass cultivars with increased lignin content in the litter produced may lead to higher carbon additions to the soil.

Soil erosion and SOC

Erosion and land degradation are accentuated through losses of soil organic carbon (SOC) [52]. The loss of the SOC pool is due primarily to three factors: (1) the reduction in plant roots (2) the increase in biological activity as soil aeration is increased by cultivation and soil temperature, and (3) increase in soil erosion that removes carbon-rich materials. To minimize negative ecological impacts of switchgrass biofuel production, SOC losses must be minimized. No-till farming has been shown to slow erosion and build SOC matter when residue inputs are sufficient [53]. These residue inputs reduce SOC loss and provide for the maintenance of soil structure and resistance from soil erosion [12]. Land maintenance has important consequences because degraded soil structure and the loss of SOC increases the greenhouse gas carbon dioxide and accelerates soil erosion losses [54][55].

Rehabilitation of degraded soil can be accomplished using appropriate bioenergy crops to improve soil productivity and restore the SOC pool. Switchgrass can restore the SOC in surface soils (0-30cm) and stabilize the soil with its deep root system (>1m) [52]. The root system of switchgrass has the potential to lower soil erosion rates 30 times in the establishment year, and 600 times in the second and third years compared to annual crop production [12][56]. Decreases in soil erosion rates result from a well-developed litter layer and increases of other carpet grasses such as fescue or smooth brome [57]. For this reason, bioenergy crops can be grown on marginal soils with low productivity to rehabilitate this degraded land. Recent estimates suggest great potential for increasing the SOC pool using biofuels. Estimates suggest up to 3 T- ha-yr⁻¹ of soil carbon can be sequestered under perennial grass biofuels like switchgrass [17] [52]. There are 10.8 Mha of severely eroded soils in the United States that may benefit from growing bioenergy crops and adoption of conservation-effective practices [52].

Carbon Sequestration

The high productivity of grassland perennials like switchgrass increases the amount of carbon sequestered in degraded soils from the extensive root systems and large amounts of leaf litter [5][58][59][60][61]. The belowground biomass of switchgrass is four to five times greater than that of corn, with the potential to input $2.2 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ into soils [52][62]. Switchgrass root systems increase the amount of SOC due to the size of the root systems, slow decomposition rates of root biomass [59][63], and root secretion of organic compounds bind soil particles and stabilize the SOC [59][64]. However, as with any productive grass population, switchgrass stands are a large carbon source due to the respiration from the extensive root systems and associated microbial communities. The microbial CO_2 emissions depend on the amount of labile carbon available in the form of leaf litter and crop residue [59] [65] [66]. For example, Al-Kaisi and Grote (2007) [59] reported annually harvested switchgrass crop systems exhibited higher soil CO_2 emissions than switchgrass crop systems harvested at five year intervals [59]. Al-Kaisi and Grote suggest difference in CO_2 emissions between the two harvesting techniques may be due to larger root biomass of individuals in the annually harvested treatment and higher microbial biomass carbon content [59]. Despite CO_2 emissions from grasslands and biofuel cropping systems from microbial and root respiration, these systems are generally viewed as net carbon sinks [58] [67] [68] [69] [70].

The degree to which switchgrass or any other biofuel can act as an agent for carbon sequestration depends on the soil environment. The soil environment includes soil quality, soil type, soil moisture, soil temperature, and the carbon to nitrogen ratio of the substrate (leaf litter and residue) [50]. For instances, the initial SOC and soil type determine how quickly switchgrass stands can sequester carbon [71]. In addition, management practices, climate, and cultivar selection may influence carbon sequestration [5]. The research of Lee, Owens, and Doolittle (2007) [61] showed that carbon sequestered at depths of 30cm to 90cm increased when manure was applied as the N source for switchgrass grown on CRP land. Frank et al. (2004) [58] reported that seasonal changes in temperature and soil moisture were the primary determinants of soil CO_2 flux in switchgrass cultivation. CO_2 flux throughout the season corresponded with changes in temperature and lower CO_2 fluxes were associated with decreased soil moisture [58]. Moreover, Al-Kaisi and Grote (2007)[59] suggest switchgrass cropping systems can potentially contribute more to soil carbon sequestration than corn-soybean rotations due to the more

extensive root system of switchgrass. Furthermore, a study conducted by Tilman et al. (2006) [17] argues that low-input high diversity (LIHD) biofuels have the greatest potential for carbon sequestration compared to monocultures. LIHD biofuels are carbon-negative because the net carbon sequestration is much greater than the CO₂ released during the biofuel production [17]. Biofuel crops will continue to sequester greater amounts of soil carbon until the system reaches equilibrium. At equilibrium, any biofuel cropping system (i.e., switchgrass) becomes a carbon reservoir [5]. It is estimated that switchgrass cropping systems have the potential to reach equilibrium around fifty years after establishment [52].

Conclusion

As consideration of switchgrass as a biofuel resource continues to develop in the future, the potential ecological implications of cultivating this crop across large sections of the central United States must be considered. These impacts can be measured by the abundance and diversity of wildlife, potential for disease and invasions, changes in soil quality, erosion, and carbon sequestration. To date, the greatest ecological consideration of the impacts of widespread switchgrass cultivation has been focused on the ability of switchgrass to sequester carbon. However, the other considerations discussed (e.g., the effects on wildlife, changing land use, disease, invasive potential, and soil quality) should also be considered when evaluating the consequences of switchgrass as a biofuel [52] [72]. One of the central tenets associated with maximizing the structural and functional characteristics of grassland ecosystems following switchgrass cultivation is the maintenance of landscape heterogeneity. Landscape heterogeneity is maximized by altered harvest rotations, no till farming, and mixed species composition. Increased structural diversity facilitates greater species abundance and species diversity because more habitat is available. Additionally, landscape heterogeneity increases the quality of the soil, and provides greater genetic variation within the community. To date, most research investigating the ecological impacts of switchgrass cultivation has been short-term, emphasizing the need for long-term assessment of impacts and consequences [42] [43]. Regardless of the species and technique, biofuel production in agricultural lands, marginal lands, and grasslands has ecosystem consequences that must be considered, but current research suggests that low-input switchgrass cultivation across a heterogeneous landscape can increase ecosystem services as well as provide economic value.

Literature Cited

- [1] Weaver JE, Fitzpatrick TJ. Ecology and relative importance of the dominants of tall-grass prairie. *Botanical Gazette* 1932; 93:113-150.
- [2] Smith MD, Knapp AK. Dominant species maintain ecosystem function with non-random species loss. *Ecology Letters* 2003; 6:509-517.
- [3] Casler MD, Vogel KP, Taliaferro CM, Wynia RL. Latitudinal adaptation of switchgrass populations. *Crop Science* 2004; 44:293-303.
- [4] Das MK, Fuentes RG, Taliaferro CM. Genetic variability and trait relationships in switchgrass. *Crop Science* 2003; 44:443-448.
- [5] Parrish DJ, Fike JH. The biology and agronomy of switchgrass for biofuels. *Critical Reviews in Plant Sciences* 2005; 24:423-459.
- [6] Porter, CL. An Analysis of Variation between Upland and Lowland Switchgrass *Panicum Virgatum* L in Central Oklahoma. *Ecology* 1966; 47:980-&.
- [7] Casler, MD. Ecotypic variation among switchgrass populations from the northern USA. *Crop Science* 2005; 45:388-398.
- [8] Qin X, Mohan T, El-Halwagi M, Cornforth G, McCarl BA. Switchgrass as an alternate feedstock for power generation: an integrated environmental, energy and economic life-cycle assessment. *Clean Techn Environ Policy* 2006; 8:233–249.
- [9] Sanderson MA, Adler PR, Boateng AA, Casler MD, Sarath G. Switchgrass as a biofuels feedstock in the USA. *Canadian Journal of Plant Science* 2006; 86:1315-1325.
- [10] Brown RA, Rosenberg NJ, Hays CJ, Easterling WE, Mearns LO. Potential production and environmental effects of switchgrass and traditional crops under current and greenhouse-altered climate in the central United States: a simulation study. *Agriculture, Ecosystems and Environment* 2000; 78:31-47.
- [11] Barney JN, DiTomaso JM, Bioclimatic predictions of habitat suitability for the biofuel switchgrass in North American under current and future climate change scenarios. *Biomass and Bioenergy* 2010; 34:124-133.
- [12] Williams PRD, Inman D, Aden A, Heath GA. Environmental and Sustainability Factors Associated With Next-Generation Biofuels in the U.S.: What Do We Really Know? *Environmental Science & Technology* 2009; 43:4763-4775.
- [13] Tilman D, Socolow R, Foley JA, Hill J, Larson E, Lynd L, et al. Beneficial Biofuels—The Food, Energy, and Environment Trilemma. *Science* 2009;325:270-271.

- [14] Bouton, J. The economic benefits of forage improvement in the United States. *Euphytica* 2007; 154:263–270.
- [15] Jessup JW. Development and status of dedicated energy crops in the United States. *In Vitro Cell.Dev.Biol.—Plant* 2009; 45:282–290.
- [16] Varvel GE, Vogel KP, Mitchell RB, Follett RF, Kimble JM. Comparison of corn and switchgrass on marginal soils for bioenergy. *Biomass and Bioenergy* 2008; 32:18-21.
- [17] Tilman D, Hill J, and Lehman C. 2006. Carbon-negative biofuels from low-input high-diversity grassland biomass. *Science*. 2006;314:1598-1600.
- [18] USDA [Internet]. Conservation Policy: Land Retirement Programs [update 2009 Feb 6, cited 2010 Jan 28]. Available from: <http://www.ers.usda.gov/Briefing/ConservationPolicy/retirement.htm>.
- [19] Claassen R, Hansen L, Peters M, Breneman V, Weinberg M, Cattaneo A, et al. Agri-Environmental Policy at the Crossroads: Guideposts on a Changing Landscape. *Agricultural Economic Report* 2001; 794:1-67.
- [20] Niemuth ND, Quamen FR, Naugle DE, Reynolds RE, Estey ME, Shaffer TL. Benefits of the Conservation Reserve Program to Grassland Bird Populations in the Prairie Pothole Region of North Dakota and South Dakota. United States Department of Agriculture Farm Service Agency 2007.
- [21] Walsh ME, Ugarte DGD, Shapouri H, Slinsky SP. Bioenergy crop production in the United States - Potential quantities, land use changes, and economic impacts on the agricultural sector. *Environmental & Resource Economics* 2003;24:313-333.
- [22] Schmer MR, Vogel KP, Mitchell RB, Moser LE, Eskridge KM, Perrin RK. Establishment stand thresholds for switchgrass grown as a bioenergy crop. *Crop Science* 2006;46:157-161.
- [23] Hoffman W, Beyea J, and Cook JH. Ecology of agricultural monocultures: Some consequences for biodiversity in biomass energy farms. In *Proceedings of the Second Biomass Conference of the Americas: Energy, Environment, Agriculture, and Industry*. Portland, Oregon. NREL/CP-200-8098. National Renewable Energy Laboratory, Golden, Colorado. 1995;1618-1627.
- [24] Gonzalez-Hernandez JL, Sarath G, Stein JM, Owens V, Gedye K, Boe A. A multiple species approach to biomass production from native herbaceous perennial feedstocks. *In Vitro Cellular & Developmental Biology-Plant* 2009;45:267-281.
- [25] Crouch JA, Beirn LA, Cortese LM, Bonos SA, Clarke BB. Anthracnose disease of switchgrass caused by the novel fungal species *Colletotrichum navitas*. *Mycological Research* 2009;113:1411-1421.

- [26] Prasifka JR, Bradshaw JD, Meagher RL, Nagoshi RN, Steffey KL, Gray ME. Development and Feeding of Fall Armyworm on *Miscanthus x giganteus* and Switchgrass. *Field and Forage Crops* 2009; 102:2154-2159.
- [27] Cornelius DR, Johnston CO. Differences in Plant Type and Reaction to Rust among Several Collections of *Panicum Virgatum* L. *Agronomy Journal* 1941; 33:115-124.
- [28] Barnett FL, Carver RF. Meiosis and pollen stainability in switchgrass, *Panicum virgatum* L. *Crop Science*. 1967;7: 301–304.
- [29] Gustafson DM, Boe A, Jin Y. Genetic variation for *Puccinia emaculata* infection in switchgrass. *Crop Science* 2003;43:755-759.
- [30] Simberloff D. Invasion Biologists and the Biofuels Boom: Cassandras or Colleagues? *Weed Science* 2008;56:867-872.
- [31] Pyke CR, Thomas R, Porter RD, Hellmann JJ, Dukes JS, Lodge DM, et al. Current practices and future opportunities for policy on climate change and invasive species. *Conservation Biology* 2008;22:585-592.
- [32] Barney JN, Ditomaso JM. Nonnative species and bioenergy: Are we cultivating the next invader? *Bioscience* 2008;58:64-70.
- [33] Bies L. The biofuels explosion: Is green energy good for wildlife? *Wildlife Society Bulletin* 2006;34:1203-1205.
- [34] Paine LK, Peterson TL, Undersander DJ, Rineer KC, Bartelt GA, Temple SA, et al. Some ecological and socio-economic considerations for biomass energy crop production. *Biomass & Bioenergy* 1996;10:231-242.
- [35] Milder JC, McNeely JA, Shames SA, Scherr SJ. Biofuels and ecoagriculture: can bioenergy production enhance landscape-scale ecosystem conservation and rural livelihoods? *International Journal of Agricultural Sustainability* 2008;6:105-121.
- [36] McCoy TD, Ryan MR, Burger LW, Kurzejeski EW. Grassland bird conservation: CP1 vs. CP2 plantings in Conservation Reserve Program fields in Missouri. *American Midland Naturalist* 2001;145:1-17.
- [37] Semere T, Slater FM. Ground flora, small mammal and bird species diversity in miscanthus (*Miscanthus x giganteus*) and reed canary-grass (*Phalaris arundinacea*) fields. *Biomass & Bioenergy* 2007;31:20-29.
- [38] Murray LD, Best LB. Short-term bird response to harvesting switchgrass for biomass in Iowa. *Journal of Wildlife Management* 2003;67:611-621.

- [39] Perlut NG, Strong AM, Donovan TM, Buckley NJ. Regional population viability of grassland songbirds: Effects of agricultural management. *Biological Conservation* 2008;141:3139-3151.
- [40] Roth AM, Sample DW, Ribic CA, Paine L, Undersander DJ, Bartelt GA. Grassland bird response to harvesting switchgrass as a biomass energy crop. *Biomass & Bioenergy* 2005;28:490-498.
- [41] Murray LD, Best LB, Jacobsen TJ, Braster ML. Potential effects on grassland birds of converting marginal cropland to switchgrass biomass production. *Biomass & Bioenergy* 2003;25:167-175.
- [42] Keshwani DR, Cheng JJ. Switchgrass for bioethanol and other value-added applications: A review. *Bioresource Technology* 2009;100:1515-1523.
- [43] Bellamy PE, Croxton PJ, Heard MS, Hinsley SA, Hulmes L, Hulmes S, et al. The impact of growing miscanthus for biomass on farmland bird populations. *Biomass & Bioenergy* 2009;33:191-199.
- [44] Nixon, E.S., and C. McMillan. The role of soil in the distribution of four grass species in Texas. *Am. Midl. Nat.* 1964;71:114-140.
- [45] Evers EW, Parsons MJ. Soil type and moisture level influence on Alamo switchgrass emergence and seedling growth. *Crop Science* 2003;43:288-294.
- [46] Barney JN, Mann JJ, Kyser GB, Blumwald E, Van Deynze A, DiTomaso JM. Tolerance of switchgrass to extreme soil moisture stress: Ecological implications. *Plant Science* 2009;177:724-732.
- [47] Johnson JMF, Barbour NW, Weyers SL. Chemical composition of crop biomass impacts its decomposition. *Soil Science Society of America Journal* 2007;71:155-162.
- [48] Christensen JH, Hewitson B, Busuioc A, Chen A, Gao X, Held I, et al. 2007: Regional Climate Projections. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor and H. L. Miller, eds.). Cambridge University Press, Cambridge, United Kingdom and New York, USA.
- [49] Knapp AK, Smith MD. 2001. Variation among biomes in temporal dynamics of aboveground primary production. *Science* 2001;291:481-484.
- [50] Bontti EE, Decant JP, Munson SM, Gathany MA, Przeszlowska A, Haddix ML, et al. Litter decomposition in grasslands of Central North America (US Great Plains). *Global Change Biology* 2009;15:1356-1363.

- [51] Murphy KL, Burke IC, Vinton MA, Lauenroth WK, Aguiar MR, Wedin DA, Virginia RA, and Lowe PN. Regional analysis of litter quality in the central grassland region of North America. *Journal of Vegetation Science*. 2002;13:395-402.
- [52] Lemus R, Lal R. Bioenergy crops and carbon sequestration. *Critical Reviews in Plant Sciences* 2005;24:1-21.
- [53] Sampson RN. Carbon Sequestration: What's the Best Approach? Presented at: CARBON: Exploring the Benefits to Farmers and Society; 2000 Aug 30; Des Moines, Iowa.
- [54] Blanco-Canqui H, Lal R. Soil and crop response to harvesting corn residue for biofuel production. *Geoderma* 2007; 141:355-362.
- [55] Fargione J, Hill J, Tilman D, Polasky S, Hawthorne P. Land clearing and the biofuel carbon debt. *Science* 2008; 319:1235-1238.
- [56] McLaughlin SB, Ugarte DGDL, Garten CT, Lynd LR, Sanderson MA, Tolbert VR, Wolf DD. High-Value Renewable Energy from Prairie Grasses. *Environmental Science & Technology*. 2002;36:2122-2129.
- [57] Mann L, Tolbert V. Soil sustainability in renewable biomass plantings. *Ambio* 2000;29:492-498.
- [58] Frank AB, Berdahl JD, Hanson JD, Liebig MA, Johnson HA. Biomass and carbon partitioning in switchgrass. *Crop Science* 2004;44:1391-1396.
- [59] Al-Kaisi MM, Grote JB. Cropping systems effects on improving soil carbon stocks of exposed subsoil. *Soil Science Society of America Journal* 2007;71:1381-1388.
- [60] ^aLee DK, Doolittle JJ, Owens VN. Soil carbon dioxide fluxes in established switchgrass land managed for biomass production. *Soil Biology & Biochemistry* 2007;39:178-186.
- [61] ^bLee DK, Owens VN, Doolittle JJ. Switchgrass and soil carbon sequestration response to ammonium nitrate, manure, and harvest frequency on conservation reserve program land. *Agronomy Journal* 2007;99:462-468.
- [62] Zan C, Fyles JM, Girourard P, Samson R, and Doan M. Carbon storage in switchgrass and short-rotation willow plantations. In: *Making a business from biomass in energy, environment, chemicals, fibers, and materials*. Proceedings of the Third Biomass Conference of the Americas: Energy, Environment, Agriculture, and Industry. August 24-29, Montr´eal, Qu´ebec, Canada. 1997; pp. 355–361. Overend, R. P. and Chornet, E., Eds., Pergamon, New York.
- [63] Puget P, Drinkwater LE. Short-Term Dynamics of Root- and Shoot-Derived Carbon from a Leguminous Green Manure. *Soil Sci. Soc. Am. J.* 2001;65:771–779.
- [64] Bronick CJ, Lal R. Soil structure and management: A review. *Geoderma* 2005;124: 3–22.

- [65] Franzluebbers AJ, Haney RL, Hons FM, and Zuberer DA. Determination of microbial biomass and nitrogen mineralization following rewetting of dried soil. *Soil Science Society of America Journal*. 1996;60: 1133–1139.
- [66] Wang WJ, Dalal RC, Moody PW, Smith CJ. Relationships of soil respiration to microbial biomass, substrate availability and clay content. *Soil Biology & Biochemistry* 2003;35: 273–284.
- [67] Frank AB, Sims PL, Bradford JA, Mielenick PC, Dugas WA, Mayeux HS. Carbon dioxide fluxes over three Great Plains Grasslands. 2001;p. 167–187. In R.F. Follett et al. (ed.) *The potential of U.S. grazing lands to sequester carbon and mitigate the greenhouse effect*. CRC Press, Boca Raton, FL.
- [68] Frank AB, Dugas WA. Carbon dioxide fluxes over a northern semiarid, mixed-grass prairie. *Agricultural and Forest Meteorology*. 2001;108: 317–326.
- [69] Sims PL, Bradford JA. Carbon dioxide fluxes in a southern plains prairie. *Agricultural and Forest Meteorology*. 2001;109: 117–134.
- [70] Suyker AE and Verma SB. Year-round observations of the net ecosystem exchange of carbon dioxide in a native tallgrass prairie. *Global Change Biology*. 2001;7: 279–289.
- [71] Garten CT, Wullschlegel SD. Soil carbon dynamics beneath switchgrass as indicated by stable isotope analysis. *Journal of Environmental Quality* 2000;29:645-653.
- [72] Raghu S, Anderson RC, Daehler CC, Davis AS, Wiedenmann RN, Simberloff D, et al. Adding biofuels to the invasive species fire? *Science* 2006;313:1742.

Figures and Tables

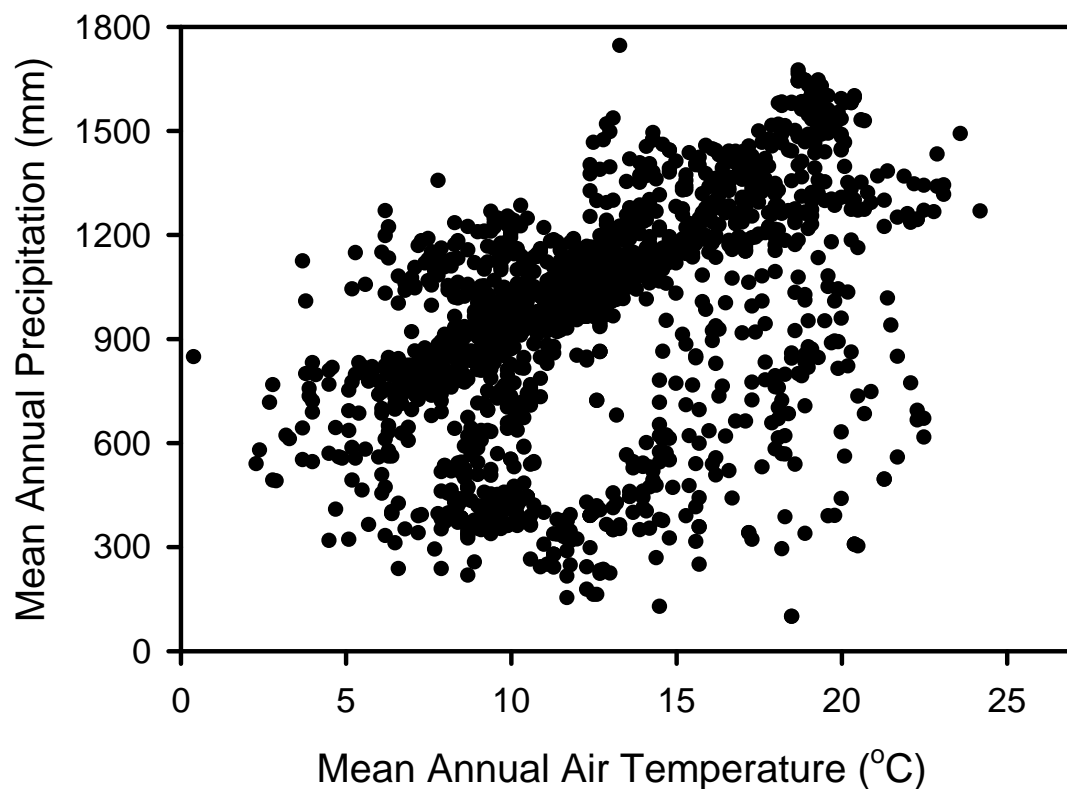


Figure 2-1 Climate distribution of switchgrass in the conterminous United States. Each data point represents a natural history collection for *Panicum virgatum* (n=1689) recorded in the Global Biodiversity Information Facility (<http://www.gbif.org/>). The climate data associated with each collection location was generated by WorldClim — Global Climate Data (<http://www.worldclim.org>).

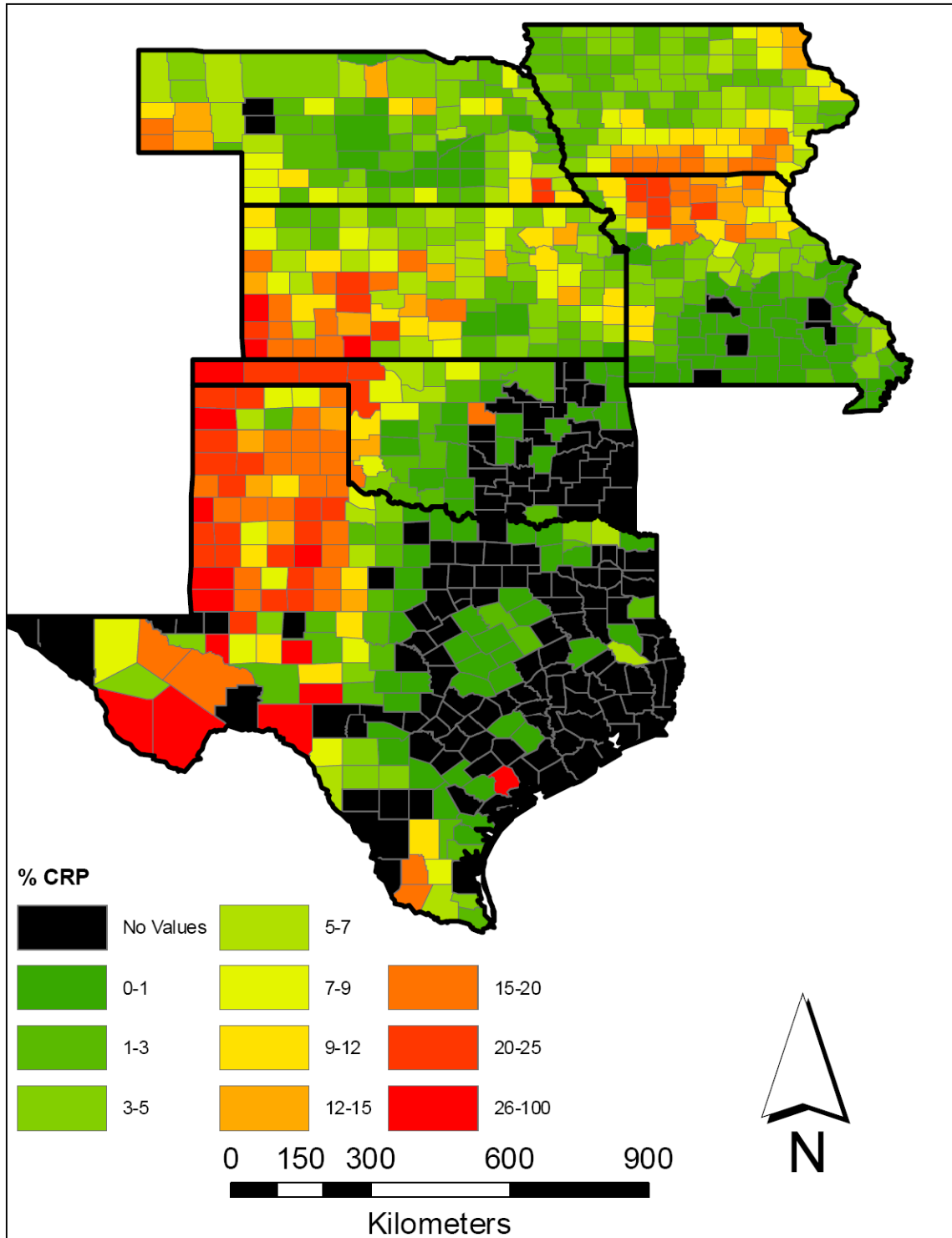


Figure 2-2 Distribution of Conservation Reserve Program (CRP) land in the Central Great Plains. Total CRP land amounted to 11.1 million acres (4.5 million ha). For each country, percent CRP lands were derived from total croplands. Data source from the Farm Service Agency (http://content.fsa.usda.gov/crpstorpt/rmepeii_r1/r1mepeii.htm).

Chapter 3 - **Ecotypic responses of switchgrass (*Panicum virgatum* L.) to altered precipitation**

Abstract

Anthropogenic climate change is projected to alter precipitation patterns, which will result in changes of water availability for plants. How dominant species within ecosystems respond to these changes can drive ecosystem responses. *Panicum virgatum* L. (switchgrass) is a common perennial C₄ (warm-season) dominant grass in the tallgrass prairie ecosystems of the Central Great Plains. We conducted an experiment in a mesocosm facility on Konza Prairie to determine the physiological and growth response of *P. virgatum* to altered precipitation regimes. Three different ecotypes of *P. virgatum* (Kansas, Oklahoma, and Texas) were subjected to three different precipitation regimes (average, -25%, +25%), based on precipitation averages for Konza Prairie. Mean maximum photosynthetic rates, stomatal conductance, transpiration, dark-adapted chlorophyll fluorescence, and water-use efficiency were lower in Kansas ecotypes. Increased precipitation treatments raised mid-day water potential and decreased water-use efficiency. Aboveground biomass responded positively to changes in precipitation (total biomass, flowering biomass, height, leaf number, etc.), while flowering times and rates were lower for Texas ecotypes, compared to the Kansas and Oklahoma ecotypes. These results indicate that precipitation and ecotype differentially affect physiological responses in this common tallgrass species. Ecotype origin was a more important driver of most physiological variables and flowering, whereas precipitation had greater control over biomass production.

Introduction

The impacts of anthropogenic climate change on terrestrial ecosystems are creating novel environments for plants. Mean annual air temperatures for the Great Plains have been projected to increase by 4°C by 2100 (Christensen et al. 2007), along with greater inter-annual and intra-annual variability in rainfall. To understand the impacts of these forecast changes on ecosystem processes, experimental manipulation of climate conditions allows for assessment of potential plant responses. Within ecosystems, dominant species have been shown to influence community structure, dynamics, invasibility, and ecosystem function (Smith & Knapp 2003; Emery & Gross 2007; Grime 1998). Dominant species generally have larger population sizes and possess larger

amounts of genetic variation than species with decreased abundance (McNaughton & Wolf 1970), which will contribute to and affect the processes of the ecosystem more than rarer species (Hillebrand et al. 2008). Populations of dominant species with large intra-specific genetic variation enables a broad range of physiological and growth responses to environmental change, and are potentially better able to respond to alterations in environmental conditions (Norberg et al. 2001, Jump & Peñuelas 2005). How dominant species, such as *Panicum virgatum*, adapt in key physiological processes to these changes can drive ecosystem responses.

Panicum virgatum L. (switchgrass) is a common perennial C₄ grass in the tallgrass prairie ecosystems of the Central Great Plains, with a broad range of adaptation to growing conditions across North America (Fig. 3-1; Parrish & Fike 2005). In the past, *P. virgatum* has been used as forage, and in the 1970's agronomic work focused on increasing its forage value and yield (Berg 1971). In the last 15 years it has been identified and studied extensively as a biofuel crop species (McLaughlin & Kszos 2005). *P. virgatum* can grow from 0.5 to 3.0 m in height with leaves that are evenly distributed throughout the plant canopy. Growth initiates late in the spring and peaks in the middle of July, with flowering starting in July and continuing until the first frost (Weaver & Fitzpatrick 1932). *P. virgatum* has been grouped into two different forms: the "upland" type, primarily from the mid and northern regions, and the "lowland" type, mainly from the southern regions of the native range. The upland type generally has a smaller size, and lower water and nitrogen requirements (Porter 1966). Lowland types are tetraploid, where upland types can be hexaploid or octoploid (Porter 1966; Casler 2005). *P. virgatum* possesses large genotypic and phenotypic variability (Casler et al. 2004; Das et al. 2004) which allows it to be broadly adapted to wide environmental and geographic range in North America (Parrish & Fike 2005).

Panicum virgatum productivity is often co-limited by nitrogen and water availability (Heaton 2004). Response to water availability varies across ecotypes, but in general, *P. virgatum* is limited more by water availability compared to other co-occurring C₄ grass species such as *Andropogon gerardii*, *Axonopus scoparius*, *Dactylis glomerata*, *Sorghastrum nutans*, and *Schizachyrium scoparium* (Knapp 1985; Knapp 1984; Stout 1992; Stout et al. 1988; Muir et al. 2001). Water availability is the dominant environmental control over individual plant tiller growth, where individuals of *P. virgatum* under water stress have been shown to have delayed inflorescence and decreased reproductive development (Sanderson & Reed 2000). Water stress has also been shown to reduce the biomass production of *P. virgatum* by up to 80% (Barney

2009). Since soil water status can affect the rate of development of *P. virgatum* (Sanderson 1992), this species can potentially be negatively affected by future climate change conditions. Predicting how species and grassland systems may respond to future climate change is often more difficult than quantifying responses to past environmental variability (Nippert et al. 2006). Precipitation variability has been shown to significantly affect physiological processes in individual plants, with impacts that translate to the ecosystem (Silletti & Knapp 2001; Knapp 2002; Fay et al. 2008; Nippert et al. 2009). To predict how ecosystems will change under climatic variability, the responses of key physiological processes in dominant plant species needs to be understood. Past studies have focused on single genotypes of species, and genetic diversity within a dominant species may have the greatest ecological impact in response to climate change (Hughes et al. 2008). Thus, in order to accurately predict the responses of ecosystems to future climate change, multiple ecotypes of a dominant species within a resource gradient should be used (Callaway et al. 2003).

We conducted an experiment to characterize the physiological responses and growth of *P. virgatum* to projected precipitation changes. By using different ecotypes growing in a gradient of soil moisture conditions, our objectives were to: (1) assess differences/similarities in key physiological traits between ecotypes across a precipitation gradient and determine plasticity of responses and (2) compare physiological responses from altered precipitation treatments to growth responses. We hypothesized: (1) physiological responses will vary between ecotypes. We predicted that ecotypes from Texas would achieve the highest biomass and CO₂ assimilation rates as precipitation increases. As precipitation decreases, the Kansas and Oklahoma ecotypes should have greater water use efficiency and higher CO₂ assimilation rates than Texas ecotypes. Texas ecotypes are generally from areas where the lowland variety is prevalent, so it should be more susceptible to water stress. (2) Varied precipitation treatments will alter the physiological responses of *P. virgatum* compared to average rainfall amounts. We predicted a general increase in biomass, lower water use efficiency, and higher CO₂ assimilation rates as precipitation increases. As water becomes less limiting, the plant is able to increase rates of assimilation and produce more biomass. (3) The physiological responses to the precipitation treatments will be reflected in plant growth responses.

Materials and Methods

Study Site & Mesocosm Facility

This research was conducted in the Rainfall Mesocosm Facility at the Konza Prairie Biological Station (KPBS). KPBS is a 3,487 ha LTER site located in northeastern KS (39.1° N, 96.9° W). This region is characterized by a mid-continental climate with cool, dry winters and warm, wet summers. Long-term annual precipitation at KPBS is 835mm (1891-2006), but 75% of the rainfall occurs during the growing season (Apr-Sept). The rainfall mesocosm facility contains 64 isolated 2.6 m³ mesocosms (Fig. 3-2). The mesocosms were constructed in 2003 using plastic-lined wood, and arranged in two, 2 x 16 arrays underneath an 11 x 25 m rainout shelter (Rainbow Plus, Stuppy Greenhouse Manufacturing Inc., North Kansas City, Missouri USA). The shelter has open walls and ends, eaves 2.4-m high to maximize air movement and heat dissipation, and a roof of clear corrugated polycarbonate (DynaGlas Plus, SPS International, San Jose, California USA) that allows > 90% light transmission. Each mesocosm ‘cell’ (1.44 m² x 1.8 m deep) contains a reconstructed soil profile from soil collected on-site. Previously, this facility was used for a precipitation variability experiment with native tallgrass prairie plant communities (Fay et al. 2008). During the summer, 2008, all relic above and belowground plant biomass was removed by hand and the top 30cm of soil was homogenized. Subsequent volunteer plants that germinated were weeded by hand, without the application of herbicide.

P. virgatum rhizomes were randomly collected in early summer, 2008, from three geographically distinct natural populations in native tallgrass prairie. These locations span a latitudinal gradient and include the Konza Prairie in north-east Kansas, the Tallgrass Prairie Preserve in north-east Oklahoma, and native tallgrass prairie in east-central Texas (USDA-ARS landholdings near Temple, TX). These locations have similar mean annual precipitation amounts, and similar environmental histories (Table 3-1). At each site, approximately 50 rhizomes of *P. virgatum* were collected from ten locations over the range of environmental conditions (different topographic positions, soil types, etc) for the site. This method of collection ensures the greatest amount of representative within-population genetic variation as possible.

The three populations were randomly assigned to the mesocosm facility, with each mesocosm cell containing a single population. Each cell was planted with rhizomes collected from each of the 10 sample locations from a single population. Rhizomes were planted with

40cm spacing, a distance to facilitate a high number of tillers per plant, but still allow competition with neighboring individuals (Sanderson & Reed 2000). During 2008, all mesocosms were watered every 3-5 days to promote establishment and minimize water stress. During spring 2009, additional individuals of *P. virgatum* from Konza Prairie and the Tallgrass Prairie Preserve were planted to replace individuals lost during the preceding winter. The mesocosms were frequently weeded over the course of the growing season to maintain *P. virgatum* species in each cell, and were watered as needed. Of the 64 cells, 21 contain individuals from Konza Prairie (KS), 16 from Tallgrass Prairie Preserve (OK), 16 from Texas, and 11 cells were empty from the removal of a previous northern ecotype.

Three different precipitation treatments were implemented based on climate change predictions for this region: contemporary average for the growing season (626.25 mm), 25% increase, and 25% decreased. Hereafter, these treatments are referred to as average, increased, and decreased. The timing of rain events was not altered, and precipitation was applied according to the average ambient rainfall interval, which is every 6 days. Rainfall was applied through a metered hand sprayer, using water from an onsite well. This water was applied at the soil surface, to minimize losses to canopy interception or runoff. Precipitation treatments were established at the end of April 2010 and continued through the first part of October 2010.

Sampling Procedure & Variables Measured

Sampling was conducted on 10 dates over the course of the growing season. For each sampling date, one individual was randomly selected from each mesocosm cell and gas exchange, dark-adapted fluorescence (F_v/F_m), and midday water potentials (Ψ_{mid}) were measured. These physiological measurements were conducted on the newest mature leaf, on the widest portion of the grass blade. For each sampling period, individuals were tagged to ensure that gas exchange, F_v/F_m , and midday water potentials (Ψ_{mid}) were conducted on the same individual-leaf, to minimize within-population variability. Since physiological measurements vary based on the time of day, measurement order for each population was randomized for each sampling period. Gas exchange measurements were conducted between 9:00-16:00 CST when solar radiation was typically above 70% of full sun levels. Soil moisture measurements were done concurrently with gas exchange and water potential measurements. Soil moisture (0-10cm) was

determined using a Hydra Probe Soil Sensor (Stevens Water Monitoring Systems, Inc) in units of water fraction by volume (wfv).

Gas exchange measurements were conducted using a LICOR 6400 IRGA with an artificial red/blue LED light source (6400-02B, LI-COR Inc., Lincoln, NE, USA). Plants were placed inside the leaf chamber and allowed to reach steady-state photosynthesis at mean ambient C_a ($400 \mu\text{mol mol}^{-1}$) and at a saturating light intensity ($2000 \mu\text{mol m}^{-2} \text{s}^{-1}$). Leaf temperature was allowed to vary with ambient daily air temperature. Relative humidity in the cuvette was maintained at ambient conditions (generally ranging from 30-50%). The variables measured included CO_2 assimilation at ambient C_a (A_{max}), stomatal conductance to water vapor (g_s), leaf-level transpiration (E), and instantaneous water use efficiency (WUE: A_{max}/E). Mid-day water potential (Ψ_{mid}) was measured concurrently with gas exchange, using a Scholander-type pressure bomb (PMS Instruments). Ψ_{mid} measurements were conducted on the same selected individual used for gas exchange measurements; however, a different leaf was used. Dark-adapted maximum photochemical efficiency (F_v/F_m) was assessed using a MINI-PAM photosynthesis yield analyzer (Heinz Walz GmbH). Measurements were conducted on the same tagged individuals within the sampling date, using the same leaf that was used for gas exchange measurements. F_v/F_m measurements were recorded during the night. Individuals were allowed to adapt for a minimum of one hour after complete darkness before any measurements were taken.

Aboveground biomass was harvested at the conclusion of the growing season (September/October). Plants were continually checked for flowering tillers, and flowering tillers were counted on seven dates. As a measure of fitness, the reproductive biomass and tiller numbers were measured. The flowering tillers from each plant were separated and weighed. The percent biomass allocated to reproduction was determined and used as a measure of fitness. Other characteristics such as flowering and non-flowering tiller height, and number of leaves per tiller were measured.

Statistical Analyses

Changes in *P. virgatum* physiological responses between ecotypes and precipitation treatments were analyzed using a mixed effects model (Proc Mixed, SAS V9.1) with the precipitation treatments and ecotypes as fixed effects and the specific mesocosm cell and date as

the random effect. Dates were treated as repeated measures and multiple comparison tests between ecotypes and treatments were done using Tukey's HSD.

Results

The precipitation treatments altered the soil moisture in this experiment. Mean soil moisture was significantly higher in the increased precipitation treatment compared to the average and decreased treatments (Fig. 3-3), and the average treatment was significantly different from the decreased treatment. Soil moisture at the beginning of the season ranged from 0.30-0.40 wfv to 0.10-0.15 wfv at the end of the season. The soil moisture for all treatments decreased over the course of the season as ambient temperature increased.

Physiology

Ecotypes and precipitation treatment responses varied significantly for many of the physiological variables (Table 3-2). A significant ecotype effect was present for carbon assimilation rates, with Kansas ecotypes showing lower mean A_{\max} compared to Oklahoma and Texas ecotypes, which did not vary significantly from each other (Fig. 3-4). Similarly, a significant ecotype effect was present for stomatal conductance, with Kansas ecotypes displaying lower mean g_s compared to the Texas and Oklahoma ecotypes (Fig. 3-4). The Texas and Oklahoma ecotypes did not differ significantly for g_s . Transpiration (E) showed an ecotype effect, similar to that of A_{\max} and g_s . Kansas ecotypes had lower transpiration rates compared to the Texas and Oklahoma ecotypes, which were not significantly different from each other (Fig. 3-4). There was significant interaction between treatments and ecotypes for water use efficiency (Table 3-2). Kansas ecotypes in the average precipitation treatment displayed significantly higher WUE compared to all three ecotypes in the increased precipitation treatment. Kansas individuals in the average precipitation treatment also had significantly higher WUE compared to the Kansas ecotypes in the decreased treatment (Fig. 3-4). All other treatment and ecotype combination were not significantly different from each other.

Mid-day water potentials (Ψ_{mid}) for all ecotypes and treatments decreased over the course of the season (Fig. 3-5). There were significant ecotype and treatment responses, but no interaction (Table 3-2). Ψ_{mid} responses to treatments showed that as soil moisture increased due to treatments, Ψ_{mid} increased significantly as well (Fig. 3-5). Differences between Ψ_{mid} responses

for ecotypes showed that Kansas ecotypes had a lower mean Ψ_{mid} of -2.1 MPa when compared to Oklahoma and Texas ecotypes which had a mean of -1.9 MPa.

Dark-adapted chlorophyll fluorescence (F_v/F_m) decreased for all ecotypes over the course of the growing season. Precipitation treatments did not significantly impact F_v/F_m , but ecotypic differences were significant (Table 3-2). Kansas ecotypes had significantly lower F_v/F_m values compared to the Oklahoma and Texas ecotypes, and Oklahoma ecotypes had significantly lower F_v/F_m values compared to Texas ecotypes (Fig. 3-6). To relate leaf biochemistry with changes in leaf gas exchange over the summer, $F_v/F_m - g_s$ response curves were compared by ecotype. As g_s values near zero, Kansas ecotypes had the lowest F_v/F_m values. Oklahoma and Texas ecotypes had similar F_v/F_m values at low g_s , but Texas ecotypes had the highest F_v/F_m values at the highest rates of g_s (Fig. 3-6).

Aboveground Biomass

Total biomass per individual was significantly different among treatments, with the highest biomass in the increased precipitation treatment (mean = 352 g/individual), and the average and decreased treatments resulting in less than 200 g/individual. The number of tillers per individual was also significantly greater for individuals in the increased precipitation treatments, while Kansas ecotypes had significantly more tillers per individual than Texas ecotypes, 69 per individual to 47 per individual respectively. However, interactions between ecotypes and treatments for tiller number and biomass were not present (Table 3-2). The exception for interactions was biomass per tiller, with Texas ecotypes in the increased treatment showing significantly higher biomass per tiller compared to all other ecotype*treatment combinations (Fig. 3-7).

Treatment and ecotype effects were significant for the percentage of biomass that was allocated to flowering culms. Individuals in the increased precipitation treatments allocated greater than 90% of their biomass into flowering tillers, where individuals in the decreased treatment allocated 77% of their biomass into flowering tillers (Fig. 3-8). Kansas and Oklahoma ecotypes allocated over 85% of their biomass into reproductive flowering tillers, while Texas ecotypes only allocated 75% of their biomass into reproductive tillers (Fig. 3-8).

Strong treatment effects were seen in flowering tiller biomass and number of leaf blades per flowering tiller (Table 3-2). Individuals in the increased treatment had a mean flowering

tiller biomass of 325 g, which was significantly greater than both the average and decreased treatments. Individuals in the increased treatment also had an average of 5.5 leaves per flowering tiller, which was significantly greater than the decreased treatment which had 4.5 leaves per flowering tiller. A significant ecotype*treatment effect was present in biomass per flowering tiller. Kansas and Texas ecotypes increased in the amount of biomass allocated to each flowering tiller as soil moisture increased. Oklahoma ecotypes did not show this trend, with the highest biomass/tiller occurring in the average treatment. Texas ecotypes had the greatest biomass per flowering tiller in the increased treatment (Fig. 3-9).

Significant ecotype effects were present in the non-flowering tiller biomass, biomass per non-flowering tiller, and leaves per non-flowering tiller (Table 3-2). Texas ecotypes had significantly greater non-flowering tiller biomass, biomass per non-flowering tiller and leaves per non-flowering tiller (4.5 leaves per non-flowering tiller to 3.1 respectively). There were no significant differences for specific leaf area (SLA) between ecotypes or treatments.

Flowering

Individuals in the increased precipitation treatment had nearly double the number of flowering tillers at the end of the growing season compared to the other precipitation treatments (Fig. 3-10). The increased precipitation treatment also flowered at a greater rate when compared to the ambient and decreased flowering rates. There were also strong ecotypic effects, with almost double the flowering tillers per individual for Kansas and Oklahoma ecotypes at the end of the growing season compared to the Texas ecotype. Both of these ecotypes flowered at a faster rate compared to the Texas ecotype, which did not begin flowering in earnest until September. Flowering tiller height was significantly greater in the increased treatment, with those individuals reaching a mean height of 129 cm.

A significant ecotype*treatment effect was seen in non-flowering tiller counts. Kansas and Texas ecotypes decreased in the amount of non-flowering tillers as the soil moisture increased, Oklahoma ecotypes displayed the opposite trend. Oklahoma ecotypes had significantly fewer non-flowering tillers in the decreased treatment compared to the other two ecotypes (Fig. 3-11). There were no significant differences for non-flowering tiller height between ecotypes or treatments.

Discussion

The overall objective of this study was to characterize the physiological responses and growth of *P. virgatum* to changes in precipitation amount. Our results showed that different precipitation treatments caused significant effects on soil moisture and corresponding plant growth and physiology. There were few interactions between ecotype and treatment, indicating that precipitation treatments and ecotype differentially affected plant responses.

Physiology

Differences in physiological responses were explained more by ecotypic variability than by the rainfalls treatments imposed. Across all precipitation treatments, the Kansas ecotypes displayed lower A_{\max} , g_s , E , F_v/F_m , and Ψ_{mid} . Kansas ecotypes did display higher WUE rates at ambient precipitation levels, suggesting adaptation to local climate conditions. Texas ecotypes did not show carbon assimilation rates higher than the other two ecotypes as hypothesized, but it was significantly higher compared to Kansas ecotype. A_{\max} , g_s , and E were tightly coupled for all ecotypes and may be similar to relationships documented previously for other dominant prairie grasses (Polley et al. 1992). We were unable to determine the ploidy of each population within ecotypes, however, lowland types are generally found in the south, and upland types found in more northern regions (Hultquist et al. 1996). The Texas and Oklahoma ecotypes may be populated with more lowland types, where the Kansas ecotype consists more of upland types. Historically it was thought that as ploidy levels increased, carbon assimilation rates increased as well (Warner et al. 1987), which would possibly explain why Kansas ecotypes had lower gas exchange rates. However, other studies have shown that carbon assimilation rates are not governed by ploidy, and differences may stem from how ecotype populations respond to stress (Wullschleger et al. 1996). Differences in physiological responses seen in this study may be attributed more to how the ecotype populations responded to water availability, than inherent differences in carbon assimilation rates based on ploidy.

The only responses affected by precipitation treatments were Ψ_{mid} and WUE, which did not support our hypothesis that gas exchange responses would decrease with lower water availability. WUE was lower in the increased treatments, and Ψ_{mid} higher in increased treatments. As water availability decreased, the photosynthetic WUE increased and Ψ_{mid} decreased to a mean of -2.2 MPa. The decreased precipitation treatment was not strong enough

to reach the critical water potential of switchgrass ($\Psi_{crit} = -3.267$; Tucker et al. 2011), and studies have shown switchgrass can maintain growth and functioning at low water potentials (Stroup et al. 2003; Knapp 1984). With more severe decreases in water availability, the WUE should increase and Ψ_{mid} should decrease if cuticular conductance is accounted for (Manzoni et al. 2011), and A_{max} , g_s , and E may then start to decline as well.

The F_v/F_m - g_s graph developed (Fig. 3-6) shows ecotypic differences in how the maximum efficiency of Photosystem II (PSII) relates to changes in leaf gas exchange. For all ecotypes, the F_v/F_m decreased as g_s decreased, but there were clear ecotypic differences. The decrease in F_v/F_m indicates a decrease in the efficiency of non-photochemical quenching (Maxwell & Johnson 2000), and is reflective of photosynthetic performance. Texas and Oklahoma ecotypes had significantly higher A_{max} compared to Kansas, and this can be seen in the F_v/F_m values at high g_s , which approach the accepted optimal value of 0.83 (Krause & Weis 1991). As g_s values decrease, Texas and Oklahoma ecotypes are able to maintain higher F_v/F_m values compared to the Kansas ecotypes, indicating a greater effect of photoinhibition on Kansas ecotypes.

Aboveground Biomass

Biomass results were affected by both precipitation treatments and ecotypic differences. Many of the biomass variables, such as biomass per individual, tillers per individual, flowering biomass per individual, and tiller height were significantly greater in the increased precipitation treatments (Table 3-2). Increased water availability allowed for greater vegetative production. This indicates that precipitation is a strong driver of how ecosystems function, as reflected in individual plant production in this study (Zhou et al. 2009; Knapp 1984; Fay et al. 2003; Fay et al. 2008).

Ecotypic differences were largely a result of the southern Texas ecotype being adapted to a longer growing season and growing in a more northern location with a shorter growing season. The change in location delayed its reproductive maturity and increased its biomass yield (Newell 1968), although the overall biomass yield was not significantly greater for Texas ecotypes in this study. Because Texas ecotypes spent a longer time in the vegetative growth stage, the % biomass allocated to reproduction was less, flowering tillers per individual were less, non-flowering tiller biomass, biomass per non-flowering tiller, and leaves per non-flowering tiller were all significantly greater for Texas ecotypes. Since the Texas ecotypes spent more time in

the vegetative stage, there were less flowering tillers which resulted in the % biomass allocated to reproductive tillers being significantly lower than Oklahoma or Kansas Ecotypes. Because Texas ecotypes had a longer period in which they vegetatively grew, they were able to take advantage of the increased precipitation treatment to produce higher biomass per tiller (Fig. 3-7), greater biomass per flowering tiller (Fig. 3-9), and high non-flowering tiller counts in all precipitation treatments (Fig. 3-11). The results of how a southern ecotype responded when grown in a more northern location are similar to what other studies have seen (Quinn 1969; Casler et al. 2004; Casler et al. 2007; Lemus et al. 2002; Berdahl et al. 2005).

Flowering

There were significant differences in flowering time and rate. Kansas and Oklahoma ecotypes flowered earlier in the season (mid-July), and at a significantly higher rate than Texas ecotypes, which initiated flowering in September (Fig. 3-10). *P. virgatum* is a highly photoperiod sensitive species (Benedict 1941), and differences in flowering dates within the species have been documented (McMillan 1965; Casler et al. 2004; Van Esbroeck et al. 2003). Sanderson & Wolf (1995) recorded very similar flowering dates for Alamo and Cave-in-Rock cultivars. Alamo cultivars flowered around the end of September and Cave-in-Rock cultivars flowered around the first week of July. The flowering time and rates seen in this study correspond with how ecotypes are known to respond to changes in latitude.

Flowering tiller heights were greatest in the increased precipitation treatments, reaching mean heights of 1.29m. These heights reflected plant heights seen in prairie remnant populations, but were lower than those found in cultivars (Das et al. 2004; Casler 2005; Alexopoulo et al. 2008). However, plant heights are variable from year to year, and in general lowland varieties have taller tillers compared to upland varieties (Alexopoulo et al. 2008). The average leaves per flowering tiller were less than what has been found on agronomic cultivars (Van Esbroeck et al. 1997), however, those have been bred to optimize yield. Tiller heights and leaves per tiller are indicative of plant aboveground biomass and correspond to the biomass results in this study.

Conclusions

The purpose of this study was to characterize the physiological responses and the growth of different ecotypes of *P. virgatum* under altered precipitation. The physiological responses of A_{\max} , g_s , E , F_v/F_m , and Ψ_{mid} in *P. virgatum* were explained by ecotypic differences. Robust responses to altered precipitation were seen in the WUE, Ψ_{mid} , and aboveground biomass variables. Ecotypic differences were also seen in several aboveground biomass variables, and most strikingly in flowering times and rates. These flowering and biomass responses can be attributed to how southern ecotypes respond when moved to a more northern region. In this study there were few interactions between ecotype and precipitation, suggesting precipitation is a strong driver of biomass production, whereas adaption of ecotypes to their local environments affects physiological processes.

Literature Cited

- Alexopoulo, E., Sharma, N., Papatheohari, Y., Christou, M., Piscioneri, I., Panoutsou, D., Pignatelli, V. 2008. Biomass yields for upland and lowland switchgrass varieties grown in the Mediterranean region. *Biomass and Bioenergy* 32:926-933.
- Barney, J.N., Mann, J.J., Kyser, G.B., Blumwald, B., Deynze, A.V., DiTomaso, J.M. 2009. Tolerance of switchgrass to extreme soil moisture stress: Ecological implications. *Plant Science* 177:724-732.
- Benedict, H.M. 1941. Effect of day length and temperature on the flowering and growth of four species of grasses. *J. Agric. Res.* 61:661–672.
- Berdahl, J.D., Frank, A.B., Krupinsky, J.M., Carr, P.M., Hanson, J.D., Johnson, H.A. 2005. Biomass yield, phenology, and survival of diverse switchgrass cultivars and experimental strains in Western North Dakota. *Agronomy Journal* 97:549–555.
- Berg, C.C. 1971. Forage yield of switchgrass (*Panicum virgatum*) in Pennsylvania. *Agronomy Journal* 63:785.
- Callaway, R.M., Pennings, S.C., Richards, C.L. 2003. Phenotypic plasticity and interactions among plants. *Ecology* 84:1115-1128.
- Casler, M.D. 2005. Ecotypic Variation among Switchgrass Populations from the Northern USA. *Crop Science* 45:388-398.
- Casler, M.D., Vogel, K.P., Taliaferro, C.M., Wynia, R.L. 2004. Latitudinal Adaptation of Switchgrass Populations. *Crop Science* 44:293–303.

- Casler, M.D., Vogel, K.P. Taliaferro, C.M., Ehlke, N.J., Berdahl, J.D., Brummer, E.C., Kallenbach, R.L., West, C.P., Mitchell, R.B. 2007. Latitudinal and longitudinal adaptation of switchgrass populations. *Crop Science* 47:2249-2260.
- Christensen, J. H., B. Hewitson, A. Busuioc, A. Chen, X. Gao, I. Held, R. Jones, R. K. Kolli, W.-T. Kwon, R. Laprise, V. Magaña Rueda, L. Mearns, C. G. Menéndez, J. Räisänen, A. Rinke, A. Sarr and P. 2007: Regional Climate Projections. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor and H. L. Miller, eds.). Cambridge University Press, Cambridge, United Kingdom and New York, USA.
- Das, M.K., Fuentes, R.G., Taliaferro, C.M. 2004 Genetic variability and trait relationships in switchgrass. *Crop Science* 44:443-448.
- Emery, S.M., Gross, K.L. 2007. Dominant species identity, not community evenness, regulates invasion in experimental grassland communities. *Ecology* 88:954-964.
- Fay, P.A., Carlisle, J.D., Knapp, A.K., Blair, J.M., Collins, S.L. 2003. Productivity responses to altered rainfall patterns in a C₄-dominated grassland. *Oecologia* 137:245–251.
- Fay, P.A., Kaufman, D.M., Nippert, J.B., Carlisle, J.D., Harper, C.W. 2008. Changes in grassland ecosystem function due to extreme rainfall events: implications for responses to climate change. *Global Change Biology* 14:1600-1608.
- Grime, J.P. 2008. Benefits of plant diversity to ecosystems: Immediate, filter and founder effects. *Journal of Ecology* 86:902-910.
- Heaton, E., Voigt, T., Long, S.P. 2004. A quantitative review comparing the yields of two candidate C₄ perennial biomass crops in relation to nitrogen, temperature, and water. *Biomass and Bioenergy* 27:21-30.

- Hillebrand, H., Bennett, D.M., Cadotte, M.W. 2008. Consequences of Dominance: A Review of Evenness Effects on Local and Regional Ecosystem Processes. 89:1510-1520.
- Hughes, A.R., Inouye, B.D., Johnson, M.T.J., Underwood, N., Vellend, M. 2008. Ecological consequences of genetic diversity. Ecology Letters 11:609-623.
- Hultquist, S.J., Vogel, K.P., Lee, D.J., Arumuganathan, K., Kaeppler, S.M. 1996. Chloroplast DNA and nuclear DNA content variations among cultivars of switchgrass, *Panicum virgatum* L. Crop Science 36:1049–1052.
- Jump, A.S., Peñuelas, J. 2005. Running to stand still: adaptation and the response of plants to rapid climate change. Ecology Letters 8:1010-1020.
- Knapp, A.K. 1984. Water relations and growth of three grasses during wet and drought years in a tallgrass prairie. Oecologia 65:35-43.
- Knapp, A.K. 1985. Effect of fire and drought on the ecophysiology of *Andropogon gerardii* and *Panicum virgatum* in a tallgrass prairie. Ecology 66:1309-1320.
- Knapp, A.K., Fay, P.A., Blair, J.M., Collins, S.L., Smith, M.D., Carlisle, J.D., Harper, C.W., Danner, B.T., Lett, M.S., McCarron, J.K. 2002. Rainfall variability, carbon cycling, and plant species diversity in mesic grassland. Science 298:2202-2205.
- Krause, G.H., Weis, E. 1991. Chlorophyll fluorescence and photosynthesis: The basics. Ann. Rev. Plant Physiol. Plant Mol. Biol. 42:313-349.
- Lemus, R., Brummer, E.C., Moore, K.J., Molstad, M.E., Burras, C.E., Barker, M.F. 2002. Biomass yield and quality of 20 switchgrass populations in southern Iowa, USA. Biomass and Bioenergy 23:433-442.

- Manzoni, S., Vico, G., Katul, G., Fay, P.A., Polley, W., Palmroth, S., Porporato, A. 2011. Optimizing stomatal conductance for maximum carbon gain under water stress: a meta-analysis across plant functional types and climates. *Functional Ecology* 25:456-467.
- Maxwell, K., Johnson, G.N. 2000. Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany* 51:659-668.
- McLaughlin, S.B., Kszos, L.N. 2005. Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. *Biomass and Bioenergy* 28:515-535.
- McMillan, C. 1965. Ecotypic differentiation within four North American prairie grasses. II. Behavioral variation within transplanted community fractions. *American Journal of Botany* 52:55-65.
- McNaughton, S.J., Wolf, L.L. 1970. Dominance and the niche in ecological systems. *Science* 167:131-139.
- Muir, J. P., Sanderson, M.A., Ocumpaugh, W.R., Jones, R.M., Reed, R.L. 2001. Biomass production of ‘Alamo’ switchgrass in response to nitrogen, phosphorus, and row spacing. *Agronomy Journal* 93:896-901.
- Newell, L.C. 1968. Effects of strain source and management practice on forage yields of two warm-season prairie grasses. *Crop Science* 8:205-210.
- Nippert, J.B., Knapp, A.K., Briggs, J.M. 2006. Intra-annual rainfall variability and grassland productivity: can the past predict the future? *Plant Ecology* 184:65-74.
- Nippert, J.B., Fay, P.A., Carlisle, J.D., Knapp, A.K., Smith, M.D. 2009. Ecophysiological responses of two dominant grasses to altered temperature and precipitation regimes. *Acta Oecologica* 35:400-408.

- Norberg, J., Swaney, D.P., Dushoff, J., Lin, J., Casagrandi, R., Levin, S.A. 2001. Phenotypic diversity and ecosystem functioning in changing environments: a theoretical framework. *Proceedings of the National Academy of Sciences, USA* 98:11376-11381.
- Parrish, D.J, Fike, J.H. 2005. The biology and agronomy of switchgrass for biofuels. *Critical Reviews in Plant Science* 24:423-459.
- Polley, H.W., Norman, J.M., Arkebauer, T.J., Walter-Shea, E.A., Greigor Jr., D.H., Bramer, B. 1992. Leaf gas exchange of *Andropogon gerardii* Vitman, *Panicum virgatum* L., and *Sorghastrum nutans* (L.) Nash in a Tallgrass Prairie. *Journal of Geophysical Research* 97:18,837-18,844.
- Porter, C.L. 1966. An Analysis of Variation Between Upland and Lowland Switchgrass, *Panicum Virgatum* L., in Central Oklahoma. *Ecology* 47:980-992.
- Quinn, J.A. 1969. Variability among High Plains populations of *Panicum virgatum*. *Bulletin of the Torrey Botanical Club* 96:20-41.
- Sanderson, M.A. 1992. Morphological development of switchgrass and kleingrass. *Agronomy Journal* 84:415-419.
- Sanderson, M.A., Reed, R.L. 2000. Switchgrass growth and development: Water, nitrogen, and plant density effects. *J. Range Manage.* 53:221-227.
- Sanderson, M.A., Wolf, D.D. 1995. Morphological development of switchgrass in diverse environments. *Agronomy Journal* 87:908-915.
- Smith, M.D., Knapp, A.K. 2003. Dominant species maintain ecosystem function with non-random species loss. *Ecology Letters* 6:509-517.

- Silletti, A.M., Knapp, A.K. 2001. Responses of the Codominant Grassland Species *Andropogon Gerardii* and *Sorghastrum Nutans* to Long-Term Manipulations of Nitrogen and Water. *American Midland Naturalist* 145:159-167.
- Stout, W.L. 1992. Water-use efficiency of grasses as affected by soil, nitrogen, and temperature. *Soil Science Society of America Journal* 56:897-902.
- Stout, W. L., G. A. Jung, and J. A. Shaffer. 1988. Effects of soil and nitrogen on water use efficiency of tall fescue and switchgrass under humid conditions. *Soil Science Society of America Journal* 52:429-434.
- Stroup, J.A., Sanderson, M.A., Muir, J.P., McFarland, M.J., Reed, R.L. 2003. Comparison of growth and performance in upland and lowland switchgrass types to water and nitrogen stress. *Bioresource Technology* 86:65-72.
- Tucker, S.S., Craine, J.M., Nippert, J.B. 2011. Physiological drought tolerance and the structuring of tallgrass prairie assemblages. *Ecosphere* 2:1-19.
- Van Esbroeck, G.A., Hussey, M.A., Sanderson, M.A. 2003. Variation between Alamo and Cave-in-Rock switchgrass in response to photoperiod extension. *Crop Science* 43:639-643.
- Van Esbroeck, G.A., Hussey, M.A., Sanderson, M.A. 1997. Leaf appearance rate and final leaf number of switchgrass cultivars. *Crop Science* 37:864-870.
- Warner, D.A., Ku, M.S.B., Edwards, G.E. 1987. Photosynthesis, leaf anatomy, and cellular constituents in the polyploidy C₄ grass *Panicum virgatum*. *Plant Physiology* 84:461-466.
- Weaver, J.E., Fitzpatrick, T.J. 1932. Ecology and Relative Importance of the Dominants of Tall-Grass Prairie. *Botanical Gazette* 93:113-150.

Wullschleger, S.D., Sanderson, M.A., McLaughlin, S.B., Biradar, D.P., Rayburn, A.L. 1996. Photosynthetic rates and ploidy levels among populations of switchgrass. *Crop Science* 2:306-312.

Zhou, X., Talley, M., Luo, Y. 2009. Biomass, litter, and soil respiration along a precipitation gradient in Southern Great Plains, USA. *Ecosystems* 12:1369–1380.

Figures and Tables

Table 3-1 General information on the sites from which each ecotype originated.

<i>Site</i>	<i>MAP (mm)</i>	<i>Temp Range (°C)</i>	<i>Latitude & Longitude</i>	<i>Area (ha)</i>
Konza Prairie	835	-2.7 — 26.6	39.10°N, 96.90°W	3,487
Tallgrass Prairie Preserve	877	-5.0 — 34.4	36.50°N, 96.25°W	15,410
Temple, TX	878	3.1 — 35.4	31.05°N, 97.34°W	178

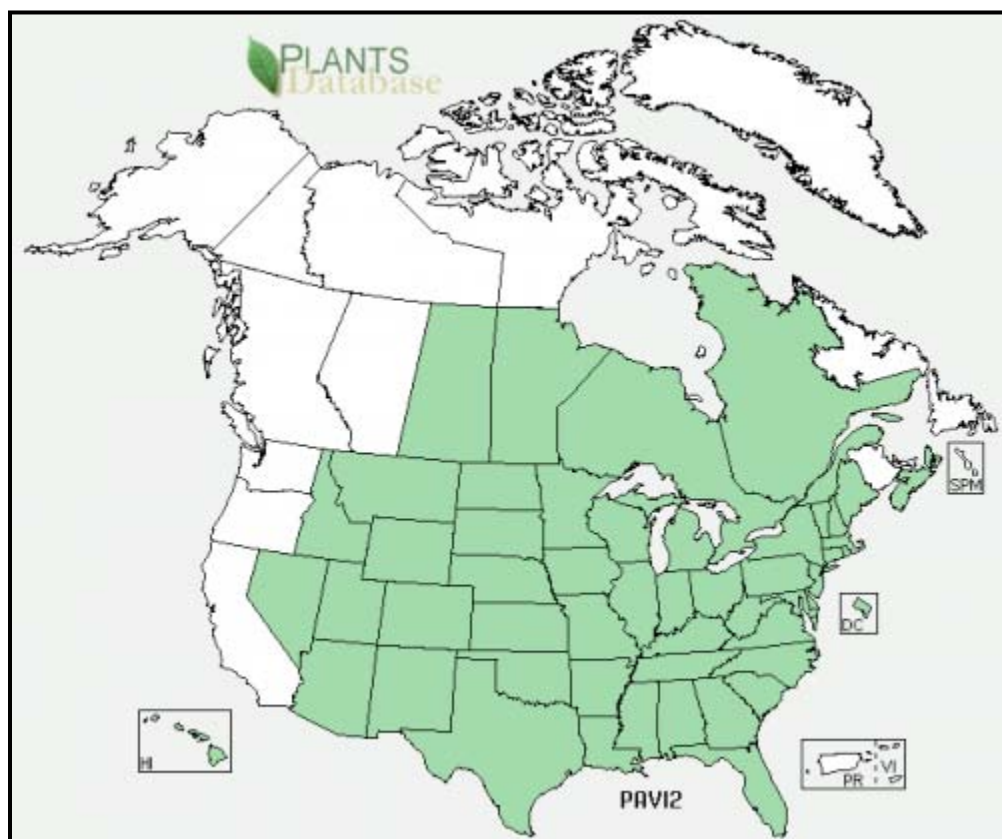


Figure 3-1 The distribution of switchgrass (*Panicum virgatum*) across North America (USDA 2009).



Figure 3-2 Rainfall Mesocosm Facility containing 64, 2.5m³ cells under an 11 x 25 m rainout shelter.

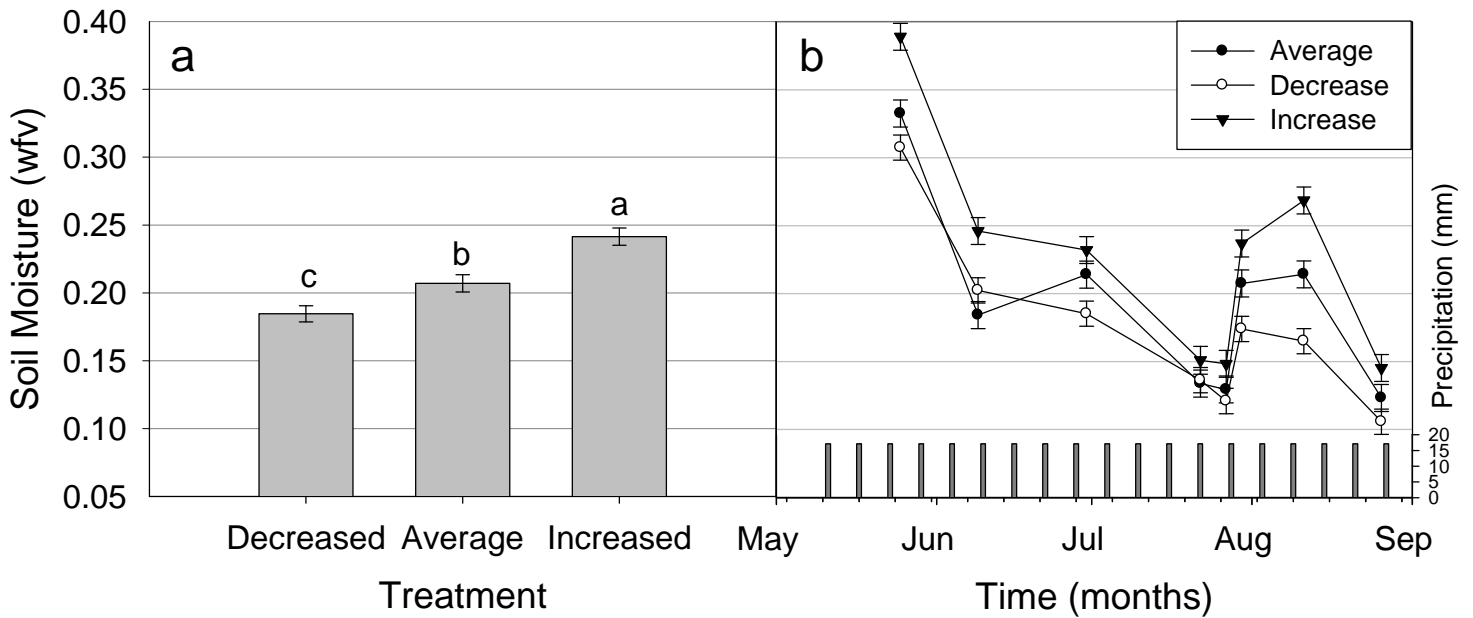


Figure 3-3 Soil moisture measurements for (a) mean soil moisture for the three treatments ($\pm 1SE$) and (b) mean soil moisture ($\pm 1SE$) for specific dates over the course of the season, separated by treatments. Bars in graph (b) display the precipitation size in mm for the

average precipitation treatment and the frequency (every 6 days). Decreased and increased treatments were 25% below and 25% above the average.

Table 3-2 ANOVA results for ecotype, treatment, and interaction effects for the variables measure. Numbers in bold show significant main effects or interaction ($p < 0.05$). Numbers in italics show marginally significant effects ($p < 0.10$).

Response	<u>Precipitation</u>		<u>Ecotype</u>		<u>Precipitation x Ecotype</u>	
	F-value	P	F-value	P	F-value	P
Soil Moisture	21.32	<0.0001	<i>2.59</i>	<i>0.0862</i>	1.74	0.1584
A_{max}	1.21	0.3088	10.65	0.0002	1.59	0.1940
g_s	0.46	0.6339	8.59	0.0007	0.87	0.4885
WUE	6.27	0.0038	0.16	0.8535	3.37	0.0165
E	1.38	0.2627	7.83	0.0012	1.12	0.3572
F_v/F_m	1.34	0.2713	7.91	0.0012	0.16	0.9565
Ψ_{mid}	10.10	0.0003	4.95	0.0116	1.81	0.1449
SLA	2.33	0.1093	0.12	0.8894	1.80	0.1450
Tillers/individ.	3.71	0.0326	3.38	0.0429	1.12	0.3584
Biomass/individ.	9.48	0.0004	2.19	0.1236	1.53	0.2104
Biomass/Tiller	4.49	0.0168	11.09	0.0001	4.31	0.0050
% allocated to reproduction	4.83	0.0127	5.99	0.0050	1.91	0.1256
Flowering Tillers/individ.	7.54	0.0015	8.48	0.0008	0.57	0.6846
Flowering Biomass/individ.	11.46	<0.0001	0.83	0.4411	1.57	0.1978
Biomass/Flowering Tiller	<i>2.86</i>	<i>0.0681</i>	13.33	<0.0001	3.51	0.0142
Flowering Tiller Height	5.12	0.0100	1.85	0.1696	1.66	0.1761
Leaves/Flowering Tiller	5.47	0.0075	2.25	0.1174	1.13	0.3555
Non-Flowering Tiller Count	3.25	0.0481	<i>2.91</i>	<i>0.0651</i>	2.87	0.0337
Non-Flowering Tiller Biomass	1.17	0.3203	4.84	0.0126	0.58	0.6795
Biomass/Non-Flowering Tiller	0.07	0.9322	15.85	<0.0001	1.73	0.1596
Non-Flowering Tiller Height	0.98	0.3824	3.05	0.0576	1.12	0.3614
Leaves/Non-Flowering Tiller	0.22	0.8069	6.70	0.0029	1.86	0.1344

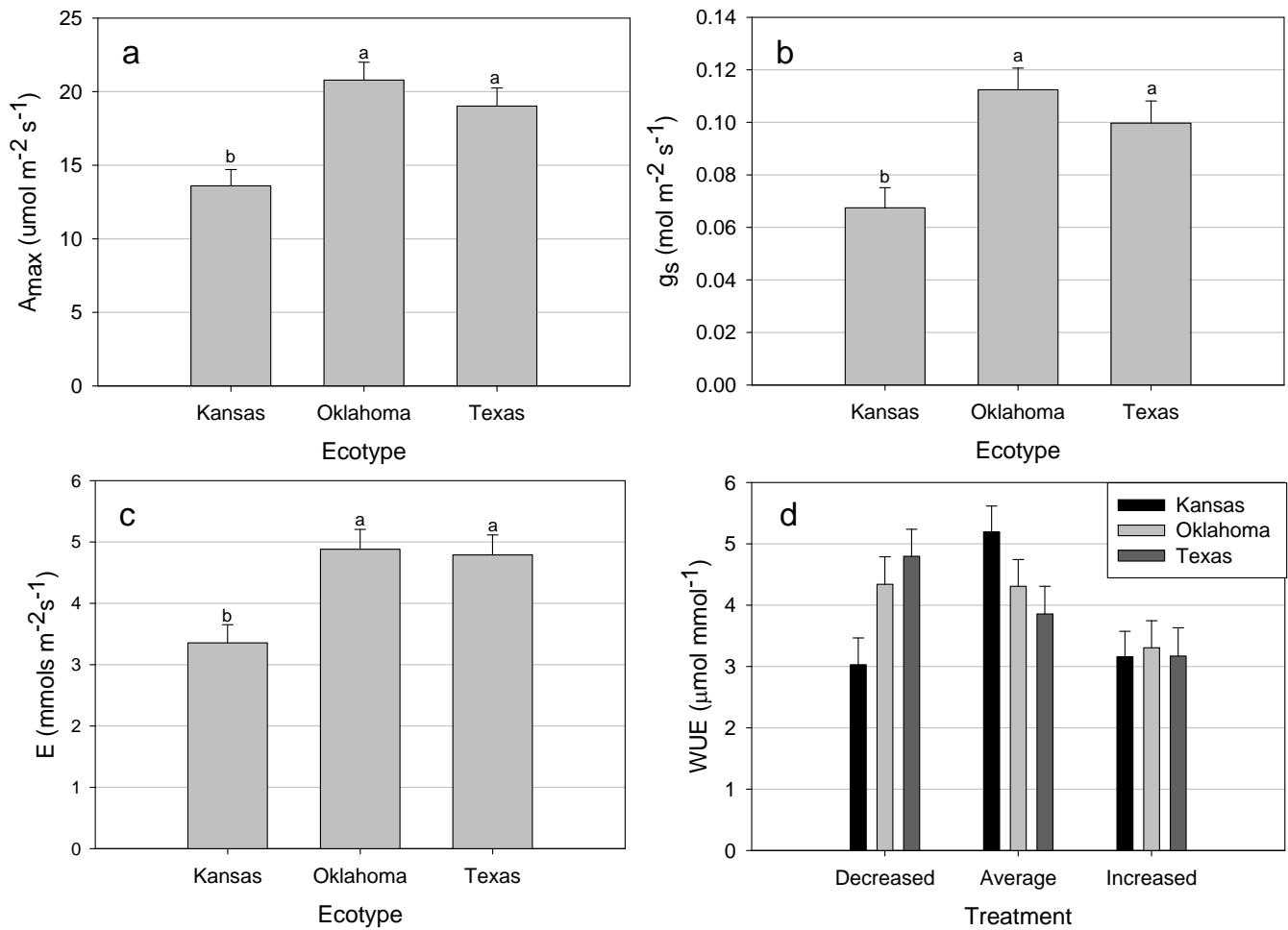


Figure 3-4 Mean gas exchange responses (± 1 SE) for ecotype and precipitation treatments. (a) Carbon assimilation rates between ecotypes (b) Stomatal conductance between ecotypes (c) Transpiration rates between ecotypes (d) WUE between ecotypes and precipitation treatments.

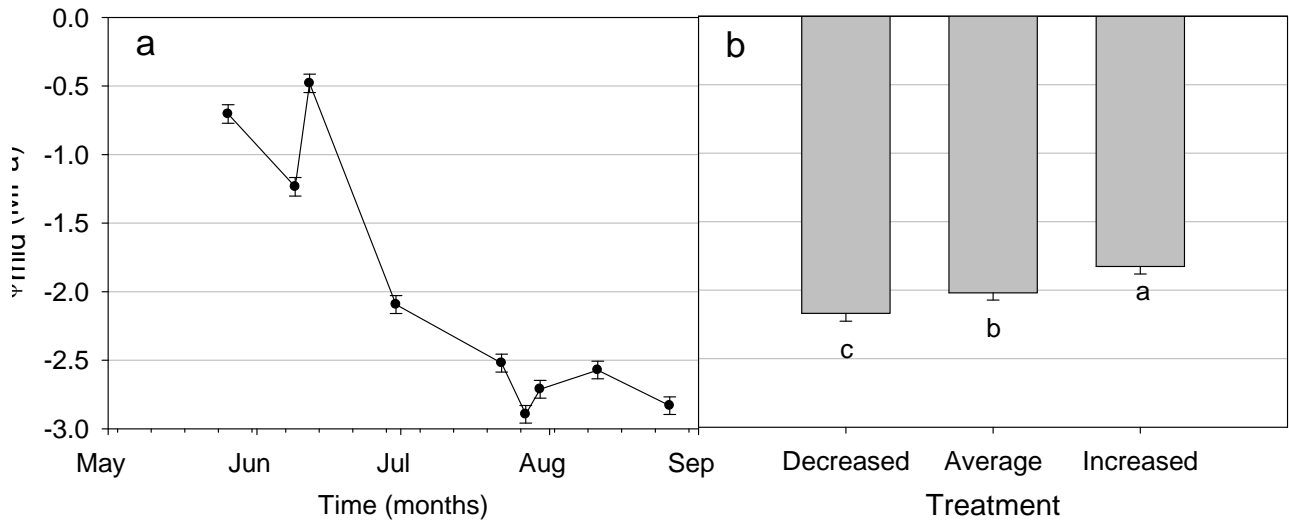


Figure 3-5 (a) Mean Ψ_{mid} responses over the course of the season. (b) Mean Ψ_{mid} ($\pm 1SE$) responses for all ecotypes in each treatment.

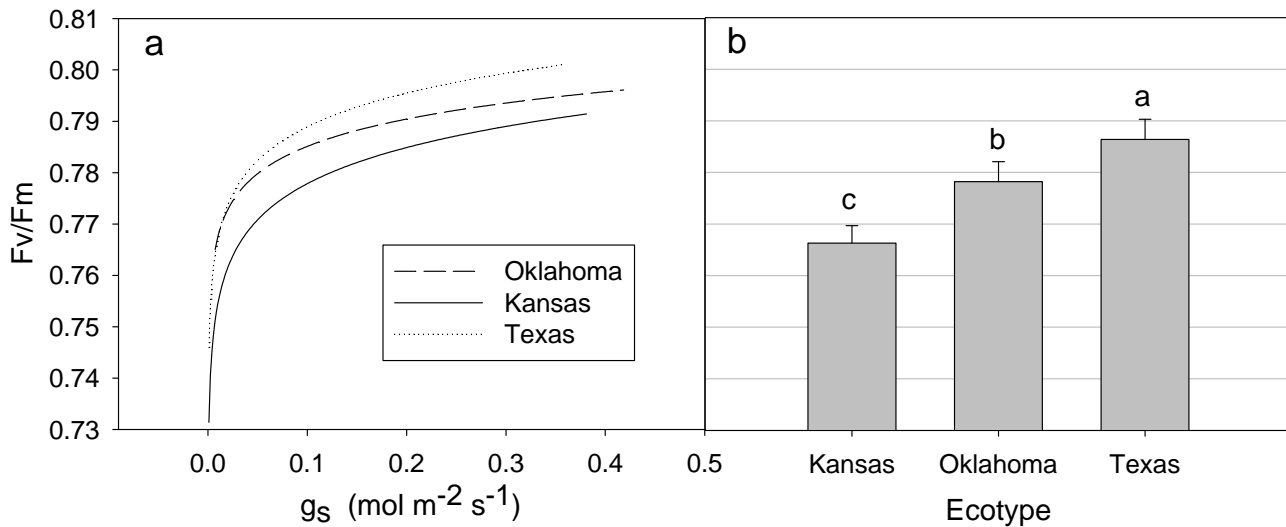


Figure 3-6 (a) Representative F_v/F_m - g_s curves for all ecotypes. Curves were fit for all the data points for the treatment or ecotype. (b) Mean F_v/F_m differences between ecotypes ($\pm 1SE$).

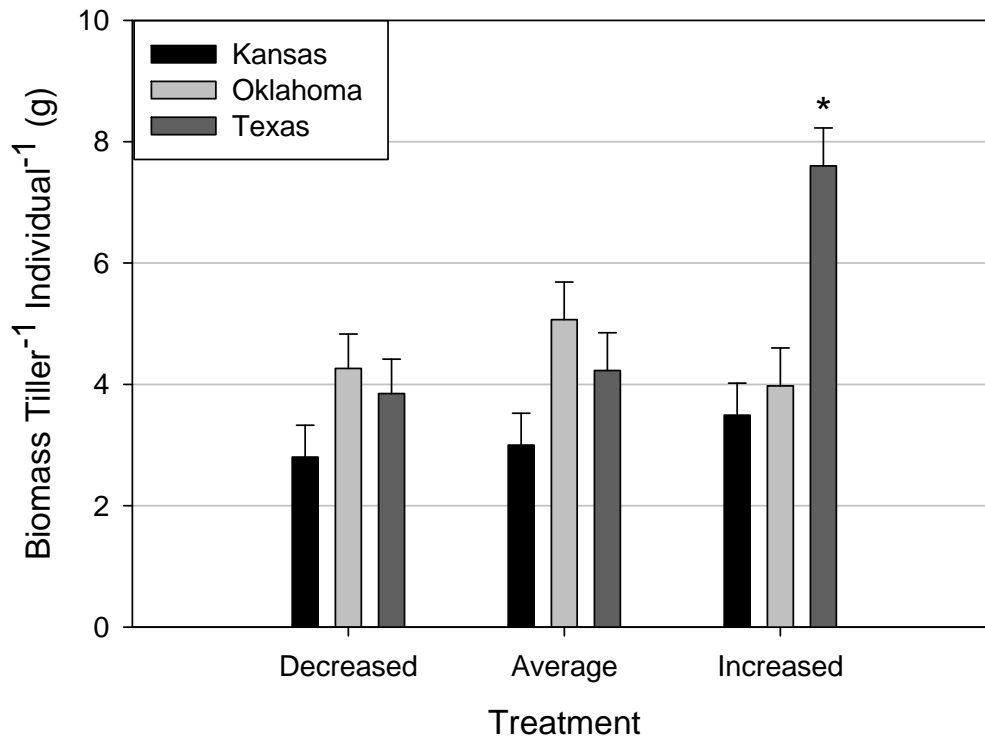


Figure 3-7 Mean biomass per tiller ($\pm 1SE$) for all ecotype and treatment combinations.

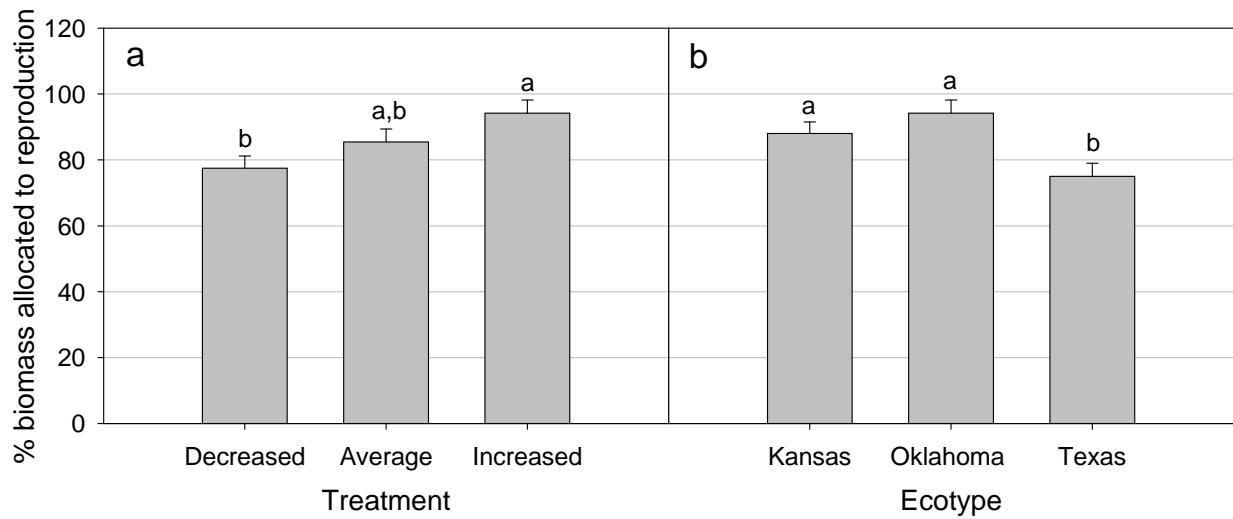


Figure 3-8 Mean percent biomass ($\pm 1SE$) allocated to flowering tillers. (a) % biomass allocation by treatment (b) % biomass allocation by ecotype.

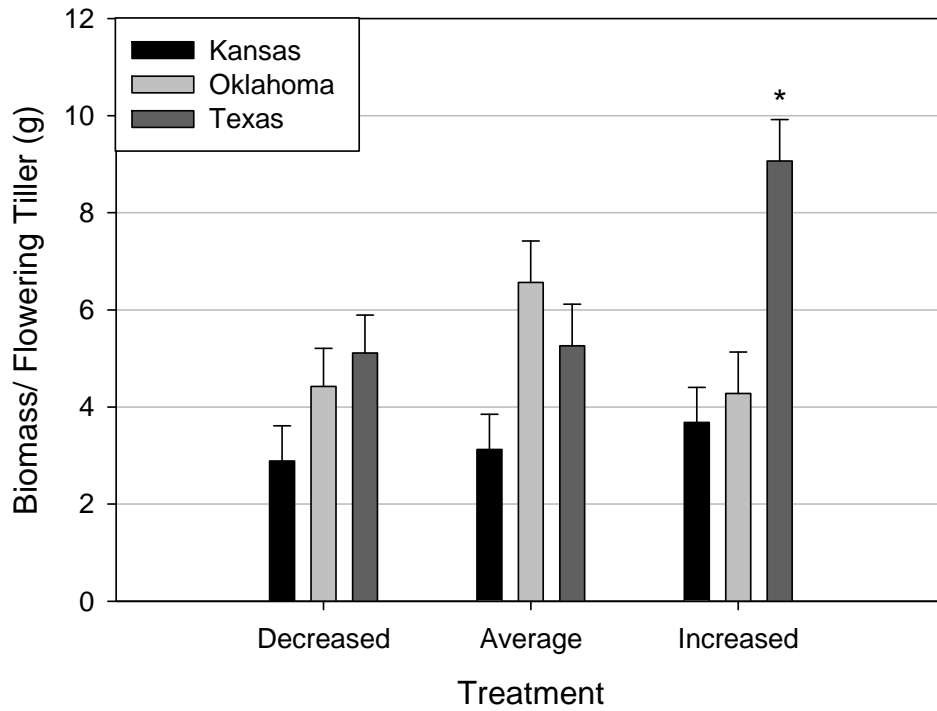


Figure 3-9 Mean biomass ($\pm 1SE$) per flowering tiller for all ecotype and treatment combinations.

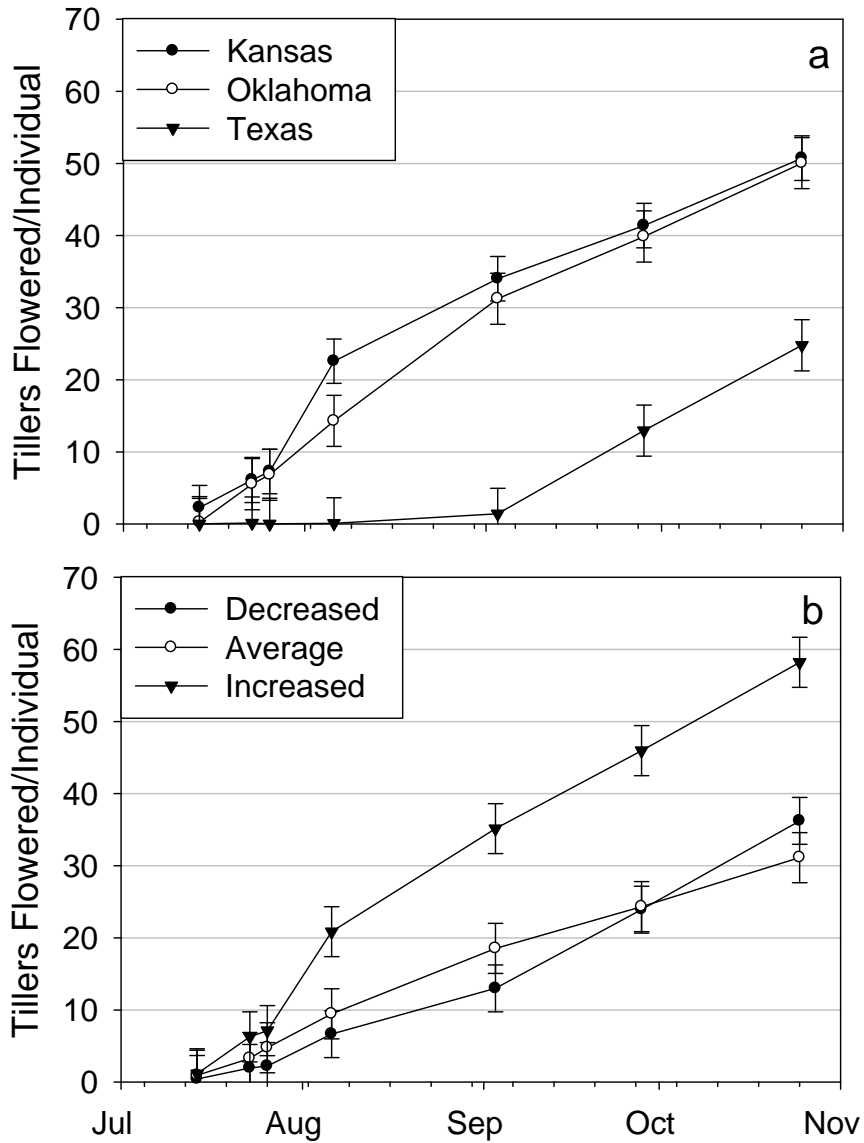


Figure 3-10 Total tillers flowered per individual over the course of the growing season. (a) Flowering tillers by ecotype (b) flowering tillers by precipitation treatment. Data points are means ($\pm 1SE$).

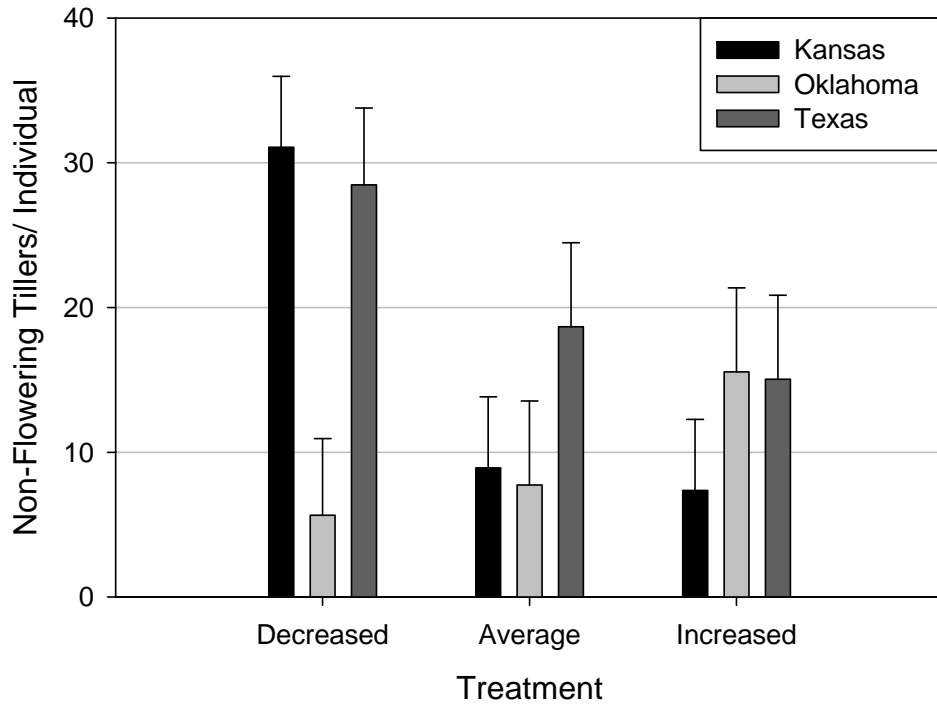


Figure 3-11 Mean number ($\pm 1SE$) of non-flowering tillers per individuals for all ecotype and treatment combinations.

Chapter 4 - Responses of switchgrass (*Panicum virgatum* L.) to passive nighttime warming

Abstract

Climate change projections predict mean annual temperature increases of 1.5-5.5°C by 2100. Daily minimum temperatures are projected to increase at a faster rate than daily maximum temperatures. Ecosystem responses to altered environments will be driven by dominant species, such as *P. virgatum* in the tallgrass prairie. *P. virgatum* is a common perennial C₄ warm-season dominant grass. We used passive nighttime temperature manipulation to assess the physiological and growth responses of *P. virgatum* to increased nighttime temperature in both upland and lowland sites. Nocturnal warming decreased daytime stomatal conductance and transpiration, increased specific leaf area, and delayed flowering in *P. virgatum*. Topographically, aboveground biomass, tiller density, and tiller weight were greater in lowland sites compared to upland sites. Biomass production responded more to topographic differences, with the main differences between upland and lowland sites being soil water status. These results indicate that while water availability is a strong driver of plant biomass production, *P. virgatum* is responsive to changes in nighttime temperature. Under greater nocturnal warming, the interaction between warming and water availability at topographic sites may create altered environments that will affect the population and community structure.

Introduction

The effects of anthropogenic climate change are creating novel environments for plants. Mean annual air temperatures for the Great Plains are projected to increase by 1.5-5.5°C by 2100 (Christensen et al. 2007; Houghton et al. 2001). Adaptation to these changes in physiological processes can drive ecosystem responses. Within ecosystems, dominant species can influence the community structure, dynamics, invasibility, and ecosystem function (Smith & Knapp 2003; Emery & Gross 2007; Grime 1998). Some dominant species change based on temperature changes, while others change based on water availability (Sherry et al. 2008; Nippert et al. 2009). Future community structure will be based on the particular dominant plant species that change, and the specific variables and degree of change (de Valpine & Harte 2001; Nippert et al. 2009). As a dominant species within the tallgrass prairie of the Central Great Plains, how

Panicum virgatum adapts in key physiological processes to these changes can drive ecosystem responses.

P. virgatum L. (switchgrass) is a common perennial warm-season C₄ grass that is native to the tallgrass prairie ecosystems in the Central Great Plains, and is adapted to a broad range of growing conditions across North America (Parrish & Fike 2005). Historically, *P. virgatum* has been used as forage, but in the last 20 years it has been identified as a potential biofuel species and studied extensively (McLaughlin & Kszos 2005, Wright et al. 2010). *P. virgatum* has a tall, erect form with leaves distributed evenly throughout the canopy, and rooting depths of up to 3 m (Porter 1966). Flowering starts in July and occurs until the first frost. Seed germination is very low, and propagation is mainly through rhizomes (Weaver & Fitzpatrick 1932, Benson & Hartnett 2006). *P. virgatum* has been grouped into two broad forms, usually based on their topographic position on the landscape (Porter 1966). The “upland” type is usually associated with higher sites topographically and from the more northern regions of its native range. The upland type is generally smaller in size, has lower water and nitrogen requirements, and found in more mesic sites. The “lowland” type is usually associated with more hydric sites and is larger than the upland types. All lowland types have been found to be tetraploid, where upland types can be hexaploid or octoploid. *P. virgatum* exhibits large genetic variability and diversity in form (Casler et al. 2004; Das et al. 2004). These traits allow it to be able to adapt to a wide range of environmental conditions and geographic ranges in North America (Parrish & Fike 2005).

Climate model projections and long term data sets have shown that the daily minimum temperatures are increasing at a greater rate than the daily maximum temperatures (Karl et al. 1991; Alward et al. 1999). Nocturnal warming exacerbates the adverse effects of soil water stress and changes plant physiological status and plant productivity (Wan et al. 2005; Zhou et al. 2007; Sherry et al. 2008), which has a direct effect on community structures (^aXu & Zhou 2005; Cross & Harte 2007). It has also been shown in other perennial grasses that nocturnal warming may weaken acclimation during water stress by altering carbon allocation between source and sink organs (^bXu & Zhou 2005). In temperature limited areas, growth may increase through increases in the growing season length. However, the magnitude of this response to warming is dependent on site (Peñuelas et al. 2004). In the case of *P. virgatum*, the photosynthetic rates remain constant across a range of temperatures under 40 °C (Knapp 1985).

While the local responses of *P. virgatum* to resource limitation have been examined for many natural populations, to our knowledge no one has examined the response of native populations of *P. virgatum* to simulated climate changes expected for the central Great Plains. In addition, much of the work done on *P. virgatum* has been performed using agronomic cultivars developed in breeding programs, with considerably less research on natural populations. Responses of these cultivars of *P. virgatum* to climate change and their agronomic impacts have been studied extensively (Hartman et al. 2011), but more work is needed to understand responses of natural *P. virgatum* populations to simulated climate change environments.

We conducted an experiment to characterize the physiological responses and growth of *P. virgatum* to increases in night-time temperature. Our objectives were to assess similarities/differences in key physiological traits between individuals in upland and lowland sites under increased night-time temperatures to determine plasticity of responses, and to compare the physiological responses to increased night-time temperatures with growth responses. We hypothesized that physiological responses would vary between individuals of *P. virgatum* in upland sites compared to lowland sites. Upland sites contain shallower soils, which translates to decreased water availability. Individuals in upland sites should have greater WUE compare to lowland sites. However, individuals in lowland sites should have great carbon assimilation rates and produce greater aboveground biomass. We also hypothesized that increased night-time temperature in the canopy would alter physiological responses of *P. virgatum*. Plants under experimental warming should exhibit reduced photosynthesis through decreases in gas exchange. Finally, we hypothesized that physiological response to increased night-time temperature would be reflected in plant growth responses.

Materials and Methods

Study site/Louvered OSC's

This research was conducted at the Konza Prairie Biological Station (KPBS). KPBS is a 3,487 ha LTER site located in northeastern KS (39.1° N, 96.9° W). The region is characterized by a mid-continental climate with cool, dry winters and warm, wet summers. Long-term annual precipitation at KPBS is 835mm, with 75% of the rainfall occurring during the growing season (Apr-Sept).

This experiment was performed in native prairie using both upland and lowland varieties of *P. virgatum*. Temperature manipulation was achieved through novel, louvered open sided chambers (Louvered OSC's; Fig. 4-1) developed by Matt Germino, Idaho State University (personal communication). The Louvered OSC's are 1m x 1m in area and contain 10.15cm slats situated at 45° angles. Slats were cut from OPTIX 36in x 48in x 0.093in Acrylic sheets (Home Depot), angled in opposite directions and meeting in the middle. The frames were adjustable to ensure the top of the frame stayed above the herbaceous canopy over the season. The Louvered OSC's allow solar energy to be accumulated under the frame during the day. At night, when the soil reradiates long-wave IR radiation, a fraction of the energy is trapped by the Louvered OSC, increasing the nighttime temperature of the grass canopy by roughly 2°C. These louvered OSCs are preferred here because they perform best in the absence of a tree canopy and under clear sky. The environment of Konza makes these louvered OSCs ideal forms of temperature manipulation.

Ten sites were chosen on Konza Prairie, across two annually burned, ungrazed watersheds. Watershed K1B had two upland, and two lowland sites, while watershed 1D had three upland and three lowland sites. Within each site, two 1m x 1m plots were staked out, with the framed and control plots randomized. Twenty plots in total were chosen, with five control plots in upland and lowland positions each, and five treatment plots in upland and lowland positions, each with a Louvered OSC's situated on top.

Sampling Procedure & Variables Measured

Temperatures for the herbaceous canopy and soil were measured using an IR thermometer (Cole-Parmer Instruments, Co.) twice during the growing season in July and August. Measurements were conducted on bare soil and the top of the herbaceous canopy within each plot. Temperatures were also recorded using DS1922L Thermocron iButtons (Embedded Data Systems, LLC) with a temperature range of -40°C to 85°C (± 0.5 °C). The iButtons were placed just above the soil surface, and recorded temperatures every ten minutes. Temperatures were measured over the growing season, from the middle of May 2010, through the middle of October 2010. Temperatures were analyzed using the mean temperature at 3:00 and 15:00 CST. These temperatures were used to assess topographic differences during peak daytime and nighttime temperatures. The iButtons were not sensitive to the temperature differences imparted

by the treatment from the Louvered OSC's, and therefore IR temperatures were used for this comparison.

Sampling was conducted over six dates over the course of the growing season. For each sampling date, one individual was randomly selected from each plot and gas exchange, dark-adapted fluorescence (F_v/F_m), and midday water potentials (Ψ_{mid}) were measured. These physiological measurements were conducted on the newest mature leaf, on the widest portion of the grass blade. For each sampling period, individuals were tagged to ensure that gas exchange and F_v/F_m , and were conducted on the same individual leaf, and midday water potentials (Ψ_{mid}) were conducted on the same individuals, using different leaf blades. This tagging helped to minimize within-population variability. Since physiological measurements vary based on the time of day, measurement order for each plot was randomized for each sampling period. Gas exchange measurements were conducted between 9:00-16:00 CST when solar radiation was typically above 70% of full sun levels. Gas exchange measurements were also conducted during the night on two separate occasions to assess the treatment effect on nighttime respiration (R_d), transpiration (E), stomatal conductance (g_s), and water use efficiency (WUE).

Gas exchange measurements were conducted using a LICOR 6400 IRGA with an artificial red/blue LED light source (6400-02B, LI-COR Inc., Lincoln, NE, USA). Plants were placed inside the leaf chamber and allowed to reach steady-state photosynthesis at ambient C_a ($400 \mu\text{mol mol}^{-1}$) and at a saturating light intensity ($2000 \mu\text{mol m}^{-2} \text{s}^{-1}$). Leaf temperature was allowed to vary with ambient daily air temperature. Relative humidity in the cuvette was maintained at ambient conditions (generally ranging from 30-50%). The variables measured included CO_2 assimilation at ambient C_a (A_{max}), stomatal conductance to water vapor (g_s), leaf-level transpiration (E), and instantaneous water use efficiency (WUE: A_{max}/E). Mid-day water potential (Ψ_{mid}) was measured concurrently with gas exchange and was done using a Scholander-type pressure bomb (PMS Instruments). Dark-adapted maximum photochemical efficiency (F_v/F_m) was conducted using a MINI-PAM photosynthesis yield analyzer (Heinz Walz GmbH). F_v/F_m measurements were recorded after individuals were allowed to adapt for a minimum of one hour after complete darkness.

Soil moisture measurements were performed concurrently with gas exchange and water potential measurements. Soil moisture (0-10cm) was determined using a Hydra Probe Soil

Sensor (Stevens Water Monitoring Systems, Inc) in units of water fraction by volume (wfv). Soil moisture was measured ten times over the summer, from May through September.

Above ground biomass was harvested at the conclusion of the growing season (September/October). A 20cm x 50cm clipping frame was used to clip two replicates within each plot. Plants were checked for flowering tillers on six dates, starting in the middle of the season. As a measure of fitness, the reproductive tiller numbers were measured. Specific leaf area (SLA) was measured at the end of the season.

Statistical Analyses

Changes in *P. virgatum* physiological responses to increased nighttime temperature were analyzed using a mixed effects model (Proc Mixed, SAS V9.1) with the temperature treatments and topographic position as fixed effects and the specific sites and date as the random effect. Multiple comparison tests between responses were done using Tukey's HSD.

Results

Soil moisture did not differ significantly ($p < 0.05$) between the control and the warming treatment. However, there were significant differences in soil moisture between the upland and the lowland sites (Table 4-1). Soil moisture increased during the first part of the growing season, and then declined from July on, with upland sites showing significantly lower soil moisture compared to lowland soils (Fig. 4-2).

Temperature

Although the target temperature of 2°C of warming was not achieved, the measurements using the IR thermometer showed a significant increase of 1°C during the night in both the canopy and on the soil surface (Fig. 4-3). Nighttime temperatures were raised to above 23°C in the canopy and above 24°C on the soil surface under the Louvered OSC's. There were no topographic differences in nighttime canopy temperature.

Air temperature measured continuously showed significant differences in daytime temperatures 1-2 cm above the soil surface between the upland and the lowland sites (Fig. 4-4). The upland sites had a significantly higher ($p < 0.0001$) mean peak daytime temperature of 31.2 °C compared to 29.6 °C at lowland sites. Topographic differences in temperature directly above the soil surface disappeared during the night.

Physiological Responses

Leaf level water potential (Ψ_{mid}) decreased over the course of the summer and there were no overall significant treatment or topographic differences (Table 4-1). However, there were topographic differences on specific dates, with upland sites showing higher Ψ_{mid} early in the summer and then lower Ψ_{w} later in the summer (Fig. 4-5). Individuals in both upland and lowland sites started out with Ψ_{w} between -0.5 to -1.0 MPa, and ended the season with Ψ_{mid} between -2.5 and -3.0 MPa.

Most daytime gas exchange measurements did not vary between treatments or topographic position (Table 4-1). Stomatal conductance and transpiration were the only variables that displayed a significant ($p < 0.05$) treatment effect. Over the course of the season, g_s decreased (Fig. 4-6) and was significantly lower in the warming treatments (Fig. 7). E also declined over the growing season (Fig. 4-6) and was significantly lower in the warming treatment as well (Fig. 4-7). A_{max} and WUE did not show any significant responses between treatments or topographic position, but like g_s and E , responses declined steadily over the growing season (Fig. 4-6). There were no significant differences for Fv/Fm responses (Table 4-1).

No significant differences were found in nighttime gas exchange measurements (Table 4-1). Nighttime transpiration values for all topography*treatment combinations had a mean of $1.6057 \text{ mmol m}^{-2}\text{s}^{-1}$ on June 15th, which was 21.9% of the daytime values. Nighttime transpiration decreased to a mean of $0.3381 \text{ mmol m}^{-2}\text{s}^{-1}$ on August 8th, which was 8.9% of the daytime values.

Aboveground biomass & flowering

Significant differences ($p < 0.05$) were present in the aboveground biomass, density of tillers, and tiller weight between the upland and the lowland sites (Table 4-1). Upland plots had a mean aboveground biomass of 268 g/m^2 , while lowland plots had more than double that of upland plots, with a mean biomass of 567 g/m^2 (Fig. 4-8). Lowland plots also had significantly more tillers per square meter than the upland plots, and significantly higher weight per tiller compared to the upland plots (Fig. 4-8).

There were few warming treatment effects, but specific leaf area (SLA) was significantly greater in the warming plots (Table 4-1; Fig. 4-9). There were also marginally significant

differences (Table 4-1) in number of tillers per square meter between the control and warming plots (Fig. 4-9), with control plots containing a higher density of tillers compared to plots with the warming treatment.

At the end of the season, the lowland plots contained marginally significantly more flowering culms per square meter than the upland plots (Table 4-1; Fig. 4-8). The lowland plots had a mean of 154 flowering culms/m², almost double that of upland plots, which had a mean of 78 flowering culms/m². Topography and treatments had significant effects on flowering times, and there was a significant interaction between them (Table 4-1). The lowland plots flowered earlier than upland plots, with 100% flowering before any other treatment*topography combination. Increased night-time temperature had a significant effect on the flowering of upland sites. The upland*Louvered OSC combination was the only one that did not have flowering occur in 100% of the plots at the end of the growing season (Fig. 4-10).

Discussion

Nighttime warming using the Louvered OSC's did not create the desired 2 °C increase of temperature in the grass canopy. However, there were still significant differences in flowering phenology and SLA between warming and control treatments. Increased nighttime temperature and topography played a significant role in the percentage of plots that flowered for *P. virgatum* individuals in the upland warming treatments. Flowering time was delayed in upland plots with warming treatments, and at the end of the season, was the only group not to have 100% of the plots flower (Fig. 4-10). These results are contrary to those found by Hovenden and others (2008) in Tasmania, Australia. They found that increases in nighttime temperature of 2 °C had a significant accelerating effect on the flowering of grasses, but was highly variable between years. They do state that southern grasslands function differently from northern grasslands, and warming causes many of the early flowering species in these grasslands to flower earlier. Other studies done in northern grasslands have shown that species that flower after peak summer temperatures delay flowering even more in response to warming, and species that flower before peak summer temperatures accelerate flowering in response to warming (Cleland et al. 2006). Sherry et al (2007) showed how a 4 °C air temperature increase accelerated the flowering of *P. virgatum* by 17 days. This work is contrary to the results found in this study. The degree of warming achieved in the study by Sherry et al (2007) was much higher than the warming

achieved in this study, so the responses in this study may be due more to intra annual variability. The lengthening of the reproductive duration may have increased, as *P. virgatum* individuals in the upland warming treatments were still flowering at the time of biomass collection. This evidence is supported by Sherry et al (2007), and could possibly lead to further drought stress under warming conditions.

Nighttime respiration and daytime carbon assimilation were not significantly different between control and warming treatments. Other studies have shown that plants subjected to nocturnal warming display increased respiration during the night, and subsequently increased photosynthesis during the day to account for the loss of carbohydrates during the night (Zhou et al. 2007; Wan et al. 2009; Niu et al. 2008). Respiration in plants under future climate change scenarios (+3.5°C) has been projected to increase by 30%, which will affect how well ecosystems can sequester carbon (Griffin et al. 2002). Along the same lines, soil respiration has been projected to increase as well under warming conditions, which will weaken the ability of ecosystems to take up and store carbon (Saleska et al. 1999; Wan et al. 2005). The lack of differences between the nighttime respiration and daytime carbon assimilation may be due to the weak treatment effect achieved or simply from high variability within the season (Zhou et al. 2007).

The only gas exchange measurements that were significant were g_s and E between the treatments (Fig. 4-7). Individuals in the warming treatment had lower g_s and E. The decrease in g_s has been documented by Xu et al. (2009), where they found g_s decreased with nocturnal warming, and was further decreased by severe and extreme water stress. The decrease in g_s from nocturnal warming can then explain the decrease in E. As the proportion of stomates that are open decrease, the amount of water transpired decreases as well. The nighttime E values ranged from 21.9% of daytime values to 8.9%. These ratios are within normal ranges found in other studies, which have ranged from 5% to as much as 30% (Snyder et al. 2003; Caird et al. 2007).

High nighttime temperatures could exacerbate plant stress from water deficits (Xu et al. 2009). There were significant differences in water availability between the upland and the lowland sites (Fig. 4-2). Upland sites had significantly lower soil moisture, and with high nighttime temperatures projected to increase stress from water deficits, individuals in these sites may be subjected to greater stresses than ones in lowland sites. Sherry et al (2008) showed that even in plots with increased precipitation, the warming effect dried out the soil and decreased

soil water availability. Precipitation predictions for the Great Plains indicate a lower frequency of rain events and greater amounts of rainfall per event (Christensen et al. 2007). Combined with warming, these conditions may lead to increased plant stress, especially in upland sites where soil water availability is lower.

Many of the differences seen in this study were between topographic positions (Table 4-1). The number of tillers/m², aboveground biomass, and tiller weight were all significantly greater for *P. virgatum* individuals in the lowland sites (Fig. 4-8). The genotype of the *P. virgatum* individuals in the upland and lowland sites is not known, however, lowland types tend to grow in the hydric sites, and the upland types in the more mesic sites. Lowland *P. virgatum* types have been shown to produce greater biomass than upland types (Lemus et al. 2002; Alexopoulou et al. 2008; Wullschleger et al. 2010), and more tillers/m² (Madakadze et al. 1998). The lowland sites have greater soil water availability, so those individuals are able to produce greater biomass. The difference in soil water availability is an important driver in biomass production, tiller density, and tiller weight (Zhou et al. 2009; Knapp 1984; Fay et al. 2003; Fay et al. 2008). One of the main treatment effects besides phenology was on plant SLA. The increase in SLA for *P. virgatum* individuals in the warming treatment may be due to a slight shading effect. The Louvered OSC's allow 90% transmission of light. The increase in SLA, or increase in area per weight may be a way for the plant to compensate for the reduced amount of light that is reaching the canopy. SLA was determined at the end of the season before the plants started to senesce. The SLA values in this study were much lower than values reported on cultivar SLA (Trócsányi et al. 2009). This may be a reflection on how cultivars are bred to maximize biomass production, and result in high SLA values.

Conclusion

The purpose of this study was to characterize the physiological and growth responses of local populations of *P. virgatum* to passive nighttime warming. Although the desired magnitude of the treatment effect was not achieved, differences were seen in flowering phenology between treatments. Individuals in upland topographic sites under the warming treatment displayed delayed flowering compared to other topographic treatment combinations. There were no significant differences in nighttime physiological measurements, and the only significant daytime physiological differences were decreased g_s and E in the warming plots. Finally,

significant differences were seen in aboveground biomass between topographic sites. Individuals in lowland sites had greater tillers m^{-2} , tiller weight, and biomass m^{-2} . Water availability based on topographic sites is a strong driver of *P. virgatum* aboveground biomass production, but nocturnal warming has the potential to impact flowering phenology, physiological responses, and exacerbate plant water stress.

Literature Cited

- Alexopoulo, E., Sharma, N., Papatheohari, Y., Christou, M., Piscioneri, I., Panoutsou, D., Pignatelli, V. 2008. Biomass yields for upland and lowland switchgrass varieties grown in the Mediterranean region. *Biomass and Bioenergy* 32:926-933.
- Alward, R.D., Detling, J.K., Milchunas, D.G. 1999. Grassland vegetation changes and nocturnal global warming. *Science* 283:229-231.
- Benson, E.J., Hartnett, D.C. 2006. The role of seed and vegetative reproduction in plant recruitment and demography in tallgrass prairie. *Plant Ecology* 187:163–177.
- Caird, M.A., Richards, J.H., Donovan, L.A. 2007. Nighttime stomatal conductance and transpiration in C₃ and C₄ plants. *Plant Physiology* 143:4-10.
- Casler, M.D., Vogel, K.P., Taliaferro, C.M., Wynia, R.L. 2004. Latitudinal Adaptation of Switchgrass Populations. *Crop Science* 44:293–303.
- Christensen, J. H., B. Hewitson, A. Busuioc, A. Chen, X. Gao, I. Held, R. Jones, R. K. Kolli, W.-T. Kwon, R. Laprise, V. Magaña Rueda, L. Mearns, C. G. Menéndez, J. Räisänen, A. Rinke, A. Sarr and P. 2007: Regional Climate Projections. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor and H. L. Miller, eds.). Cambridge University Press, Cambridge, United Kingdom and New York, USA.
- Cleland, E.E., Chiariello, N.R., Loarie, S.R., Mooney, H.A., Field, C.B. 2006. Diverse responses of phenology to global changes in a grassland ecosystem. *Proceedings of the National Academy of Sciences of the United States of America* 103:13740-13744.

- Cross, M.S., Harte, J. 2007. Compensatory responses to loss of warming-sensitive plant species. *Ecology* 88:740-748.
- Das, M.K., Fuentes, R.G., Taliaferro, C.M. 2004. Genetic variability and trait relationships in switchgrass. *Crop Science* 44:443-448.
- De Valpine, P., Harte, J. 2001. Plant responses to experimental warming in a montane meadow. *Ecology* 82:637-648.
- Emery, S.M., Gross, K.L. 2007. Dominant species identity, not community evenness, regulates invasion in experimental grassland communities. *Ecology* 88:954-964.
- Fay, P.A., Carlisle, J.D., Knapp, A.K., Blair, J.M., Collins, S.L. 2003. Productivity responses to altered rainfall patterns in a C₄-dominated grassland. *Oecologia* 137:245–251.
- Fay, P.A., Kaufman, D.M., Nippert, J.B., Carlisle, J.D., Harper, C.W. 2008. Changes in grassland ecosystem function due to extreme rainfall events: implications for responses to climate change. *Global Change Biology* 14:1600-1608.
- Griffin, K.L., Turnbull, M., Murthy, R., Lin, G., Adams, J., Farnsworth, B., Mahato, T., Bazin, G., Potasnak, M., Berry, J.E. 2002. Leaf respiration is differentially affected by leaf vs. stand-level night-time warming. *Global Change Biology* 8:479-485.
- Grime, J.P. 2008. Benefits of plant diversity to ecosystems: Immediate, filter and founder effects. *Journal of Ecology* 86:902-910.
- Hartman, J.C., Nippert, J.B., Orozco, R.A., Springer, C.J. 2011. Potential ecological impacts of switchgrass (*Panicum virgatum* L.) cultivation in the Central Great Plains, USA. *Biomass and Bioenergy*.

- Houghton, J. 2001. The science of global warming. *Interdisciplinary Science Reviews* 26:247-257.
- Hovenden, M.J., Williams, A.L., Pedersen, J.K., Vander Schoor, J.K., Willis, K.E. 2008. Elevated CO₂ and warming impacts on flowering phenology in a southern Australian grassland are related to flowering time but not growth form, origin, or longevity. *Australian Journal of Botany* 56:630-643.
- Karl, T.R., Kukla, G., Razuvayev, V.N., Changery, M.J., Quayle, R.G., Heim Jr., R.R., Easterling, D.R., Bin Fu, C. 1991. Global warming: Evidence for asymmetrical diurnal temperature change. *Geophysical Research Letters* 18:2253-2256.
- Knapp, A.K. 1984. Water relations and growth of three grasses during wet and drought years in a tallgrass prairie. *Oecologia* 65:35-43.
- Knapp, A. K. 1985. Effect of fire and drought on the ecophysiology of *Andropogon gerardii* and *Panicum virgatum* in a tallgrass prairie. *Ecology* 66:1309-1320.
- Lemus, R., Brummer, E.C., Moore, K.J., Molstad, M.E., Burras, C.E., Barker, M.F. 2002. Biomass yield and quality of 20 switchgrass populations in southern Iowa, USA. *Biomass and Bioenergy* 23:433-442.
- Madakadze, I., Coulman, B.E., Stewart, K., Peterson, P., Samson, R., Smith, D.L. 1998. Phenology and tiller characteristics of big bluestem and switchgrass cultivars in a short growing season area. *Agronomy Journal* 90:489-495.
- McLaughlin, S.B., Kszos, L.N. 2005. Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. *Biomass and Bioenergy* 28:515-535.

- Nippert, J.B., P.A. Fay, J.D. Carlisle, A.K. Knapp, and M.D. Smith. 2009. Ecophysiological responses of two dominant grasses to altered temperature and precipitation regimes. *Acta Oecologica* 35:400-408.
- Niu, S., Li, Z., Xia, J., Han, Y., Wu, M., Wan, S. 2008. Climatic warming changes plant photosynthesis and its temperature dependence in a temperate steppe of northern China. *Environmental and Experimental Botany* 63:91-101.
- Parrish, D.J, Fike, J.H. 2005. The Biology and Agronomy of Switchgrass for Biofuels. *Critical Reviews in Plant Science* 24: 423-459. Peñuelas J., C. Gordon, L. Llorens, T. Nielsen, A. Tietema, C. Beier, et al. 2004. Nonintrusive field experiments show different plant responses to warming and drought among sites, seasons, and species in a North-South European gradient. *Ecosystems* 7:598-612.
- Porter, C.L. 1966. An Analysis of Variation Between Upland and Lowland Switchgrass, *Panicum Virgatum* L., in Central Oklahoma. *Ecology* 47:980-992.
- Rose, L.W. IV, Das, M.K., Taliaferro, C.M. 2008. Estimation of genetic variability and heritability for biofuel feedstock yield in several populations of switchgrass. *Annual of Applied Biology* 1:1-17.
- Saleska, S.R., Harte, J., Torn, M.S. 1999. The effect of experimental ecosystem warming on CO₂ fluxes in a montane meadow. *Global Change Biology* 5:125-141.
- Sherry, R.A., Zhou, X., Gu, S., Arnone III, J.A., Schimel, D.S., Verburg, P.S., Wallace, L.L., Luo Y. 2007. Divergence of reproductive phenology under climate warming. *Proceedings of the National Academy of Sciences of the United States of America* 104:198-202.

- Sherry, R.A, Weng, E., Arnone III, J.A., Johnson, D.W., Schimel, D.S., Verburg, P.S., Wallace, L.L., Luo, Y. 2008. Lagged effects of experimental warming and doubled precipitation on annual and seasonal aboveground biomass production in a tallgrass prairie. *Global Change Biology* 14:2923-2936.
- Smith, M.D., Knapp, A.K. 2003. Dominant species maintain ecosystem function with non-random species loss. *Ecology Letters* 6:509–517.
- Snyder, K.A, Richards, J.H., Donovan, L.A. 2003. Night-time conductance in C3 and C4 species: do plants lose water at night? *Journal of Experimental Botany* 54:861-865.
- Trócsányi, Z.K., Fieldsend, A.F., Wolf, D.D. 2009. Yield and canopy characteristics of switchgrass (*Panicum virgatum* L.) as influenced by cutting management. *Biomass and Bioenergy* 33:442-448.
- Wan, S., Hui, D., Wallace, L., Luo, Y. 2005. Direct and indirect effects of experimental warming on ecosystem carbon processes in a tallgrass prairie. *Global Biogeochemical Cycles* 19:1-13.
- Wan, S., Xia, J., Liu, W., Niu, S. 2009. Photosynthetic overcompensation under nocturnal warming enhances grassland carbon sequestration. *Ecology* 90: 2700-2710.
- Weaver, J.E., Fitzpatrick, T.J. 1932. Ecology and Relative Importance of the Dominants of Tall-Grass Prairie. *Botanical Gazette* 93:113-150.
- Wright, L., Turhollow, A. 2010. Switchgrass selection as a “model” bioenergy crop: A history of the process. *Biomass and Bioenergy* 34:851-868.
- Wullschleger, S.D., Davis, E.B., Borsuk, M.E., Gunderson, C.A., Lynd, L.R. 2010. Biomass production in switchgrass across the United States: Database description and determinants of yield. *Agronomy Journal* 102:1158-1168.

- ^aXu, Z.Z., Zhou G.S. 2005a. Effects of water stress and high nocturnal temperature on photosynthesis and nitrogen level of a perennial grass *Leymus chinensis*. *Plant and Soil* 269:131-139.
- ^bXu, Z.Z., Zhou G.S. 2005b. Effects of water stress and nocturnal temperature on carbon allocation in the perennial grass, *Leymus chinensis*. *Physiologia Plantarum* 123:272-280.
- Xu, Z.Z., Zhou, G.S., Shimizu, H. 2009. Effects of soil drought with nocturnal warming on leaf stomatal traits and mesophyll cell ultrastructure of a perennial grass. *Crop Science* 49:1843-1851.
- Zhou, X., Liu, X., Wallace, L.L., Luo Y. 2007. Photosynthetic and respiratory acclimation to experimental warming for four species in a Tallgrass Prairie ecosystem. *Journal of Integrative Plant Biology* 49:270-281.
- Zhou, X., Talley, M., Luo, Y. 2009. Biomass, litter, and soil respiration along a precipitation gradient in Southern Great Plains, USA. *Ecosystems* 12:1369-1380.

Figures and Tables

Table 4-1 ANOVA results for topographic position, treatment, and interaction effects for the variables measured. Numbers in bold show significant main effects or interactions ($p \leq 0.05$). Numbers in italics indicate marginal significance ($p \leq 0.10$).

Response Variable	<u>Treatment</u>		<u>Topography</u>		<u>Interaction</u>	
	F	p-value	F	p-value	F	p-value
Soil Moisture	0.50	0.4906	6.28	0.0234	0.11	0.7499
A _{max} (day)	1.27	0.2756	0.50	0.4895	0.02	0.8991
g _s (day)	<i>4.27</i>	<i>0.0549</i>	2.90	0.1076	0.05	0.8192
E (day)	<i>4.12</i>	<i>0.0591</i>	<i>3.22</i>	<i>0.0914</i>	0.07	0.7909
WUE (day)	0.28	0.6068	2.12	0.1651	0.02	0.8889
Ψ _{mid}	0.23	0.6382	0.42	0.5244	2.01	0.1753
Fv/Fm	<i>3.72</i>	<i>0.0711</i>	1.37	0.2581	1.84	0.1933
Rd (night)	0.27	0.6101	0.26	0.6177	0.96	0.3419
g _s (night)	0.01	0.9049	0.08	0.7841	0.07	0.7979
E (night)	1.92	0.1843	3.02	0.1014	0.26	0.6196
WUE (night)	0.03	0.8660	2.74	0.1172	0.03	0.8580
SLA	10.57	0.0050	1.83	0.1953	0.01	0.9332
Tillers/m ²	<i>3.43</i>	<i>0.0824</i>	5.21	0.0365	0.28	0.6028
Flowering Tillers/m ²	0.72	0.4097	<i>3.58</i>	<i>0.0767</i>	0.00	0.9804
Flowering Time	2.73	0.0251	2.73	0.0251	2.91	0.0183
Aboveground Biomass/m ²	0.99	0.3353	7.58	0.0142	0.59	0.4534
Tiller Weight (g)	0.74	0.4032	8.35	0.0107	0.04	0.8457
% Flowering Tillers	0.00	0.1466	2.00	0.1763	0.78	0.3895
IR Canopy Temp (night)	6.09	0.0252	0.26	0.6154	0.07	0.8011
IR Soil Temp (night)	5.49	0.0324	<i>4.08</i>	<i>0.0604</i>	0.21	0.6532
IR Canopy Temp (day)	1.31	0.2697	0.22	0.6441	0.00	0.9648
IR Soil Temp (day)	0.00	0.9658	0.03	0.8707	0.38	0.5451
Soil Temp (SWC meter day)	0.00	0.9882	0.06	0.8080	0.00	0.9904



Figure 4-1 A louvered OSC as developed by Germino (personal communication). The optix plexi-glass slats reduce the loss of re-radiated daytime heat that occurs during the night, increasing nighttime temperature in the canopy.

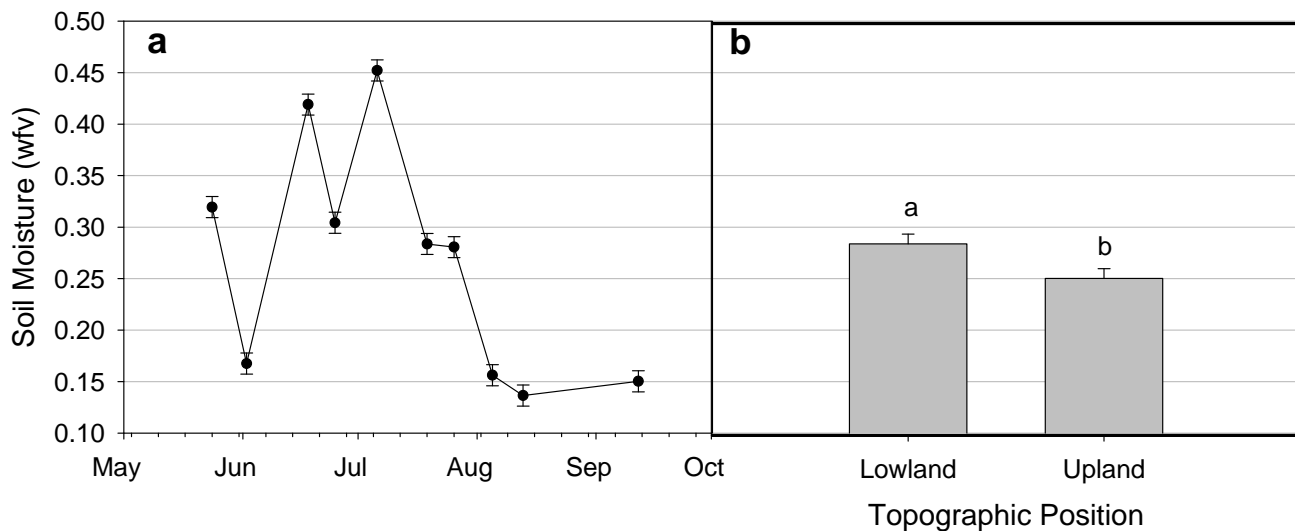


Figure 4-2 Soil moisture (0-10cm) (a) over the course of the growing season (b) between topographic positions. Soil moisture increased until July, and then decreased the rest of the season. Soil moisture was significantly lower in the upland soils compared to the lowland soils. Soil moisture is expressed as water fraction by volume (wfv).

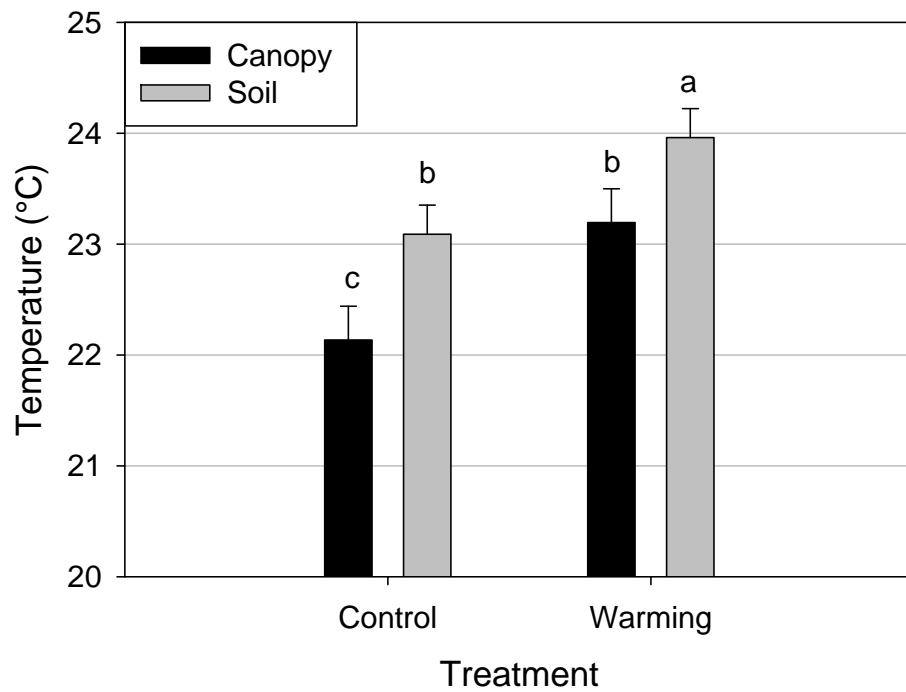


Figure 4-3 Mean nighttime temperatures between the control and Louvered OSC treatments ($\pm 1SE$). Black bars represent the herbaceous canopy temperature. Grey bars represent soil surface temperatures. The Louvered OSC increased the temperature of both the canopy and the soil surface.

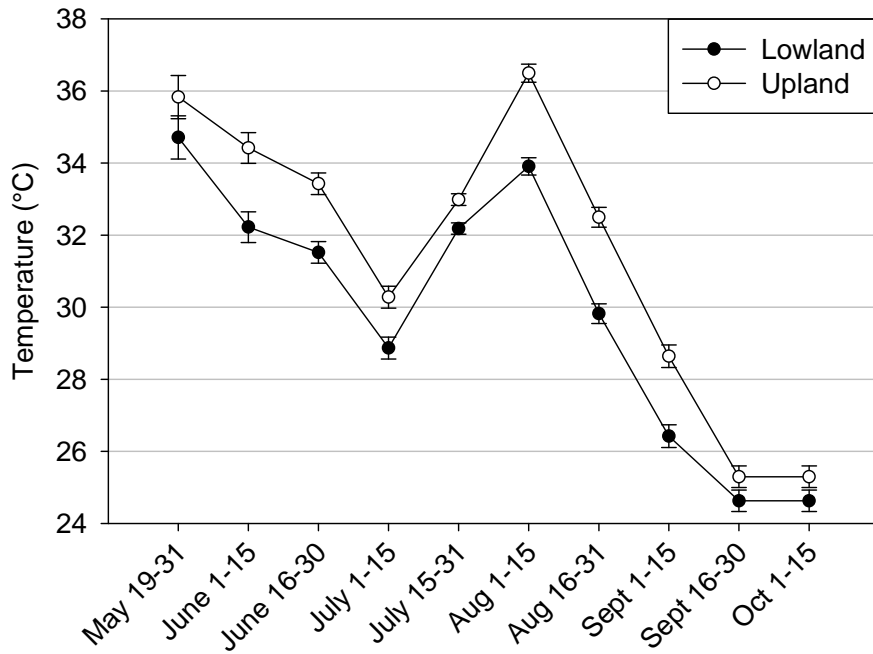


Figure 4-4 Mean daytime air temperatures ($\pm 1SE$) for upland and lowland sites. Air temperatures were taken 1-2 cm above the soil surface and averaged over two week periods. Upland sites had significantly higher peak daytime temperatures compared to lowland sites.

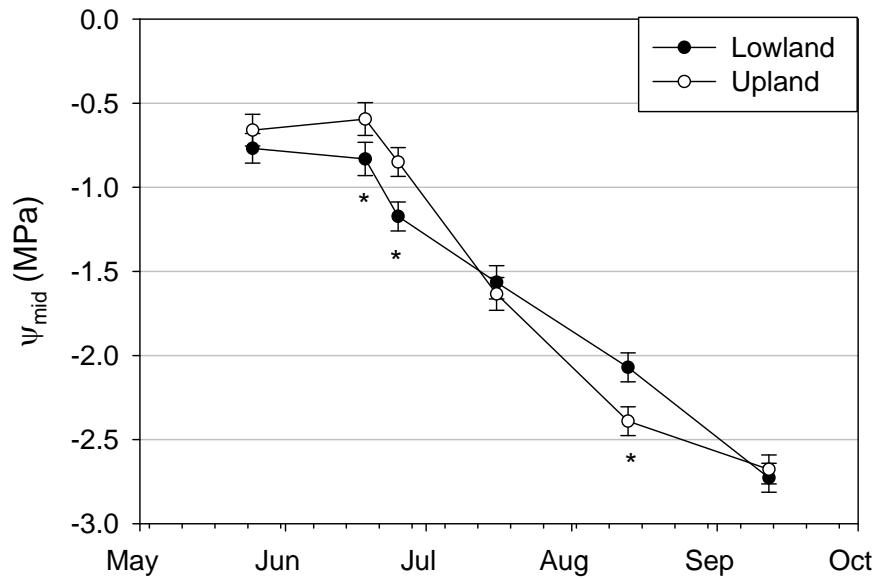


Figure 4-5 Mean (± 1 SE) mid-day leaf level water potential (Ψ_{mid}) for upland and lowland sites over the course of the summer. Significant topographic differences, as indicated by asterisks, occurred during June and August.

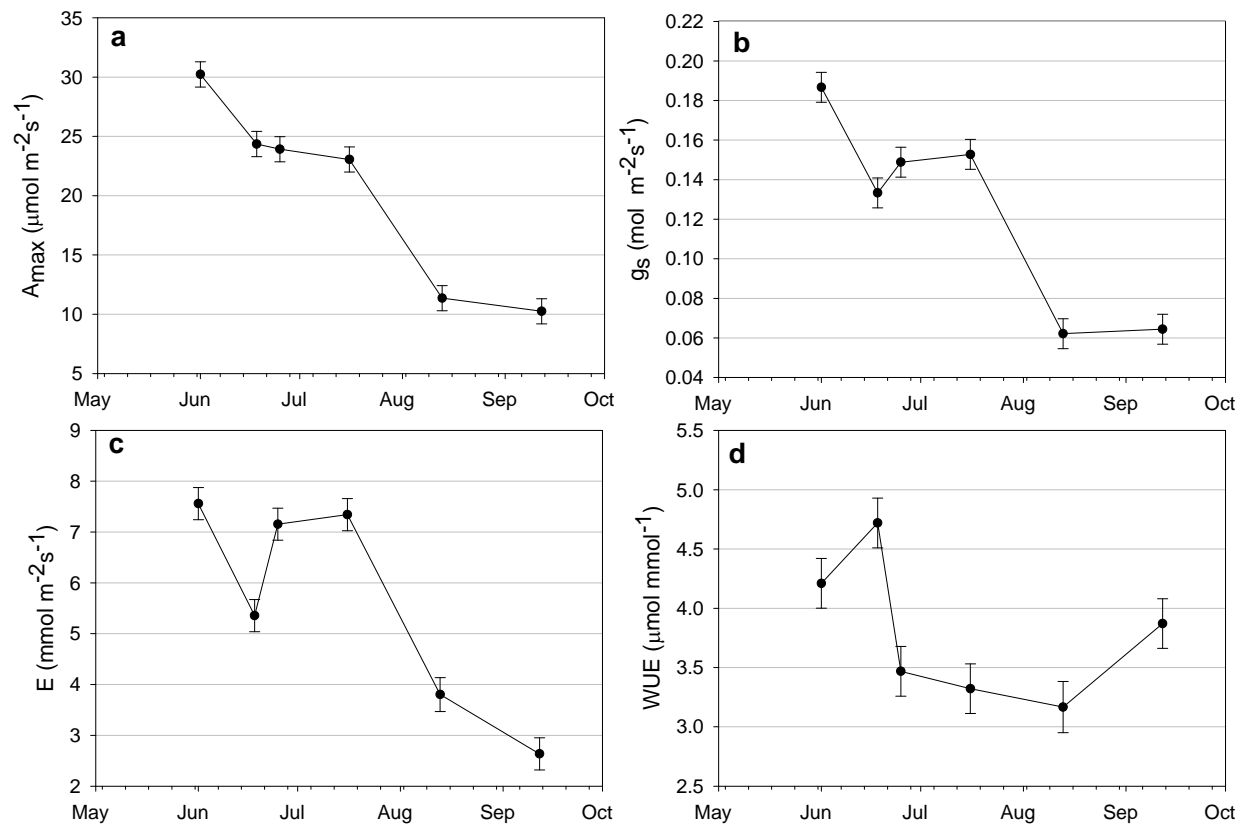


Figure 4-6 Physiological responses of (a) carbon assimilation (b) stomatal conductance (c) transpiration and (d) water use efficiency over the growing season. Each point is the overall mean response ($\pm 1\text{SE}$) for the sample date for all treatments and topographic positions.

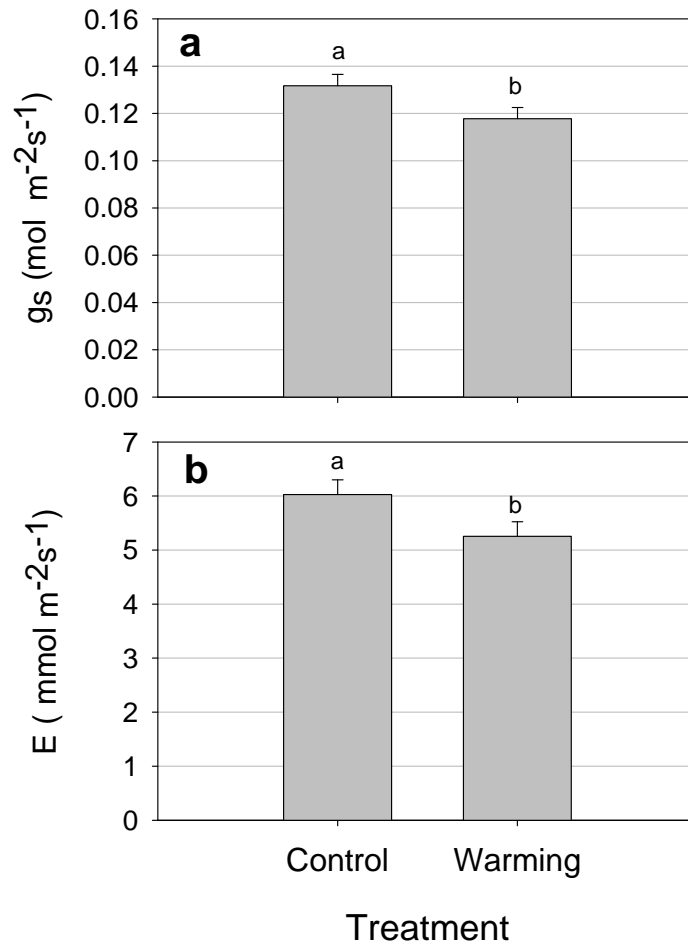


Figure 4-7 Physiological responses of (a) stomatal conductance between treatments (b) transpiration between treatments. Each is a marginally significant ($p < 0.10$) overall mean response ($\pm 1\text{SE}$).

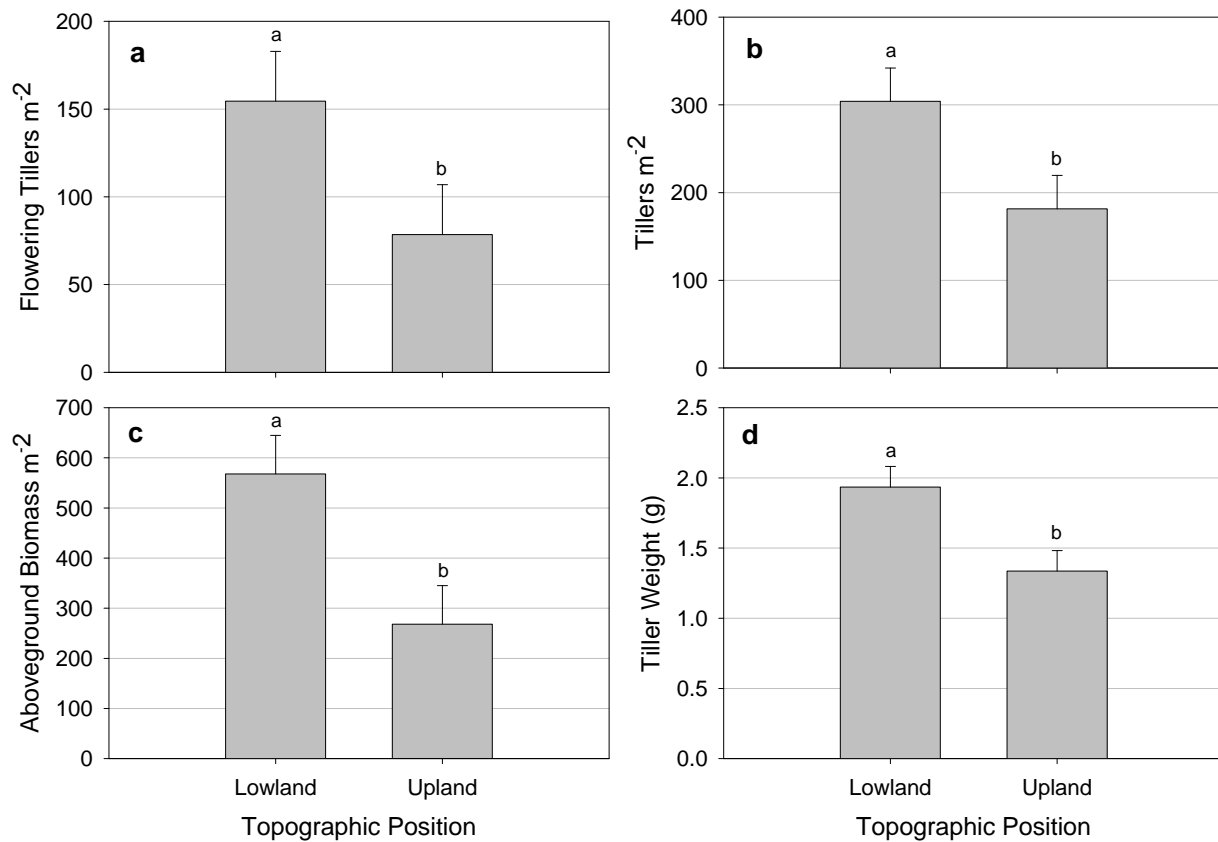


Figure 4-8 Mean responses ($\pm 1SE$) for (a) flowering tiller density (b) tiller density (c) aboveground biomass and (d) tiller weight. Tiller density, aboveground biomass and tiller weight are all significantly ($p < 0.05$) lower in the upland sites. Flowering tiller density is marginally significant with increased flowering tillers in the lowland site.

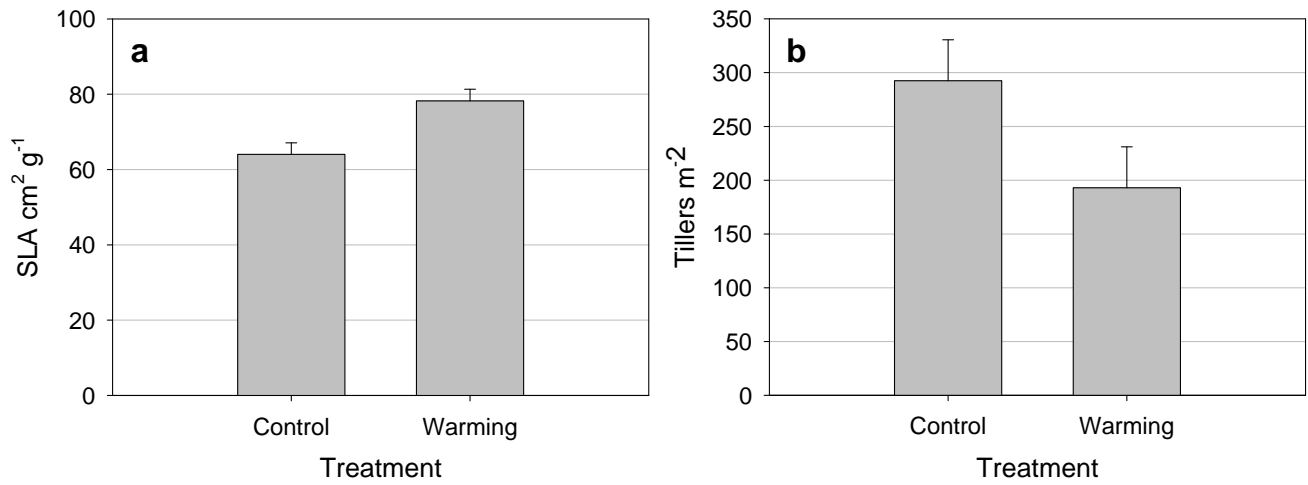


Figure 4-9 Mean responses ($\pm 1\text{SE}$) for (a) specific leaf area (SLA) and (b) tiller density for the control and warming treatments. SLA is significantly greater ($p < 0.05$) in warming treatments and tiller density is marginally significant between control and warming treatments.

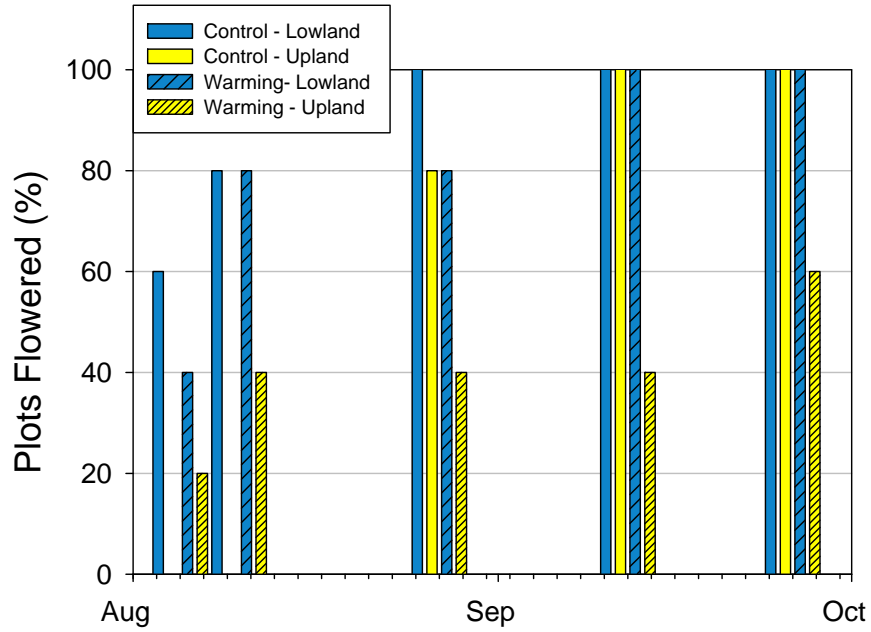


Figure 4-10 The percentage of plots flowered for each sampling date during the 2010 growing season. Plots in the upland sites under the warming treatment did not completely flower at the end of the season compared to the other treatment*topography plots.

Chapter 5 - Conclusions

P. virgatum is an important common perennial warm-season C₄ grass that is native to the tallgrass prairie. The species is co-dominant within grassland ecosystems, and is widely adapted to a large environmental and geographic range (Fig. 2-1, Fig. 3-1). As a dominant species, it has larger population sizes and contains larger amounts of genetic variation (McNaughton & Wolf 1970), which will contribute to and affect the processes of the ecosystem more than rarer species (Hillebrand et al. 2008). *P. virgatum* possesses large phenotypic and genotypic variability, and has high annual productivity across its large habitat range (Sanderson et al. 2006). These traits make it important for both future responses of ecosystems to climate change, and as a potential biofuel species.

Anthropogenic climate change is expected to dramatically alter the natural fluctuations in precipitation and temperature (Easterling et al. 2000; Houghton et al. 2001; Alley et al. 2003; Alley et al. 2007). Increased inter and intra annual variability in rainfall is expected, with mean annual air temperatures rising 4°C by the year 2100 (Christensen et al. 2007). Plants are able to respond to the current levels of climate variability, but we need to be able to better predict how plants will respond to predicted climate change scenarios. The goal of this thesis was to characterize the physiological and growth responses of different ecotypes of *P. virgatum* to predicted climate change conditions, and to address the ecological consequences of switchgrass cultivation for biofuel.

In chapter 2, I discussed the ecological consequences of implementing widespread cultivation of switchgrass for biofuel. Much of the focus has been on using switchgrass to produce biomass for biofuel, and to sequester carbon underground (Sanderson et al. 2006; Qin et al. 2006). However, I argued that other aspects such as increasing the potential for invasibility, outbreaks of disease, and habitat suitability need to be taken into account. I concluded that to minimize the negative ecological impacts of switchgrass cultivation, maintenance of landscape heterogeneity is a key factor. By altering harvest rotations, using mixed species composition, and implementing no-till farming, the resulting structural diversity will increase species abundance and soil quality. Future directions include more long term studies to determine the impacts and consequences of switchgrass cultivation (Bellamy et al. 2009; Keshwani & Cheng 2009).

The first experiment, detailed in Chapter 3, and was conducted using a novel outdoor mesocosm facility to determine the physiological and growth responses of *P. virgatum* to altered precipitation regimes. Three different ecotypes of *P. virgatum* (Kansas, Oklahoma, Texas) were subjected to three different precipitation regimes (ambient, -25%, +25%). Many of the leaf level physiological responses, such as A_{\max} , g_s , E , F_v/F_m , and Ψ_{mid} were explained by the differences between ecotypes. One of the largest ecotypic differences was in the flowering times and rates. The Texas ecotype initiated flowering at a later date, and at a lesser rate than the Kansas or Oklahoma ecotypes. This is indicative of southern ecotypes moving towards more northern locations (Casler et al. 2004; Casler 2005). If growing seasons were to lengthen due to climate change effects, it would create the possibility of these southern ecotypes drifting north (Casler et al. 2007). The precipitation treatments altered WUE, Ψ_{mid} , and many of the aboveground biomass measurements. There were few interactions between precipitation and ecotype, which leads me to conclude that precipitation is a strong driver of aboveground biomass production, while the adaptation of the ecotype to their native environments affects physiological processes. Future research should include a variety of ecotypes in a range of latitudinal and longitudinal locations to address genotype x location interactions (Casler et al. 2007). Although change in total rainfall is one aspect of predicted climate change, studies on variability in precipitation frequency would elicit a greater understanding of how species, and ultimately ecosystems, will respond to climate change (Fay et al. 2008; Hughes & Diaz 2008;).

The second experiment, in Chapter 4 used Louvered open sided chambers (Louvered OSC's) to create a passive nighttime warming effect on local populations of *P. virgatum* at Konza Prairie. Like the first experiment, the purpose was to characterize the physiological and growth responses to nocturnal warming. Although the desired treatment effect was not achieved during the study, one of the main differences between treatments was in the flowering phenology. The results of this study contradicted other experiments that show grassland species, such as *P. virgatum*, that initiate flowering before peak summer temperatures accelerate their flowering in response to warming (Cleland et al. 2006; Sherry et al. 2007). Flowering phenology is highly variable in switchgrass (Quinn & Wetherington 2002), and the small treatment effect was not enough to effectively accelerate flowering. Many of the differences seen in this study were related to aboveground biomass responses between upland and lowland sites. With higher soil water content, and lower daytime mean temperatures, the effects of increased nighttime

temperate may be lessened in the lowland sites compared to upland sites (Sherry et al. 2008). In the end, the treatment was not very effective, but under significantly increased nighttime temperatures, the differences between *P. virgatum* in lowland and upland sites may become even starker, potentially impacting flowering phenology, physiological responses, and increasing water stress (Ku et al. 1978; Sherry et al. 2007; Xu et al. 2009).

The Central Great Plains region of the United States will continue to be impacted by anthropogenic climate change. Implementation of widespread switchgrass cultivation requires careful management practices to increase heterogeneity and minimize negative ecological consequences. Increased temperatures and precipitation variability are likely to alter the physiology, growth, and phenology of grassland species. Although cultivars of *P. virgatum* have been studied extensively for feedstock and biofuels, there is less knowledge in how natural ecotypes respond to resource alterations. To increase our ability to predict responses of species to climate change, more research needs to be done using multiple and mixed assemblages of genotypes to gradients of environmental variability.

Literature Cited

- Alley, R. B., Marotzke, J., Nordhaus, W.D., Overpeck, J.T., Peteet, D.M., Pielke, R.A., Pierrehumbert, R.T., Rhines, P.B., Stocker, T.F., Talley, L.D., Wallace, J.M. 2003. Abrupt climate change. *Science* 299:2005-2010.
- Alley, R.B., et al. *Climate Change 2007: The Physical Science Basis, Summary for Policy Makers*. 2007. Geneva, Switzerland, IPCC Secretariat. Working Group I Fourth Assessment Report of the Intergovernmental Panel on Climate Change. 2-2-2007.
- Bellamy, P.E., Croxton, .P.J., Heard, M.S., Hinsley, S.A., Hulmes, L., Hulmes, S., Nuttall, P., Pywell, R.F., Rothery, P. 2009. The impact of growing miscanthus for biomass on farmland bird populations. *Biomass & Bioenergy* 33:191-199.
- Casler, M.D. 2005. Ecotypic variation among switchgrass populations from the Northern USA. *Crop Science* 45:388-398.
- Casler, M.D., Vogel, K.P., Taliaferro, C.M., Wynia, R.L. 2004. Latitudinal adaptation of switchgrass populations. *Crop Science* 44:293–303.
- Casler, M.D., Vogel, K.P. Taliaferro, C.M., Ehlke, N.J., Berdahl, J.D., Brummer, E.C., Kallenbach, R.L., West, C.P., Mitchell, R.B. 2007. Latitudinal and longitudinal adaptation of switchgrass populations. *Crop Science* 47:2249-2260.
- Cleland, E.E., Chiariello, N.R., Loarie, S.R., Mooney, H.A., Field, C.B. 2006. Diverse responses of phenology to global changes in a grassland ecosystem. *Proceedings of the National Academy of Sciences of the United States of America* 103:13740-13744.

- Christensen, J. H., B. Hewitson, A. Busuioc, A. Chen, X. Gao, I. Held, R. Jones, R. K. Kolli, W.-T. Kwon, R. Laprise, V. Magaña Rueda, L. Mearns, C. G. Menéndez, J. Räisänen, A. Rinke, A. Sarr and P. 2007: Regional Climate Projections. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor and H. L. Miller, eds.). Cambridge University Press, Cambridge, United Kingdom and New York, USA.
- Easterling, D. R., Meehl, G.A., Parmesan, C., Changnon, S.A., Karl, T.R., Mearns, L.O. 2000. Climate extremes: Observations, modeling, and impacts. *Science* 289:2068-2074.
- Fay, P.A., Kaufman, D.M., Nippert, J.B., Carlisle, J.D., Harper, C.W. 2008. Changes in grassland ecosystem function due to extreme rainfall events: implications for responses to climate change. *Global Change Biology* 14:1600-1608.
- Hillebrand, H., Bennett, D.M., Cadotte, M.W. 2008. Consequences of dominance: A review of evenness effects on local and regional ecosystem processes. *Ecology* 89:1510-1520.
- Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K., Johnson, C.A., eds. 2001. *Climate change 2001: the scientific basis. Contributions of working groups I to the third assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge.
- Hughes, M.K., Diaz, H.F. 2008. Climate variability and change in the drylands of Western North America. *Global and Planetary Change* 64:111–118.
- Keshwani, D.R., Cheng, J.J. 2009. Switchgrass for bioethanol and other value-added applications: A review. *Bioresource Technology* 100:1515-1523.

- Ku, S.B., Edwards, G.E., Smith, D. 1978. Photosynthesis and nonstructural carbohydrate concentration in leaf blades of *Panicum virgatum* as affected by night temperature. *Canadian Journal of Botany* 56:63-68.
- McNaughton, S.J., Wolf, L.L. 1970. Dominance and the niche in ecological systems. *Science* 167:131-139.
- Sanderson, M.A., Adler, P.R., Boateng, A.A., Casler, M.D., Sarath, G. 2006. Switchgrass as a biofuels feedstock in the USA. *Canadian Journal of Plant Science* 86:1315-1325.
- Sherry, R.A., Zhou, X., Gu, S., Arnone III, J.A., Schimel, D.S., Verburg, P.S., Wallace, L.L., Luo Y. 2007. Divergence of reproductive phenology under climate warming. *Proceedings of the National Academy of Sciences of the United States of America* 104:198-202.
- Sherry, R.A., Weng, E., Arnone III, J.A., Johnson, D.W., Schimel, D.S., Verburg, P.S., Wallace, L.L., Luo, Y. 2008. Lagged effects of experimental warming and doubled precipitation on annual and seasonal aboveground biomass production in a tallgrass prairie. *Global Change Biology* 14:2923–2936.
- Qin, X., Mohan, T., El-Halwagi, M., Cornforth, G., McCarl, B.A. 2006. Switchgrass as an alternate feedstock for power generation: an integrated environmental, energy and economic life-cycle assessment. *Clean Techn Environ Policy* 8:233–249.
- Quinn, J.A., Wetherington, J.D. 2002. Genetic variability and phenotypic plasticity in flowering phenology in populations of two grasses. *Journal of the Torrey Botanical Society* 129:96-106.
- Xu, Z.Z., Zhou, G.S., Shimizu, H. 2009. Effects of soil drought with nocturnal warming on leaf stomatal traits and mesophyll cell ultrastructure of a perennial grass. *Crop Science* 49:1843-1851.