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Plant Diversity Influences the Structure and Function of a Restored Prairie and its Responses to Added Disturbances

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PLANT DIVERSITY INFLUENCES THE STRUCTURE AND FUNCTION OF A RESTORED PRAIRIE AND ITS RESPONSES TO ADDED DISTURBANCES

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PLANT DIVERSITY INFLUENCES THE STRUCTURE AND FUNCTION OF A RESTORED PRAIRIE AND ITS RESPONSES TO ADDED DISTURBANCES

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As global warming, the human conversion of natural landscapes to agricultural use, and widespread biodiversity losses continue to alter the ecosystems we depend on, an understanding of the relationship between ecosystem structure, composition, and function is needed to maintain valuable ecosystem states and their associated functions. Research testing the limits of an ecosystem's ability to maintain essential structure and functioning under disturbance conditions can aid in this goal.

In this study, I measured the relationship between plant diversity, community structure and functional traits, and their responses to added disturbances. I added disturbances representing those either caused or intensified by human activity to a prairie restoration planted at multiple levels of diversity and measured subsequent variation in ecosystem traits. My research scales up traditional 1mx1m-plot studies to test whether plant diversity can produce grassland ecosystems that are resilient to disturbances, as suggested by small-plot experiments.

Variation in ecosystem functional traits (including functional composition, nutrient cycling, invasion resistance, and plant growth strategy) was calculated using ANOVA, linear-mixed-effects regression models, post-hoc tests, and two-sample comparisons. Community structural variation was calculated via Bray-Curtis dissimilarity and PERMANOVA.

Vegetation structure and composition was more diverse in high-diversity plots, and ecosystem functional traits generally less variable in response to added disturbances. Invader counts were also lower in high-diversity plots. These patterns suggest that plant diversity can maintain ecosystem structure and function through disturbance events and limit biological invasion. Uncontrolled effects including weather and soil nutrient gradients also influenced vegetation structure and function. These effects were often more significant than diversity or disturbance treatments in structuring ecosystem traits. These results suggest that investing in biodiversity at the outset may aid the establishment of desired ecosystem states that are resilient to disturbance and help avoid costly invasive species management later on. However, system responses to disturbance are influenced by existing environmental conditions which should be accounted for when attempting to plan or conserve desired ecosystem states.

"[Our] well-being is far more intertwined with the rest of the biota than many of us would be inclined to believe"

-Thomas Lovejoy

DEDICATION

To you, wherever your travels have taken you. Thank you for telling me I was worth it.

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To my dad, who helped design my rainout shelters and brought me Benadryl when I couldn't breathe. To my brothers Ben and John, who were the most reliable and good-natured volunteers anyone could hope for. To my grandfather David, whose construction assistance helped me to finish what I started. I'm proud to be his granddaughter. To Chris Helzer, whose conversation and advice continue to prove invaluable. To Craig Allen, for hiring and advising me, and making this study possible. To my classmate, Hannah, for starting me on this work. To Hugh, whose intelligence, work ethic, and tireless optimism should come standard in every human being. To Jess, whose data exploration saved the day in my proposal defense! And to Zac, Lark, Nick, my mother and grandmother, Bryan and Rachel, Emma, and all of my family, friends, and classmates who have comforted, housed, and mentored me over the past two years. Your stalwart support are inspirational.

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CHAPTER 1: INTRODUCTION TO GRASSLAND SYSTEMS, THREATS TO GRASSLAND FUNCTIONING, AND THE BIODIVERSITY-ECOSYSTEM FUNCTION HYPOTHESIS

I. Introduction

Grassland ecosystems have adapted over thousands to millions of years in semi-arid regions around the globe, and their loss puts the functions performed by these systems at risk when they are replaced. Grasslands provide services that humanity depends on, including nutrient cycling, water storage and filtration, and biomass production (Wall et al. 2015), yet their existence is threatened by changes in global climate and the expansion of agriculture (Sanderson et al. 2002). A quantitative understanding of the effect of the stresses brought about by continued anthropogenic climate change and agricultural development is crucial for predicting future change and protecting remnant and restored grasslands worldwide.

At the most fundamental biotic level, the biodiversity of an ecosystem is a defining pattern which influences its function and response to perturbations and disturbances (Zavaleta et al. 2010; Kreyling et al. 2008), and which may contribute to its survival in the face of climate change (Jentsch et al. 2011). One conceptual framework which has been used to describe this relationship between diversity and ecosystem continuity is the Biodiversity-Ecosystem Function hypothesis (BEF). The BEF hypothesis (Schulze and Mooney 1993; Tilman and Downing 1994) predicts that species diversity is in itself an underlying source of ecosystem function and continuity in the face

of environmental stress, ultimately contributing to the resilience of these systems to environmental changes (Cortina et al. 2006).

To test the relationship between one form of biodiversity, namely plant diversity, and grassland response to disturbance, I conducted a study from 2015-2016 measuring several structural and functional traits in a tallgrass prairie restoration in central Nebraska and their variation in response to the planted biodiversity levels and to added disturbances. I tested two basic hypotheses; first, that a relationship exists between plant diversity and associated ecosystem pattern and process, and second, that this relationship extends to the response of ecosystem structural and functional traits to disturbance. These two hypotheses underpin the specific hypotheses tested in subsequent chapters.

II. Background

My research focused on the question, "How does plant diversity contribute to ecosystem structure, function, and ecosystem responses to disturbance?" by measuring traits representing ecosystem structure and function both before and after the addition of multiple disturbances in a restoration planted at multiple levels of diversity. Ecosystem pattern and process are mutually reinforcing, and feedbacks between the two may act to either stabilize the system or to push the system toward a new state following disturbance (Beisner et al. 2003). Measuring patterns in ecosystem structure and functional traits and their relationship to added disturbance help to predict which ecosystem properties are most vulnerable to disturbance, thereby reducing uncertainty in maintaining these systems under increased environmental stress.

For the remainder of this chapter, I first outline the key concepts driving my research, including grassland system characteristics, threats to grasslands, and the role of biodiversity in maintaining grassland structure and function. I then review the key ecological concepts I use to frame my research design, specifically the biodiversity-ecosystem function (BEF) hypothesis (Schulze and Mooney 1993; Brose and Hillebrand 2016) and ecological resilience. Finally, I describe the study conducted to address uncertainty surrounding the response of grassland systems to disturbance and describe the structure of the thesis.

GRASSLANDS AND GRASSLAND THREATS

Grassland ecosystems contribute globally to biodiversity and ecosystem service provisioning and are threatened by human activity. Climate change, eutrophication from the burning of fossil fuels and agricultural production, and the use of grasslands for grazing and hay production may constrain the ability of grasslands to persist in a functional state (Clark and Tilman 2008).

The benefits of functional grasslands are well-documented: for example, grassland soils are known to be a significant carbon sink with the potential to reduce atmospheric carbon dioxide levels and mitigate climate change (Seastedt and Knapp 1993; Lal 2004). These soils formed over millions of years via interactions between fire, grazing, and plant growth strategies that favored belowground growth (Cushman and Jones, 2004). To avoid dessication and promote regrowth following grazing, prairie grasses and forbs evolved extensive root systems that can reach depths of three meters or more (Weaver 1965). This belowground primary production, occurring in semi-arid regions with limited decomposition (the average ratio of live biomass to soil organic

matter in grasslands globally is 1:10, compared with 1:0.7 in the average forest soil; Anderson1992), results in an estimated 604 petagrams ($Pg=10^{15}g$) of carbon storage in grassland soils, compared with an estimated 498 petagrams in woodland soils (Coleman et al. 2004; Gibson 2009). Studies describing the production of stable organic matter from litter inputs via microbial transformation (Kallenbach et al. 2016, Cotrufo et al. 2015) reinforce the value of deep, organic grassland soils as carbon sinks. Biodiversity is key to maintaining these soils, yet the transformation of diverse, deep-rooted grassland vegetation to large-scale row-crop agriculture and suburban development has led to the widespread loss of grassland soils. Soil organic horizons have declined in the U.S. Midwest from an average of a few meters to several centimeters, and these eroded materials contribute not only to a loss of fertility and carbon storage potential, but also to aquatic eutrophication, siltation, and other environmental harms (Pimentel and Burgess 2013).

Grasslands have only recently become highly fragmented. In North America, prairies benefitted over the past ten thousand years from grazing interactions and deliberate management that encouraged grassland expansion, including burning by Native American communities (Weaver 1965; Cushman and Jones 2004). However, settlement and agricultural expansion in the Great Plains in the late 1800s destroyed much of the native vegetation in the interest of crop production (Samson and Knopf 1994). Today only one percent of the original North American tallgrass prairie remains (Kaul et al. 2011), often in small, fragmented plots that are especially vulnerable to continued loss (Samson and Knopf 2004). This pattern is not unique to North America. Globally, 37% of remaining grassland ecosystems are in small, isolated patches, and

ecosystem services such as habitat provisioning and nutrient cycling are affected by this fragmentation (White et al. 2000). Present-day grassland management including the removal of large, migrating herds of grazing animals (McGranahan et al. 2013), the reduction of fire as a management tool, and the planting of windbreaks and failure to prevent woody encroachment into grasslands (Twidwell et al. 2014), are all threats to the persistence of this system (Samson et al. 2004; Gibson 2009)

Restoration is increasingly required to maintain grassland ecosystems and their associated services (Suding et al. 2011). Restorations face unique challenges; not only must they overcome the legacy effects of agriculture and other human management, including excess soil nitrogen and losses of soil microbial symbionts (Riggs and Hobbie 2016), they must also withstand the increasing frequency and intensity of stresses arising from global climate change, including ongoing and widespread nitrification and significant changes in weather (Radeloff et al. 2015) without losing their essential structure and functions.

There is some evidence that sufficiently large or repeated disturbances can overcome the beneficial effects of diversity by degrading a system's ability to maintain its essential structure and function in the face of disturbances (Villnas et al. 2013). These studies range from post-disturbance inventories of natural systems (Li et al. 2007) to global nutrient enrichment experiments conducted by the Nutrient Network (Hautier et al. 2014) and ongoing drought and eutrophication experiments (De Boek et al. 2010). Studies that address the impact of global disturbances on natural ecosystems are crucial to our understanding of how to create climate-resilient ecosystems.

Grassland systems worldwide are affected by a number of disturbances which may impact their resilience to disturbance. Much of the loss of prairies and their related services occurred rapidly as native grasses and forbs were plowed under in favor of row-crop agriculture (Weaver 1965). In the remaining prairies, state shifts from grassland to woodland are occurring as cyclical disturbances, such as large-scale grazing by buffalo and periodic burning, have been removed or drastically reduced (Twidwell et al. 2014). Restoration, if attempted, frequently fails when the grassland is invaded by non-native, unsown grasses and forbs which outcompete native species and limit grassland productivity (Going et al. 2009).

In this study, I added disturbances to a tallgrass prairie restoration and measured the response of the grassland community, at low and high levels of planted diversity, to these added disturbances. I selected disturbances representing those currently affecting grassland systems (Radeloff et al. 2013). Adding disturbance treatments to both low and high diversity plots provided information about how these disturbances affect the tallgrass prairie system differentially based on community diversity.

Drought

To simulate drought, I built 5mx2.5m rainout shelters in the center of each of four low-diversity and four high-diversity plots in the spring of 2015. This rain-interception treatment became the base disturbance treatment to which all other experimental treatments were added. The IPCC reports that droughts are increasing in intensity and duration at a global scale (2014), and models predict increasingly severe drought over mid-latitude regions where the majority of grasslands exist today (Dai 2010). Chronic water stress represents a long-term disturbance whose effects become more pronounced

as the disturbance persists (Lake 2013). Water stress may limit the functioning of an ecosystem to the point where it is unable to tolerate additional disturbances.

Grassland vegetation evolved in semi-arid climates, and is tolerant to drought conditions due to the development of specific vegetative adaptations such as waxy cuticles that resist dessication (Cushman and Jones 2004), as well as the C4 photosynthetic pathway which limits evapotranspiration and photorespiration in dry climates (Gibson 2009). We may therefore expect mixed responses to drought simulations. There is evidence that drought may be especially detrimental to plant community structure and function in mid-summer, when temperature extremes may increase transpiration and exacerbate the effects of water stress (De Boek et al. 2011). This increased water stress may lower the tolerance of grassland vegetation to additional stresses, and so although water stress alone may not cause large variation in community structure or function, it may prove detrimental to sustained ecosystem structure and function when combined with additional disturbances.

Grassland soils are also vulnerable to drought stress, as soil microbes that drive ecosystem nutrient cycling and litter decomposition respond quickly and negatively to water stress (Schimel et al. 2016). Water-stressed plots may therefore demonstrate lower decomposition rates and litter turnover as soil microbes die or become dormant in the face of drought.

Water stress is compounded in soils that have little or no litter cover, as exposed soils dry and experience large fluctuations in temperature, a situation that may limit seedling establishment in warm or hot climates (Heady 1992). Thus, over time, water

stress may contribute to changes in vegetation composition and significantly alter the structure and function of grasslands.

Nitrogen addition

Grassland systems world-wide are continually stressed by eutrophication from nitrogen deposition and agricultural runoff (deVries et al. 2014). By reducing species diversity and shifting competitive dominance from C4 to C3 pathway grases (Galloway et al. 2004), eutrophication may erode system resilience, or the grasslands' ability to withstand additional disturbances (Hautier et al. 2014). Studies of reproduction and nutrient loading have found significant alterations in tallgrass prairie bud bank dynamics in response to nitrogen deposition (Dalgeish et al. 2008), indicating that the demography of these systems may be profoundly altered by ongoing nitrogen deposition, thus changing the structure and function of the system. Competitive interactions among many species for common limiting resources, especially nitrogen, contributes to the biodiversity of these systems, and studies indicate that chronic eutrophication reliably reduces biodiversity (Harpole et al. 2016; Clark and Tilman 2008).

Biomass harvesting

I cut all standing biomass in two disturbance-treatment subplots within each low-diversity and high-diversity whole-plot in early July of 2015 and 2016. I timed this treatment to coincide with peak biomass production, when most hay meadows are harvested. In 2015, biomass removal was applied to one disturbance subplot within each whole-plot, while in 2016 I applied the treatment to both the already-established biomass removal plot and to a combined treatment of biomass removal and eutrophication.

Remnant prairies in the Midwest are often hayed, as haying is considered a management alternative to more intensive practices including prescribed fire or grazing (Helzer 2011). Although periodic biomass removal can result in an increase in species and structural diversity (Collins 1998; Helzer 2010), frequent disturbances including can potentially shift competitive dynamics (Silvertown et al. 2016; Villnas et al. 2013) by favoring fast-growing annual or biennial species over slower-growing perennials (Grime 1979). Additionally, the effects of persistent disturbances may amplify over time, making a 'ramp' disturbance whose effects become more severe as the disturbance continues (Lake 2013). Large shifts in species cover can significantly alter the nutrient cycling, water balance, soil structure, and other functions of grassland systems (Diaz et al. 2005). Many remnant prairies in the Midwest are managed as haymeadows; therefore biomass removal is a significant anthropogenic disturbance affecting grassland systems in my study region.

THE BEF HYPOTHESIS AND NICHE COMPLEMENTARITY

Efforts to maintain and increase grassland functioning in the face of these and other persistent disturbances have, in recent decades, begun to focus on the maintenance of a diverse range of native species (Folke et al. 2004). In the early 1990s, this focus on biodiversity as a method for improving ecosystem functioning was articulated in the formal construction of the biodiversity-ecosystem function (BEF) hypothesis. The BEF hypothesis states that higher levels of biodiversity contributes directly to the sustained maintenance of more ecosystem functions and support those functions at higher levels (Tilman et al. 2006). Experimental research in marine systems (Lefcheck et al. 2016) and grassland mesocosms (Bradford et al. 2002) demonstrate that diverse systems with many

overlapping species functional traits can withstand the effects of multiple environmental stresses, including conditions such as drought and increased nutrient loads (Jentsch et al. 2011).

Studies linking biodiversity and ecosystem functioning have proliferated in recent decades (Risser 1995; Isbell et al. 2011) as researchers seek to define the role of biodiversity in maintaining ecosystem structure and function in the face of changing climate regimes. Increased biodiversity positively correlates with the number of simultaneous functions an ecosystem can maintain (Zavaleta et al. 2010, Gamfeldt et al. 2008), and may contribute to the continued provision of those functions in a changing environment (Suding et al. 2008).

Niche complementarity is one mechanism hypothesized to link biodiversity to increased ecosystem functioning. Niche complementarity denotes the process by which the interaction of multiple species creates a cohesive community, allowing for multiple species to coexist and function at a higher level as a unit than any single species within that community could alone (Loreau et al. 2001). Evidence for niche complementarity includes productive overyielding - where the primary productivity of a system is higher at high diversity levels than the maximum productivity of each species in that system grown in monoculture would predict (Tilman et al. 2014). Niche complementarity is essentially a conflation of the theories of niche partitioning and facilitation (Cardinale et al. 2007). Niche partitioning accounts for the coexistence of many species by positing that groups of species evolve different resource acquisition strategies, habitat preferences, and lifestyles, thereby avoiding direct competition. By evolving to become partial rather than direct competitors, niche partitioning allows many species in a community to more-or-

less stably coexist (Hutchinson 1959; Whittaker 1965). Facilitation, meanwhile, hypothesizes that species interacting with one another under harsh conditions contribute to one another's survival and therefore the maintenance of the community as a whole (Brooker et al. 2008). Facilitation was long ignored by ecologists in favor of competitive, individualistic theories of species interactions (Gleason 1926), but has gained traction as more experimental research into community stability and resilience is conducted. Some studies in this field have demonstrated that under harsh abiotic conditions facilitation becomes more important than competition in structuring communities, while relatively low levels of abiotic stress allow for more competitive interactions (Maestre et al. 2009).

In support of BEF and niche complementarity hypotheses, studies conducted in experimental plots of varying species richness indicate that complementarity effects arising from higher levels of biodiversity are real and increase over time (Cardinale et al. 2007; Zavaleta et al. 2010). In mesocosms, research demonstrates that higher-diversity plant communities both maintain their productivity in the face of extreme drought (Kreyling et al. 2008; Jentsch et al. 2011) and regain high levels of productivity more quickly than their low-diversity counterparts following drought (Vogel et al. 2012).

In addition to increased primary productivity via overyielding (Tilman et al. 2001), biodiversity can offer protection from biological invasion (Fargione and Tilman 2005). This is likely a side effect of the increased competition for limiting resources at high levels of biodiversity. As a diverse range of species co-evolve over several generations to exploit the full range of available resources (Ashton et al. 2010), community stability is enhanced as niches available for invading species decline (Going et al. 2009; Wedin 1999). In experimental restorations, higher seeding richness enables

restored plant communities to successfully establish by outcompeting non-target species (Foster et al. 2015; Piper 2015); this effect is even more important than seeding density in establishing diverse prairie restorations that are resistant to biological invasions (Carter and Blair 2012; Nemec 2012).

Biodiversity also allows ecosystems to sustain multiple functions closer to their maximum potential than do monocultures or plots with only a few species (Zavaleta et al. 2010). Studies conducted at the scale of several hectares and at higher levels of species richness show that niche complementarity positively influences the ability of ecosystems to persist through time and in the face of a variety of disturbances (Gamfeldt et al. 2008; Isbell et al. 2015; Tilman et al. 2012).

Niche complementarity has been tested in ecological research comparing the level of functioning and resilience to environmental disturbances of monoculture and multi-species assemblages (Cardinale and Palmer 2002; Jentsch et al. 2011). These studies are often conducted under the umbrella of biodiversity-ecosystem function experiments (Brose and Hillebrand 2016; Suding et al. 2008; Zavaleta et al. 2010), which compare species assemblages at multiple levels of diversity to assess whether biodiversity impacts the ability of ecosystems to sustain multiple ecological functions in the face of altered growth conditions.

ECOLOGICAL RESILIENCE

Ecosystem structure and function are not constant through time; rather, they undergo a natural range of fluctuation within which they maintain features recognizable as belonging to that system (Carpenter et al. 2001). When environmental stresses push these processes beyond this natural range of variation, the system may ‘flip’ into a new,

potentially undesirable state (Lake 2013). The ability of a system to withstand disturbances while maintaining its essential structure and functions and without shifting into a new state is known as ecological resilience (Holling 1973). Because of its role in maintaining key ecosystem processes, biodiversity has emerged as a defining feature of multi-functional ecosystems that are resilient to environmental stresses (Cardinale et al. 2007; Risser 1995; Suding et al. 2008).

Ecological resilience is distinct from engineering resilience (how quickly a system returns to a defined equilibrium following disturbance) (MacGillivray and Grime 1995). While a central component of engineering resilience is stability, ecological resilience focuses on flexibility, or the ability of a system to re-organize and adapt to change without significantly altering its underlying structuring processes (Allen et al. 2014). To quantify ecosystem resilience, researchers first define the parameters being measured: namely the resilience *of* what, *to* what (Carpenter et al. 2001; Cumming et al. 2005). The ecological resilience of a system depends on both the range of natural variation within which a system can maintain its defining processes (the domain of attraction) and the inertia of the components comprising the system, or rather, how easily they change in response to a perturbation or disturbance (Carpenter et al. 2001). A disturbance or perturbation may degrade the resilience of a system by shrinking its domain of attraction (i.e. when biodiversity loss removes functional redundancy, making it more likely that the next disturbance or species loss will permanently alter system dynamics; Suding et al. 2008) or by pushing components of the ecosystem past the threshold of their current state, making it possible for the ecosystem to re-organize around a new domain of attraction (Thrush et al. 2009).

As climate change continues to alter long-term weather patterns and increase the occurrence of extreme weather events (IPCC 2014), increasing the resilience of desired ecosystem states to new climate regimes has become a key objective for land managers and policy makers hoping to maintain the services these ecosystems provide. Enhancing ecosystem resilience requires the ability to measure it, and so the questions of how to measure resilience and what mechanisms underpin system resilience to external disturbances and perturbations are among the most important in ecology today (Sutherland et al. 2013).

In this study, ecosystem components representing the properties of ecosystem resilience (the domain of attraction and system inertia) include system structure (e.g., does the species mixture resemble a grassland system? How many species are there compared with the diversity needed to provide the functions and services of a grassland system?), and the variation in functional traits in response to added disturbances (e.g., what is the magnitude of variation in plant growth strategy and nutrient cycling?). By adding disturbances and measuring variation in these metrics, I attempt to define the boundaries of system function and determine what parameters holding the system in its current state are most vulnerable to disturbance.

Assessing the variation in a range of parameters representing ecosystem structure and function is one method for measuring whether a system is losing its resilience before any major shift actually occurs (Litzow and Hunsicker 2016; Villnas et al. 2013), as certain parameters may vary drastically when the system is stressed beyond its average tolerance (Scheffer et al. 2009). Studies focused on systemic resilience typically measure several proxies of ecological function, including primary productivity, invasion

resistance, and nutrient and water cycling, among others. This strategy has been employed in long-term mesocosm experiments (Zavaleta et al. 2010), but larger-scale experiments in more realistic restoration settings are needed to evaluate the reliability of patterns found in these small, tightly-controlled experiments.

Field research testing community resilience to disturbance is still developing, however, and faces significant challenges in design and interpretation. The majority of experiments designed to assess the impact of disturbances on community resilience are 1m x 1m mesocosms planted in artificially low levels of diversity in comparison with natural plant communities (Jentsch et al. 2007; Stewart et al. 2013; Beierkuhnlein and Nesshoever 2006). Many studies conducted at scales larger than square-meter mesocosms do not support the idea that increased variability is visible prior to a state shift (Burthe et al. 2016). This study attempts to bridge the gap between small-scale, tightly controlled experiments and large-scale replicated studies by scaling up the types of research typically conducted on small test plots of limited diversity (Zavaleta et al. 2010; Tilman et al. 2014) by introducing a controlled set of disturbances to disturbance plots established within a larger restoration planted at distinct levels of biodiversity.

While it is likely that disturbances affect ecosystems differently when in combination than when encountered separately, few studies have tested the compound effects of multiple disturbances on ecosystem structure and function (Kreyling et al. 2007), and post-hoc monitoring of disturbance events offer no experimental control (Li et al. 2007). Most ecosystems encounter multiple disturbances acting at different scales and intensities (Lake 2013), and the combined stress of these disturbances may outweigh the beneficial effects of diversity in otherwise healthy ecosystems. Studies performed in

field-scale settings that include the complex dynamics of natural systems are needed (Villnas et al. 2013; Thrush et al. 2009).

III. Study goals and thesis overview

My thesis explores the relationship between plant diversity and metrics of community structure, community function, and their responses to added disturbance. To test the relationship between biodiversity and ecosystem responses to disturbance, I measured traits representing aspects of ecosystem structure and function in a tallgrass restoration planted at three distinct levels of biodiversity (Figure 1.1). Metrics used in this study include traits representing community structure, such as plant diversity, frequency, and cover, ground cover, and soil characteristics such as soil chemistry and microbial biomass. Functional traits measured in this study include measurements related to primary productivity, nutrient cycling, and invasion resistance, and their relative responses to added disturbance at multiple levels of biodiversity (Table 1.1).

In chapter two, I describe community structural traits and their variation due to the experimental treatments of plant diversity levels and added disturbances. Chapter 3 investigates the interaction between plant diversity, disturbance, and plant growth strategy by measuring the variation in specific leaf area and chlorophyll content of representative species. In chapter 4, I test the effects of plant diversity and added disturbances on traits related to nutrient cycling, specifically soil respiration and litter decomposition. In chapter 5, I look at the relationship between plant diversity, experimental disturbance additions, and the prevalence of invasive species in my study site. Finally, in chapter 6 I synthesize the results of this project and discuss implications

for ecosystem management and future research directions. While this study focuses on a tallgrass restoration in central Nebraska, the relationships discussed between biodiversity and ecosystem function have implications for grassland systems worldwide.

IV. Literature Cited

Allen, C.R., Angeler, D.G., Garmestani, A.S., Gunderson, L.H., Holling, C.S. 2014. Panarchy: theory and application. Ecosystems **17**:578-589.

Anderson, J.M. 1992. Responses of soils to climate change. Advances in Ecological Research **22**: 163-210.

Ashton, I.W., Miller, A.E., Bowman, W.D., Suding, K.N. 2010. Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. Ecology **91**: 3252-60.

Beierkuhnlein C, Nesshoever C. 2006. Biodiversity experiments—artificial constructions or heuristic tools? Progress in Botany **67**:486–535.

Beisner, B.E., Haydon, D.T., Cuddington, K. 2003. Alternative stable states in ecology. Frontiers in Ecology and the Environment **1**: 3376-382.

Bradford, M.A., Jones, T.H., Bardgett, R.D., Black, H.I.J., Boag, B., Bonkowski, M., Cook, R., Eggers, T., Gange, A.C, Grayston, S.J., Kandeler, E., McCaig, A.E., Newington, J.E., Prosser, J.I., Setala, H., Staddon, P.L., Tordoff, G.M., Tscherko, D., Lawton, J.H. Impacts of soil faunal community composition on model grassland ecosystems. Science **298**: 615-618.

Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler, G., Liancourt, P., Tielborger, K., Travis, J.M., Anthelme, F., Armas, C., Coll, L., Corcket, S.D., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C.L., Saccone, P., Schiffers, K., Seifan, M., Touzard, B., Michalet, R. 2008. Facilitation in plant communities: the past, the present, and the future. Journal of Ecology **96**:681-686.

Brose, U., & Hillebrand, H. 2016. Biodiversity and ecosystem functioning in dynamic landscapes. Philosophical Transactions of the Royal Society B: Biological Sciences **371**:1694.

Burthe, S.J., Henrys, P.A., Mackay, E.B., Spears, B.M., Campbell, R., Carvalho, L., Dudley, B., Gunn, I.D.M., Johns, D.G., Maberly, S.C., May, L., Newell, M.A., Wanless, S., Winfield, I.J., Thackeray, S.J., Daunt, F. 2016. Do early warning

indicators consistently predict nonlinear change in long-term ecological data? Journal of Applied Ecology **53**: 666-676.

Cardinale BJ, Palmer MA, Collins SL. 2002. Species diversity increases ecosystem functioning through interspecific facilitation. Nature **415**:426–429.

Cardinale, B.J., Wright, J.P., Cadotte, M.W., Carroll, I.T., Hector, A., Srivastava, D.S., Loreau, M., Weis, J.J. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. Proceedings of the National Academy of Science **104**:18123-18128.

Carpenter, S., Walker, B., Anderies, J., Abel, N. 2001. From metaphor to measurement; resilience of what to what? Ecosystems **4**: 765-781.

Carter, D., Blair, John M. 2012. High richness and dense seeding enhance grassland restoration establishment but have little effect on drought response. Ecological Applications **22**: 1308–1319.

Clark. M., Tilman, David. 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature Letters **451**:7.

Coleman, D.C., Crossley, D.A. Jr., Hendrix, P.F. 2004. *Fundamentals of Soil Ecology*. Amsterdam: Elsevier.

Collins, S. L. 1998. Modulation of diversity by grazing and mowing in native tallgrass prairie. Science **280**(5364), 745–747.

Cortina, J., Toma, F., Jaime, M., Valdecantos, A., Pe, M., Rey, U., Carlos, J. 2006. Ecosystem structure, function, and restoration success: Are they related ? Journal for Nature Conservation **14**: 152–160.

Cotrufo, M. F., Soong, J. L., Horton, A. J., Campbell, E. E., Haddix, M. L., Wall, D. H., & Parton, W. J. 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nature Geoscience **8**(10): 776–779.

Cumming, G. S., Barnes, G., Perz, S., Schmink, M., Sieving, K.E., Southworth, J., Binford, M., Holt, R.D., Stickler, C., Van Holt, T. 2005. An exploratory framework for the empirical measurement of resilience. Ecosystems **8**: 975–987.

Cushman, Ruth C., Jones, Stephen R. 2004. *Peterson Field Guides: The North American Prairie.* New York: Houghton Mifflin Harcourt.

Dai, Aiguo. 2010. Drought under global warming: a review. Wiley Interdisciplinary Reviews: Climate Change **2**: 45-65.

Dalgeish, H.J., Kula, A.R., Hartnett, D.C., Sandercock, B.K. 2008. Responses of two bunchgrasses to nitrogen addition in tallgrass prairie: the role of bud bank demography. American Journal of Botany **95**:672-680.

De Boeck, H. J., Dreesen, F. E., Janssens, I. A., Nijs, I. 2011. Whole-system responses of experimental plant communities to climate extremes imposed in different seasons. New Phytologist **189**: 806–817.

Díaz, S., Lavorel, S., Chapin, F. S., Paula, I. I. I., Diego, A.T., & Karl, E. G. 2005. Chapter 7, Functional Diversity: At the crossroads between ecosystem functioning and environmental filters. In *Terrestrial Ecosystems in a Changing World,* J.G. Canadell, D.E. Pataki, L.F. Pitelka, Eds. Berlin: Springer-Verlag, pp 81-91.

Fargione, J. E., Tilman, D. 2005. Diversity decreases invasion via both sampling and complementarity effects. Ecology Letters **8**: 604-611.

Folke, C., Carpenter, S., Walker, B., Scheffer, M., Elmqvist, T., Gunderson, L., Holling, C.S. 2004. Regime shifts, resilience, and biodiversity in ecosystem management. Annual Review of Ecology, Evolution, and Systematics **35**: 557-581.

Foster, B., Houseman, G., Hall, D., Hinman, S. 2015. Does tallgrass prairie restoration enhance the invasion resistance of post-agricultural lands? Biological Invasions **17**: 3579-3590.

Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., VoRoSmarty, C.J. 2004. Nitrogen cycles: past, present, and future. Biogeochemistry **70**: 123-226.

Gamfeldt, L., Hillebrand, H., Jonsson, P. R. 2008. Multiple functions increase the importance of biodiversity for overall ecosystem functioning. *Ecology*, **89**: 1223–1231.

Gibson, David J. 2009. *Grasses and grassland ecology*. New York: Oxford University Press.

Gleason, H.A. 1926. The individualistic concept of the plant association. Bulletin of the Torrey Botanical Club **53**:1.

Grime, J. P. 1979. *Plant Strategies and Vegetation Processes*. Chichester: Wiley.

Harpole, W.S., Sullivan, L.L., Lind, E.M., Firn, J., Adler, P.B., Borer, E.T., Chase, J., Fay, P.A., Hautier, Y., Hillebrand, H., MacDougall, A.S., Seabloom, E.W., Williams, R., Bakker, J.D., Cadotte, M.W., Chaneton, E.J., Chu, C., Cleland, E.E., D'Antonio, C., Davies, K.F., Gruner, D.S., Hagenah, N., Kirkman, K., Knops, J.M., LaPierre, K.J., McCulley, R.L., Moore, J.L, Morgan, J.W., Prober, S.M., Risch, A.C., Schuetz, M., Stevens, C.J., Wragg, P.D. 2016. Addition of Multiple Limiting Resources Reduces Grassland Diversity. Nature **537**(7618): 93-96.

Hautier et al. 2014. Eutrophication weakens stabilizing effects of diversity in natural grasslands. Nature **508**:521-526.

Heady, H.F., Bartolome, J.W., Pitt, M.D., Savelle, G.D., Stroud, M.C. 1992. California prairie. In *Natural grasslands: introduction and western hemisphere*, R.D. Coupland, Ed. Amsterdam: Elsevier, vol. 8A, pp. 313-335.

Helzer, Chris. 2011. Using defoliation of dominant grasses to increase prairie plant diversity. The Prairie Ecologist. http://prairieecologist.com/2011/03/15/using-defoliation-of-dominant-grasses-to-increase-prairie-plant-diversity (Accessed 8 May 2015).

Helzer, Chris. 2010. *The Ecology and Management of Prairies in the Central United States.* Iowa City, IA: University of Iowa Press.

Holling, C. S. 1973. Resilience and stability of ecological systems. Annual Review of Ecological Systems **4**: 1-23.

Huntly, N.J., Kane, M.D. *NSF Long-term ecological research program description (LTER*). NSF Division of Environmental Biology (Accessed 3 January 2017).

Hutchinson, G.E. 1959. Homage to Santa Rosalia, or why are there so many kinds of animals? The American Naturalist **870**: 145-159.

IPCC. 2014. *Climate Change 2014: Impacts, Adaptations and Vulnerability. Summary for Policymakers.* Cambridge: Cambridge University Press.

Isbell, F. 2011. High plant diversity is needed to maintain ecosystem services. Nature **477**: 199-U96.

Jentsch A, Kreyling J, Beierkuhnlein C. 2007. A new generation of climate change experiments: events, not trends. Frontiers Ecology and Environment **5**:365–74.

Jentsch, A, Grant, K., Nagy, L., Schloter, M., Wo, J., Kreyling, J., Hein, R., Otieno, D., Sing, B.K., Elmer, M., Lara, M., Pritsch, K., Stadler, J., Mirzae, H., Rascher, U. 2011. Climate extremes initiate ecosystem-regulating functions while maintaining productivity. Journal of Ecology **99**: 689-702.

Kallenbach, C. M., Grandy, A., Frey, S. D. 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nature Communications **7**: 13630

Kaul, Robert B., David Sutherland, Steven Rolfsmeier. 2011. *The Flora of Nebraska, 2nd ed.* Lincoln: University of Nebraska Press.

Kreyling, J., Wenigmann, M., Beierkuhnlein, C., Jentsch, A. 2008. Effects of Extreme Weather Events on Plant Productivity and Tissue Die-Back are modified by community composition. Ecosystems **11**:752-763.

Lake, Philip S. 2013. Resistance, Resilience and Restoration. Ecological Management & Restoration **14**: 20-24.

Lal, R. 2004. Soil carbon sequestration to mitigate climate change. Geoderma **123**: 1-22.

Lavorel, S., McIntyre, S., Landsberg, J., Forbes, T.D.A. 1997. Plant functional classifications: from general groups to specific groups based on response to disturbance. Trends in Ecology and Evolution **12**: 474-478.

Lefcheck, J. S., Byrnes, J. E. K., Isbell, F., Gamfeldt, L., Griffin, J. N., Eisenhauer, N., Duffy, J. E. 2015. Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. Nature Communications **6**: 6936.

Li, J., Duggin, J.A., Loneragan, W.A. 2007. Grassland responses to multiple disturbances on the New England Tablelands in NSW, Australia. Plant Ecology **193**:39.

Litzow, M.A., Hunsicker, M.E. 2017. Early warning signals, nonlinearity, and signs of hysteresis in real ecosystems. Ecosphere **7**(12): e01614

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A., Raffaellil, D., Schmid, B., Tilman, D., Wardle, D.A. 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. Science **294**: 5543.

Maestre, F.T., Callaway, R.M., Valladares, F., Lortie, C.J. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. Journal of Ecology **97**:199-205.

MacGillivray, C.W., Grime, J.P., Integrated Screening Program Team. 1995. Testing Predictions of the Resistance and Resilience of Vegetation Subjected to Extreme Events. Functional Ecology **9**:4.

McGranahan, D.A., Brown, P.W., Schulte, L.A., Tyndall, J.C. A historical primer on the US farm bill: supply management and conservation policy. Journal of Soil and Water Conservation **68**(3): 67-73.

Nebraska Game and Parks Commission. 2011. *The Nebraska natural legacy project: State Wildlife Action Plan, 2nd ed.* Lincoln, Nebraska, NGPC.

Nemec, Kristine. 2012. *The relationship between diversity, seeding density, and ecological functions in tallgrass prairie restorations*. Dissertation. University of Nebraska-Lincoln.

NRCS Web Soil Survey. USDA. https://websoilsurvey.nrcs.usda.gov/app/ (Accessed 16 Jan. 2017).

Pimentel D., Burgess, M. 2013. Soil erosion threatens food production. Agriculture **3**:443-463.

Piper, J.K. 2014. Incrementally rich seeding treatments in tallgrass prairie restoration. Ecological Restoration **32**: 396-406.

Price, Katilyn. 2015. Plant diversity affects performance of invasive thistles in restored Nebraska grasslands. Thesis.

Radeloff, Volker C., Williams, John W., Bateman, Brooke L., Burke, Kevin D., et al. 2015. The rise of novelty in ecosystems. *Ecological Applications* **25**: 2051-2068.

Riggs, C.E., Hobbie, S.E. 2016. Mechanisms driving the soil organic matter decomposition response to nitrogen enrichment in grassland soils. Soil Biology and Biochemistry **9**:54-65.

Risser, Paul G. 1995. Biodiversity and Ecosystem Function. Conservation Biology **9**: 742-746.

Samson, F.B., Knopf, F.L., Ostlie, W.R. 2004. Great Plains ecosystems: past, present and future. Wildlife Society Bulletin **32**(1): 6-15.

Sanderston, E.W., Jaiteh, M., Levy, M.A., Redford, K.H., Wannebo, A.V., Woolmfer, G. 2002. The human footprint and the last of the wild. BioScience **52**(10): 891-904.

Scheffer, M., Rinaldi, S. 2000. Minimal models of top-down control of phytoplankton. Freshwater Biology **45**: 265-283.

Scheffer, M., Bascompte, J., Brock, W.A., Brovkin, V., Carpenter, S.R., Dakos, V., Held, H., van Nes, E.H., Rietkerk, M., Sugihara, G.. 2009. Early-warning signals for critical transitions. Nature **461**(3): 53-59.

Schulze, E.D., Mooney, H.A. 1993. *Biodiversity and Ecosystem Function.* New York: Springer-Verlag.

Smith, D., Henderson, K., Houseal, G., Williams, D. 2010. *Tallgrass Prairie Center Guide to Prairie Restoration in the Upper Midwest.* Des Moines: University of Iowa Press.

Stewart, R.I.A., Dossena, M., Bohan, D.A., Jeppesen, E., Kordas, R.L., Ledger, M.E., Meerhoff, M., Moss, B., Mulder, C., Shurin, J.B., Suttle, B., Thompson, R., Trimmer, M., Woodward, G. 2013. Chapter 2: Mesocosm Experiments as a Tool for Ecological Climate-Change Research, In *Advances in Ecological Research*, Woodward, G., O'Gorman, E.J. (Eds.). Elsevier Academic Press **48**:71-181.

Suding, K. N., Ashton, I.W., Bechtold, H., Bowman, W.D., Mobley, M.L., Winkleman, R.W. 2008. Plant and microbe contributions to community resilience in a directionally changing environment. Ecological Monographs **78**: 313-329.

Suding, K. N. 2011. Toward an era of restoration in ecology: successes, failures, and opportunities ahead. Annual Reviews in Ecology and Evolutionary Systems **42**:465-487.

Sutherland, W.J., Freckleton, R.P., Godfray, C.J., Bessinger, S.R., Benton, T., Cameron, D.D., Carmel, Y., Coomes, D.A., Coulson, T., Emmerson, M.C., Hails, R.S., Hays, G.C., Hodgson, D.J., Hutchings, M.J., Johnson, D., Jones, J.P.G., Keeling, M.J., Kokko, H., Kunin, W.E., Lambin, X., Lewis, O.T., Malhi, Y., Mieszkowsda, N., Milner-Gulland, E.J., Norris, K., Phillimore, A.B., Purves, D.W., Reid, J.M, Reuman, D.C., Thompson, K., Travis, J.M.J., Turnbull, L.A., Wardle, D.A.., Wiegand, T. 2013. Identification of 100 fundamental ecological questions. Journal of Ecology **101**: 58-67.

Thrush, S. F., Hewitt, J. E., Dayton, P. K., Coco, G., Lohrer, A. M., Norkko, A., Norkko, J., Chiantore, M. 2009. Forecasting the limits of resilience: integrating empirical research with theory. Proceedings of the Royal Society B **276**: 3209–3217.

Tilman, D., Isbell, F., Cowles, J. M. 2014. Biodiversity and Ecosystem Functioning. Annual Reviews in Ecology and Evolutionary Systems **45**:471-93.

Tilman, D., Peter B. Reich, P.B., Knops, J.M.H. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature **44**:04742.

Tilman, D., Reich, P. B., Knops, J., Wedin, D., Mielke, T., Lehman, C. 2001. Diversity and productivity in a long-term grassland experiment. Science, New Series **294**:5543.

Twidwell, D., Rogers, W.E., Fuhlendorf, S.D., Wonkka, C.L., Engle, D.M., Weir, J.R., Kreuter, U.P., Taylor, C.A. Jr. 2013. The rising Great Plains fire campaign: citizens' response to woody plant encroachment. Frontiers in Ecology and the Environment **11**:e64-e71.

Villnas, A., Norkko, J., Hietanen, S., Josefson, A.B., Lukkari, K., Norkko, A. 2013. The role of recurrent disturbances for ecosystem multifunctionality. Ecology **94**: 2275-2287.

Vogel, A., Scherer-Lorenzen, M., Weigelt, A. 2012. Grassland Resistance and Resilience after Drought Depends on Management Intensity and Species Richness. PLoS One. 7(5): e36992

deVries, W., Goodale, C., Erisman, J.W., Hettelingh, J. 2014. Impacts of Nitrogen Deposition on Ecosystem Services in Interaction with Other Nutrients, Air Pollutants and Climate Change. In *Nitrogen Deposition, Critical Loads, and Biodiversity*, A. Sutton et al., Eds. New York: Springer.

Wall, D. H., Nielsen, U. N., Six, J. 2015. Soil biodiversity and human health, Nature **528**(7580):69-76.

Weaver, J. E. 1965. *Native Vegetation of Nebraska*. Lincoln: University of Nebraska Press.

Wedin, David A. 1999. Nitrogen availability, plant-soil feedbacks and grassland stability. VIth International Rangeland Congress Proceedings **1**: 193-197.

White, R., Murray, S., Rohweder, M. 2000. *Pilot analysis of global ecosystems: grassland ecosystems technical report.* Washington, D.C.: World Resources Institute.

Whittaker, R.H. 1965. Dominance and Diversity in Land Plant Communities. *Science* **147**:3655.

Zavaleta, E. S., Pasari, J. R., Hulvey, K. B., & Tilman, G. D. 2010. Sustaining multiple ecosystem functions in grassland communities requires higher biodiversity. Proceedings of the National Academy of Sciences of the United States of America, **107**: 1443–1446.

V. Tables and Figures

Table 1.1. Ecological traits measured in a study of the relationship between ecosystem diversity, structure, function, and changes in structure and function in response to added disturbances. Measurements of these traits were collected in 2015 and 2016 in a tallgrass prairie restoration in central Nebraska, planted in 60m x 60m plots of low, medium, and high diversity (Fig. 1.1). Disturbances were added to 1m x 2m subplots within rainout shelters. Treatments were rainout shelter only, rainout plus ammonia-nitrate fertilizer addition, rainout plus biomass removal, or a combination of all three.

Ecosystem Structure		**Dates Measured**
1	Plant structure	
	height, cover, bareground	June 2015/16
	community composition	June 2015/16
2	Soil structure	
	soil organic matter, pH, nitrogen content	June 2015/16
	soil microbial biomass	June 2015/16
Ecosystem Function		
1	Plant growth strategy	
	specific leaf area	July 2016
	leaf chlorophyll content	July 2016
2	Nutrient cycling	
	litter decomposition	June-Nov 2015/16
	soil microbial respiration	June 2016
3	Invasion resistance	
	whole-plot surveys for invasive species presence	July 2016
	subplot surveys of invasive species cover	June 2015/16

Figure 1.1. A map of the study site in the Platte River Prairies restoration in central Nebraska. The site is located 10km south of Wood River, NE and was planted in 2010 in twelve 60m x 60m plots of either a Big Bluestem monoculture, a mid-diversity mixture of grasses and forbs, or a high-diversity mix of grasses and forbs. Monoculture plots have established with a few recruited native species and are referred to as low-diversity throughout the study.

CHAPTER 2: PLANT DIVERSITY CONTRIBUTES TO GRASSLAND STRUCTURE AND ITS RESPONSE TO DISTURBANCE

I. Introduction

Vegetation diversity influences grassland ecosystem processes by providing a variety of structural and functional traits (Gibson 2009; Isbell et al. 2011; Kohler et al. 2017). Diversity in plant structure and functioning can increase the number of functions operating simultaneously in a system and the level at which those functions operate, measured as a percent of the potential maximum output for that system (Lefcheck et al. 2016; Diaz et al. 2005). Increased diversity also provides a buffer against losses of system function in response to disturbance via imbrication, as overlapping species traits reduce the likelihood that the loss of one or a few individuals will cause a large change in ecosystem function (Kang et al. 2015). This relationship between biodiversity and ecosystem functioning, known as the Biodiversity-Ecosystem Function hypothesis (Schulze and Mooney 1993; Tilman and Downing 1994) suggests that biodiversity itself is a driving force in maintaining ecosystem continuity.

Biodiversity is a result of multiple environmental 'filters' that constrain the establishment of species, including species dispersal, establishment, and competition for resources (Diaz et al. 2005). Today, seed dispersal is not limited by traditional modes of transport, as human activities can spread propagules from any area of the world to another (Wilson et al. 2015). For example, the grassland restoration plots used in this study were planted by restoration professionals who prepared and sowed a chosen seed mix. Though the majority of seed dispersal was constrained by management choices, outside propagules were not prevented from establishing within the restoration, nor were

seedlings establishing from the existing soil seed bank. Following seed and propagule dispersal, other filters lead to species sorting, as certain plants respond more or less favorably to environmental filters including disturbance, environmental constraints, and competition for resources (Leibold et al. 2004).

Biodiversity is a key variable underlying ecosystem functioning. Experimental research in grasslands indicates that reductions in plant community diversity limit ecosystem multi-functionality (Isbell et al. 2015). Long-term research has shown that grassland plant demography can shift rapidly in response to changes in the environment (Silvertown et al. 2006), and shifts in species dominance may lead to broader changes in nutrient cycling, faunal habitat provisioning, and other system functions (Diaz et al. 2005). Measuring changes in plant community composition can help to predict changes in ecosystem function when the historical community structure and functioning of the system is known (Kohler et al. 2017).

Ground cover is partly influenced by biodiversity, and changes in ground cover can also impact system multi-functionality (Berendse et al. 2015). Very low and very high litter cover may inhibit microbial activity (Gibson 2009), while intermediate levels of litter input (these values are context-specific) provide the nutrients needed to maintain soil functioning without overwhelming the system. Evenly distributed, deep litter reduces soil temperatures, holds in soil moisture, and can enhance seedling establishment in dry hot climates by protecting seeds from herbivory and wide fluctuations in temperature and moisture (Heady et al. 1992), though these effects are highly stochastic and influenced by a range of mitigating factors, including seed size and surface moisture availability (Bascompte and Rodriguez 2000; Gibson 2009). Higher levels of bare ground can result

in greater soil erosion (Lal 2001), large temperature fluctuations at the soil surface, and lower rates of seedling establishment when seeds are exposed to wide temperature and moisture variability.

Functional diversity provides a connection between species diversity and variation in ecosystem function, and can be measured with indexes of both functional richness and functional diversity (Mason et al. 2005). Though various methods for measuring these indices have been proposed (Ricotta et al. 2014), groupings should correspond with significant differences in functional traits. For my study, I used a very broad trait grouping: categorizing species into cool-season grasses, warm-season grasses, and forbs, which correlate with broad differences in phenology and mode of resource acquisition. These categories are commonly used in analyses of plant functional groups (Tilman et al. 1997) and capture relevant variation in traits among species related to ecosystem function.

Increasing biodiversity is positively correlated with a wider range of ecosystem functions, and increased diversity in vegetation structure and functional groupings provides one link between plant species diversity and increased ecosystem function (Diaz et al. 2007). In this study, I test the link between species diversity and measurements of ecosystem structural characteristics, including functional group diversity, the range in vegetation height, bareground, and litter cover and depth. If there is no relationship between diversity and variety of structure and function, but a positive relationship is found between biodiversity and function, this would suggest that functional group and structural diversity are not an important link between biodiversity and function. On the other hand, if there is a positive correlation between species diversity and system

structural and functional diversity, then this relationship could be an important link between species richness and function.

Testing the response of ecosystem structure to experimental manipulations is one method of measuring the relationship between diversity and community structure and functional traits. I applied this strategy to a grassland restoration planted at low and high diversity. I hypothesized that species richness would decline in response to added disturbances due to the selective effects of different disturbances on plant growth strategies, and that the average cover of each functional group would shift depending on the treatment applied (i.e., in favor of more competitive species in response to nitrogen treatments, or more ephemeral, weedy species in biomass removal treatments) (Grime 1979). I also hypothesized that the variation in structure would decline in response to added disturbance. Specifically, I predicted that the range in vegetation heights would decline in response to added disturbances (Hautier et al. 2014) and that bareground would increase across all added disturbances. I predicted that litter cover and depth would vary primarily by year, mainly due to a prescribed burn six weeks before the start of my study which removed all bareground cover, but would vary secondarily by treatment effects. Finally, I hypothesized that soil characteristics would vary mainly by planted diversity; specifically, I predicted that microbial biomass would greater in high-diversity compared with low-diversity plots. Finally, I predicted that there would be a smaller or insignificant response to disturbance across all of measures of ecosystem structure at high diversity compared with low-diversity restoration plots (Isbell et al. 2015).

II. Methods

STUDY SITE

This study took place at the Platte River Prairies, owned by the Nature Conservancy in south-central Nebraska. The site, 10km south of Wood River, Nebraska (40°44'37.8"N 98°35'23.9"), is located within the Central Platte River ecosystem, identified as a Biologically Unique Landscape by the Nebraska Game and Parks Commission (NGPC 2011). Soils at the site include Wann loam, rarely flooded; Caruso loam rarely flooded; and Bolent-Clamux complex, occasionally flooded (NRCS).

In 2010, The Nature Conservancy seeded twelve 60m x 60m plots in native tallgrass prairie, with four plots each of Big Bluestem (*Andropogon gerardii*) monoculture, mid-diversity, and high-diversity seed mixes (Nemec 2013). The diversity treatments have established with significant differences between monoculture and high-diversity species richness (34 vs. 73 species, respectively; Price 2015). The 'monoculture' plots have accumulated a number of additional species and are referred to as low-diversity plots throughout this study. Functional groups represented within the whole-plots include C3 (cool-season) grasses, C4 (warm-season) grasses, and both leguminous and non-leguminous forbs. This grouping is a very broad generalization of growth types and was chosen for ease of categorization and notable differences in growth periods, reproduction, and nutrient acquisition represented by each group, differences which may influence their response to disturbance (Lavorel et al. 1997).

The site is maintained via burning; the most recent burn was in March of 2015, six weeks before the start of the study, with no additional management during the course of this study. The burn removed all biomass cover at the beginning of the research period, and so large differences in vegetation and litter cover are apparent by year.

The research site covers an environmental gradient with increasing soil organic matter (percent weight lost on ignition) from north to south (Figure 2.1). This gradient correlates with other soil chemical and functional traits, including soil respiration (Solvita CO2 Burst test, ppmC) and soil pH (Figure 2.2). There is also a significant gradient in nitrate (ppm, KCL-extractable NO_3^-) from southwest to northeast (Figure 2.3). These gradients were included as fixed effects in statistical analyses.

Precipitation and temperature during the course of this study varied significantly by year. Precipitation in 2015 was much higher than in 2016, with a major peak in June (25cm total, mostly occurring in a single event). 2016 rainfall was more evenly distributed, with the highest rainfall totals in April (16cm) and July (15cm), and low rainfall in other months (Figure 2.4). Average maximum and minimum temperatures were higher in 2016, with sustained higher average temperatures across the spring, summer, and fall (Figure 2.4). The minimum temperatures in January 2015 and 2016 were -19.44ºC and -23.3ºC, respectively, and maximum temperatures in July 2015 and 2016 were 35ºC and 38.3ºC, respectively.

TREATMENTS

In May of 2015, I constructed 2.5 x 5m rainout shelters in the center of each low-diversity and high-diversity research plot. Beneath each shelter, I established four 1m x 2m plots of additional experimental disturbances, with 50cm spacing between each treatment. These disturbance treatments consisted of either no additional treatment, biomass removal (cutting biomass down to 4-8cm height during the first week of July), nitrogen fertilizer addition (30g of inorganic 34-0-0 dry ammonium nitrate fertilizer added twice per summer, first in mid-June and then six weeks following the first

treatment for a rate of 10gNH4NO3/m-1/summer), or a combination of biomass removal and nitrogen addition (Figure 2.5).

Treatments were chosen to represent current threats to grasslands. Rainout shelters were built to impose water stress to the vegetation, which would potentially lower their threshold of resilience to additional disturbances. Drought is expected to be exacerbated in the mid-latitudes where the majority of grassland systems exist in coming decades (IPCC 2014), and in conjunction with increases in summer temperatures poses an increasingly large threat to grasslands (de Boek et al. 2011). Beneath rainout shelters, four 1m x 2m disturbance plots were established with either no additional treatments, nitrogen addition, biomass removal, or a combination of the two. Nitrogen addition (via inorganic nitrogen fertilizer) was used to represent the widespread terrestrial eutrophication arising largely from agricultural drift and fossil fuel burning (Suding et al. 2008; Tilman et al. 2008). Biomass removal simulated haying, a common management strategy in prairies throughout the United States. Haying is considered a management alternative to grazing or fire (Smith et al. 2010), and may alter ecosystem function by removing dominant species at peak growth and reducing the amount of biomass left on the field for subsequent growing seasons.

DATA COLLECTION

Soil collection occurred at two scales across all monoculture and high-diversity whole-plots in early June of 2015 and 2016. Temperatures averaged 19.7^{o}C and 19.4^{o}C on sampling dates, and sampling occurred no less than 48 hours after any rainfall event. At the 60m x 60m plot scale I collected three composite cores, each made up of three sub-samples dug to 20cm with a hand auger. At the 1m x 2m disturbance treatment scale,

along with two 2m x 1m plots near the rainout shelters with no added disturbance treatments, I collected a single composite core made up of three sub-samples. Samples were collected in the first week of June 2015 and 2016.

All soil samples were stored at 2^{o}C prior to analysis. Analyses were conducted by Ward Labs, an agricultural testing lab located in Kearney, Nebraska. Whole-plot cores were analyzed for total soil organic matter (SOM), KCL-extractable nitrate (NO_3^-, ppm), microbial biomass carbon (ppmC), and microbial respiration (ppmC, Solvita Burst test). Subplot cores were analyzed for pH, total soil organic matter, total organic carbon, total organic nitrogen, soil nitrate, and microbial biomass carbon in 2015, and total SOM, nitrate, and microbial respiration and biomass in 2016. Soil organic matter was measured as percent lost on ignition via combustion tests; soil inorganic nitrogen as the ppm KCL-extractable nitrate per sample, and estimated microbial biomass C via a chloroform-extraction method.

Soil moisture was recorded via a hand-held moisture meter which recorded moisture in the top 10cm of soils, and via moisture access tubes dug to 30cm and 50cm in the center of each rainout plot. Soil moisture readings were collected four times in June-August 2015 at the edge, 25cm inside, 50cm inside, and at the center of each subplot as well as within moisture access tubes (Figure 2.6).

Vegetation sampling also occurred at two scales in mid-June of 2015 and 2016. At the 60m x 60m scale, vegetation sampling followed a systematic random-start transect sampling method (Elzinga et al. 1998). Four north-south transects were evenly spaced across each 60m x 60m plot, and eight 50cm x 50cm quadrats were sampled along each transect using a random start and an eight-meter spacing following the random start. This

method was intended to maximize the distance between each quadrat and minime the likelihood of double-sampling large clonal species. At the 1m x 2m plot scale, disturbance treatment plots were also sampled using to a systematic-random design. Each disturbance plot was divided into eight 50cmx50cm quadrats and four of those quadrats randomly selected and surveyed.

Variables recorded for each quadrat include the cover (Daubenmire cover class method, Damgaard 2014), frequency, and range of heights (the tallest and shortest individuals of each species within each quadrat, in cm) for each species. Adults and seedlings were measured separately and adults only used in analysis. Additional measurements collected for each quadrat include percent bare ground, litter cover, and litter depth (Elzinga et al. 1998). Together, these variables allow for a statistical representation of the aboveground community structure.

ANALYSIS

Analysis of plant and soil variables was conducted for both the whole-plot and disturbance treatment scales. At the 60m x 60m scale, quadrat cover values were averaged by transect (four transects, with eight quadrats per transect) using the Daubenmire midpoint cover values (Elzinga et al. 1998). At the disturbance treatment level (2m x 1m), percent cover values were averaged by treatment plot. Scripts and data used for analysis (program R; R Core Team) are included in Appendix I.

Response variables were assessed for variation by the explanatory variables of sampling location, sampling year, diversity treatments, disturbance treatments, and the gradients in soil organic matter and nitrogen. Disturbance treatments were not used as

explanatory variables in 2015, as data was gathered prior to disturbance additions. Analyses for 2016 data included disturbance treatments as explanatory variables.

I first calculated mean bareground, litter cover, and litter depth by diversity (low or high), disturbance treatments, and distance from river, a variable which was strongly related to gradients in both soil pH and soil organic matter. To determine whether any variation among treatment groups was statistically significant, I conducted an ANOVA which included sampling location as a grouping factor to account for random variation by location in the field (Zuur et al. 2007). Finally, to find the variables most predictive of variation in bare ground and litter cover, I constructed a global model including all measured parameters:

[M2] Percent bareground or Litter cover ~ distance from river + diversity + year + biomass removal + nitrogen addition + (1|site),

where distance from river is distance from the edge of the Platte River to the center of each plot in meters, year is the sampling year, and (1|site) a random-effects variable specifying random variation by whole-plot. I began with this global model and used automated stepwise backward selection (*step* function in R), which removes insignificant parameters and reports the best-fit model determined via AICc values, a statistic which compares the goodness-of-fit of models via their explanatory power and the number of parameters used.

Soil microbial biomass carbon, an indicator of total soil microbial biomass, was measured in response to both diversity and disturbance treatments. Soil microbial biomass is one of the few soil variables measured in this study that changes quickly (over

weeks to months) and may provide a link between the relatively rapid changes in plant growth and slower-changing soil characteristics (Schimel et al. 2007; Bach et al. 2012). Soil chemistry is slow-changing relative to the duration of this study (Snapp and Morrone 2008); therefore, average soil organic matter, nitrate, and pH were included as explanatory variables in this study. Variation in soil microbial biomass was tested using the linear contrast model:

[M3] soil microbial C (ppmC) ~ diversity + rainout + distance + biomass removal + nitrogen addition + soil organic matter + kcl-N + (1|block),

where soil microbial C is the average microbial biomass carbon per soil sample, and (1|block) is a random-effects variable accounting for unknown variation by plot row, west to east. I began with this global model and again used stepwise backward selection to remove insignificant parameters and report the best-fit model using AICc values.

Next, I calculated community composition, which included species richness (the number of species per sample) and abundance (percent cover estimated from Daubenmire cover class values) for both large and small-scale measurements. In addition to these species-level diversity measures, I grouped cover midpoint values into six broad categories for an assessment of relative cover by functional type: sown and unsown C3 (cool-season) grasses, sown and unsown C4 (warm-season) grasses, and sown and unsown forbs.

Variation in species richness was measured by sampling year, diversity, and rainout shelter effects using Welch's two-sample T-tests. Next, to calculate variation among group species composition I conducted Bray-Curtis dissimilarity tests on the relative cove values of species and functional groups for both the 60m x 60m and the 1m

x 2m scales. PERMANOVA was used to determine the principal sources of variation among groups determined using the resulting dissimilarity matrices (Buttiegeg and Ramette 2014). The dissimilarity matrices from the species and functional group analyses were used as the response variables, and explanatory variables included year, diversity, disturbance treatments, and soil chemistry variables. Finally, I plotted community data in multi-dimensional space using bounded canonical correspondence, a constrained version of multi-dimensional plotting where group differences are displayed along given sources of variation (variables were chosen from PERMANOVA) (Anderson and Willis 2003; Legendre and Anderson 1999). Species were also grouped by sown/unsown status and functional type (C3 grasses, C4 grasses, forbs), resulting in six broad functional groups, and community composition assessed in the same manner as with individual species.

I also calculated the range in heights for each species in each quadrat, and then compared the range in height by diversity and disturbance treatments using ANOVA. I then calculated the difference in vegetation heights among sampling sites using Bray-Curtis dissimilarity tests. The resulting dissimilarity index values were plotted by diversity and disturbance treatments to visualize the direction and strength of significant parameters on variation in vegetation height.

III. Results

Site characteristics varied primarily by large-scale parameters, including sampling year (correlating primarily with time since fire and summer rainfall), diversity level, and existing soil gradients. Bareground and litter cover varied mostly with the large-scale variables of distance from river, year, and diversity treatments. In contrast with my initial hypotheses, no experimental treatment had a measurable effect on bareground or litter

cover. Bareground was responsive to only one significant predictor - distance from river, which was not included in my initial hypotheses. This result may be a result of the variation in soil organic matter and nitrogen content, as soils near the Platte were sandier and had lower moisture content than plots further from the river (Figure 2.6). Soil microbial biomass also varied mainly by existing site gradients, with soil organic matter positively correlated with microbial biomass.

Variation in vegetation height and species composition (species richness and percent cover) varied significantly by diversity, as expected due to the initial biodiversity plantings. The range in vegetation height, calculated only for 2016, varied significantly by diversity and marginally significantly by rainout shelter effects, with a larger range in vegetation height at high diversity and a lower range in vegetation height beneath rainout shelters at both low and high diversity. Community composition varied significantly by both year and diversity at the whole-plot scale, and at the disturbance-addition subplot scale added disturbance treatments were not associated with variation in community composition, diversity, or evenness. Functional group diversity was also predicted mainly by large-scale effects, which included distance from river, diversity treatments, site nitrogen, and rainout shelters providing the majority of variation.

Structure and soil gradients

Year and diversity were the largest sources of variation for bare ground and litter cover. In 2015, bareground averaged 20% at low diversity and 0.85% in high-diversity plots. In 2016, bareground averaged 0.33% for high diversity and 11% for low-diversity plots (Table 2.1). Within sampling years, there was no significant variation by any parameter in 2015, while in 2016 both bareground and litter cover varied by diversity.

This is primarily due to the prescribed burn in March of 2015 which removed all ground cover. ANOVA analysis of variation among treatments in 2016 found that the only significant differences in bareground in 2016 were among treatments ($p=0.01$) in high diversity 2m x 1m treatment subplots, with no single treatment predicting this difference (p values all > 0.1).

The best model describing variation in bareground, chosen via backward selection from [M2], was:

[M4] Percent bareground ~ distance from river + year + (1|site),

Year had the largest effect on bareground. Distance from river was a significant parameter in 2016, with increasing bareground correlated with distance from the Platte River (Figure 2.7). Both nitrogen addition and diversity treatments were marginally significant predictors of bareground in 2016 ($p=0.07$ and $p=0.058$, respectively), with nitrogen addition associated with higher bareground and high diversity associated with lower bareground.

Litter cover showed opposite variation from bareground. In 2015, litter cover was nearly zero for all treatments and diversity levels, while in 2016 litter cover was very high among all treatments and diversity levels (Table 2.2). Litter depth also changed by year. In 2015, litter depth did not vary significantly from zero, while in 2016 average litter depth averaged around 32cm in low-diversity and 36cm in high-diversity plots. This variation by year is largely due to the prescribed burn in March 2015 which removed nearly all litter that year. No single factor was predictive of percent litter cover, as the best model chosen using backwards selection from the global model [M2] was:

[M5] Litter cover ~ distance from river + diversity + year + (1|site)

where 'Litter cover' represents the average litter cover, excluding quadrats where it was not measured. My results suggest that litter cover is a much more stochastic variable than bareground (Bascompte and Rodriguez 2011), with more parameters and interactions remaining in the model, and thus more potential sources of variation.

The best model describing variation in soil microbial biomass, chosen through stepwise backward selection to find the best-fit model, was:

[M5] soil microbial C ~ diversity + distance + (1|block).

Soil microbial biomass varied significantly with diversity and distance from river, with no other predictive variables. Soil samples were collected in mid-diversity as well as the low and high diversity plots for whole-plot measurements. Microbial biomass C in mid-diversity treatments did not significantly vary from the group mean. Increasing distance from river was significantly associated with decreasing microbial biomass carbon ($p=0.015$), in contrast with overall soil organic matter which increased with distance from river.

Plant diversity and community composition

At the whole-plot scale, species richness and diversity varied significantly by diversity treatment and by rainout shelter presence, but not by year. A Welch's two-sample t-test indicated a large difference in average species richness by diversity (an average of 5.78 species in low-diversity and 21.45 species in high-diversity quadrats, $p=<2.2e-16$). Variation by rainout shelter effects was also significant – in high-diversity plots, whole-plot samples averaged 14 species per transect while rainout shelters

averaged 11 species per subplot. This difference is likely due to a difference in observational scales (entire plots versus 2.5m x 5m rainout shelter units), and could be a source of variation in functional traits measured that were observed within rainout shelters.

The species with the highest average cover across all quadrats was the C4-grass *Andropogon gerardii*, as expected due to its high dominance in tallgrass prairie and in the planting mix used for low-diversity plots. Though *Andropogon gerardii* was dominant at both low and high diversity, it was less dominant in high-diversity plots as other C4 grasses provided competition (Tables 2.3 and 2.4 for species occurrence and average cover). The native forbs from the sunflower and goldenrod genuses were also dominant across all plots, including low-diversity plots initially planted as monocultures.

The average range in height varied significantly by diversity level, with marginally significant effects of rainout shelters. No other treatment or site parameters were significant (Table 2.5). Within disturbance-treatment subplots, evenness in vegetation did not vary significantly by any experimental treatment, including diversity. This lack of variation among disturbance treatments indicates that the underlying source of variation in vegetation height is diversity, and to a lesser extent rainout shelter effects.

For community composition, the dominance of large-scale site effects was also apparent. Bray-Curtis dissimilarity index values revealed that community composition differed most strongly by planted diversity. A PERMANOVA (permutational ANOVA run on non-parametric distance data, such as the Bray-Curtis matrix) (Buttiegeg and Ramette 2014) successfully identified significant sources of variation, explaining about 63% of the overall variation in community composition. Significant parameters were

diversity, year, rainout shelter effects, distance from river, and site nitrogen (Table 2.6). These explanatory variables were used to perform plotting of community composition in multidimensional space using a bounded technique (canonical correspondence analysis; Anderson and Willis 2003) (Figure 2.8). Within disturbance-treatment subplots, no treatment was predictive of variation in species richness or evenness, and species evenness values did not differ significantly in response to any diversity or disturbance treatment.

For species functional groups, the most important explanatory variables were again large-scale factors, with diversity, year, rainout shelter effects, distance from river, and site nitrogen all significant parameters (Figure 2.9). Year was the largest single factor determining community composition, explaining 73% of the variation in functional composition. With year providing the bulk of variation, the explanatory variables of all significant variables together account for 83% of the variation in functional community composition (Table 2.7). At the added-disturbance subplot scale no treatment variable provided significant variation.

IV. Discussion

To measure the impact of biodiversity in mediating the response of a tallgrass prairie restoration to added disturbances, indices of vegetation structure and diversity were collected and compared by a number of possible explanatory variables. Variation in these indicators represent the confluence of several interacting factors operating at the whole-plot and disturbance-addition scales. At the whole-plot scale, changes in vegetation structure and diversity were related to the known parameters of site management, including planted diversity and periodic maintenance, including prescribed

burning. They also represent the known but uncontrolled factors of year and an underlying soil chemistry gradient corresponding with distance to the Platte River.

Disturbance treatments added another source of known, controlled variation within the larger-scale variation of planted diversity. The addition of rainout shelters, biomass removal, and nitrogen addition represent known climatic and land management factors that affect natural grasslands today (Kohler et al. 2017). Droughts of increasing length and intensity are expected to affect mid-latitudes in the coming century (Scheffield and Wood 2008), and ongoing eutrophication from fossil fuel burning and conventional agriculture continually affects grassland structure and function (Tilman et al. 2014; Harpole et al. 2014). Biomass removal represents the use of grasslands for hay production, which may unintentionally shift vegetation structure to favor more fast-growing species and may support and hinder grassland function.

Time was a significant source of variation across most response variables, with sampling year standing in for time since fire and variation in summer rainfall. This was especially notable in the ground cover variables of bareground and litter cover. The shift in ground cover across diversity levels and treatments from 2015 to 2016 represents the effect of site maintenance (in this case, prescribed burning) which maintains grassland structure and function in the absence of natural disturbance (Twidwell et al. 2013). Another source of variation captured by the 'year' factor was variance in weather pattern. 2015 had higher than average rainfall for the season, which stimulated a large amount of biomass growth. Biomass was not removed via fire or any other treatment in 2016, leading to very low bareground and extensive, deep litter cover in 2016.

The site soil organic matter and nitrogen gradients were also notable. Site nitrogen

varied more strongly from west to east than simply by distance from river, making this gradient distinct from the distance from river, soil organic matter, and soil pH variables. Soil microbial biomass did not vary significantly by the site nitrogen variable, but was positively related to soil organic matter. It is not surprising that soil microbial biomass is correlated with the organic matter gradient, as microbial detritus is a contributor to SOM (Kallenbach et al. 2016; Cotrufo et al. 2015). However, this data does provide corroboration for the relationship between microbial abundance and soil organic matter (Bradford et al. 2013) in a large-scale field setting with much more uncontrolled variation than typical mesocosm tests.

Community composition for individual-species and functional-group measurements varied mainly by diversity, year, and in the case of functional groupings, average soil nitrate. Rainout shelter effects were also a source of variation in species diversity and evenness. While these effects could be a result of increased water stress, the lower species diversity and increased species evenness measured in these plots may simply be an artifact of the smaller observational scale. Within rainout shelters, no treatment caused significant variation. Site inorganic nitrate levels, averaged by sampling plot, played a small but significant role in shaping plant community structure across diversity levels. From this data, it appears that species composition does respond to average soil nitrogen at the site level and to a lesser extent to added nitrogen (ammonia) treatments.

The lack of variation in community composition by added disturbances may be explained by one or both of the following hypotheses: first, there might be some complementary interactions occurring at both the low and high diversity systems that

provide a general resistance to added disturbances at both low and high diversity. Second, the size and intensity of experimental disturbance additions in comparison with the size of the whole-plots may have been too small to create notable impacts because the surrounding, relatively undisturbed vegetation was able to take advantage of the disruptions introduced at the disturbance-plot scales and compensate for any change within the disturbance addition subplots. Current research is mixed in its support of these hypotheses. Some research suggests that relatively rapid and long-term responses can occur in response to even low levels of eutrophication (Harpole et al. 2014; Tilman et al. 2014), which suggests that the relatively small scale or intensity of my disturbance additions is not necessarily the reason for the lack of variation in my field study. Other studies support the idea that inherent system properties arising from species interactions support the maintenance of system structure in the face of disturbance (Jentsch et al. 2013; Isbell et al. 2011), which supports my hypothesis that at both levels of diversity there were complementary interactions among species that limited the effects of disturbance treatments.

Implementing experimental disturbances within larger restoration plots was an attempt to scale up mesocosm studies showing the impact of biodiversity and community resistance to disturbance to a larger, less controlled field setting. The results of this study indicate that even at low diversities, the established community structure at large scales is fairly resistant to change from targeted disturbances and likely requires more prolonged or intense disturbances to exhibit a measurable structural response. This lack of variation may have been partly the result of low replication (n=8) which likely affected our ability to discern patterns among disturbance treatments if they did indeed exist. While studies

of community response at small scales and low levels of diversity show fairly rapid community-level responses (as in Zavaleta et al. 2010), those effects appear to be mitigated when disturbances are implemented in the center of large, well-established plant communities with a higher resistance to change.

V. Conclusion

A central hypothesis motivating this study is that diversity in vegetation structure and functional groupings provides an intermediate step connecting plant species diversity to the maintenance of ecosystem functions over time. The data collected in this study indicate that planted diversity correlates with a significant amount of variation in several measures of ecosystem structure which may impact functioning, including the amount of bareground (with lower bareground at high diversity), range in vegetation heights (with greater variety in vegetation height at high diversity), and functional diversity (with more functional groups represented at high diversity). Notably, none of these variables responded to added disturbances, with the exception of rainout shelters, which served to simplify community diversity and narrow the range of vegetation heights. The large variation these parameters at both diversity levels in response to large-scale, uncontrolled factors including year and soil gradients, shows that species diversity and structure is responsive to environmental gradients, and this responsiveness may have an effect on the functional capacity of the system.

If diversity and community structure are, in fact, significant drivers of ecosystem function, and not driven by only a few dominant species, we can predict from these results that there is likely to be very little variation in functional traits measured due to added disturbances, but larger amounts of variation by diversity, time since disturbance,

and site gradients. If variation in community structure and composition is not a significant factor driving ecosystem function, then we might in fact find large differences in measurements of functional traits independent of the small or nonexistent variation in species and functional groupings measured in this study.

V1. Literature Cited

Anderson, M. J., & Willis, T. J. 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology **84**:511-525.

Bach, E. M., Baer, S. G., Six, J. 2012. Plant and Soil Responses to High and Low Diversity Grassland Restoration Practices. Environmental Management **49**:412-424.

Bascompte, J. and Rodríguez, M.A. 2000. Self-disturbance as a source of spatiotemporal heterogeneity: the case of the tallgrass prairie. Journal of Theoretical Biology **204**(2), 153–164.

Berendse, F., van Ruijven, J., Jongejans, E., Keesstra, S. 2015. Loss of plant species diversity reduces soil erosion resistance. Ecosystems **18**: 881-888.

Borcard, D., Gillet, F., Legendre, P. 2011. *Numerical Ecology with R*. Springer, New York.

Bradford, M. A., Keiser, A. D., Davies, C. A., Mersmann, C. A., & Strickland, M. S. 2013. Empirical evidence that soil carbon formation from plant inputs is positively related to microbial growth. Biogeochemistry **113**:271–281.

Buttigieg PL, Ramette A. 2014. A Guide to Statistical Analysis in Microbial Ecology: a community-focused, living review of multivariate data analyses. FEMS Microbiol Ecol. **90**: 543–550.

Clark. M., Tilman, David. 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature Letters **451**:7.

Cotrufo, M. F., Soong, J. L., Horton, A. J., Campbell, E. E., Haddix, M. L., Wall, D. H., & Parton, W. J. 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nature Geoscience **8**:776–779.

Damgaard, C. 2014. Estimating mean plant cover from different types of cover data: a coherent statistical framework. Ecosphere, **5**: 1–7.

De Boeck, H. J., Dreesen, F. E., Janssens, I. A., Nijs, I. 2011. Whole-system responses of experimental plant communities to climate extremes imposed in different seasons. New Phytologist **189**: 806–817.

Díaz, S., Lavorel, S., Chapin, F. S., Paula, I. I. I., Diego, a T., & Karl, E. G. 2005. Chapter 7, Functional Diversity: At the crossroads between ecosystem functioning and environmental filters. In *Terrestrial Ecosystems in a Changing World*, J.G. Canadell, D.E. Pataki, L.F. Pitelka, Eds. Berlin: Springer-Verlag, pp 81-91.

Elzinga, C.L., Salzer, D.W., Willoughby, J.W. 1998. *Measuring and monitoring plant populations.* U.S. Bureau of Land Management Papers **17**.

Gibson, David J. 2009. *Grasses and grassland ecology*. Oxford Univ. Press: New York.

Grime, J. P. 1979. *Plant Strategies and Vegetation Processes*. Wiley: Chichester.

Harpole, W. Stanley, Lauren L. Sullivan, Eric M. Lind, Jennifer Firn, Peter B. Adler, Elizabeth T. Borer, Jonathan Chase, et al. 2016. Addition of Multiple Limiting Resources Reduces Grassland Diversity. Nature **537**: 93-96

Hautier et al. 2014. Eutrophication weakens stabilizing effects of diversity in natural grasslands. Nature **508**:521-526.

Heady, H.F., Bartolome, J.W., Pitt, M.D., Savelle, G.D., Stroud, M.C. 1992. California prairie. In *Natural grasslands: introduction and western hemisphere*, R.D. Coupland, Ed. Vol. 8A, pp. 313-335. Amsterdam: Elsevier.

IPCC. 2014. *Climate Change 2014: Impacts, Adaptations and Vulnerability. Summary for Policymakers.* Cambridge: Cambridge University Press.

Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W. S., Reich, P. B., Scherer-Lorenzen, M., Schimel, B., Tilman, D., van Ruijven, J., Weigell, A., Wilsev, B.J., Zavaleta, E.S., Loreau, M. 2011. High plant diversity is needed to maintain ecosystem services. Nature **477**(7363): 199–202.

Isbell, F., Craven, D., Connolly, J., Loreau, M., Schmid, B., Beierkuhnlein, C., Bezemer, T.M., Bonin, C., Bruelheide, H., de Luca, E., Ebeling, A., Griffin, J.N, Guo, Q., Hautier, Y., Hector, A., Jentsch, A., Kreyling, J., Lanta, V., Manning, P., Meyer, S.T., Mori, A.S., Naeem, S., Niklaus, P.A., Polley, H.W., Reich, P.B., Roscher, C., Seabloom, E.W., Smith, M.D., Thakur, M.P., Tilman, D., Tracy, B.F., van der Putten, W.H., van Ruijven, J., Weigelt, A., Weisser, W.W., Wilsey, B., Eisenhauer, N. 2015. Biodiversity increases the resistance of ecosystem productivity to climate extremes. Nature **526**(7574): 574–577.

Jentsch, A., Grant, K., Nagy, L., Schloter, M., Wo, J., Kreyling, J., Hein, R., Otieno, D., Sing, B.K., Elmer, M., Lara, M., Pritsch, K., Stadler, J., Mirzae, H., Rascher, U. 2011. Climate extremes initiate ecosystem regulating functions while maintaining productivity. Journal of Ecology **99**: 689-702.

Kallenbach, C. M., Grandy, A., & Frey, S. D. 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nature Communications **7**: 13630.

Kang, S., Ma, W., Li, F.Y., Zhang, Q., Niu, J., ding, Y., Han, F., Sun, X. 2015. Functional diversity instead of species redundancy determines community stability in a typical steppe of inner Mongolia. PLoS One **10**(12): e0145605.

Kohler, M., Devaux, C., Grigulis, K., Leitinger, G., Lavorel, S., Tappeiner, U. 2017. Plant functional assemblages as indicators of the resilience of grassland ecosystem service provision. Ecological Indicators **73**:118-127

Lal, R. 2016. Soil degradation by erosion. Land Degradation & Development **12**: 519–539.

Lavorel, S., McIntyre, S., Landsberg, J., Forbes, T.D.A. 1997. Plant functional classifications: from general groups to specific groups based on response to disturbance. Trends in Ecology and Evolution **12**: 474-478.

Lefcheck, J. S., Byrnes, J. E. K., Isbell, F., Gamfeldt, L., Griffin, J. N., Eisenhauer, N., Duffy, J. E. 2015. Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. Nature Communications **6**: 6936.

Legendre, P., Anderson, M.J. 1999. Distance-Based Redundancy Analysis: Testing Multispecies Responses in Multifactorial Ecological Experiments. Ecological Monographs **16**:1.

Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., Holt, R.D., Shurin, J.B., Law, R., Tilman, D., Loreau, M., Gonzalez, A. 2004. The metacommunity concept: a framework for multi-scale community ecology. Ecology Letters **7**:601-613.

Mason, N.W.H., Mouillot, D., Lee, W.G., Wilson, J.B. 2005. Functional richness, functional evenness and functional divergence: the primary components of functional diversity. Oikos **111**: 112-118.

McArdle, B.H. and M.J. Anderson. 2001. Fitting multivariate models to community data: A comment on distance-based redundancy analysis. Ecology **82**: 290–297.

Nebraska Game and Parks Commission. 2011. *The Nebraska natural legacy project: State Wildlife Action Plan, 2nd ed.* Lincoln, Nebraska, NGPC.

NRCS Web Soil Survey. USDA. https://websoilsurvey.nrcs.usda.gov/app/. (Accessed 16 Jan. 2017).

Price, Katilyn. 2015. *Plant diversity affects performance of invasive thistles in restored Nebraska grasslands*. Thesis.

Ricotta, C., Celesti-Grapow, L., Kuhn, I., Rapson, G., Pysek, P., LaSorte, F.A., Thompson, K. 2014. Geographical constraints are stronger than invasion patterns for European urban floras. PLoS One **9**: e85661.

Schimel, J., Balser, T. C., Wallenstein, M., Wallenstein, M. 2007. Microbial Stress-Response Physiology and Its Implications for Ecosystem Function. Ecology **88**: 1386–1394.

Schulze, E.D., Mooney, H.A. 1993. *Biodiversity and Ecosystem Function.* New York: Springer-Verlag.

Sheffield, J., & Wood, E. F. 2008. Projected changes in drought occurrence under future global warming from multi-model, multi-scenario, IPCC AR4 simulations. Climate Dynamics **31**(1), 79–105.

Silvertown, J., Poulton, P., Johnston, E., Edwards, G., Heard, M., Biss, P.M. 2006. The park grass experiment 1856-2006: its contribution to ecology. Journal of Ecology **94**:801-814.

Smith, D., Henderson, K., Houseal, G., Williams, D. 2010. *Tallgrass Prairie Center Guide to Prairie Restoration in the Upper Midwest.* Des Moines: University of Iowa Press.

Snapp, S.S. and Morrone, V.L. Soil Quality Assessment. In *Soil Science: A Step-by-Step Field Analysis*. Logsdon, S., Clay, D., Moore, D., Tsegaye, T., Eds. Soil Science Society of America.

Suding, K. N. 2011. Toward an Era of Restoration in Ecology: Successes, Failures, and Opportunities Ahead. Annual Reviews in Ecology and Evolutionary Systems **42**:465-487.

Tilman, D., Downing, J.A. 1994. Biodiversity and stability in grasslands. Nature **367**: 363-365.

Tilman, D., Isbell, F., Cowles, J. M. 2014. Biodiversity and Ecosystem Functioning. Annual Reviews of Ecological Systems **45**:471-93.

Twidwell, D., Rogers, W.E., Fuhlendorf, S.D. , Wonkka, C.L., Engle, D.M., Weir, J.R., Kreuter, U.P., Taylor, C.A. Jr. 2013. The rising Great Plains fire campaign: citizens' response to woody plant encroachment. Frontiers in Ecology and the Environment **11**:e64-e71.

Wilson, J.R.U., Dormontt, E.E., Prentis, P.J., Lowe, A.J., Richardson, D.M. 2009. Something in the way you move: dispersal pathways affect invasion success. Trends in Ecology and Evolution **24**(3): 136-144.

Zuur, A.F., Ieno, E.N., Smith, G.M. 2007. *Analyzing Ecological Data.* New York: Springer Science.

VII. Tables and Figures

TABLES

Table 2.1. Average percent bareground, measured via quadrat-transect surveys for the Platte River Prairies research site. Bareground is summarized by year (2015, 2016) and diversity(1=low diversity, 2=high diversity). n=number of quadrats in which bareground was observed, mean, sd, and se are all % values. Summary statistics are calculated across all quadrats, including those where no bareground was observed. The large difference in bareground *n* by year is primarily due to a prescribed burn in March 2015 which removed nearly all litter cover from previous years.

diversity	*year*	*n*	*mean*	*sd*	*se*
1	2015	229	19.42	1.244	1.16
1	2016	17	0.84	5.22	0.05
2	2015	158	11.39	16.00	1.06
2	2016	22	0.33	1.55	0.05

Table 2.2. Average percent litter cover (*mean_cover*) and average maximum litter depth(*mean_depth*, cm) from quadrat-transect surveys a the Platte River Prairies research site. Litter cover and depth is summarized here by year (2015, 2016) and diversity (1=low diversity, 2=high diversity). n=number of quadrats where litter cover was observed. Mean cover, sd, and se are percent values. Mean depth, sd, and se are in cm. Litter cover varied significantly between years due to a prescribed burn in March 2015 which removed nearly all litter cover and standing biomass from the field.

diversity	*year*	*n*	*mean_cover*	*sd*	*se*	*mean_depth*	*sd*	*se*
1	2015	24	2.17	12.43	0.82	18.46	31.05	6.34
1	2016	210	88.20	10.61	0.70	22.38	7.24	0.49
2	2015	13	0.31	1.78	0.11	7.154	20.40	5.66
2	2016	213	85.97	20.99	1.40	25.21	9.087	0.62

Table 2.3. Species frequency and percent cover where present for adults surveyed in 2016 at the Platte River Prairies restoration site. Frequency is the number of quadrats in which the species appeared (out of 224 50cm x 50cm quadrats), and average cover is the percent canopy cover, estimated from Daubenmire cover class values, in quadrats where the species occurs.

Taxa (Adults)	*Frequency*	*Average cover*
A1	1	2.50000
Ambrosia_artemisifolia	1	15.000000
Asclepias_sp	1	37.500000
Asclepias_verticillata	1	15.000000
Calamagrostis_canadensis	1	2.500000
Chenopodium_alban	1	2.500000
Cirsium_arvense	1	15.000000
Desmodium_canadense	1	14.166667
Echinacea_purpurea	1	2.500000
Elylmus_trachycaulus	1	8.897849
Elymus_virginicus	1	15.000000
Unid_Forb1	1	15.000000
Unid_Bunchgrass1	1	15.000000
Helianthus_petiolaris	1	2.500000
Hordeum_jubatum	1	37.500000
Melilotus_albus	1	2.500000
Muhlenbergia_racemosa	1	15.000000
Onosmodium_molle	1	2.500000
Penstemon_gracilis	1	15.000000
Physalis_virginiana	1	2.500000
Plantago_patagonica	1	2.500000
Rumex_crispus	1	15.000000
Shizacyrium_scoparium	1	15.000000
Thlaspi_arvense	1	2.500000
Trifolium_campestris	1	2.500000
Unid_Leguminous	1	15.000000
Unid_Herbaceous_Forb	1	15.000000
Unid_Herbaceous_Forb	1	2.500000
Unid_C3grass	2	2.500000
Bromus_inermis	2	8.750000

Capsella_bursa_pastoris	2	8.750000
Conium_maculatum	2	8.750000
Helianthus_grosseserratus	2	8.750000
Hyperium_perforatum	2	2.500000
Ulmus_sp	2	8.750000
Unid_Forb2	2	8.750000
Ambrosia_trifida	3	10.833333
Bromus_japonicus	3	6.666667
Cirsium_vulgare	3	10.833333
Conyza_canadensis	3	2.500000
Helianthus_serriola	3	10.833333
Penstemon_grandiflorus	3	2.500000
Verbena_hastata	3	10.833333
Dalea_candida	4	10.312500
Desmathus_illinoense	4	8.750000
Erigeron_annuus	4	8.750000
Unid_Forb3	4	11.250000
Unid_Bunchgrass2	4	12.812500
Poa_compressa	4	5.625000
Solanum_sp	4	5.625000
Acer_sp	5	10.000000
Physalis_longifolia	5	2.500000
Chamaecrista_fasciculata	6	2.500000
Dalea_purpurea	6	10.416667
Desmodium_illinoense	7	12.916667
Eupatorium_altissimum	7	18.214286
Silphium_integrifolium	7	24.821429
Symphyotrichum_novae_angliae	7	16.428571
Coreopsis_tinctoria	8	5.625000
Unid_Forb4	8	15.937500
Lythrum_salicaria	8	5.989583
Melilotus_sp	9	12.175926
Carex_gravida	10	17.916667
Chenopodium_pratericola	10	5.000000
Heliopsis_helianthoides	10	13.000000
Asclepias_syriaca	11	7.045455
Helianthus_annuus	11	4.204545
Ciralt_seed	12	10.625000
Cornus_sp	12	5.625000
Koeleria_macrantha	12	15.104167

Verbena_stricta	12	5.104167
Lactuca_sp	13	7.147436
Sonchus_arvensis	13	6.346154
Ambrosia_psilostachya	14	6.071429
Poa_pratensis	15	23.000000
Eragrostis_trichodes	16	15.000000
Glycyrrhiza_lepidota	16	20.091146
Rudbeckia_hirta	16	3.671875
Astragalus_canadensis	17	16.813725
Pascopyrum_smithii	18	7.638889
Penstemon_digitalis	20	29.250000
Artemisia_ludoviciana	21	26.645692
Sporobolus_compositus	22	11.931818
Symphyotrichum_lanceolatum	23	6.847826
Sphenopholis_obtusata	24	4.236111
Solidago_pauciflorus	30	10.541667
Elymus_trachycaulus	31	8.897849
Solidago_missouriensis	33	12.438131
Helianthus_pauciflorus	35	11.946429
Ratibida_columnifera	37	8.984234
Cirsium_altissima	40	10.864583
Carex_brevior	43	12.199612
Schizacyrium_scoparium	47	17.273936
Aster_ericoides	50	11.437500
Panicum_virgatum	51	13.819444
Lotus_unifoliatus	56	7.934311
Monarda_fistulosa	60	18.623115
Helianthus_maximiliani	64	16.129557
Achillea_millefolium	65	15.841117
Setaria_sp	67	8.132196
Sorgastrum_nutans	67	20.662402
Elymus_canadensis	69	12.345411
Taraxacum_officinale	72	6.168981
Solidago_gigantea	75	13.200273
bare	96	14.961589
litter	99	73.709115
Solidago_canadensis	108	18.278715
Andropogon_gerardii	145	41.251090

Table 2.4. Species frequency and percent cover for seedlings surveyed in 2016. Frequency is the number of quadrats out of 224 total in which seedlings were counted. Seedling frequency was low in comparison with adults, and seedlings were not included in data analysis due to small sample size.

Taxa (seedlings)	Frequency	Average cover
Ambtri_seed	1	2.500000
Asla_seed	1	2.500000
Assyr_seed	1	2.500000
Bromus_seed	1	2.500000
Chamfasc_seed	1	2.500000
Chenalb_seed	1	2.500000
Callirhoe_involucrata	1	2.500000
Conmac_seed	1	2.500000
Desmoill_seed	1	2.500000
Eltra_seed	1	2.500000
Erigerann_seed	1	2.500000
forb_seed	1	2.500000
Hehe_seed	1	2.500000
Hepet_seed	1	2.500000
Pasmi_seed	1	2.500000
Pendi_seed	1	2.500000
Pengran_seed	1	15.000000
Pruvulg_seed	1	2.500000
Tricamp_seed	1	2.500000
Verbatha_seed	1	2.500000
Asteric_seed	2	2.500000
Cortinct_seed	2	2.500000
Hean_seed	2	2.500000
Physavir_seed	2	2.500000
Silin_seed	2	8.750000
unk_seedlings	2	2.500000
Vestri_seed	2	2.500000
Ambart_seed	3	2.500000
Cornus_seed	4	2.500000
Lysal_seed	4	6.666667
Poa_seed	4	2.500000
Soncharv_seed	4	5.625000
Ambpsi_seed	5	7.500000
Elyca_seed	5	6.000000

Glyle_seed	6	2.500000
Lotun_seed	6	2.500000
Sonu_seed	6	12.708333
Achmil_seed	7	4.285714
Rudhi_seed	7	2.500000
Setaria_seed	7	2.500000
Meli_seed	8	3.802083
Ciralt_seed	12	10.625000
seedlings	12	4.062500
Sopa_seed	13	5.865385
Hepa_seed	14	3.392857
Chenoprat_seed	15	3.035714
Hemax_seed	15	6.500000
Sogi_seed	16	5.494792
Somi_seed	16	5.234375
Ange_seed	21	9.895692
Taof_seed	26	4.302885
Mofi_seed	33	3.952020
Soca_seed	34	4.240196

Table 2.5. PERMANOVA results for variance in plant community height ranges (measured as the difference between shortest and tallest individuals of each species) by treatment type, calculated from Bray-Curtis dissimilarity index. The BC index was conducted on a community height matrix excluding seedlings. Significant correlations marked with *. Diversity and rainout shelters are the only sources of variation among treatments, together explaining 35% of the variation in vegetation height.

adonis(formula = ht.dist ~ diversity + rainout + rain + biomass + nitro, data = ht.meta, permutations = 999, strata = ht.meta$site)

Permutation: free
Number of permutations: 999
Terms added sequentially (first to last)

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
diversity	1	6.0942	6.0942	35.495	0.31097	0.001 ***
rainout	1	0.7866	0.7866	4.581	0.04014	0.001 ***
rain	1	0.0777	0.0777	0.452	0.00396	0.951
biomass	1	0.1652	0.1652	0.962	0.00843	0.402
nitro	1	0.1117	0.1117	0.650	0.00570	0.753
Residuals	72	12.3619	0.1717		0.63080	
Total	77	19.5972			1.00000	

Table 2.6. PERMANOVA results for variation in community composition calculated via Bray-Curtis dissimilarity index, for all quadrats sampled in 2015 and 2016. Significant correlations marked with an *. Diversity and year together explain over 55% of the total variance in species composition, while rainout shelter effects, distance from river, and site nitrogen level explain a small but significant portion of variation (1.37%, 1.55%, and 2.47%, respectively. Individual treatments of nitrogen addition and biomass removal were not significant predictors of species composition. The model is overall fairly successful, explaining roughly 62% of community variation along five main axes, and over 50% of the variation along two main parameters.

adonis(formula = a.comm.bc ~ diversity + year + rainout + biomass + nitro + dist + n + om, data = meta, permutations = 999, strata = meta$site)

Blocks: strata
Permutation: free
Number of permutations: 999
Terms added sequentially (first to last)

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
diversity	1	8.9902	8.9902	106.520	0.28941	0.001	***
year	1	8.6065	8.6065	101.974	0.27706	0.001	***
rainout	1	0.4255	0.4255	5.041	0.01370	0.002	**
biomass	1	0.1500	0.1500	1.778	0.00483	0.114	
nitro	1	0.0303	0.0303	0.359	0.00098	0.921	
dist	1	0.4440	0.4440	5.261	0.01429	0.001	***
n	1	0.7696	0.2565	3.040	0.01555	0.001	***
om	1	0.1567	0.1567	1.850	0.00505	0.001	***
Residuals	138	11.6470	0.0844		0.37912		
Total	147	31.0632			1.00000		

Figure 2.1. Gradient in soil organic matter (SOM, percent mass lost on ignition) measured from three composite soil cores collected at the 60m x 60m plot scale at the Platte River Prairies research prairie in early June 2016. SOM is generally higher nearer the road to the south and lower near the Platte River to the north. Darker shades indicate higher SOM values.

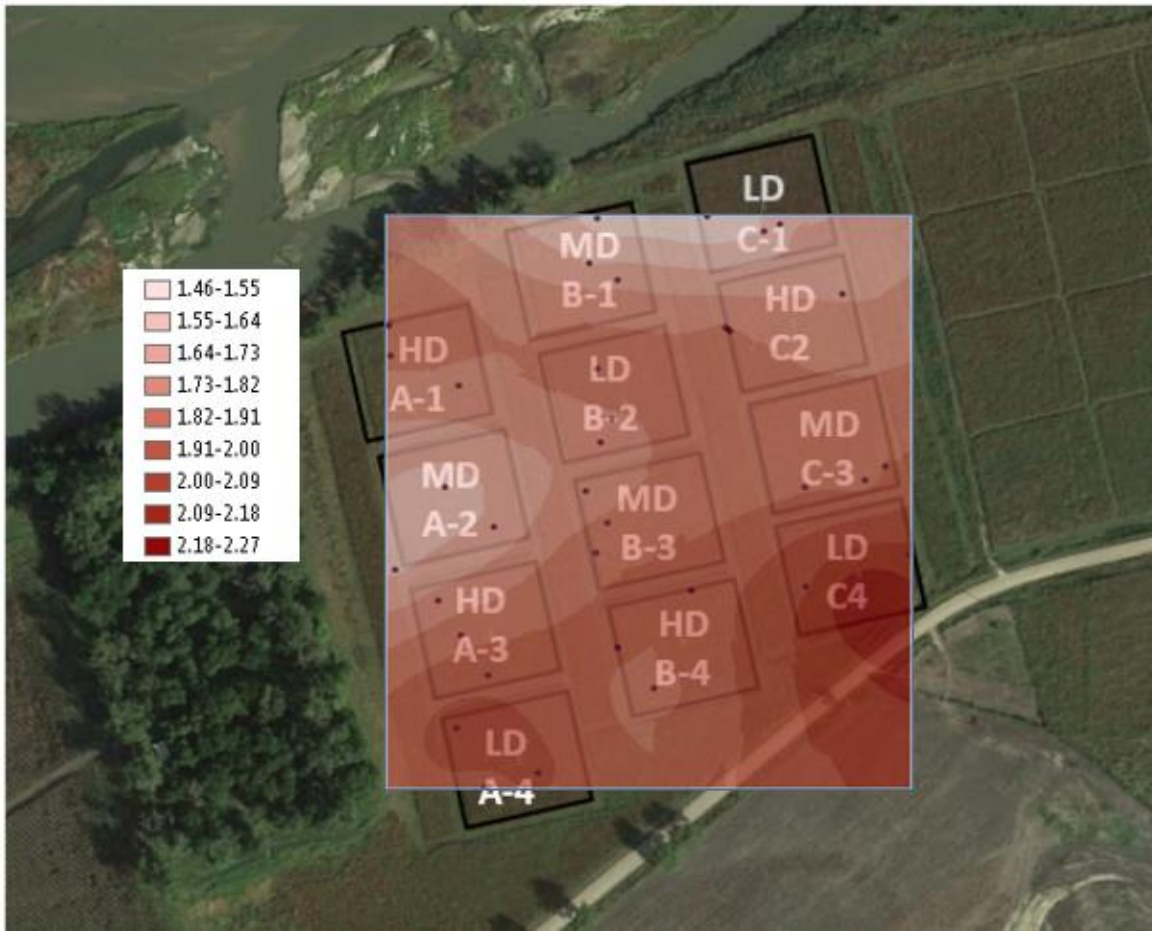


Figure 2.2. Gradient in soil pH by distance from river (slope = 0.006/meter, R^2=0.654, p=2.11e^{-12}), measured from whole-plot scale composite soil cores collected at the Platte River Prairies research prairie in early June 2015. The increase in pH with increasing distance from river indicates that soil pH is highest to the south and lowest to the north, nearest to the Platte River. The grey shading indicates the 95% confidence interval around mean pH values.

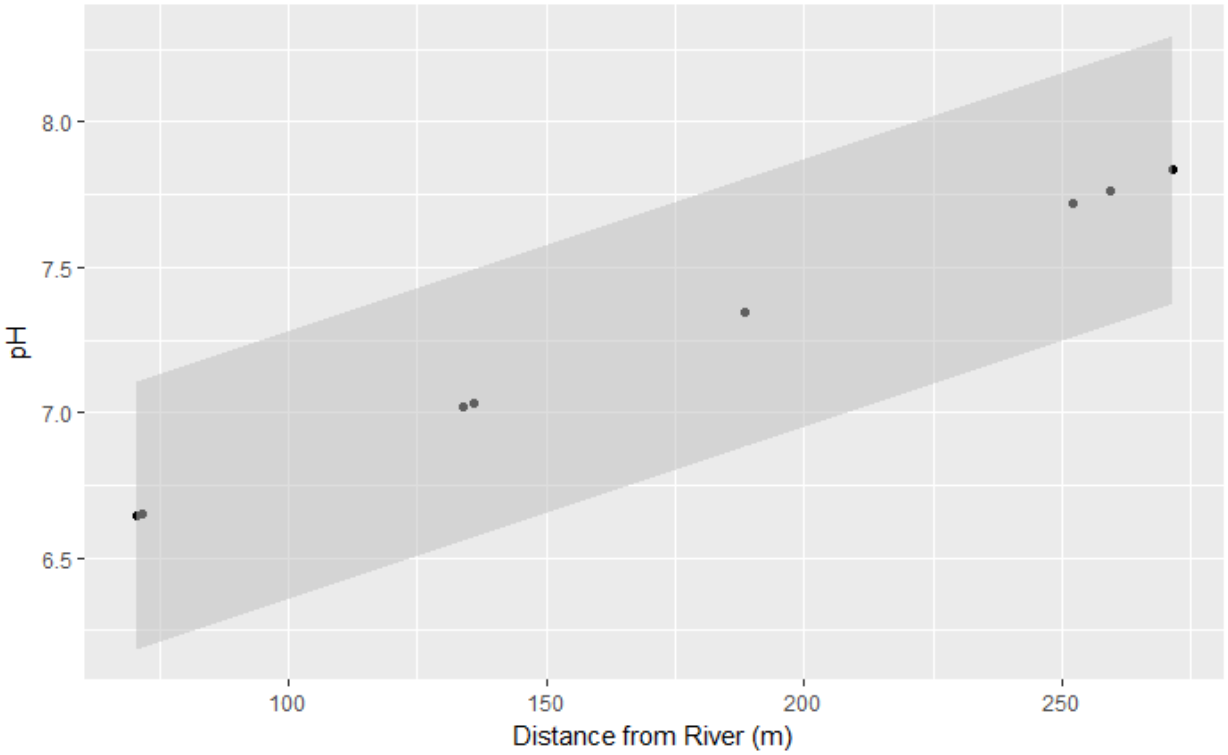


Figure 2.3. Soil nitrate (ppm KCL-extractable NO_3^-) gradient, measured from 3 composite soil cores per 60m x 60m plot, collected at the Platte River Prairies research prairie in early June 2016. The decline in soil nitrate from west to east indicates that soil nitrate is highest to the west and lowest to the east of the research plots.

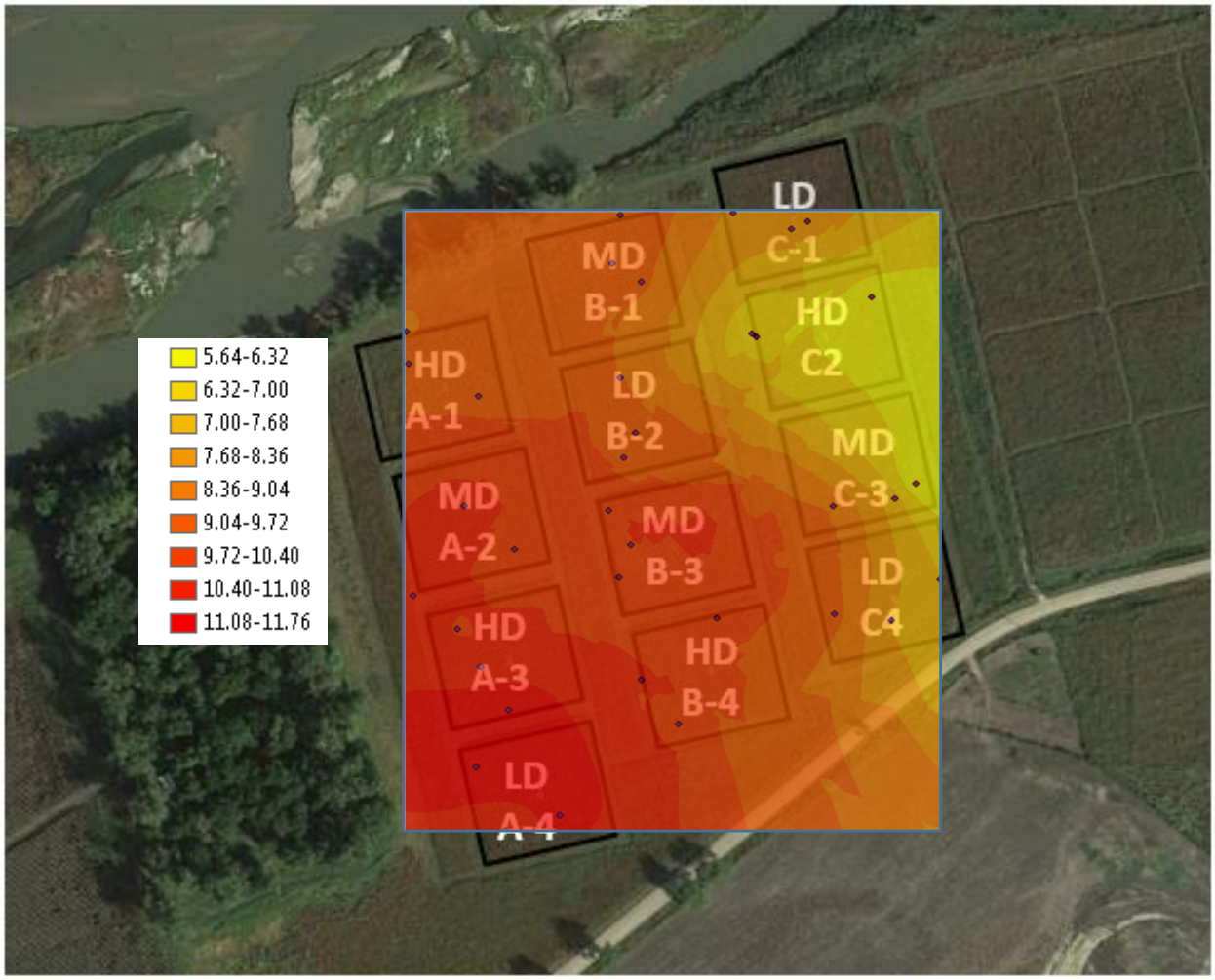


Figure 2.4. Average rainfall (cm) by month for the years 2015 and 2016 (top) and average maximum temperatures (cm) for the years 2015 and 2016 (bottom). Weather data is from the Hansen weather station, approx. 19km ESE of the research site. Maximum temperatures in 2015 were lower and dropped off much more quickly than in 2016. Rainfall in 2015 was much higher across May – July than in 2016, leading to long-term flooding of the research site during the month of June.

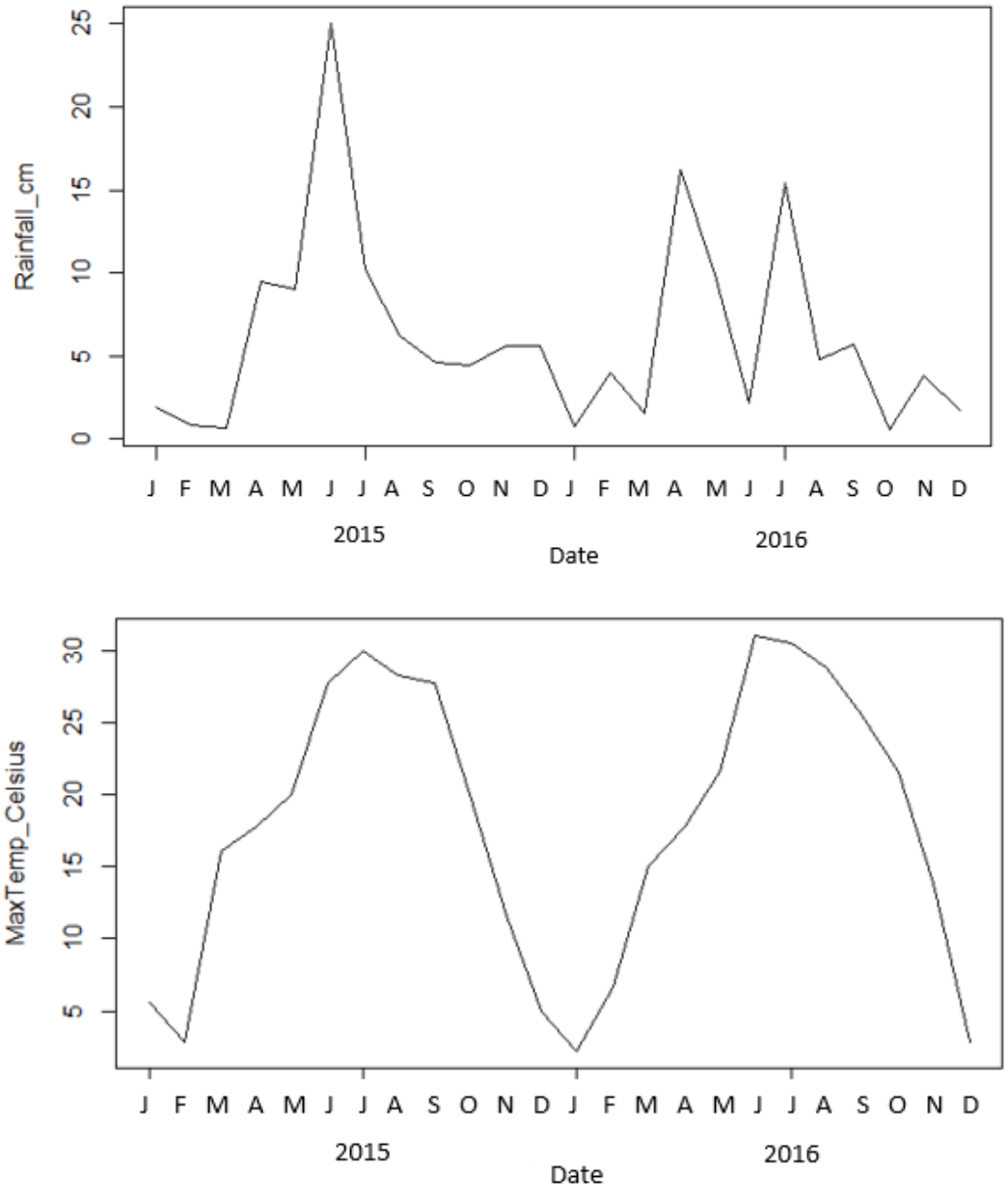


Figure 2.5. Soil moisture varies predominantly by distance from river (r^2 = 0.04, p-value = 3.21e-8) and by depth in soil profile (r^2 = 0.43, p-value = 2.2e-16). Other significant varibles include sampling date, rainout shelters, and distance from the edge of the plot; however, as these are strongly stochastic their effects are not shown here. Soil moisture values were collected using a hand-held soil moisture meter across four sampling dates in June, July, and August 2015, measuring depths of 10, 30, and 50cm and locations in each subplot of 0cm from edge, 25cm from edge, 50cm from edge, or in the center of the subplots. Soil moisture readings for 30cm and 50cm depths were collected via moisture access tubes lined with PVC, corked with rubber corks and covered with cans; however, soil moisture at that depth was high enough that readings frequently neared 100%.

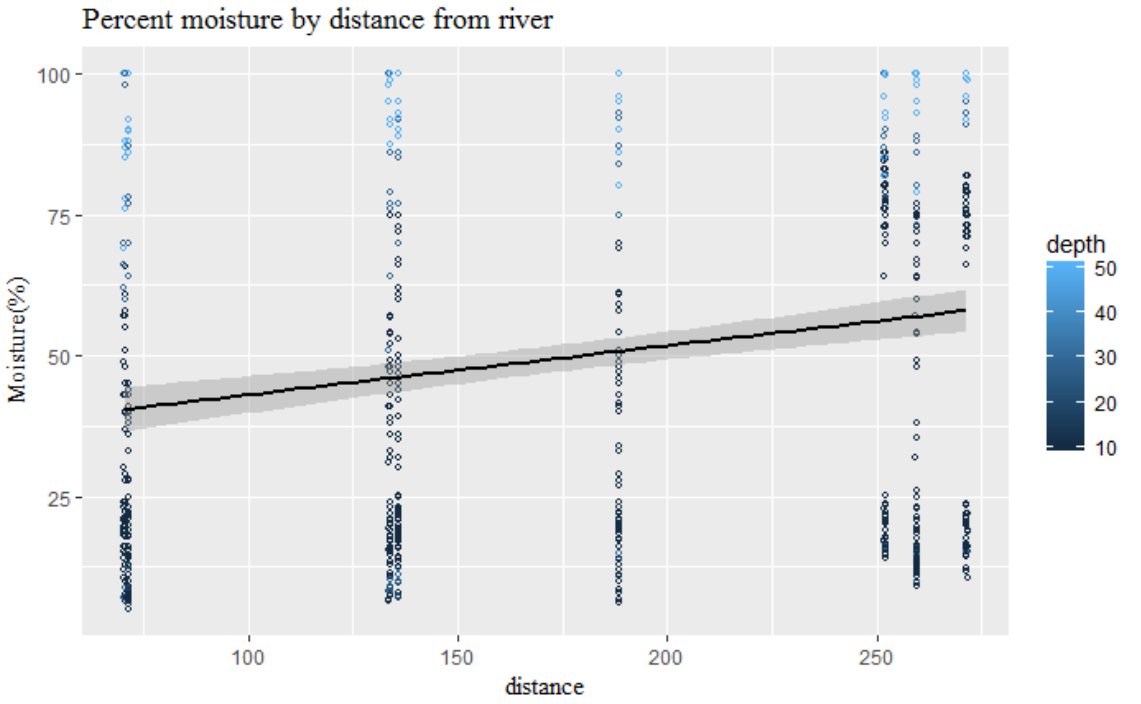


Figure 2.6. A diagram of the experimental disturbance treatments added to the center of each low-diversity and high-diversity research plot in May 2015. The subplot treatments are all 2mx1m, and samples were collected 50cm within the border of each of these plots to minimize overlapping treatment effects. Comparison plots indicate plots sampled outside rainout shelters.

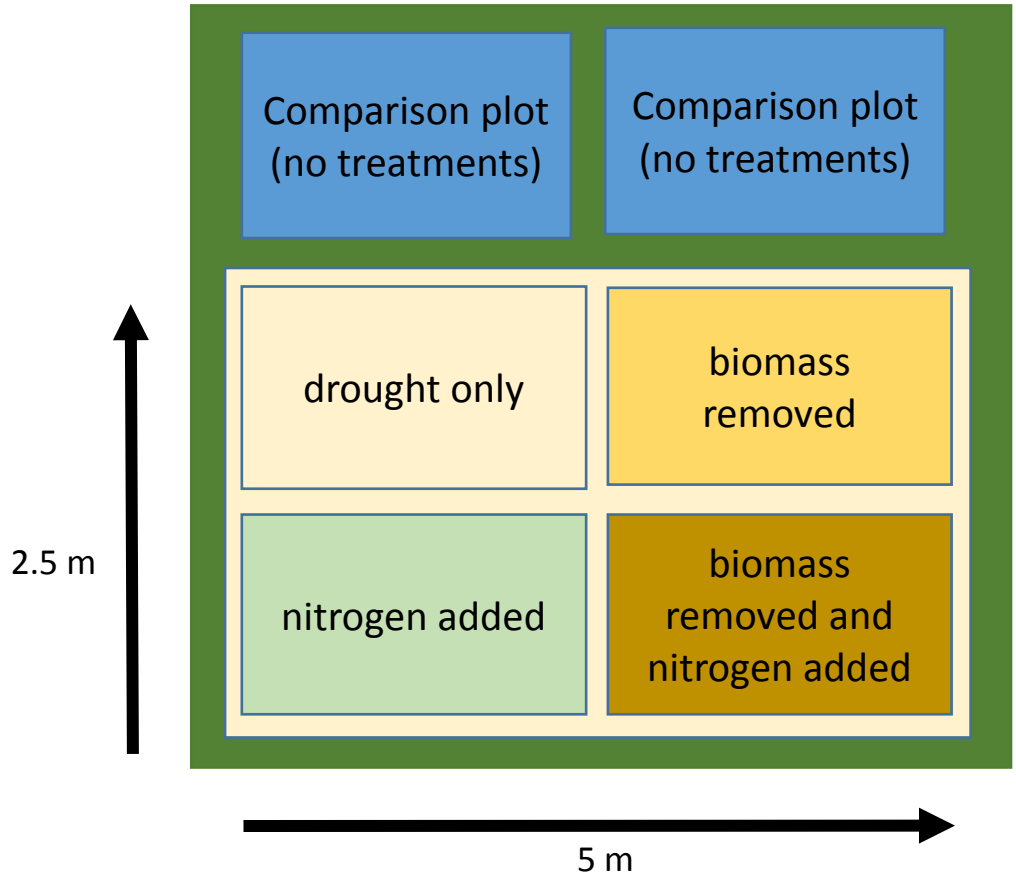


Figure 2.7. Bareground varies significantly by distance from river. Bareground was averaged from four transects across each low-diversity and high-diversity plot (eight 50cm x 50cm quadrats per transect). Shown here is the linear fit for percent bareground ~ distance (m), with the 95% confidence interval shown. Intercept =4.3781, Slope = 0.0655, P= 0.0208.

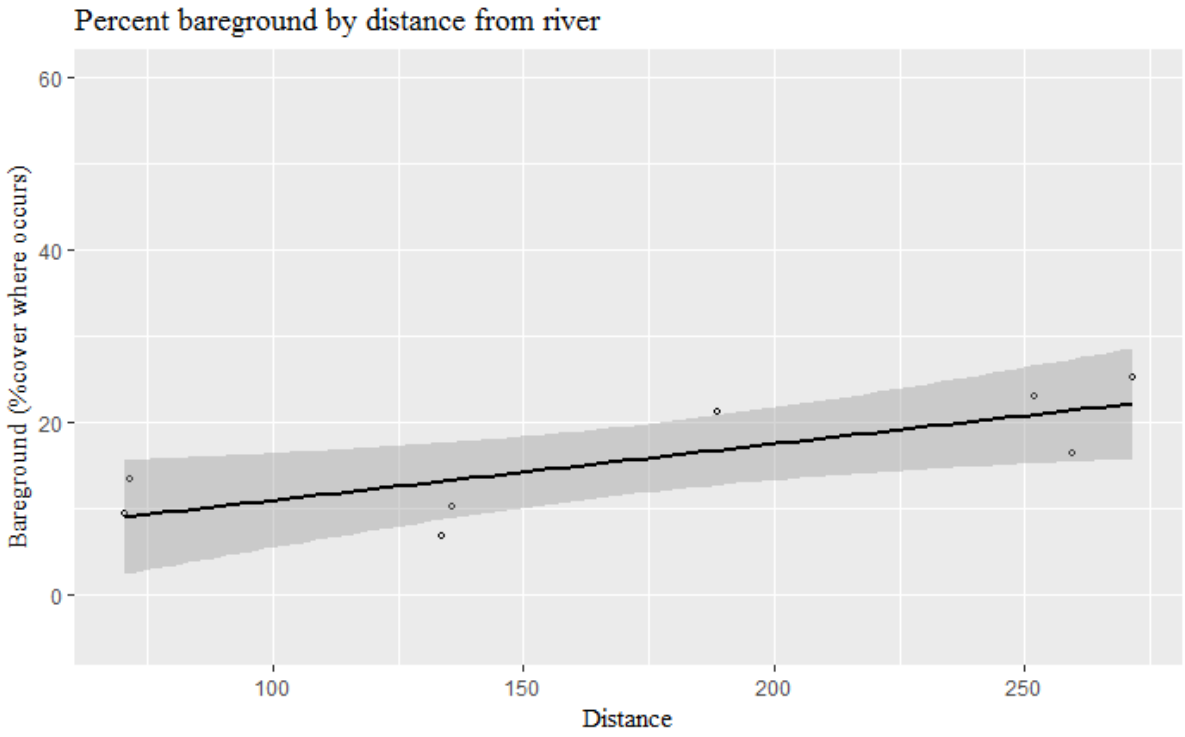


Figure 2.8. Community composition across all quadrats sampled (448 total quadrats per year, sampled in mid-to-late June 2015 and 2016) varies by diversity, year, rainout shelter effects, distance from river, soil organic matter, and soil inorganic nitrogen gradients. Dissimilarity among quadrats was determined via a Bray-Curtis dissimilarity analysis, followed by a PERMANOVA to determine significant sources of variation (Table 2.6). Finally, distances were plotted using Canonical Correspondence to visualize the magnitude and direction of variation among parameters. Year, diversity, and distance from Platte River were by far the largest sources of variation, with, rainout shelters, organic matter, and nitrate gradients playing minor roles.

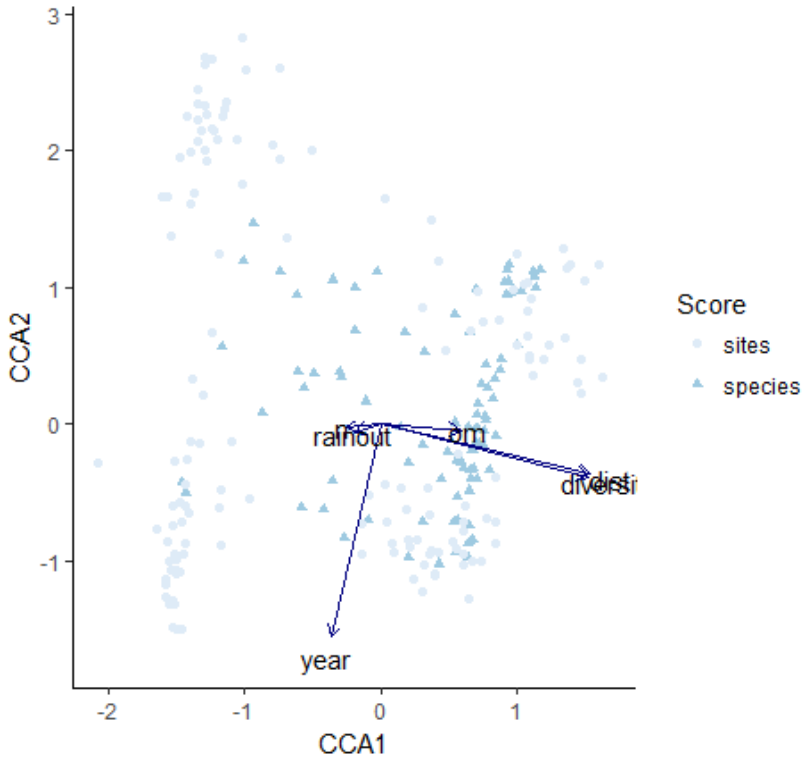
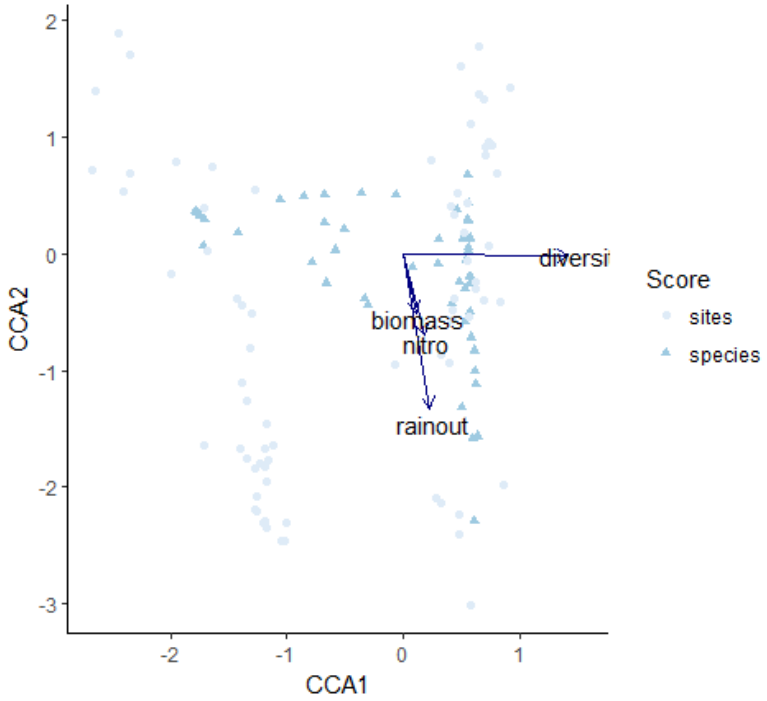


Figure 2.9. Vegetation structure (range in vegetation heights by species) varies by diversity, rainout shelter effects, and secondarily by the disturbance treatments of biomass removal and nitrogen addition. Principal sources of variation were determined first through a Bray-Curtis dissimilarity analysis of the range in variation height, followed by a PERMANOVA to establish main sources of variation. Variation was then plotted along the main sources of variation in multi-dimensional space using CCA to allow for the display of multiple significant parameters.



CHAPTER 3: PLANT DIVERSITY CONTRIBUTES TO PLANT GROWTH STRATEGY AND ITS RESPONSE TO DISTURBANCE

I. Introduction

As a driver of nutrient cycling, carbon sequestration, habitat provisioning, and other ecosystem services, primary productivity is one of the most-studied ecosystem functions tied to system diversity (Diaz et al. 2005; Jentsch et al. 2011; Brose and Hillebrand 2016). Net biomass production (g dry weight) is the typical measure of primary productivity used in these studies (Lefcheck et al. 2015: Tilman et al. 2006), with research demonstrating decreases in biomass production in response to competitive stress (Suding et al. 2008), the maintenance of biomass production via systemic responses to environmental pressures (Kreyling et al. 2008, Jentsch et al. 2011), and the relationship between primary productivity and changes in nutrient inputs (Tilman et al. 2001). In this study, I did not collect absolute values of primary productivity, focusing instead on two metrics of plant growth strategy that mediate primary productivity, namely specific leaf area and leaf chlorophyll content (Useche and Shipley 2010; James and Drenovsky 2007).

Plasticity in individual responses to stress provides the flexibility necessary for ecosystems to persist in the face of disturbance (Levins 1968). Studies that find rapid changes in specific leaf area in response to environmental variation suggest that individuals are able to alter growth strategy in response to limiting resources in grasslands, including light, water, and soil nutrients (Useche and Shipley 2010). The changes in plasticity of individual traits to survive in multi-species assemblages may also

allow those individuals to better withstand environmental stresses, thus lowering the system's vulnerability to disturbance.

Specific leaf area (SLA, ratio of leaf area to leaf dry mass), is a measure of vegetation growth strategy that has been identified as a key contributor to higher relative growth rates. Higher relative growth rates, measured as SLA, have been shown to promote invasive species success (Lake and Leishman 2004). SLA responds to multiple factors, including light and nutrient availability. Under light limitation, leaves may become broader and thinner, resulting in a higher area-to-mass ratio (Hoffman et al. 2005). Conversely, when nutrients and water are limiting, plants devote more resources to survival and fewer resources to growth, and any new leaves will likely be smaller, resulting in a decrease in average specific leaf area (Diaz et al. 2005; James and Drenovsky 2007). In this study, I predicted that specific leaf area would be higher in high-diversity plots compared with low-diversity plots due to variation in canopy structure and a more limiting light environment, and that specific leaf area would decrease in response to all added disturbances due to the increased physiological stress the treatments impose, which may lead to reduced resources allocated toward growth.

Leaf chlorophyll content provides a measure of leaf nitrogen concentration and correlates with both the relative growth rate (Shipley et al. 2006) and nutrient cycling in vegetation (Malavasi and Malavasi et al. 1999). As such, leaf chlorophyll represents the potential of the plant community to take up nutrients to form biomass. In this study, I predicted that leaf chlorophyll content would be higher in high-diversity relative to low-diversity plots due to complementarity and competitive effects, as species are both supported by a diversity of plant species while also competing for light (Gibson 2009). I

predicted that leaf chlorophyll content would increase in response to nitrogen additions as a result of readily-available inorganic ammonia as plants devote more energy to light harvesting in order to utilize this increase in resources, and that leaf chlorophyll content would decrease in response to biomass removal treatments due to the reduced leaf area available for photosynthesis following cutting. Finally, I predicted that variation in chlorophyll content would be smaller for high-diversity than for low-diversity whole-plots.

Specific leaf area and chlorophyll content represent different aspects of the same functional trait; namely, plant growth strategy, and there is some evidence that variation in leaf nitrogen concentration is largely driven by differences in specific leaf area (Hoffman et al. 2005). Deviations from a strong positive correlation in the responses of these two variables to added disturbances could indicate that each is more responsive to different stresses and may help to pinpoint key sources of vulnerability in primary productivity to added disturbance.

II. Methods

STUDY SITE

This study took place at the Platte River Prairies, owned by the Nature Conservancy in south-central Nebraska. The site, 10km south of Wood River, Nebraska (40°44'37.8"N 98°35'23.9"), is located within the Central Platte River ecosystem, identified as a Biologically Unique Landscape by the Nebraska Game and Parks Commission (NGPC 2011). Soils at the site include Wann loam, rarely flooded; Caruso loam rarely flooded; and Bolent-Clamux complex, occasionally flooded (NRCS).

In 2010, The Nature Conservancy seeded twelve 60m x 60m plots in native tallgrass prairie, with four plots each of Big Bluestem (*Andropogon gerardii*) monoculture, mid-diversity, and high-diversity seed mixes (Nemec 2013). The diversity treatments have established with significant differences between monoculture and high-diversity species richness (34 vs. 73 species, respectively; Price 2015). The 'monoculture' plots have accumulated a number of additional species and are referred to as low-diversity plots throughout this study. Functional groups represented within the whole-plots include C3 (cool-season) grasses, C4 (warm-season) grasses, and both leguminous and non-leguminous forbs. This grouping is a very broad generalization of species groups and was chosen for ease of categorization and correspondence with large differences in phenology and nutrient acquisition represented by each group, differences which may influence their responses to disturbance (Lavorel et al. 1997).

The site is maintained via burning; the most recent burn was in March of 2015, six weeks before the start of the study, with no additional management during the course of this study. The burn removed all biomass cover at the beginning of the research period, and so large differences exist in vegetation and litter cover by year.

The research site covers an environmental gradient with increasing soil organic matter (percent mass lost on ignition) from north to south. This gradient correlates with other soil chemical and functional traits, including soil respiration (Solvita CO2 Burst test, ppmC) and soil pH. There is also a significant gradient in nitrate (ppm KCL-extractable NO_3^-) from southwest to northeast from southwest to northeast. These gradients were included as fixed effects in statistical analyses.

Precipitation and temperature during the course of this study varied significantly by year. Precipitation in 2015 was much higher than in 2016, with a major peak in June (25cm total, mostly occurring in a single event). 2016 was more evenly distributed, with the highest rainfall totals in April (16cm) and July (15cm) and low rainfall in other months. Average maximum and minimum temperatures were higher in 2016, with sustained higher average temperatures across the spring, summer, and fall. The minimum temperatures in January 2015 and 2016 were -19.44^{o}C and -23.3^{o}C, respectively, and maximum temperatures in July 2015 and 2016 were 35^{o}C and 38.3^{o}C.

TREATMENTS

In May of 2015, I constructed eight 2.5 x 5m rainout shelters in the center of each low-diversity and high-diversity research plot. Beneath each shelter, I established four 1m x 2m plots of additional experimental disturbances, with 50cm spacing between each treatment. These disturbance treatments consisted of either rainout shelter only, biomass removal (cutting biomass down to 4-8cm height during the first week of July), nitrogen fertilizer addition (30g of inorganic 34-0-0 dry ammonium nitrate fertilizer added twice per summer, first in mid-June and then six weeks following the first treatment for a rate of 10gNH_4NO_3./m-1/summer), or a combination of biomass removal and nitrogen addition (Figure 3.1).

Treatments were chosen to represent current threats to grasslands. Rainout shelters were built to impose a water shortage, which could lower the threshold of vegetation resilience to additional disturbances. Drought is expected to be exacerbated in the mid-latitudes where the majority of grassland systems exist in coming decades (IPCC 2014), and in conjunction with increases in summer temperatures poses an increasingly

large threat to grasslands (de Boek et al. 2011). The disturbance plots were established beneath rainout shelters. Nitrogen addition represented the widespread terrestrial eutrophication arising largely from agricultural drift and fossil fuel burning (Suding et al. 2008; Tilman et al. 2008). Biomass removal simulated haying, a common management strategy in prairies throughout the United States. Haying is considered a management alternative to grazing or fire (Smith et al. 2010), and may alter ecosystem function by removing dominant species at peak growth and reducing the amount of biomass left on the field for subsequent growing seasons.

DATA COLLECTION

To test the impact of diversity, soil resources, and disturbance treatments on the relative growth rate of vegetation in both high-diversity and monoculture plots, data on specific leaf area and leaf chlorophyll content were collected in early July of 2016. This data, coupled with soil data collected in 2015 and 2016, was used to measure the effect of differing levels of biodiversity and added disturbance on plant growth strategy.

Soil collection occurred at both the whole-plot and disturbance treatment subplot scales across all monoculture and high-diversity plots in the first week of June of 2015 and 2016. Temperatures averaged 19.4^{o} and 19.7^{o}C on sampling dates, and sampling occurred no less than 48 hours after any rainfall event. Soil samples were collected from three randomly-selected locations at the whole-plot scale for all twelve diversity treatments in June 2016. At the 60m x 60m plot scale I collected three composite cores, each made up of three sub-samples dug to 20cm with a hand auger. At the 1m x 2m disturbance treatment scale, along with two 2m x 1m plots established near the rainout shelters with no added disturbance treatments, I collected a single composite core made

up of three sub-samples. All soil samples were stored at 2^{o}C until analysis, and were processed at Ward Labs, an agricultural lab specializing in soil and plant samples located in Kearney, Nebraska. Soil moisture was recorded via a hand-held moisture meter which recorded moisture in the top 10cm of soils, and via moisture access tubes at 30cm and 50cm depth in the center of each rainout plot. Soil moisture readings were collected four times in June-August 2015 at the edge, 25cm in, 50cm in, and at the center of each subplot as well as from moisture access tubes.

Vegetation surveys within disturbance addition subplots were conducted according to a systematic random design. Each subplot within the rainout shelter was divided into eight 50cmx50cm quadrats and four of those quadrats randomly selected for measurement. Variables recorded include cover class (Daubenmire cover method; Elzinga et al. 1998), frequency, and height (shortest and tallest individuals of each species, in cm) of each species within each quadrat. Bareground and litter cover and height were also recorded. Representative species were selected from this survey as those with the greatest frequency and cover across all plots, to develop a survey of plant growth strategy across treatment types.

In 2016, samples for specific leaf area analysis were collected in early July from five individuals of each representative species within each subplot by collecting one leaf, fully emerged and about 1/3 from the crown, from each individual for analysis. Samples were kept moist and on ice until processing. SLA was calculated as leaf area per unit of leaf dry mass (cm/g) (Wilson et al. 1999), resulting in a standardized ratio that was comparable across samples. Leaf area measurements were obtained by scanning fresh samples using a desktop scanner and measuring area using the ImageJ image processing

software. Leaf dry mass was obtained by weighing samples to the nearest hundredth of a gram after drying in a 37^{o}C drying oven for 72 hours.

Leaf chlorophyll content was measured two weeks before and two weeks following the final disturbance treatments of nitrogen addition and biomass removal, in July of 2016. Measurements were collected using a hand-held CCM-300 chlorophyll meter, which uses a fluorescence technique to provide reliable estimates of leaf chlorophyll content (mg chlorophyll/m^2 tissue biomass). One leaf of five individuals of each representative species present in each subplot was measured. Leaves were selected about one-third from the top of each individual (or one-third from the end of the leaf in the case of grasses), and were uniformly green and free of disease to minimize non-random variation among samples.

ANALYSIS

Soil

Strong variation in soil chemistry could influence the responses of vegetation to biodiversity and disturbance treatments; therefore, soil organic matter (SOM), soil nitrate (ppm KCL-extractable NO_3^-), and pH were calculated for samples at both the whole-plot and disturbance-addition subplot scales. Soil microbial biomass was measured using chloroform-extraction, and respiration was also measured via a modified substrate-induced respiration test (Solvita, inc.). All samples were processed by Ward Labs, an agricultural testing firm located in Kearney, Nebraska. Significant variation in soil chemistry and microbial biomass by distance from river was calculated using linear regressions. Soil moisture values were also collected via a handheld soil moisture meter and variation assessed by the variable distance (m) from river. Soil chemical analyses

revealed strong soil gradients, with inceasing organic matter, pH, and moisture with increasing SOM with distance from the Platte River, and increasing soil nitrate with distance west to east.

Specific leaf area and leaf chlorophyll content

Specific leaf area (SLA) and leaf chlorophyll content may be helpful for describing variation in growth strategy among members of the same species growing in different conditions, as is the case in this study (Wilson et al. 1999; Shipley 2006). To assess SLA, a set of representative species was chosen to compare across treatment plots, including members of the *Helianthus*, (Sunflower) genus, the *Solidago* (Goldenrod) genus, the C4 photosynthetic pathway grasses *Andropogon gerardii, Sorghastrum nutans,* and *Panicum virgatum,* and a common forb *Mondarda fistulosa* (Wild Bergamot). The Goldenrod species *Solidago gigantea* and *Solidago canadensis* were combined for this analysis due to their occupation of the same niche space in different plots. The varieties of *Andropogon gerardii* planted in high-diversity and low-diversity plots differed, as nursery seed was used to augment low-diversity plantings in order to achieve a high enough seeding density for restoration. Some variation among diversity plots is related to this difference in variety.

The effects of diversity, environment, and experimental treatments were tested using the parameters of planted diversity, soil resources, control versus rainout-treated subplots, and the effects of treatments applied within rainout shelters. To include the effects of each of these sources of variation, a single global model of the possible interactions between treatments and site conditions that were expected to influence plant growth strategy was created. Stepwise backward selection was used to remove

parameters that were not predictive, and selection stopped when the highest-weight model was found (Borcard et al. 2011). The resulting models were assessed for their statistical and biological significance (Zuur et al. 2007). Variation among group means was calculated using Tukey's HSD posterior testing to assess the significance of variation introduced by statistically significant parameters.

Global models were tested for individual species and by subplot, using a species-weighted average chlorophyll content calculated for each subplot. I predicted that leaf chlorophyll content would be more affected by the nutrient use efficiency of the system and to the relative availability of mineralizable soil nitrogen, while leaf area would be more likely affected by competition for light and space. Individual species were also expected to vary depending on life strategies; therefore, slightly different models were expected to perform better for specific leaf area and leaf chlorophyll content.

III. Results

Specific Leaf Area

For the full set of representative species, SLA was assessed using the species-weighted average for each subplot (sum of the weighted SLA of each species, determined as the SLA/weighted cover of each species sampled in the plot). The global model for whole-group SLA was:

[M1] SLA ~ diversity*rainout*nitrogen addition*biomass removal*nitrate*SOM + (1|site),

where diversity is the planted diversity (low or high), rainout indicates the presence of rainout shelters (separating control from treated plots), nitrogen addition is a factor describing whether the subplot has had nitrogen added, biomass removal is a factor

describing whether the subplot had biomass removed the previous year, and site nitrate and SOM are the average nitrate and organic matter concentrations for each rainout shelter, and (1|site) is a random-effects variable controlling for random variation by sampling site. This model was parsed using stepwise backward selection to find the best-fitting model.

No models were significantly better than the global model, and no parameters could individually predict variation in group means. However, ANOVA revealed significant variation by diversity level and rainout shelter effects, and a TukeyHSD posterior test of group means revealed that high diversity treatments and rainout-shelter treated subplots both had significantly higher average specific leaf area (Table 3.1 and Figure 3.2). Reduced UV light resulting from the rainout shelter roofing material may have led to higher relative SLA beneath rainout shelters as compared with control plots. Additionally, soil moisture did not differ significantly by rainout shelters for the top 10cm of soil (average 3% decrease in average soil moisture, p-value 0.09), and at deeper depths soil was consistently near 100%. The rainout structures therefore may be imposing light stress more than water stress, leading to increased SLA.

Individual species common to low-diversity and high-diversity plots were also analyzed for variation. The relative influence of explanatory parameters for *Andropogon gerardii* was tested using the global model, and as with the group-weighted SLA, no simpler model could be found. ANOVA was conducted to identify variation in group means among treatment groups, and several treatments were found to vary significantly in average SLA, including diversity, biomass removal, and nitrogen addition. A TukeyHSD posterior test of group means showed that average SLA was significantly

higher at high diversity compared with monoculture whole-plots, and in biomass-removal treatment subplots. High-diversity, nitrogen-addition subplots also showed significantly higher SLA than low-diversity nitrogen addition subplots (Table 3.1 and Figure 3.3).

For the *Solidago* genus (*canadensis* and *gigantea)*, once again no best model could be chosen from the global model. No single parameter was predictive of changes in Solidago; rather, an interaction of multiple terms was considered the best predictor. ANOVA to test variation among treatment groups revealed significant variation in group means by diversity and rainout shelter effects. TukeyHSD revealed significantly higher average SLA values at high diversity compared with low-diversity plots, and higher SLA beneath rainout shelters compared with outside shelters (Table 3.2). Within high-diversity plots, nitrogen addition subplot averages had marginally higher mean SLA ($p=0.06$), and within low-diversity plots there was a more significant variation between group means for rainout shelter compared with control plots (Table 3.1 and Figure 3.4).

Analysis of species unique to high-diversity plots revealed revealed no significant variation among *Helianthus* species (*maximiliani* and *pauciflorus*) in response to any explanatory variables, nor among samples of *Monarda fistulosa*. The C4 grasses *Sorgastrum nutans* and *Panicum virgatum,* meanwhile, did not vary by rainout shelter or any treatment groups, but ANOVA did reveal significant variation by soil organic matter and soil nitrate concentrations. A linear regression to identify the direction of the variation revealed a strong negative correlation between soil nitrate levels and SLA ($r=0.88$, $p=0.003$), and a positive correlation between soil organic matter and SLA ($r=-0.13$, $p=0.002$) for the C4 grass group.

Leaf Chlorophyll Content

Leaf chlorophyll measurements were collected following the addition of the full set of treatments; therefore, we can therefore compare the full factorial design of nitrogen addition, biomass removal, and their combination as potential predictors of leaf chlorophyll content. To test the effects of possible explanatory variables on the species-weighted average of all representative species by subplot, the global model:

[M2] CLA ~ diversity*rainout*nitrogen addition*biomass removal*SOM*nitrate + (1|site)

was constructed, where diversity is the planted diversity (low or high), rainout indicates the presence of rainout shelters (separating control from treated subplots), nitrogen addition is a factor describing whether the subplot has had nitrogen added, biomass removal is a factor describing whether the subplot had biomass removal treatments, and site nitrate and SOM are the average nitrate and organic matter concentrations for each rainout shelter. (1|site) is a random-effects variable controlling for unknown causes of variation by sampling site. This model was parsed using stepwise backward selection to find the best-fitting model.

No single parameter was a strong predictor of leaf chlorophyll content, and backwards selection found no simpler model could be constructed from the global model. A Tukey HSD posterior test run for the full set of treatment variables revealed slightly higher group chlorophyll by nitrogen addition, with no other individual parameter proving important. This effect was driven entirely by variation in high-diversity plots, as low-diversity subplots showed no variation in response to nitrogen addition. Biomass removal alone was not a significant predictor of variation at either diversity level, and the

interaction of nitrogen addition and biomass removal was significant for high-diversity, but not low-diversity plots (Table 3.3 and Figure 3.5). Linear regression tests revealed no significant relationship between soil organic matter, nitrate, and average subplot leaf chlorophyll.

For the C4 grass *Andropogon gerardii* (Big Bluestem), backwards selection from the global model again found that no simplification was possible; however, the parameters diversity, rainout shelter, and the interaction of diversity and biomass removal were significant predictors of variation in leaf chlorophyll (Table 3.2). Nitrogen addition was a marginally significant parameter ($p=0.07$). No variation by soil organic matter or nitrate levels was apparent. A TukeyHSD to compare differences in group means by treatment found significantly higher leaf chlorophyll in high-diversity plots, higher leaf chlorohpyll beneath rainout shelters, and higher leaf chlorophyll in nitrogen addition plots (Table 3.4). The difference in group means by rainout shelter vs control subplots was nearly twice as large in low-diversity plots compared with high-diversity plots, while the difference in group means by nitrogen addition was nearly the same at both low and high diversity (Table 3.3 and Figure 3.6).

The forbs *Helianthus maximiliani* and *pauciflorus* (Maximilian's and Stiff sunflower), no significant variation was apparent except by nitrogen addition treatments, noted in the global linear model, with the difference in group means assessed using a TukeyHSD test (Table 3.3; Figure 3.7).

For the forbs *Solidago canadensis and gigantea* (Canada and Giant Goldenrod), backwards selection once again could not simplify the global model. Assessment of the global model revealed a marginally significant effect of rainout shelters ($p=0.06$). All

other significant parameters were interactive effects, including the interaction of diversity and rainout shelters, diversity and biomass removal, diversity and nitrogen addition, and some 3-way interactions among variables (Table 3.2). Posterior testing suggests that many of these effects may be largely driven by variation in group means by diversity. TukeyHSD posterior tests revealed significant differences in mean chlorohphyll by diversity and biomass removal, but not by rainout shelter. There was also significantly lower chlorohpyll in biomass removal subplots at low-diversity, but not at high-diversity plots (Table 3.3 and Figure 3.8).

For the forb *Monarda fistulosa* (Wild Bergamot), the global model minus the diversity parameter was used to test variation. Once again, the model could not be simplified, though the global model revealed significantly higher leaf chlorophyll with rainout shelter treatments and lower leaf chlorophyll with biomass removal treatments (Table 3.2). ANOVA to test for variation in group means confirmed that there was significant variation by each treatment effect, with rainout shelter, nitrogen addition, and biomass removal groups all showing significant variation. A TukeyHSD test to compare group means showed significantly higher leaf chlorophyll beneath rainout shelters compared with control plots, and significantly lower average chlorophyll contents in both nitrogen addition and biomass removal treatments (Table 3.3 and Figure 3.9).

Finally, the forb *Glycyrrhiza lepidota* (wild licorice) was measured in the B4 restoration plot. The B4 plot contained several unique species and had higher average soil nitrogen and soil organic matter than most of the restoration plots. The global model used to test variation in *Glycyrrhiza lepidota* was:

[M3] Chlorophyll ~ rainout*nitrogen addition*biomass removal,

as these were the only parameters which varied among treatment groups. Once again, the global model for leaf chlorophyll could not be simplified; however, ANOVA revealed significant variation by rainout shelter, nitrogen addition, and biomass removal treatments. A TukeyHSD posterior test revealed that leaf chlorophyll was significantly lower with rainout shelters and biomass removal treatments, while nitrogen addition treatments had significantly higher average chlorophyll (Table 3.3 and Figure 3.10).

IV. Discussion

There is some evidence that changes in plant growth strategy mediates relative growth rate and alters both the demographic and functional characteristics of an ecosystem under stressful conditions of environmental stress (Grime 1979). This variation in growth strategy results in measurable variation in specific leaf area and leaf chlorophyll content that can be used to assess how plant species alter their resource allocation in response to environmental pressures. Species that are not able to alter growth strategy in response to disturbance may be more at risk of being lost from the system under repeated disturbances, leading to demographic shifts (Useche and Shipley 2010). Reduced soil nitrogen in grasslands tends to favor native species that have adapted to nutrient limitations for this reason, as periodic reductions in nitrogen availability disproportionately impact invaders and shift competitive dominance toward native species (Lake and Leishman 2004).

I predicted that the variation in chlorophyll and specific leaf area group means by treatment effects would be larger for low-diversity compared with high-diversity plots. This hypothesis was partially supported by the data. First, *Andropogon gerardii* did show

nearly double the increase in leaf chlorophyll content in response to rainout shelter treatments at low-diversity compared with high diversity plots. *Andropogon* in low-diversity plots also had nearly double the increase in leaf chlorophyll in response to nitrogen addition treatments, and nearly double the loss in leaf chlorophyll in biomass removal treatments. The reduction in *Solidago* leaf chlorophyll by biomass removal treatment was also nearly double in low-diversity than in high-diversity plots, and was not significantly different from zero in high-diversity plots. Complicating this picture, however, are the species-weighted community averages of chlorophyll content. Chlorophyll content showed more significant variation in response to rainout shelter and treatment effects for high-diversity than low-diversity subplots in these species-averaged subplot values. This reversal of trends at the group level may reflect the greater variety in species composition among high-diversity compared with low-diversity plots. The lower replication at this higher level of observation (ie, each subplot is a single observation while at the individual-species level each leaf is a single observation) may make stochastic patterns appear more significant in high-diversity plots compared with the relatively constant low-diversity plots which contained, on average, only one or two species per subplot.

Leaf chlorophyll of species present in only high-diversity plots varied as expected in response to treatments. *Helianthus* leaf chlorophyll increased significantly by nitrogen addition. *Monarda fistulosa* chlorophyll increased with rainout treatment and decreased with the nitrogen addition and biomass removal treatments. Meanwhile, *Glycyrrhiza lepidota* decreased with rainout shelters, decreased with biomass removal, increased with

nitrogen addition, and was not significantly different from the rainout-shelter only treatment with the nitrogen addition and biomass removal treatment.

These patterns in leaf chlorophyll reflect the biotic stresses added by each of the disturbance treatments. Rainout shelters, intended to simulate drought conditions, may have impacted light conditions more than water, reducing light and heat stress at the hottest periods of the day and allowing individuals to grow more quickly and invest more in tissue production than those outside rainout shelters. *Glycyrrhiza*, the exception to this rule, had reduced leaf chlorophyll beneath rainout shelters. Nitrogen addition, meanwhile, provided a flush of readily available nutrients that may have offset other stresses and allowed for more rapid growth. Finally, biomass removal imposed an acute stressor, similar to grazing, that typically forces plants to allocate more resources to belowground growth, temporarily reducing aboveground productivity (Cushman and Jones 2004).

Specific leaf area showed much smaller variation in general, though the patterns that were apparent were similar to those seen for leaf chlorophyll. In the species-weighted subplots, as well as the *Andropogon gerardii* and *Solidago* groups, specific leaf area was higher at high diversity, and for subplots specific leaf area also increased with rainout shelter treatments. For *Andropogon gerardii*, specific leaf area increased a similar amount for both low and high-diversity plots in response to biomass removal, and *Solidago* species showed some increase in specific leaf area in response to rainout shelter treatments, though this pattern was not apparent within diversity levels. The increase in specific leaf area in response to biomass removal for *Andropogon* makes intuitive sense when considering that these measurements were collected a year post-disturbance, and biomass removal had reduced competition for space aboveground. I would expect this

increase in specific leaf area to shrink as time post-disturbance increases and vegetation once again becomes more crowded.

These results indicate that diversity and rainout shelter effects were the primary sources of variation in both specific leaf area and leaf chlorophyll content. In the case of specific leaf area, these effects were often the only significant effects, with significant variation among treatments apparent only in the very common *Andropogon* and *Solidago* taxa and for the species-averaged subplot data. Leaf chlorophyll content varied more by specific treatments, primarily nitrogen addition.

The larger variation in community-weighted chlorophyll content and specific leaf area at high-diversity compared with low-diversity plots was somewhat surprising, but previous research has shown that more diverse communities are sometimes more sensitive to disturbances than their low-diversity counterparts (McCann 2000). However, the variation at high diversity represents the average of multiple species with much wider natural ranges in leaf chlorophyll and SLA values than the Solidago and Andropogon which dominated the low-diversity plots. The clear directionality of variation in low-diversity plots (Figures 3.2 and 3.6), compared with the more mixed variation in high-diversity plots, shows the influence of multiple interacting species at the high-diversity plots which simply did not occur at low-diversity plots.

The larger differences measured in leaf chlorophyll compared with specific leaf area may indicate that leaf chlorophyll is more responsive to disturbance treatments; however, it is more likely that the more significant variation in leaf chlorophyll is due instead to the timing of measurements. Because SLA sampling occurred before final treatments were implemented, variation in SLA was a result of disturbance treatments

added in summer 2015 and one of the two nitrogen addition treatments in 2016, while measurements of leaf chlorophyll reflect the full set of disturbance treatments added in both 2015 and 2016. Therefore, the magnitude of effects ought to be larger for chlorophyll than for SLA assuming the two metrics are both responsive to treatments.

Some difficulties plagued this analysis. Most importantly, a low level of replication for each treatment, especially in species unique to high-diversity plots, led to an inability to accurately predict the magnitude of effects of linear contrasts and to simplify my global model to find useful predictive parameters. Nevertheless, tests of variation among groups by treatment, including ANOVA to test for significant variation among group means and TukeyHSD to assess the pairwise differences in group means, identified several sources of variation in response to treatments. These results suggest that variation among treatment groups is occurring, but greater replication and more years of continued disturbance treatments and measurements is needed to determine the true magnitude of effects of our various treatments.

V. Conclusion

In general, plant growth strategy is sensitive to disturbance at both low and high levels of diversity. I expected the magnitude of variation in both leaf chlorophyll and specific leaf area in response to treatments to be higher in low-diversity than in high-diversity plots. This pattern was apparent for the leaf chlorophyll content values for the *Andropogon* and *Solidago* genuses, which were measured in both plots. Variation in specific leaf area was of a similar magnitude at both low and high-diversity plots. Patterns of variation in SLA and chlorophyll partially confirmed my initial hypotheses; I

expected nitrogen addition to increase specific leaf area and leaf chlorophyll content, which it generally did. I also expected that biomass removal plots would have lower specific leaf area and leaf chlorophyll contents, which was not entirely the case. While leaf chlorophyll content generally declined in response to biomass removal, biomass removal plots showed a slight increase in specific leaf area. If plants were space-limited, then an increase in SLA following biomass removal may be a logical outcome.

Although the variation among treatments in high-diversity plots was quite large, there is some evidence that a diverse community with a variety of responses to disturbance may be more resilient to changes in system states than a less diverse community with more muted responses, even though the low-diversity community may be initially more resistant to change (Risser 1995; Smith et al. 2009). Individual species within the more diverse community show less overall variation by disturbance treatment and may therefore be more able to survive external stresses and maintain system continuity following disturbance events. This lack of individual variability may reflect the overall higher tolerance to stress that plants growing in multi-species assemblages have developed in order to live in more competitive conditions than those growing with few other species.

VI. Literature Cited

Altman, N., Krzywinski, M. 2015. Points of significance: split plot design. Nature Methods **12**:165-166.

De Boeck, H. J., Dreesen, F. E., Janssens, I. A., Nijs, I. 2011. Whole-system responses of experimental plant communities to climate extremes imposed in different seasons. New Phytologist **189**: 806–817.

Borcard, D., Gillet, F., Legendre, Legendre, P. 2011. *Numerical Ecology with R*. New York: Springer.

Brose, U., Hillebrand, H. 2016. Biodiversity and ecosystem functioning in dynamic landscapes. Philosophical Transactions of the Royal Society B: Biological Sciences **371**:1694.

Cardinale B.J., Palmer M.A., Collins S.L. 2002. Species diversity increases ecosystem functioning through interspecific facilitation. Nature **415**:426–429.

Cardinale, B.J., Wright, J.P., Cadotte, M.W., Carroll, I.T., Hector, A., Srivastava, D.S., Loreau, M., Weis, J.J. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. Proceedings of the National Academy of Science **104**(46): 18123-18128.

Clark. M., Tilman, David. 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature Letters **451**:7.

Cushman, Ruth C., Jones, Stephen R. 2004. *Peterson Field Guides: The North American Prairie.* Houghton Mifflin Harcourt.

Díaz, S., Lavorel, S., Chapin, F. S., Paula, I. I. I., Diego, A.T., & Karl, E. G. 2005. Chapter 7, Functional Diversity: At the crossroads between ecosystem functioning and environmental filters. In *Terrestrial Ecosystems in a Changing World,* J.G. Canadell, D.E. Pataki, L.F. Pitelka, Eds. Berlin: Springer-Verlag, pp 81-91.

Elzinga, C.L., Salzer, D.W., Willoughby, J.W. 1998. *Measuring and monitoring plant populations.* U.S. Bureau of Land Management Papers, Paper 17.

Gibson, David J. 2009. *Grasses and grassland ecology*. New York: Oxford University Press.

Hoffmann, W. A., Franco, A. C., Moreira, M. Z., & Haridasan, M. 2005. Specific leaf area explains differences in leaf traits between congeneric savanna and forest trees. Functional Ecology **19**: 932–940.

IPCC. 2014. *Climate Change 2014: Impacts, Adaptations and Vulnerability. Summary for Policymakers.* Cambridge: Cambridge University Press.

James, J. J., Drenovsky, R. E. 2007. A Basis for Relative Growth Rate Differences Between Native and Invasive Forb Seedlings. *Rangeland Ecology & Management,* **60**: 395–400.

Jentsch, A., Grant, K., Nagy, L., Schloter, M., Wo, J., Kreyling, J., Hein, R., Otieno, D., Sing, B.K., Elmer, M., Lara, M., Pritsch, K., Stadler, J., Mirzae, H., Rascher, U. 2011. Climate extremes initiate ecosystem regulating functions while maintaining productivity. Journal of Ecology **99**: 689-702.

Lake, J.C., Leishman, M.R. 2004. Invasion success of exotic plants in natural ecosystems: the role of disturbance, plant attributes and freedom from herbivores. Biological Conservation **117**:215-226.

Lavorel, S., McIntyre, S., Landsberg, J., Forbes, T.D.A. 1997. Plant functional classifications: from general groups to specific groups based on response to disturbance. Trends in Ecology and Evolution **12**: 474-478.

Lefcheck, J. S., Byrnes, J. E. K., Isbell, F., Gamfeldt, L., Griffin, J. N., Eisenhauer, N., Duffy, J. E. 2015. Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. Nature Communications **6**: 6936.

Levins, R. 1968. *Evolution in changing environments*. Princeton, NJ: Princeton University Press.

Liang, J., Zhou, M., Tobin, P.C., McGuire, A.D., Reich, P.B. 2015. Biodiversity influences plant productivity through niche-efficiency. Proceedings of the National Academy of Sciences **112**: 5738-5743.

Malavasi, U. C., Malavasi, M. M. 2001. Leaf characteristics and chlorophyll concentration of Schyzolobium parahybum and Hymenaea stilbocarpa seedlings grown in different light regimes. Tree Physiology **21**: 701–703.

McCann, K.S. 2000. The diversity-stability debate. Nature **405**: 228-233.

Naes, T., Aastveit, A.H., Sahni, N.S. 2007. Analysis of split-plot designs: an overview and comparison of methods. Quality and Reliability in Engineering International **23**:801-820.

Nebraska Game and Parks Commission. 2011. *The Nebraska natural legacy project: a comprehensive wildlife conservation strategy*. Lincoln, Nebraska, USA.

NRCS Web Soil Survey. USDA. https://websoilsurvey.nrcs.usda.gov/app/ (Accessed 16 Jan. 2017).

Price, Katilyn. 2015. Plant diversity affects performance of invasive thistles in restored Nebraska grasslands. Thesis.

R Core Team. 2013. *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria.

.

Risser, Paul G. 1995. Biodiversity and Ecosystem Function. Conservation Biology **9**: 742-746.

Shipley, B. 2006. Net assimilation rate, specific leaf area and leaf mass ratio: which is most closely correlated with relative growth rate? A meta-analysis. Functional Ecology **20**:565-574.

Smith, D., Henderson, K., Houseal, G., Williams, D. 2010. *Tallgrass Prairie Center Guide to Prairie Restoration in the Upper Midwest.* Des Moines: University of Iowa Press.

Smith, M.D., Knapp, A.K., Collins, S.L. 2009. A framework for assessing ecosystem dynamics in response to chronic resource alterations induced by global change. Ecology **30**:3279-3289.

Suding, K. N., Ashton, I.W., Bechtold, H., Bowman, W.D., Mobley, M.L., Winkleman, R.W. 2008. Plant and microbe contributions to community resilience in a directionally changing environment. Ecological Monographs **78**: 313-329.

Suding, K. N. 2011. Toward an Era of Restoration in Ecology: Successes, Failures, and Opportunities Ahead. Annual Reviews in Ecology and Evolutionary Systems **42**:465-487.

Tilman, D., Reich, P. B., Knops, J., Wedin, D., Mielke, T., Lehman, C. 2001. Diversity and Productivity in a Long-Term Grassland Experiment. Science, New Series **294**:5543.

Useche, A., Shipley, B. 2010. Interspecific correlates of plasticity in relative growth rate following a decrease in nitrogen availability. Annals of Botany **105**: 333–339. https://doi.org/10.1093/aob/mcp284.

Wilson, Peter J., Thompson, Ken, Hodgson, John G. 1999. Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. New Phytologist **143**:1.

Zuur, A.F., Ieno, E.N., Smith, G.M. 2007. *Analyzing Ecological Data.* New York: Springer Science.

VI. Tables and Figures

TABLES

Table 3.1. Significant variation in mean specific leaf area by treatment type, describing the variation in group means among experimental treatments. Contrasts derived from post-hoc TukeyHSD 95% significance tests of variation calculated from linear mixed-effects models which included all treatment and soil chemistry parameters and parsed via backwards selection to remove insignificant parameters. HD=high diversity plots, LD=low-diversity plots, Rainout=rainout shelter, Bio. removal=biomass removal.

Taxonomic group	Group contrasts	Difference	P-value
Species-weighted subplot average	HD :: LD	0.083	0.012
	Rainout :: Control (ignoring diversity level)	0.166	1.59e-05
	Rainout :: Control LD	0.125	0.043
	Rainout :: Control HD	0.215	0.0004
Andropogon gerardii	HD :: LD	0.153	2.25e-05
	Biomass removal (ignoring diversity)	0.0889	0.049
	Bio. removal :: no Bio. removal LD	0.1	0.348
	Bio. removal :: no Bio. removal HD	0.07	0.687
Solidago spp.	HD :: LD	0.139	0.002
	Rainout :: Control (ignoring diversity)	0.204	4.42e-05
	Rainout :: Control LD	0.204	0.004
	Rainout :: Control HD	0.213	0.059

Table 3.2. Significant parameters predicting variation in leaf chlorophyll content derived from linear mixed-effects models which included all treatment and soil chemistry parameters and parsed via backwards selection to remove insignificant parameters. Species-weighted subplot average represents the average chlorophyll by subplot, weighted by the cover of each species measured.

Taxonomic group	Significant Parameters	Regression coefficient	P-value
Species-weighted subplot average	NA	NA	NA
Andropogon gerardii	HD	262.27	<2e-16
Andropogon gerardii	Rainout	41.76	0.017
Andropogon gerardii	HD*biomass removal	64.07	0.018
Helianthus spp.	N addition	28.6	0.049
Solidago spp.	HD*rainout	657.936	0.003
	HD*N addition	-475.825	0.034
	HD*biomass removal	-645.224	0.024
Monarda fistulosa	Rainout	159.53	<2e-16
	Biomass removal	-78.6	1.03e-05
Glycyrrhiza lepidota	Rainout	-49.00	0.034
	Biomass removal	-91.4	0.001

Table 3.3. Significant variation in group mean chlorophyll content (mg chlorophyll/m^2 leaf tissue) by treatment type, describing the variation in group means among experimental treatments derived from a TukeyHSD 95% significance test of variation among group means. '::' indicates a comparison among measurement groups. 'Difference' indicates the increase or decrease in leaf chlorophyll (mg/m^2) from the first group to the second group in the comparison. Only significant differences are reported here.

Taxa	Group contrasts	Difference	P-value
Species-weighted subplot average	N addition :: no N addition (ignoring diversity level)	41.783	0.003
	N addition :: no N addition HD	62.051	0.009
	N addition :: no N addition LD	18.889	0.747
	N addition + Biomass removal HD	-61.151	0.027
	N addition + Biomass removal LD	4.416	0.996
Andropogon gerardii	HD :: LD	16.132	0.03
	Rainout :: Control (ignoring diversity level)	33.360	6.2e-06
	Rainout :: Control HD	23.912	0.037
	Ranout :: Control LD	54.742	0.001
	N addition : no N addition (ignoring diversity level)	33.898	1.08e-05
	N addition :: no N addition HD	35.073	0.001
	N addition :: no N addition LD	32.556	0.046
Helianthus spp.	N addition :: no N addition (ignoring diversity level)	28.6	0.049
Solidago spp.	HD :: LD	45.351	4.8e-06
	Biomass removal :: no biomass removal (ignoring diversity level)	-51.971	4.8e-06
	Biomass rem.:: no biomass rem. HD	-33.429	0.234
	Biomass rem. :: no biomass rem. LD	-63.649	0.00006
Monarda fistulosa	Rainout :: Control	23.046	0.001
	N addition :: no N addition	-20.852	0.022
	Biomass removal :: no biomass removal	-52.269	0.0002
Glycyrrhiza lepidota	Rainout :: Control	-69.033	0.0002
	N addition :: no N addition	44.916	0.013
	Biomass removal :: no biomass removal	-62.693	0.001

Figure 3.1. A diagram of the experimental disturbance treatments added to the center of each low-diversity and high-diversity research plot in May 2015. Rainout shelters are 5m x 2.5m, and treated subplots are all 2mx1m. Samples were collected 50cm within the border of each of these plots to minimize accidental measurement of neigboring treatment effects. Comparison plots are plots sampled outside rainout shelters with no disturbances added.

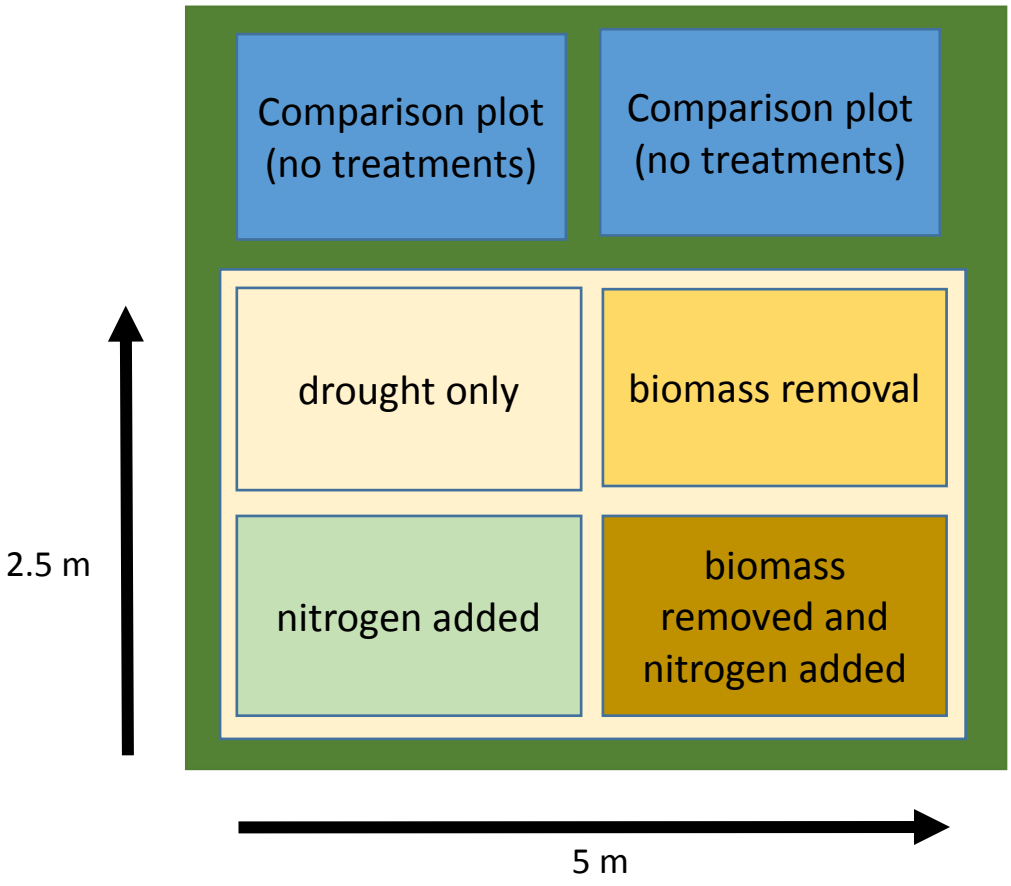


Figure 3.2. Figure 1. Species-weighted subplot specific leaf area (SLA) by diversity (graph 1 = LD, graph 2 = HD) and treatment effects (Ctrl=control, R=rainout-shelter only, R+N=rainout shelter plus nitrogen addition, R+B=rainout shelter plus biomass removal). Red dots and vertical bars represent the mean +/- 1 standard error. Open dots indicate the subplot average SLA values, measured from samples collected in early July 2016 from all treatment and control plots in low and high-diversity plots.

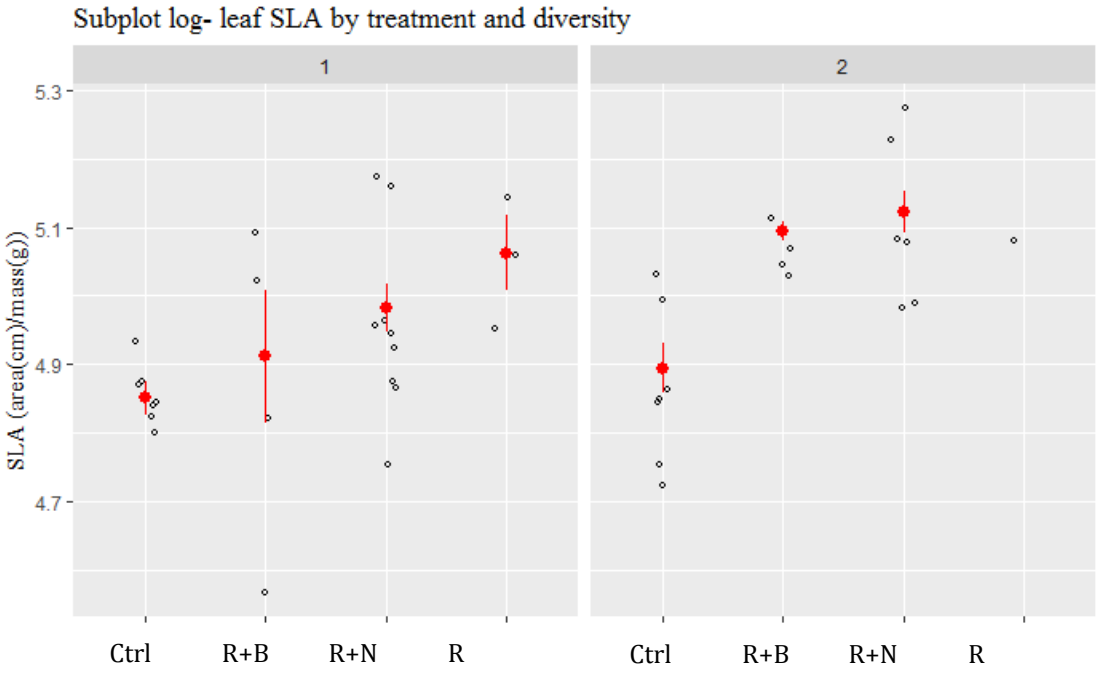


Figure 3.3. *Andropogon gerardii* specific leaf area varies by diversity and biomass removal (1=no biomass removal, 2=biomass removal), but within diversity levels specific leaf area does not differ significantly by biomass removal treatment. Red dots and vertical bars represent the mean +/- 1 standard error. Open dots indicate individual samples, collected in early July 2016 from all treatment and control plots in low and high-diversity plots.

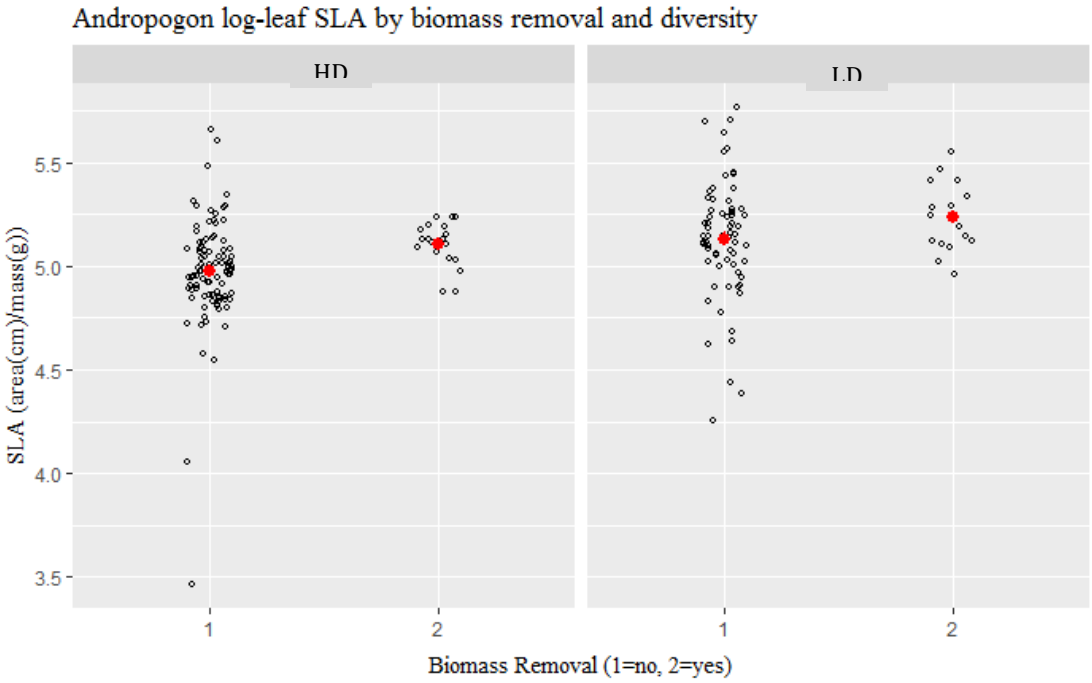


Figure 3.4. *Solidago spp*. specific leaf area varies by rainout shelter and diversity effects. Red dots and error bars represent the mean +/- 1 standard error. Open dots indicate individual samples, collected in early July 2016 from all treatment and control plots in low and high-diversity plots.

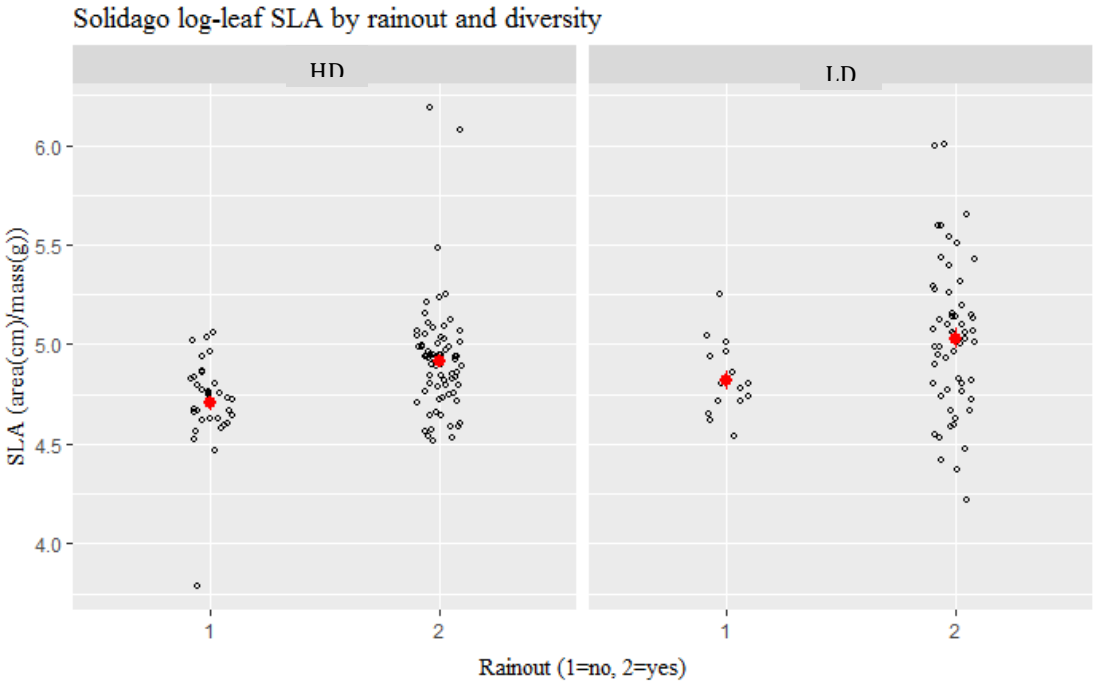


Figure 3.5. Species-weighted subplot leaf chlorophyll varies significantly by diversity and treatment effects (Ctrl=control, R+B=rainout + biomass removal, R+N=rainout+nitrogen addition, R+N+B=rainout+biomass removal+nitrogen addition, R=rainout only). Red dots and error bars represent the mean +/- 1 standard error. Open dots indicate subplot average chlorophyll, weighted by species cover, collected in early July 2016 from all treatment and control plots in low and high-diversity plots.

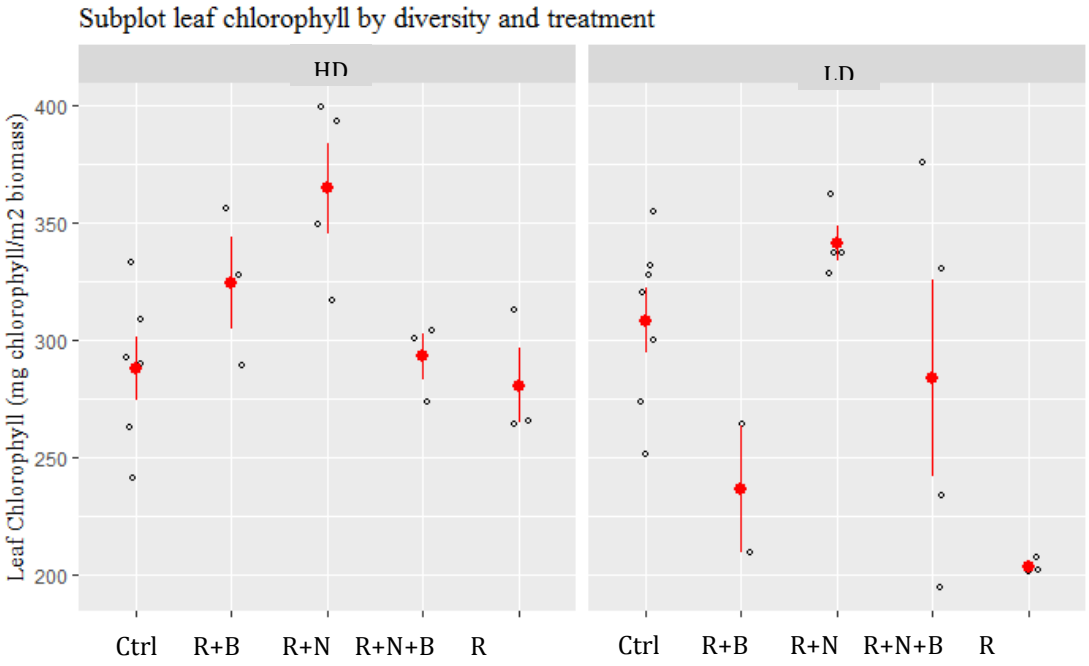


Figure 3.6. *Andropogon gerardii* leaf chlorophyll (mg/m^2, measured using hand-held fluorometer) varies significantly by rainout shelter, nitrogen addition, and diversity effects (graph 1 = LD, graph 2 = HD). Treatment codes: Ctrl=control, R=rainout shelter only, R+N=rainout+nitrogen addition. Red dots and error bars represent the mean +/- 1 standard error. Open dots indicate individual samples, collected in early July 2016 from all treatment and control plots in low and high-diversity plots.

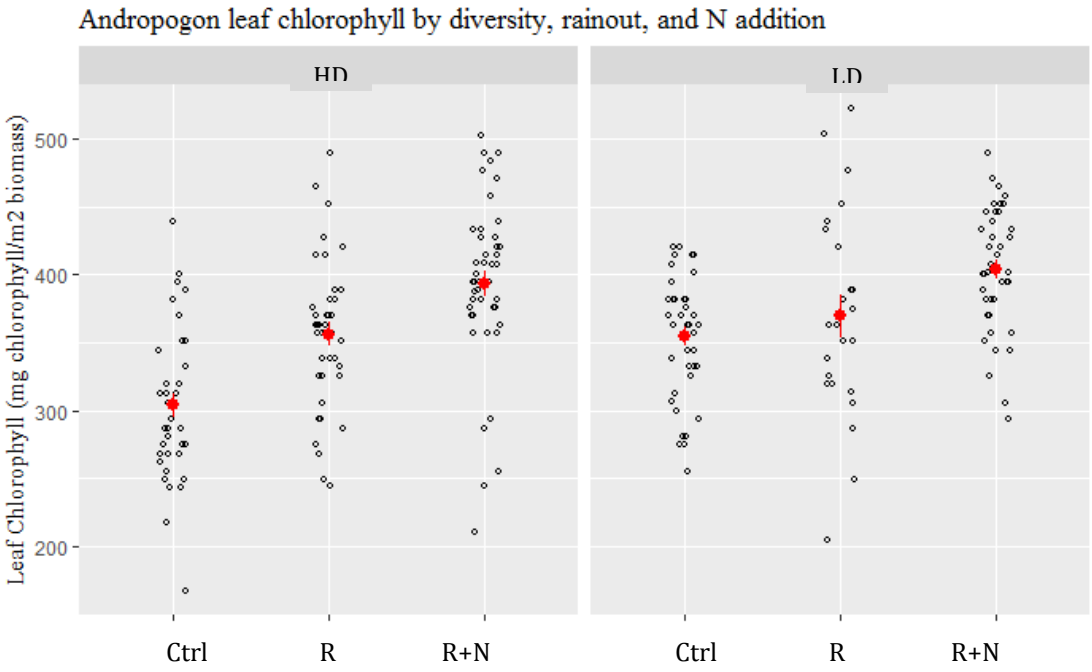


Figure 3.7. *Helianthus spp.* (Sunflower genus) leaf chlorophyll content (mg/m^2, measured using hand-held fluorometer) varies by nitrogen addition. Red dots and vertical bars represent the mean +/- 1 standard error. Open dots indicate individual samples, collected in early July 2016 from all treatment and control plots in low and high-diversity plots.

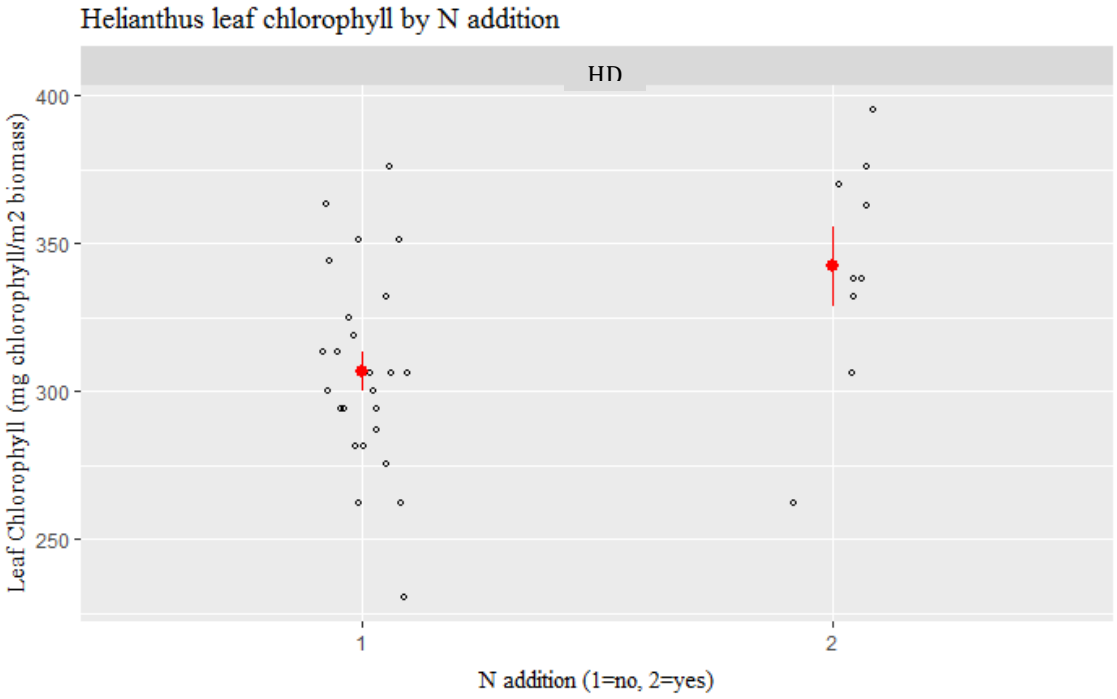


Figure 3.8. Solidago leaf chlorophyll (mg/m^2, measured using hand-held fluorometer) varies significantly by biomass removal and diversity treatments (graph 1=LD, graph 2=HD). Red dots and vertical bars represent the mean +/- 1 standard error. Open dots indicate individual samples, collected in early July 2016 from all treatment and control plots in low and high-diversity plots.

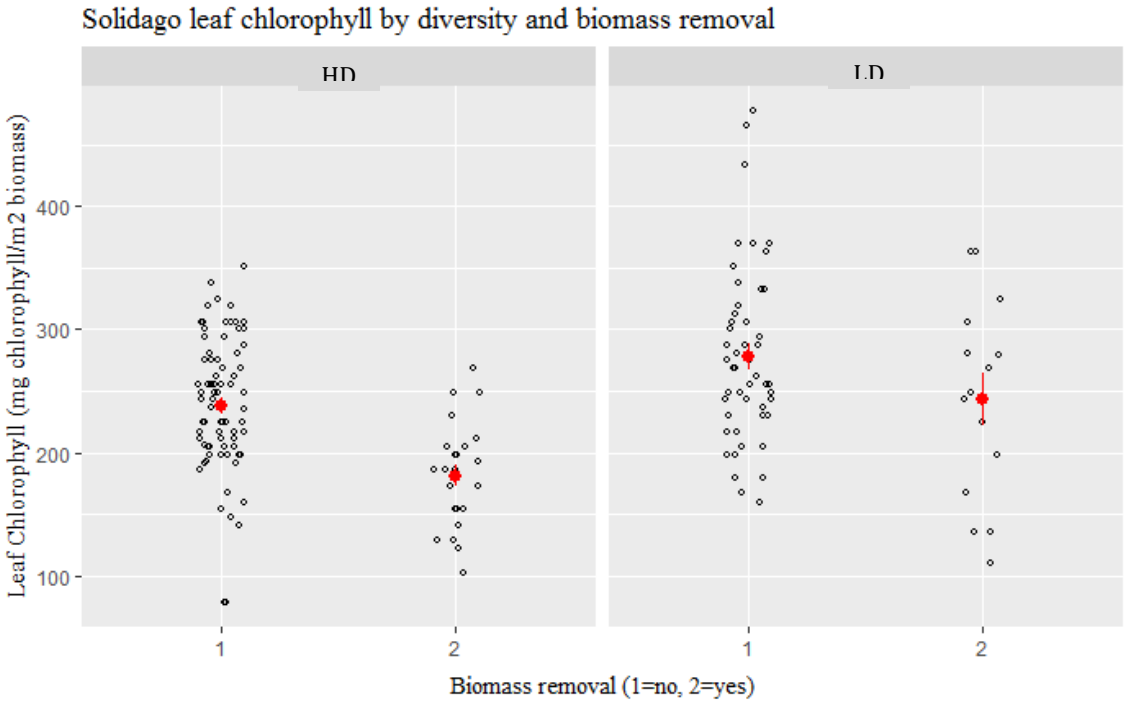


Figure 3.9. *Monarda fistulosa* is only present in high-diversity plots, and its leaf chlorophyll (mg/m^2, measured using hand-held fluorometer) varies by rainout shelter and treatment effects. Treatment codes: Ctrl=control, R=rainout-only, R+N=rainout + nitrogen addition, R+N+B=rainout + N addition + biomass removal. Red dots and vertical bars represent the mean +/- 1 standard error. Open dots indicate individual samples, collected in early July 2016 from all treatment and control plots in low and high-diversity plots.

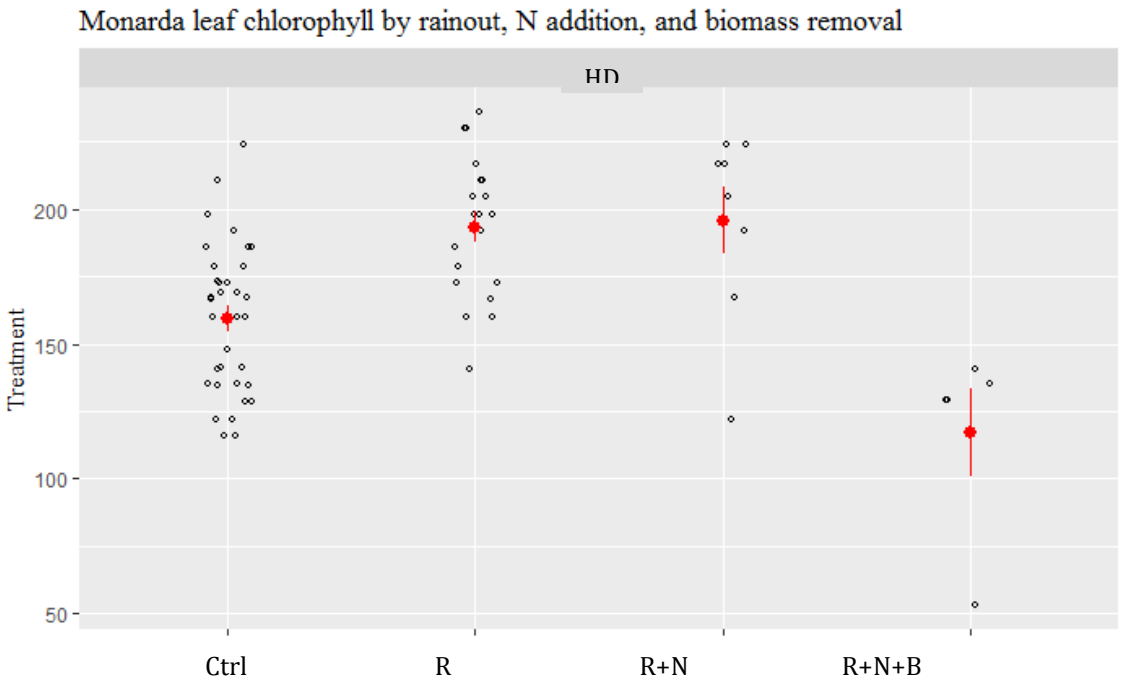
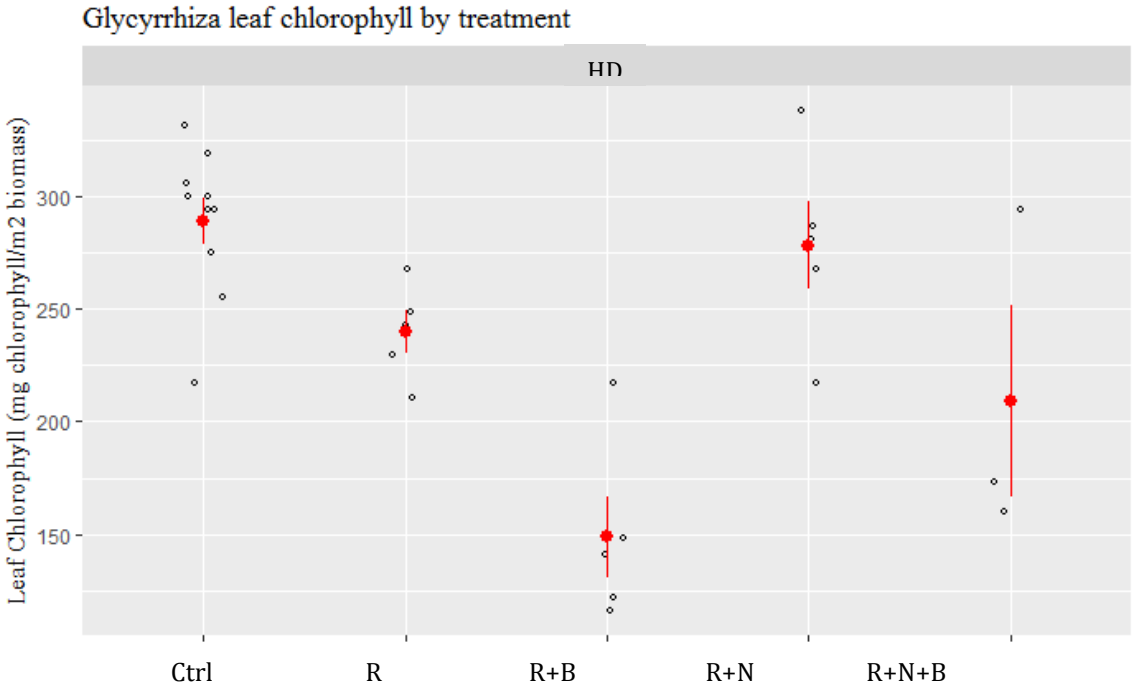


Figure 3.10. *Glycyrrhiza lepidota* is present only in high-diversity plots, and its leaf chlorophyll (mg/m^2, measured using hand-held fluorometer) varies by rainout shelter and treatment effects. Treatment codes: Ctrl=control, R=rainout-only, R+B=rainout + biomass removal, R+N=rainout + nitrogen addition, R+N+B=rainout + nitrogen addition + biomass removal. Red dots and vertical bars represent the mean +/- 1 standard error. Open dots indicate individual samples, collected in early July 2016 from all treatment and control plots in low and high-diversity plots.



CHAPTER 4: PLANT DIVERSITY CONTRIBUTES TO NUTRIENT CYCLING AND ITS RESPONSE TO DISTURBANCE

I. Introduction

Nutrient cycling in grasslands contributes to ecosystem stability and the provisioning of key ecosystem services, from waste processing to carbon sequestration (Leman and Kleber 2015; Gibson 2009). The ability of systems to maintain this function is related to a number of factors, including biodiversity and management (Wickings et al. 2010). Carbon cycling in grasslands is a result of interactions between above- and below-ground systems which contribute to soil formation, ecosystem fertility, and ultimately sustained productivity and water and nutrient cycling (Sylvain and Wall 2011). In this study, I measured the impacts of altered levels of biodiversity and added disturbances on carbon cycling in the near-surface soil system. Specifically, I studied the variation in litter decomposition and soil microbial activity in response to the parameters of diversity and disturbance treatment in a tallgrass prairie restoration in central Nebraska planted in 60m x 60m plots of low or high diversity.

In their foundational discussion of carbon cycling research, Singh and Gupta (1977) note that ‘a consideration of decomposition and soil respiration seems inevitable’ in understanding the nutrient cycling of a particular ecosystem. Though methods for measuring these parameters have changed, testing the interactions between litter decomposition and soil decomposer communities remains central to an understanding of nutrient cycling in ecosystems. Litter inputs to the soil system are an interface between the aboveground and belowground systems, and the rate of decomposition signals how

quickly nutrients from decaying plant material may be incorporated into the soil system, whether through fragmentation, leaching, fungal digestion, and other methods of litter breakdown. Plant litter traits are important determinants of litter decomposition rates and soil nutrient content in the upper 5-10cm of soil (Cornelissen et al. 1999; Wickings et al. 2010), and deeper in the soil profile organic matter formation and microbial activity are largely driven by plant root exudates (deVries and Caruso 2016). Recently, it has been recognized that the chemical recalcitrance of litter inputs may not be predictive of their stability within soils, as labile carbon is often consumed with higher efficiency by the microbial community, leading to greater microbial biomass formation and lower soil nutrient loss from mineralization or leaching (Cotrufo et al. 2015).

Soil microbial respiration, meanwhile, is a direct measure of the activity of soil microbes, including both bacteria and fungi. This measure includes the potential processing of litter inputs, but also other inputs including root exudates (Leman and Kleber 2015). Soil respiration rates are indicators of soil fertility (ie, the availability of nutrients accessible via microbial processing; Haney et al. 2008) as well as the overall physical and biological structure of the oil, as soil biological activity builds soils via particle aggregation (Evanylo and McGuinn 2009) and increases the overall biotic support potential of the soil system.

Soil microbial activity is strongly influenced not only by plant community composition, but also by the physiological response of individual plants to environmental stresses (Bardgett and van der Putten 2014; de Vries and Caruso 2016) via alterations in root exudates and physical chemical composition. It is therefore possible for changes in the aboveground plant community to significantly affect microbial community structure

and function as well as the rate of litter decomposition and its incorporation into the soil food web (Diaz et al. 2005).

In this study, I predicted that litter mass loss and soil respiration would be higher overall in high-diversity than in low-diversity plots due to the increased amount and variety of nutrient inputs to the system. Among disturbance treatment subplots, I predicted that litter mass loss would be greater in the comparison plots than in subplots beneath rainout shelters, and that nitrogen addition treatments would correspond with greater mass loss while biomass removal would correspond with lower mass loss compared with the rainout-only treatments due to dry conditions in biomass removal plots. For soil respiration measured at the subplot scale, I predicted that soil respiration would be higher in high-diversity plots, higher within rainout shelters than in comparison plots, and lower in nitrogen addition plots than in any other treatment subplot due to reduced plant root exudates. At the whole-plot (60m x 60m) scale, I predicted that soil respiration would increase with increasing soil organic matter and decrease with increasing soil nitrate levels.

II. Methods

STUDY SITE

This study took place at the Platte River Prairies, a Nature Conservancy prairie restoration in south-central Nebraska. The site, 10km south of Wood River (40°44'37.8"N 98°35'23.9"), is located within the Central Platte River ecosystem, which is identified as a Biologically Unique Landscape by the Nebraska Game and Parks Commission (NGPC 2011). Soils at the site include Wann loam, rarely flooded; Caruso loam rarely flooded; and Bolent-Clamux complex, occasionally flooded (NRCS).

In 2010, The Nature Conservancy seeded twelve 60m x 60m plots in native tallgrass prairie, with four plots each of Big Bluestem (*Andropogon gerardii*) monoculture, mid-diversity, and high-diversity seed mixes (Nemec 2013). The diversity treatments have established with significant differences between monoculture and high-diversity species richness (34 vs. 73 species, respectively; Price 2015). The 'monoculture' plots have accumulated a number of additional species and are referred to as low-diversity plots throughout this study. Functional groups represented within the whole-plots include C3 (cool-season) grasses, C4 (warm-season) grasses, and both leguminous and non-leguminous forbs. This grouping is a very broad generalization of plant types and was chosen for ease of categorization and the large differences in phenology and nutrient acquisition represented by each group, which may influence their response to disturbance (Lavorel et al. 1997).

The site is maintained via burning; the most recent burn was in March of 2015, six weeks before the start of the study, with no additional management during the course of this study. The burn removed all biomass cover at the beginning of the research period, leading to significant differences in bareground and litter cover by year.

The research site covers an environmental gradient with increasing soil organic matter (percent mass lost on ignition) from north to south (Figure 4.1). This gradient correlates with other soil chemical and functional traits, including soil respiration (Solvita CO2 Burst test, ppmC) and soil pH (Figure 4.2). There is also a significant gradient in nitrate (ppm, KCL-extractable NO_3-) from southwest to northeast (Figure 4.3). These gradients were included as fixed effects in statistical analyses.

Precipitation and temperature during the course of this study varied significantly by year. Precipitation in 2015 was much higher than in 2016, with a major peak in June (25cm total, mostly occurring in a single event). 2016 rainfall was more evenly distributed, with the highest rainfall totals in April (16cm) and July (15cm), and low rainfall in other months (Figure 4.4). Average maximum and minimum temperatures were higher in 2016, with sustained higher average temperatures across the spring, summer, and fall (Figure 4.4). The minimum temperatures in January 2015 and 2016 were -19.44^{o}C and -23.3^{o}C, respectively, and maximum temperatures in July 2015 and 2016 were 35^{o}C and 38.3^{o}C.

TREATMENTS

In May of 2015, I constructed 2.5 x 5m rainout shelters in the center of each low-diversity and high-diversity research plot. Beneath each shelter, I established four 1m x 2m plots of additional experimental disturbances, with 50cm spacing between each treatment. These disturbance treatments consisted of either no additional treatment, biomass removal (cutting biomass down to 4-8cm height during the first week of July), nitrogen fertilizer addition (30g of inorganic 34-0-0 dry ammonium nitrate fertilizer added twice per summer, first in mid-June and then six weeks following the first treatment for a rate of 10gNH_4NO_3/ m-1/summer), or a combination of biomass removal and nitrogen addition to (Figure 4.5).

Treatments were chosen to represent current threats to grasslands. Rainout shelters were built to impose water stress to the vegetation, which would potentially lower their threshold of resilience to additional disturbances. Drought is expected to be exacerbated in the mid-latitudes where the majority of grassland systems exist in coming

decades (IPCC 2014), and in conjunction with increases in summer temperatures poses an increasingly large threat to grasslands (de Boek et al. 2011). Beneath rainout shelters, four 1m x 2m disturbance plots were established with either no additional treatments, nitrogen addition, biomass removal, or a combination of the two. Nitrogen addition (inorganic nitrogen ammonia fertilizer) was used to represent the widespread terrestrial eutrophication arising largely from agricultural drift and fossil fuel burning (Suding et al. 2008; Tilman et al. 2008). Biomass removal simulated haying, a common management strategy in prairies throughout the United States. Haying is considered an alternative to grazing or fire (Smith et al. 2010), and may alter ecosystem function by removing dominant species at peak growth and reducing the amount of biomass left on the field for subsequent growing seasons.

DATA COLLECTION

Multiple variables related to nutrient cycling were measured in disturbance subplots, including soil organic matter and inorganic nitrogen levels, soil microbial respiration, and litter decomposition. These data were gathered via litter decomposition trials and soil collection.

Soil moisture was recorded via a hand-held moisture meter which recorded moisture in the top 10cm of soils, and via moisture access tubes dug to 30cm and 50cm in the center of each rainout plot. Soil moisture was measured four times in June-August 2015 from the edge, 25cm within, 50cm within, and at the center of each subplot as well as from moisture access tubes in the center of each shelter (Figure 4.6).

Soil collection occurred at the disturbance-addition subplot scale in 2015 and at both the whole-plot and disturbance-addition scales in 2016. Temperatures averaged 19.4

and 19.7^{o}C on sampling dates, and sampling occurred no less than 48 hours after any rainfall event. In 2016. A single composite core composed of three sub-samples (top 20cm of soil, collected using a hand auger) was collected within each disturbance-treatment subplot for all high-diversity and low-diversity plots.

Soil samples were stored at 2^{o}C pending analysis, and analyses were conducted by Ward Labs, an agricultural testing lab in Kearney, Nebraska. In 2015, disturbance-treatment soil samples were analyzed for pH, total soil organic matter, total organic carbon, total organic nitrogen, soil nitrate, microbial biomass carbon, and soil respiration. In 2016, both whole-plot and subplot soil cores were analyzed for total SOM, KCL-extractable nitrate, microbial biomass carbon, and microbial respiration. Soil organic matter was measured as percent lost on ignition via combustion tests; soil inorganic nitrogen as the parts per million KCL-extractable nitrate per sample, and estimated microbial biomass C via a chloroform-extraction method. Soil respiration was measured via the Solvita CO2-Burst test (Haney et al. 2008), which measures the flush of carbon emitted following the rewetting of air-dried soil samples as a proxy for the total potential microbial activity within the soil.

20cm x 20cm litterbags were constructed out of 1mm wire mesh and filled with 3g of mixed *Solidago canadensis* and *gigantea* (two common Goldenrod species) leaves, collected from full-grown individuals within the restoration plots. Collected leaves were fully-extended, in the upper third of the plants, and uniformly green and free of disease. Leaves were air-dried for at least 10 days before weighing and sealing in litterbags. Litterbags were numbered, and two litterbags placed in each 2mx1m disturbance subplot and in each 2m x 1m comparison plot. Litterbags were placed in subplots on 3 July and

collected 1 October 2015, and placed in subplots on 26 June and collected 1 December 2016. Because not enough intact bags were recovered in 2016 to conduct statistical analyses, this sample was removed from the data set.

ANALYSIS

Data files and scripts used in statistical analyses (R Core Team) are included in Appendix I. Variation in soil respiration and litter decomposition was assessed via multi-model inference (Burnham and Anderson 2007), using a set of *a priori* linear mixed-effects models containing variables which I predicted would play a role in controlling the variation in litter decomposition and soil respiration (Grueber et al. 2011). No interaction effects were specified in these models due to the limited sample size.

The response variable of litter mass loss was log-transformed to fit a normal distribution for analysis. To measure the variation in litter mass loss by potential explanatory variables, three sets of models were constructed. The first model set hypothesized that treatment effects were the primary determinants of variation in litter decomposition. This 'treatment-effects' model set consisted of the following:

[M1.1] mass loss ~ rainout + nitrogen + biomass + (1|site)

[M1.2] mass loss ~ diversity + (1|site)

[M1.3] mass loss ~ diversity + rainout + (1|site)

[M1.4] mass loss ~ rainout + (1|site),

where 'mass loss' is the percent weight lost, in dry weight, at the end of the incubation period, 'rainout' indicates the 2mx1m subplots with only the rainout-shelter treatment, 'nitrogen' indicates the 2mx1m subplots with nitrogen added, 'biomass' indicates the 2mx1m subplots receiving the biomass removal treatment, and 'diversity'

indicates the large-scale treatment of diversity level. (1|site) is a random-effects variable that accounts for random variation by whole-plot.

Models for site-only effects included:

[M1.5] mass loss ~ bare + KCL-N + SOM + (1|site)

[M1.6] mass loss ~ dist + (1|site)

[M1.7] mass loss ~ SOM + (1|site),

where 'dist' refers to the distance, in meters, from the edge of the Platte River to the center of each set of experimental treatment plots, 'bare' refers to the average percent bareground in each subplot, 'pH' refers to the average soil pH of each subplot, and 'SOM' refers to the average total soil organic matter content measured for each subplot. SOM, soil moisture, and distance variables were never included in the same model due to the high level of correlation (r=0.8) between these three variables.

Finally, I constructed a set of mixed-parameter models, where large-scale parameters from both site and location were included to see whether a mix of site and treatment effects was the most predictive:

[M1.8] mass loss ~ diversity + dist + (1|site)

[M1.9] mass loss ~ rainout + dist + (1|site)

[M1.10] mass loss ~ diversity + bare + KCL-N + SOM + (1|site).

A final, random-effects-only model was included in the model set to test whether any models performed better or worse than random:

[M.R] mass loss ~ 1 + (1|site).

Soil respiration was tested according to a similar group of models, though the parameters were expected to influence soil microbial activity in different ways

(decomposition was predicted to be more influenced by aboveground variables, and soil respiration was predicted to be more influenced by belowground variables). Sampling year was included in every model due to the statistically significant variance in soil respiration by year. This set included:

Treatment models:

[M2.1] respiration ~ year + rain + nitro + biomass + (1|site)

[M2.2] respiration ~ year + diversity + (1|site)

[M2.3] respiration ~ year + diversity + rainout + (1|site)

[M2.4] respiration ~ year + rainout + (1|site);

Site models:

[M2.5] respiration ~ year + dist + bare + KCL-N + (1|site)

[M2.6] respiration ~ year + dist + (1|site)

[M2.7] respiration ~ year + diversity + bare + KCL-N + SOM + (1|site)

[M2.8]respiration ~ year + avg.som + (1|site);

Mixed-parameter models:

[M2.9] respiration ~ year + diversity + dist + (1|site)

[M2.10] respiration ~ year + rainout + dist + (1|site).

Following the determination of the best-fit models for each response variable, the best models were evaluated using linear-mixed-effects regression and significant parameters reported. Where significant parameters were continuous, individual correlation tests were performed to determine the linear correlation between the response and explanatory variables. Where significant parameters were categorical, I performed least-squares means tests to calculate the variation in group means by the explanatory variable and reported the variation in group means.

III. Results

One model supplied 96% of the AICc model weights for litter mass loss; that model was M1.4 (mass loss ~ rainout). The next-best model had a delta-AICc value of 6.48 (delta-AICc describes how much less explanatory weight the model has than the top model) and included an additional parameter, and so was not included in the model set (Grueber et al. 2011). The only models which performed better than random were those containing treatment effects. All site-effects models performed worse than random. A least-squares means test of the variation in litter mass loss by rainout shelter showed an average mass loss of 32.23% outside of rainout shelters and 44.21% within rainout shelters (Table 4.1). Variation in litter mass loss by rainout shelter, diversity, and additional subplot treatments within rainout shelters were visualized using dotplots with group means and standard error added (Figures 4.7 and 4.8).

The best-fit model for soil respiration was M2.7 (year + diversity + bare + KCL-N + avg.som), with 100% of the AICc model weight. All parameters in this model were significant, with bareground, average SOM, average soil nitrate, and diversity all positively correlated with soil respiration. 2015 had over double the average soil respiration than did 2016, accounting for the majority of variation among soil samples (Table 4.2 for correlations among continuous variables, Table 4.3 for least-squares means comparisons of group means by categorical variables). Variation in soil respiration by year and diversity were visualized using dotplots with group means and standard error (Figure 4.9), and variation by soil variables (bareground, nitrate, and soil organic matter) visualized with a plot of the linear regression between these variables (Figure 4.10).

IV. Discussion

The largest measured sources of variation in litter mass loss and soil respiration in this study match the predicted sources of variation - for litter decomposition, the aboveground treatment effect of rainout shelters was the most significant predictive parameter. Soil respiration, meanwhile, was most related to uncontrolled site characteristics.

Although belowground soil characteristics can significantly influence litter decomposition (Cleveland et al. 2014; Cotrufo et al. 2013), my litterbags were placed on the soil surface and had less contact with the belowground decomposer community compared to litter decomposition tests which bury litterbags in the soil or place litter on the soil surface without a mesh bag. Partially due to this effect, treatment effects were more important than soil gradients in predicting variation in litter decomposition in my study.

Rainout shelters provided the largest treatment effect by far for litter decomposition, with mass loss rates a third higher beneath rainout shelters than outside rainout shelters. This variation is the opposite of what I predicted given that rainout shelters were intended to intercept rainfall and thereby reduce moisture and biological activity within the shelters. The most likely explanation for this opposite effect is that rainout shelters actually provided the inverse of the treatment they were designed to impose; by providing some protection from sun exposure, they limited transpiration and increased overall moisture levels beneath shelters. This effect was visually noticeable in July and August, when vegetation appeared greener within shelters than without. Additionally, simply blocking vertical flow from rainfall did not limit lateral flow of moisture when the field flooded following heavy rains, nor did it impede belowground

movement of water (moisture tubes inserted into the center of rainout shelters and test subplots outside rainout shelters showed standing water at 30cm depth for almost the entire summer in both 2015 and 2016). If we accept that rainout shelters were holding in more moisture than they were impeding, it is logical that decomposition may be higher within the shelters than without.

The contribution of uncontrolled soil nutrient gradients to variation in soil respiration followed the pattern I expected to see, as a greater supply of limiting resources in general corresponds with increased biological activity. More interesting to my study of the relationship between biodiversity and ecosystem function is the clear positive relationship between increased biodiversity and soil respiration. The large increases in soil respiration with increased diversity has been shown to emerge through a number of mechanisms, including the increased diversity of root exudates from high-diversity plants which cultivate a more diverse and active soil microbial community (Zak et al. 2014). Soil samples were collected in early June in 2015 and 2016, respectively, which is early in the season for soil activity; however, due to a greater species richness, high-diversity plots may become active earlier in the season, therefore stimulating soil activity earlier in the season.

The reduction in soil respiration in 2016 may be related to the increased soil cover provided by a much more extensive and deep litter cover in 2016 compared with 2015 (Table 4.4). This extensive litter cover led to lower soil temperatures and slower plant growth in 2016 compared with 2015. Litter cover has been associated with large fluctuations in soil activity in various studies (Gibson 2009). This variable fluctuates

widely among years depending on the amount of growth the previous year and what treatments have been implemented to remove standing dead biomass.

The two indices of nutrient cycling I measured for this study, soil respiration and litter decomposition, are widely used to assess the nutrient cycling capacities of ecosystems (Singh and Gupta 1977). Litter decomposition is one mechanism by which the products of plant primary productivity cycle into the belowground system, and soil respiration is a measurement indicating the biological activity of the soil and its ability to process those nutrients.

Nutrient cycling was controlled by several parameters in this study. Litter decomposition was predominantly influenced by rainout shelter effects, which is most likely a proxy for the moisture and temperature control provided by the shelters. Shelter roofs were 4-5 feet above the soil surface and constructed from a clear roofing material that allows 99% of visible light to pass through; however, it is possible that the roofs created a greenhouse effect via shading and maintaining a more constant temperature, creating more ideal conditions for litter decomposition compared with the unstable moisture and temperature regimes outside of the rainout shelters.

V. Conclusion

Soil respiration was influenced by both site and treatment effects, with plant diversity and pre-existing soil nutrient gradients responsible for the variation in soil respiration. Higher levels of planted diversity were positively correlated with soil respiration rates. This correlation indicates that plant diversity can have a direct effect on soil microbial activity independent of soil characteristics, and suggests that planting a

diverse array of native species may contribute to increasing belowground as well as aboveground biological activity.

I am interested in this link between plant diversity and soil biological activity primarily because increased soil biological activity may lead to an increase in soil nutrient mineralization, greater primary productivity, and ultimately, higher rates of soil formation in prairie restorations (Oades 1982; Golchin et al. 1994; Cotrufo et al. 2013). Establishing a clear link between biodiversity and soil formation could encourage more diverse restoration plantings and help to establish restorations with stronger internal feedbacks which lead to self-maintaining systems that are both lower-maintenance and provide the associated ecosystem services, including carbon sequestration (Horwath 2015) and water storage (Hudson 1994) which humans and other species require.

VI. Literature Cited

Bardgett, R.D., van der Putten, W.H. 2014. Belowground biodiversity and ecosystem functioning. Nature **515**: 505-511.

De Boeck, H. J., Dreesen, F. E., Janssens, I. A., Nijs, I. 2011. Whole-system responses of experimental plant communities to climate extremes imposed in different seasons. New Phytologist **189**: 806–817.

Burnham, K.P., Anderson, D.R. 2007. *Model selection and multimodel inference: a practical information-theoretic approach (2nd ed)*. New York: Springer-Verlag.

Clark, C. M., & Tilman, D. 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature **451**(7179): 712–5.

Cleveland, C.C., Reed, S.C., Keller, A.B., Nemergut, D.R., O'Neill, S.P., Ostertag, R. Vitousek, P.M. 2014. Litter quality versus soil community controls over decomposition: a quantitative analysis. Oecologia **174**(1): 283-94.

Cornelissen, J.H.C., Perez-Harguindeguy, N., Diaz, S., Grime, J.P., Marzano, B., Cabido, M., Vendramini, F., Cerabolini, B. 1999. Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents. New Phytologist **143**: 191-200.

Cotrufo, M. F., Wallenstein, M. D., & Boot, C. M. 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization : do labile plant inputs form stable soil organic matter? Global Change Biology **19**(4): 988-95.

Díaz, S., Lavorel, S., Chapin, F. S., Paula, I. I. I., Diego, a T., Karl, E. G. 2005. Chapter 7, Functional Diversity: At the crossroads between ecosystem functioning and environmental filters. In: *Terrestrial Ecosystems in a Changing World*, Canadell, J.G., Pataki, D.E., Pitelka, L.F., Eds. New York: Springer.

Evanylo, G., McGuinn, R. 2009. Agricultural management practices and soil quality: measuring, assessing, and comparing laboratory and field test kit indicators of soil quality attributes. Virginia Cooperative Extension **452**: 400.

Fierer, N. 2003. Stress ecology and the dynamics of microbial communities and processes in the soil. Dissertation. University of California.

Gibson, David J. 2009. *Grasses and grassland ecology*. New York: Oxford University Press.

Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P. 1994. Study of free and occluded particulate organic matter in soils by solid-state 13c CP/MAS NMR spectroscopy and scanning electron microscopy. Australian Journal of Soil Research **32**: 285-309.

Grueber, C.E., Nakagawa, S., Laws, R.J., Jamieson, I.G. 2011. Multimodel inference in ecology and evolution: challenges and solutions. Journal of Evolutionary Biology **24**:699-711.

Haney, R.L., Brinton, W.H., Evans, E. Estimating soil carbon, nitrogen, and phosphorous mineralization from short-term carbon dioxide respiration. Communications in Soil Science and Plant Analysis **39**: 2706-2720.

Horwath, W. 2015. Chapter 12: Carbon cycling: the dynamics and formation of organic matter. In *Soil Microbiology, Ecology, and Biochemistry*, Eldor A. Paul, Ed. London: Academic Press.

Hudson, B.D. 1994. Soil organic matter and available water capacity. Journal of Soil and Water Conservation **49**(2): 189-194.

IPCC. 2014. *Climate Change 2014: Impacts, Adaptations and Vulnerability. Summary for Policymakers*. Cambridge: Cambridge University Press.

Kallenbach, C. M., Grandy, A., & Frey, S. D. 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nature Communications **7**: 13630.

Lehmann, J., Kleber, M. 2015. The contentious nature of soil organic matter. *Nature* **528**(7580): 60-68.

Nebraska Game and Parks Commission. 2011. *The Nebraska natural legacy project: State Wildlife Action Plan, 2nd ed.* Lincoln, Nebraska, NGPC.

NRCS Web Soil Survey. USDA. https://websoilsurvey.nrcs.usda.gov/app/ (Accessed 16 Jan. 2017).

Oades, J.M. 1984. Soil organic matter and structural stability: mechanisms and implications for management. Plant Soil **76**: 319-337.

Price, Katilyn. 2015. Plant diversity affects performance of invasive thistles in restored Nebraska grasslands. Thesis.

Singh, J.S., Gupta, S.R. 1977. Plant decomposition and soil respiration in terrestrial ecosystems. Botanical Review **43**:449-496.

Suding, K. N. 2011. Toward an Era of Restoration in Ecology: Successes, Failures, and Opportunities Ahead. Annual Reviews in Ecology and Evolutionary Systems **42**:465-487.

Smith, D., Henderson, K., Houseal, G., Williams, D. 2010. *Tallgrass Prairie Center Guide to Prairie Restoration in the Upper Midwest.* Des Moines: University of Iowa Press.

Sylvain, Z.A., Wall, D.H. 2011. Linking soil biodiversity and vegetation: implications for a changing planet. American Journal of Botany **98**:517-27.

de Vries, F.T., Caruso, T. 2016. Eating from the same plate? Revisiting the role of labile carbon inputs in the soil food web. Soil Biology and Biochemistry **302**: 4-9.

Wickings, K., Grandy, A.S., Reed, S., Cleveland, C. 2011. Management intensity alters decomposition via biological pathways. Biochemistry **104**: 365-379.

Zak, D. R., Holmes, W. E., White, D. C., Peacock, A. D., Tilman, D. 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology **84**(8): 2042–2050.

VI1. Tables and Figures

TABLES

Table 4.1. Least-squares means table of mean mass loss by rainout shelter vs. control plots. Litterbags were filled with 3g air-dried *Solidago* leaves, incubated on the soil surface for 100 days, then collected, dried in a 37ºC oven for72 hours, and reweighed to get an estimate of percent mass loss. Two litterbags were incubated per experimental subplot. Not all litterbags were able to be re-collected at the end of the incubation period.

	Estimate	*SE*	*DF*	*t-value*	*Lower CI*	*Upper CI*	*p-value*
no rainout	32.23	2.69	7.1	11.98	25.9	38.6	<2e-16
rainout	44.21	1.98	2.1	22.34	36.2	52.2	0.001

Standardized Within-Group Residuals:

Min	*Q1*	*Med*	*Q3*	*Max*
-1.99472591	-0.64607626	0.07971314	0.38611066	3.21428794

Number of Observations: 79

Number of Groups: 3

Table 4.2. Correlations between significant continuous variables and soil respiration (ppmCO2) for soils samples collected in 2015 and 2016 across low, medium, and high levels of planted diversity. 'Parameters' here are continuous variables extracted from the best-fitting model, chosen via AICc, from a set of potential models describing variation in soil respiration (Solvita CO2 Burst test). Reported values are the linear regressions and associated probabilities. Average percent bareground, soil nitrate (ppm KCL-N) and soil organic matter (%SOM) are all positively correlated with soil respiration.

Parameter	R^2	*t-value*	*P-value*
Bareground	0.46	2.79	0.005
KCL-nitrate	0.24	3.32	0.001
SOM	0.08	-2.29	0.023

Table 4.3. Least-squares means table of differences in average soil respiration (ppmCO2, Solvita CO2 Burst test). 'Parameters' here are categorical variables extracted from the best-fitting model, chosen via AICc, from a set of potential models describing variation in soil respiration (Solvita CO2 Burst test). Reported values are the linear regressions and associated probabilities. Year was highly significant, with twice the rate of soil respiration in 2015 than in 2016. Diversity was more mixed; however, high diversity had significantly higher rates of soil respiration than low or mid-diversity.

Parameter	*Estimate*	*SE*	*DF*	*t-value*	*Lower CI*	*Upper CI*	*p-value*
year 2015	61.52	3.35	2.6	18.36	49.7	73.3	9e-04
year 2016	30.36	3.33	2.5	9.13	18.4	42.3	0.006
diversity hd	52.63	2.93	4.1	17.95	44.6	60.7	2e-16
diversity md	32.89	6.05	55.9	5.44	20.8	45.0	1e-04
diversity ld	39.95	2.94	4.2	13.58	31.9	48.0	2e-16

Table 4.4. Percent litter cover and average maximum litter depth by year and diversity. The large difference in litter cover and depth by year may partially account for the lower soil respiration values measured in 2016.

diversity	*year*	*n*	*mean_litter*	*sd*	*se*	*max_depth*	*sd*	*se*
1	2015	24	0.4583	0.658	0.1343	18.46	31.05	6.339
1	2016	24	91.07	6.963	1.421	22.38	7.243	0.4998
2	2015	17	1.218	3.068	0.7441	7.154	20.4	5.659
2	2016	23	86.98	10.06	2.098	25.21	9.087	0.6227

FIGURES

Figure 4.1. Gradient in soil organic matter (percent mass lost on ignition), measured from 20cm-deep composite soil cores (3 cores per composite core, 3 composite cores per 60m x 60m plot) collected at the Platte River Prairies research prairie in early June 2015. SOM organic matter is highest to the south near the road, and lowest to the north near the Platte River. Darker shades indicate higher SOM values.

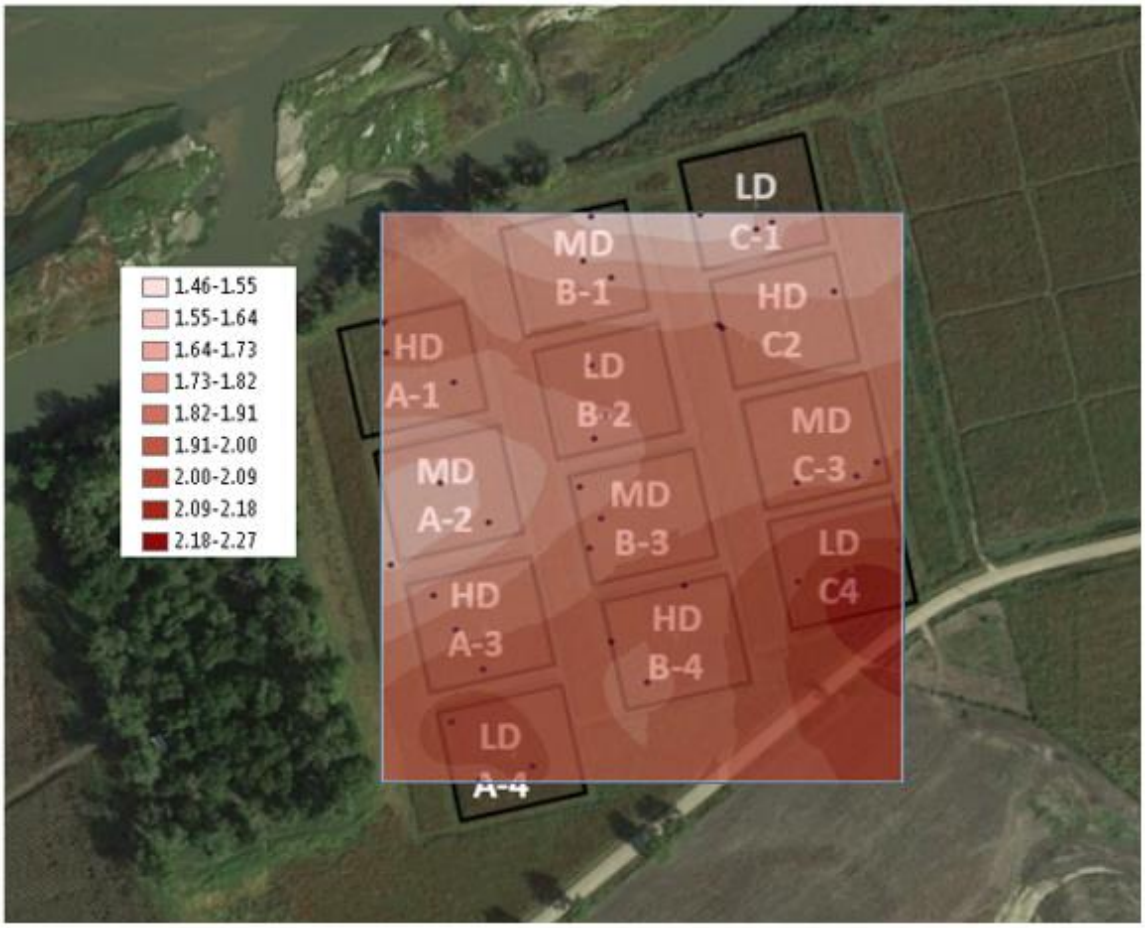


Figure 4.2. Gradient in soil pH by distance from river (slope = 0.006/meter, R^2=0.654, p=2.11e^{-12}), measured from whole-plot scale composite soil cores collected at the Platte River Prairies research prairie in early June 2015. The increase in pH with increasing distance from river indicates that soil pH is highest to the south and lowest to the north, nearest to the Platte River. The grey shading indicates the 95% confidence interval around mean pH values.

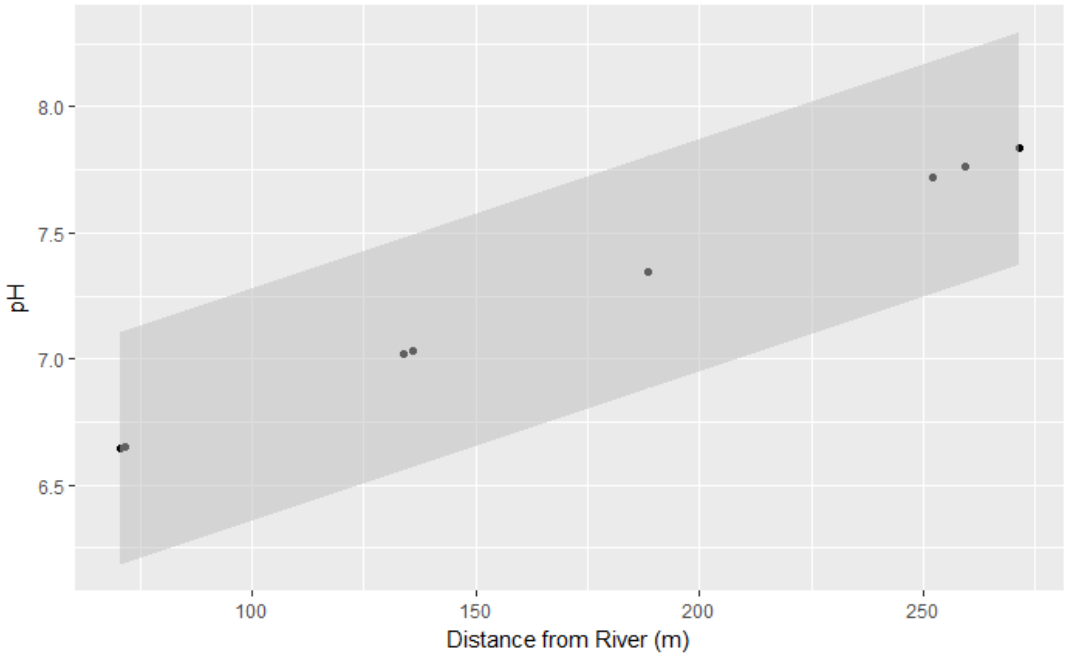


Figure 4.3. Soil nitrate (ppm KCL-extractable NO_3^-) gradient, measured from 3 composite soil cores (3 sub-cores from top 20cm of soil) collected at the Platte River Prairies research site in early June 2015. The decline in soil nitrate indicates that soil nitrate is highest to the west and lowest to the east of the research plots.

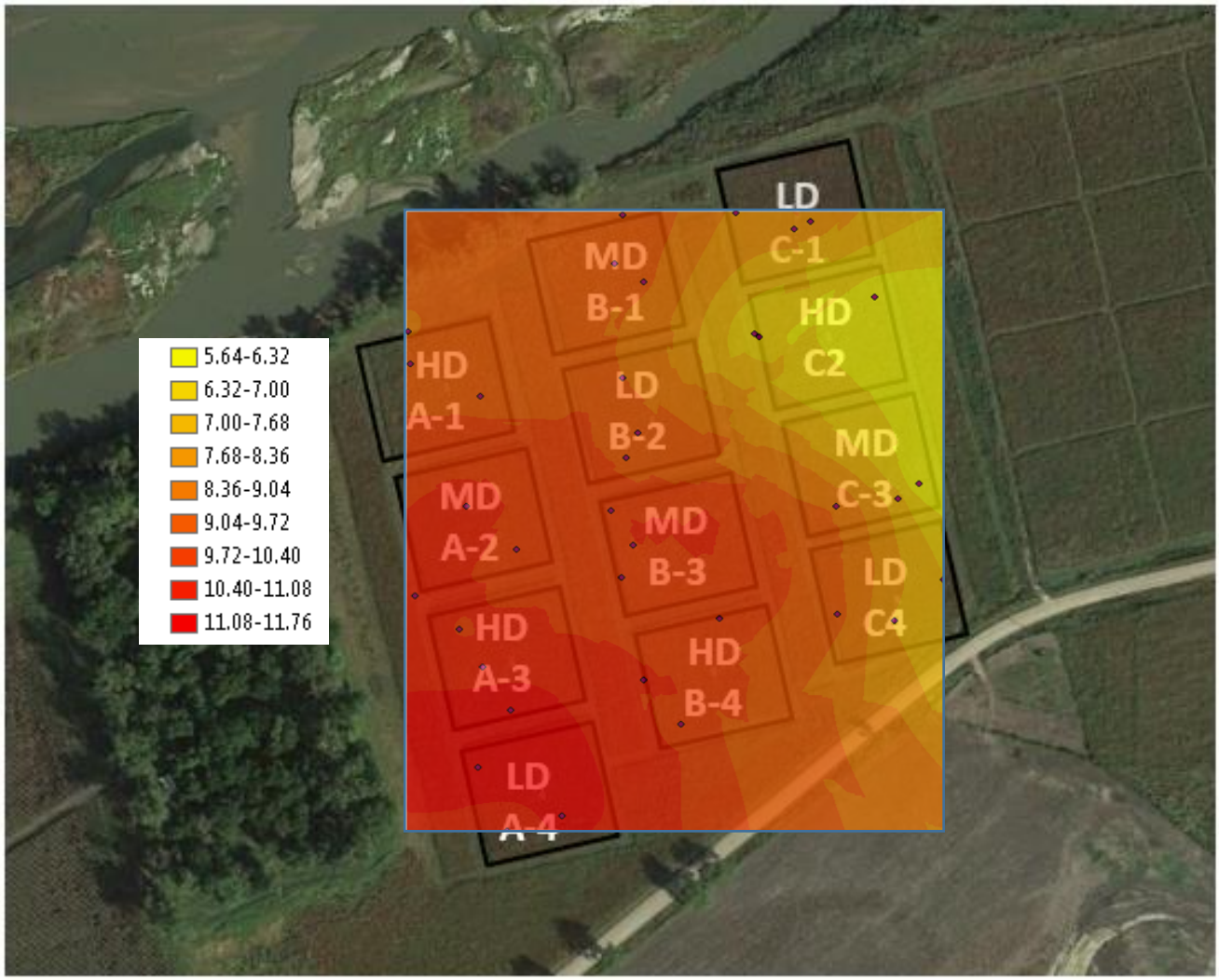


Figure 4.4. Average rainfall (cm) by month for the years 2015 and 2016 (top) and average maximum temperatures (cm) for the years 2015 and 2016 (bottom). Weather data is from the Hansen weather station, approx. 19km ESE of the research site. Maximum temperatures in 2015 were lower and dropped off much more quickly than in 2016. Rainfall in 2015 was much higher across May – July than in 2016, leading to long-term flooding of the research site during the month of June.

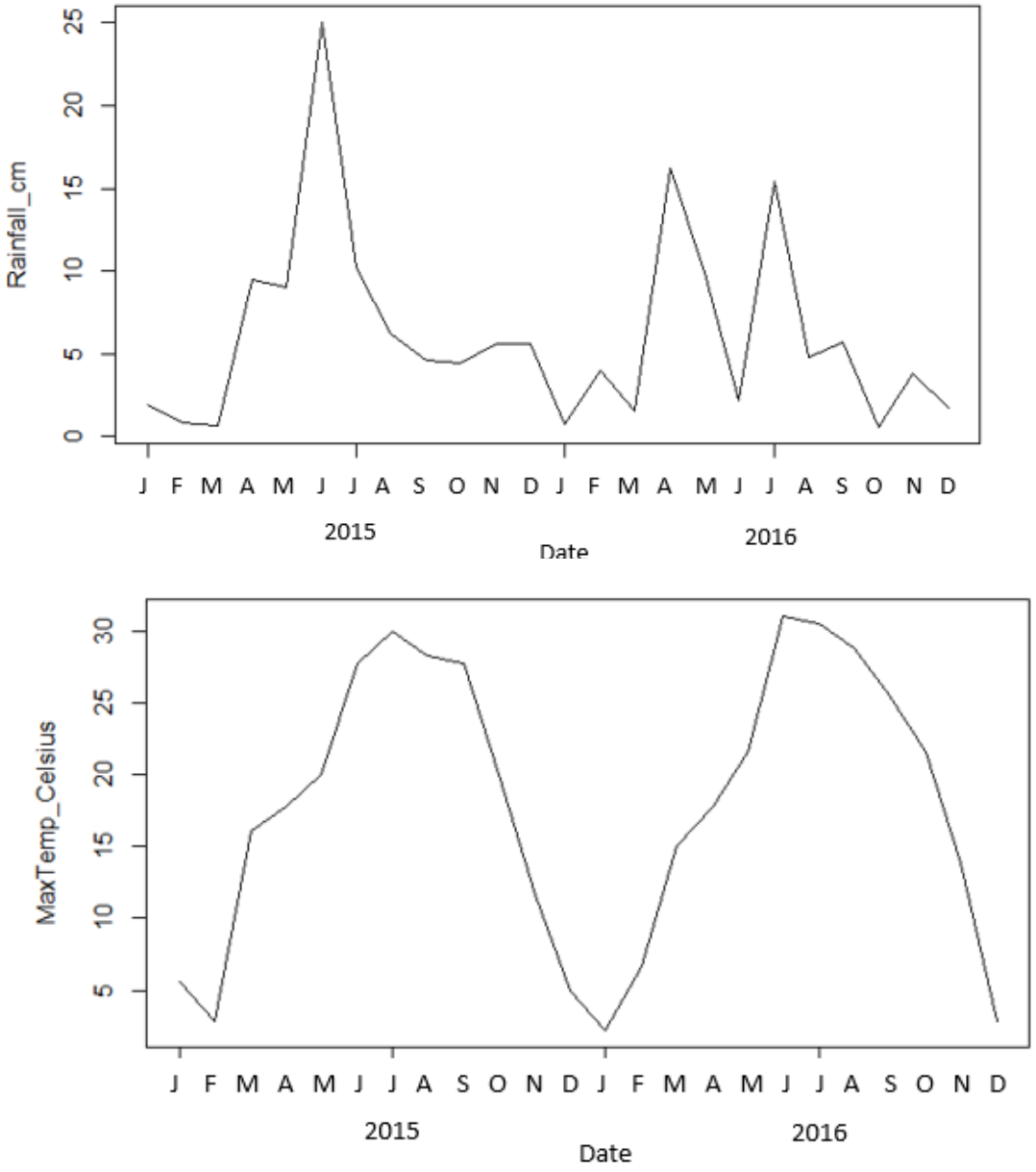


Figure 4.5. A diagram of the experimental disturbance treatments added to the center of each low-diversity and high-diversity research plot in May 2015. Rainout shelters were 2.5m x 5m, and subplot treatments were 2mx1m. Samples were collected 50cm within the border of each of these plots to minimize overlapping treatment effects. Comparison plots indicate plots sampled outside rainout shelters that had no treatments added.

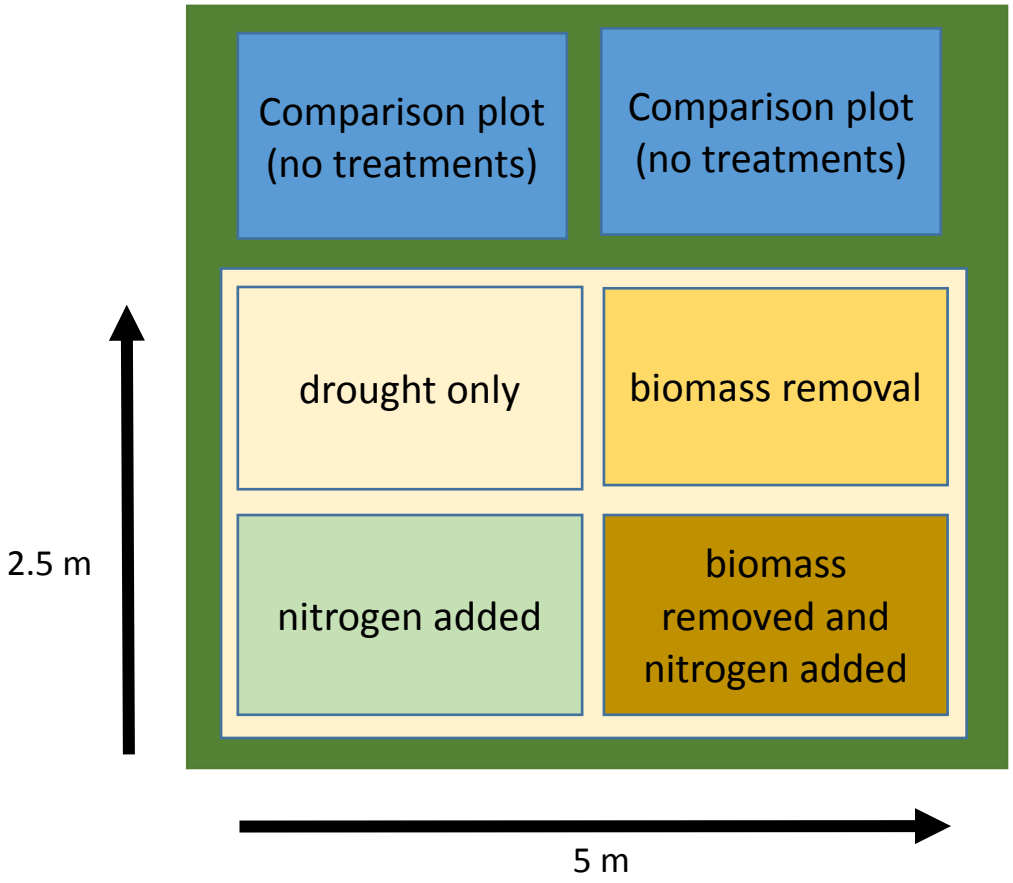


Figure 4.6. Soil moisture varies predominantly by distance from river (r2 = 0.04, p-value = 3.21e-8) and by depth in soil profile (r^2 = 0.43, p-value = 2.2e-16). Other significant varibles include sampling date, rainout shelters, and and distance from the edge of the plot; however, as these are strongly stochastic their effects are not shown here. Soil moisture values were collected using a hand-held soil moisture meter across four sampling dates in June, July, and August 2015, measuring depths of 10, 30, and 50cm and locations in each subplot of 0cm from edge, 25cm from edge, 50cm from edge, or in the center of the subplots. Soil moisture readings for 30cm and 50cm depths were collected via moisture access tubes lined with PVC, corked with rubber corks and covered with cans; however, soil moisture at that depth was high enough that readings frequently neared 100%.

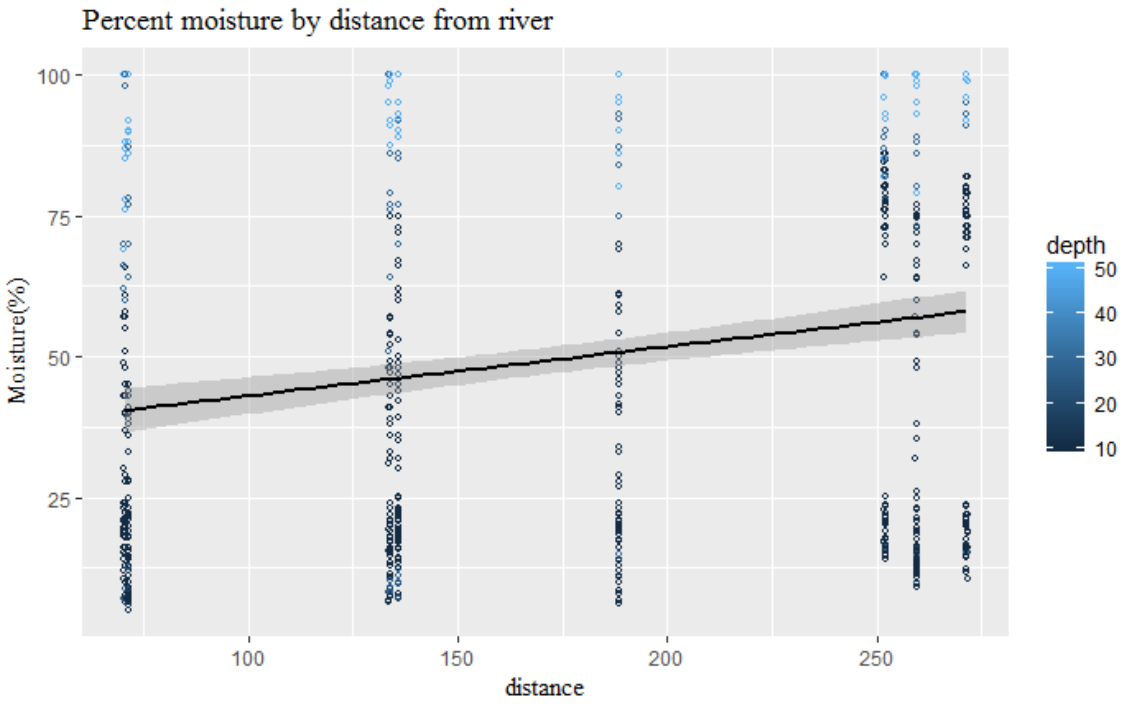


Figure 4.7. Variation in litter mass loss (% dry weight lost from initial bag construction to the end of incubation, measured to the nearest hundredth of a gram) by diversity and disturbance treatments added within the larger experimental treatment of diversity (ld=low-diversity, hd=high-diversity). Litter mass loss was fairly uniform across treatments added within rainout shelters in high-diversity plots, and varied among treatments added within rainout shelters in low-diversity plots; however, this variation was too small to be statistically significant. Red circles and error bars indicate group means and standard errors for untransformed mass loss data. Open dots indicate individual litter bag mass loss values. Treatment codes: Ctrl=Control plots, R=Rainout shelter, R+B=Rainout+biomass removal, R+N=Rainout plus nitrogen addition, R+N+B=Rainout+nitrogen addition+biomass removal.

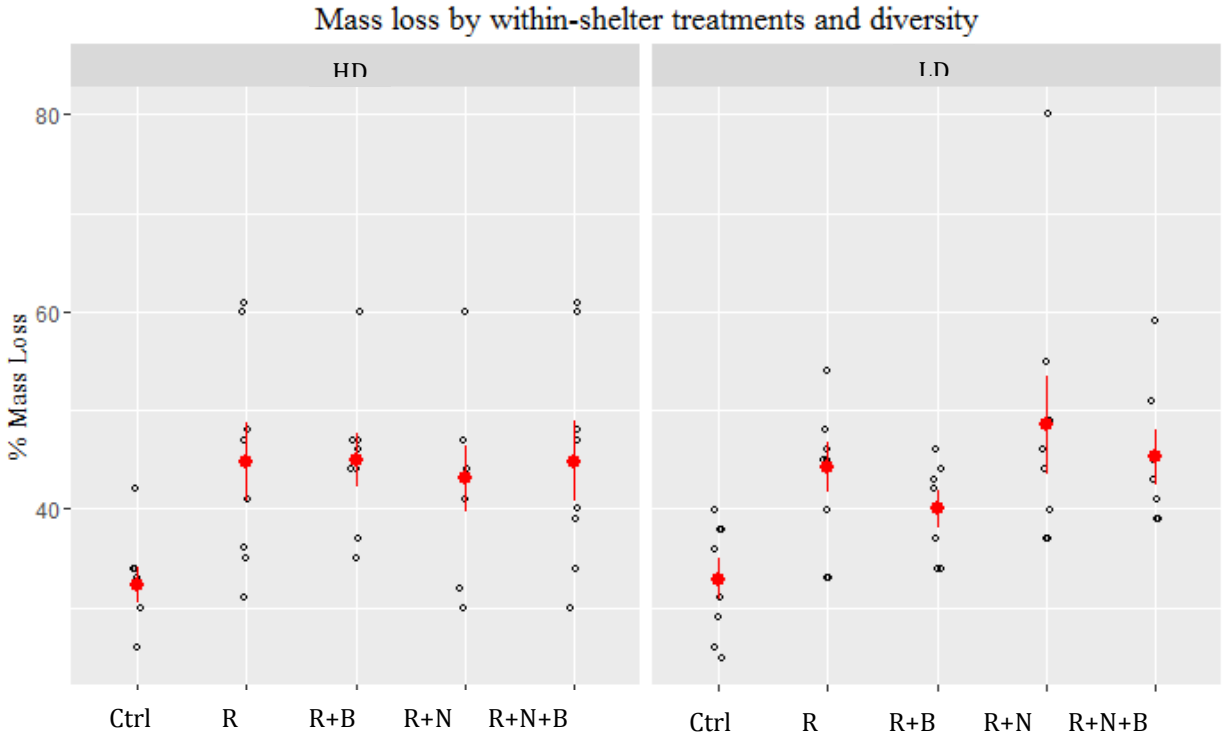


Figure 4.8. Litter mass loss (% dry weight lost from initial bag construction to the end of incubation, measured to the nearest hundredth of a gram) by rainout shelter and diversity treatments only. Red circles and error bars indicate group means and standard errors for untransformed mass loss data. Open dots indicate individual mass loss values. Variation in mass loss is not significant by diversity; however, variation is highly significant by rainout shelter effects. The effect of rainout shelters was the only significant source of variation among litter decomposition across all of the disturbance plots. Treatment codes: 1=no rainout shelter, 2=rainout shelter.

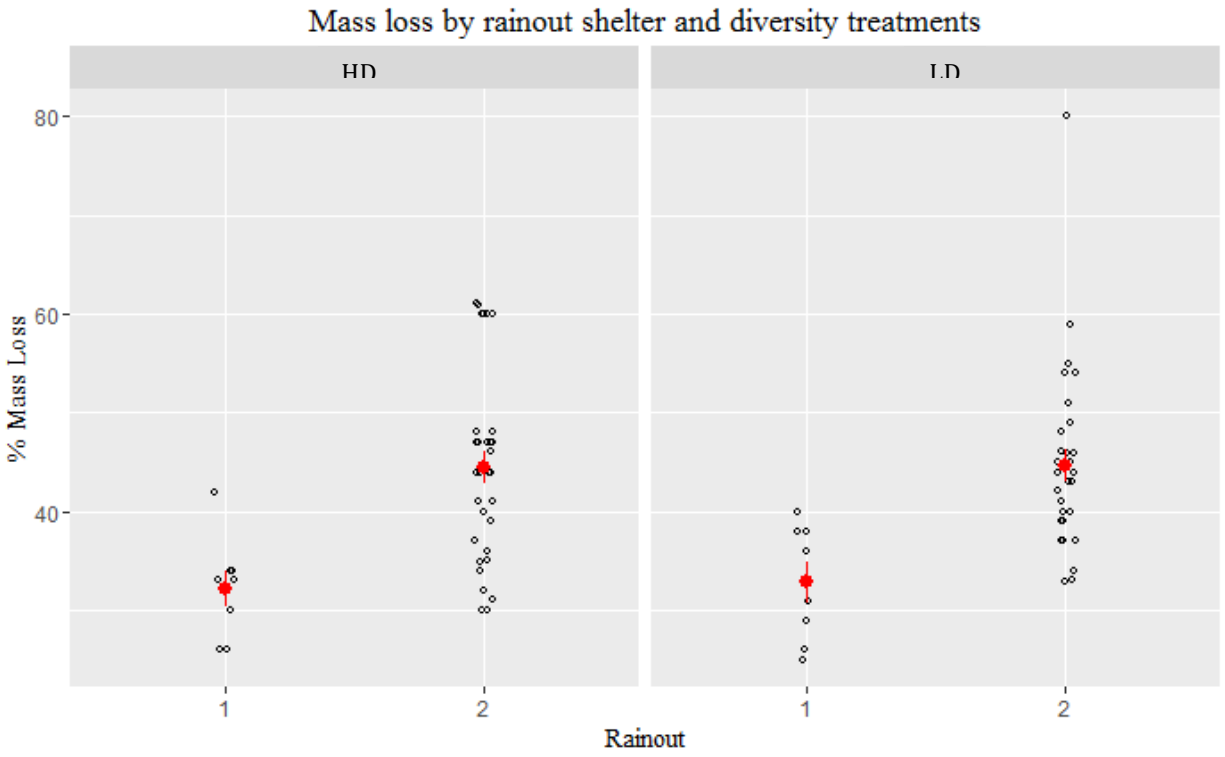


Figure 4.9. Variation in soil respiration (ppmCO2) by year and diversity, the main sources of variation among treatment variables. Variation is significant by both diversity and year. No mid-diversity soil samples were collected in 2015, and in 2016 mid-diversity respiration values overlapped with low- and high-diversity plot values. Red circles and error bars indicate group means and standard error. Open dots indicate individual soil respiration samples.

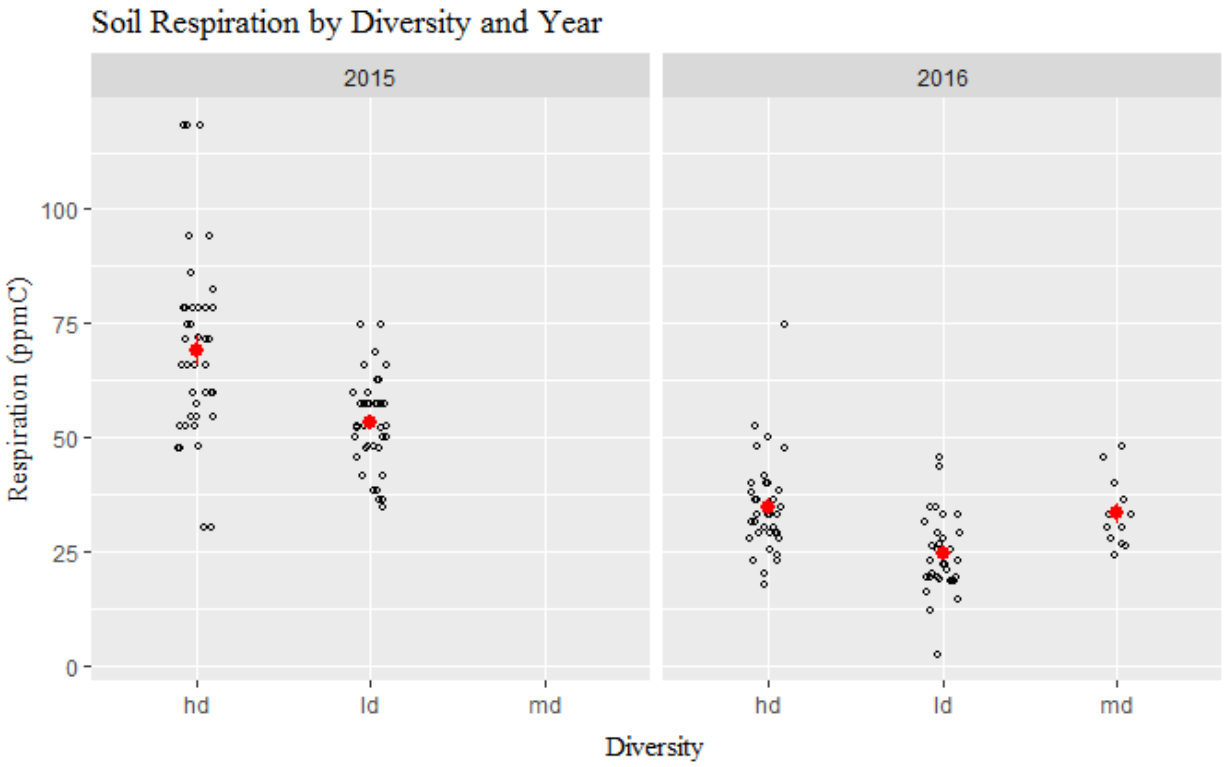
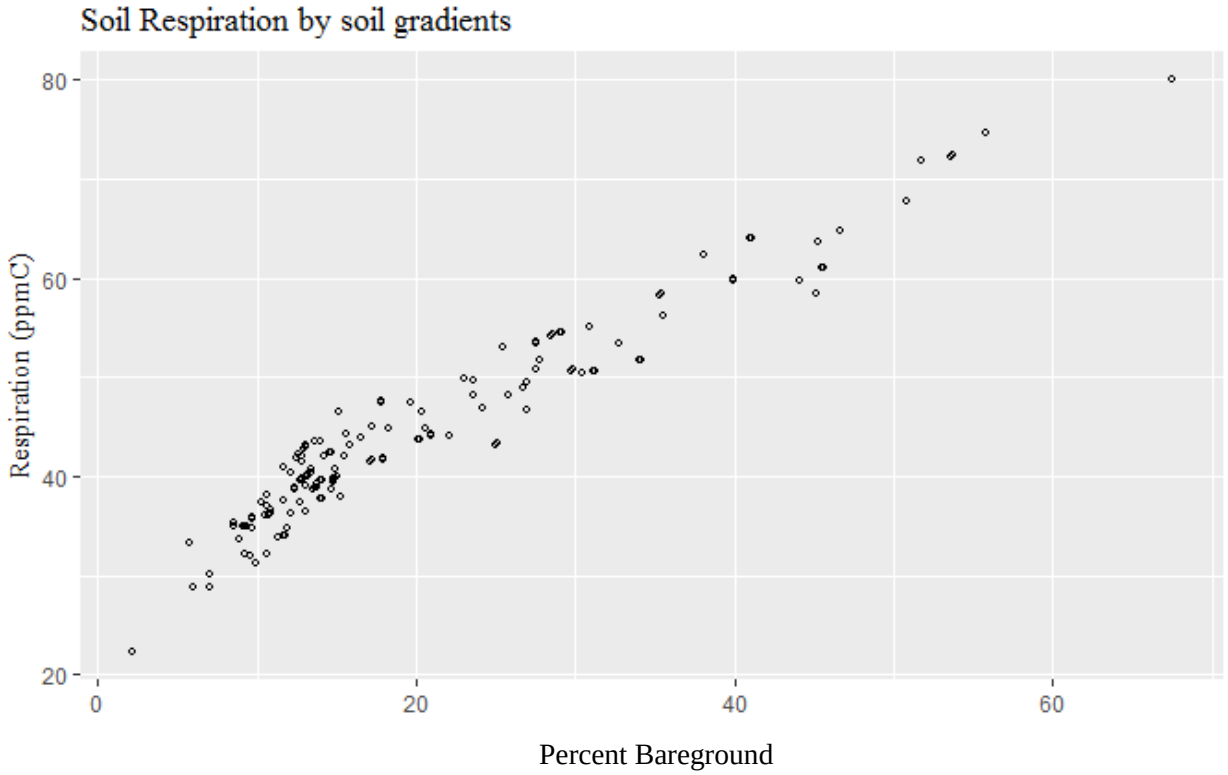


Figure 4.10. Variation in soil respiration (ppmCO2) plotted against the interaction of explanatory variables percent bareground, soil nitrate (ppm NO_3^-), and soil organic matter (%mass lost on ignition), the main uncontrolled site variables contributing to variation in soil respiration. Soil respiration was measured in 2015 and 2016. Dots represent individual samples; chart is for visualization purposes and does not include a regression line.



CHAPTER 5: PLANT DIVERSITY CONTRIBUTES TO INVASION RESISTANCE

I. Introduction

Biological invasion, as both a symptom and driver of biodiversity loss and the loss of functioning in natural systems, is one of the most disruptive ecological forces today (Risser 1995). However, the establishment of a high level of plant biodiversity from the outset may protect ecosystems from invasion (Foster et al. 2015). This protective mechanism occurs through both complementarity and sampling effects, which are both enhanced at higher levels of biodiversity. Complementarity effects develop over time as species in a community evolve to become partial rather than direct competitors, allowing many species to coexist and fully utilize all available resources in a system (Loreau et al. 2001) and reducing the ability for invaders to occupy the system. Sampling effects directly enhance invasion resistance as more diverse plots are more likely to contain the most productive native species that will dominate both space and resource availability and outcompete invasive species (Fargione and Tilman 2005). In restorations, higher seeding richness has been shown to improve species establishment by outcompeting unsown species (Piper 2015). This effect is even more important than seeding density in establishing invasion-resistant communities (Carter and Blair 2012; Nemec 2012).

The tallgrass prairie ecosystem is threatened by multiple types of invaders which may diminish its functioning. Cool-season (C3-pathway) grasses invade open ground and shift the timing of peak biomass production and senescence earlier in the year, leaving grasslands more vulnerable to fires in mid to late summer. Tree and shrub invaders, including Eastern Redcedar in the southern and eastern Great Plains, are leading to state

shifts in many grasslands, with significant associated alterations to water and carbon cycling. Finally, herbaceous invaders, including thistles, outcompete native species for resources, reproduce rapidly, and can lead to significant losses in soil stability and associated service provisioning as native communities shift to fast-growing invasive forbs (Helzer 2010). Ongoing and significant intervention is often necessary to mitigate the effects of these invaders (Kennedy et al. 2002; D'Antonio and Chambers 2006).

A large number of small-scale invisibility studies indicate that when biodiversity is supported from the outset, restorations may be less vulnerable to invasion; however, at large scales this effect may reverse (Powell et al. 2011). While research indicates a positive relationship between biodiversity and resistance to invasion at small scales, abiotic factors including increased soil nutrients from both agricultural runoff and fossil fuel combustion (Hautier et al. 2014) may increase invasive species success even in highly diverse communities by increasing the total resources available for invasive species (Suding et al. 2004; Zeiter and Stampfli 2012). A tallgrass prairie restoration established on previously cropped land may not be sufficiently protected from invasion even at high levels of biodiversity due to land-use history and ongoing eutrophication from human activity.

In addition to long-term variations in site characteristics, disturbances and short-term fluctuations in resource availability may significantly affect the ability of biodiversity to mitigate biological invasion. Frequent disturbances, including drought, biomass removal (via grazing or haying), and eutrophication from nitrogen deposition and agricultural runoff are thought to reduce the protective effect of biodiversity by

decreasing the competitive tolerance of the native community (Villnas et al. 2013), but this hypothesis has not been widely tested in grasslands.

To test the relationship between invasion and potential controlling factors including diversity, soil resources, and added disturbances, I collected soil cores in early June of 2015 and 2016 to assess soil chemistry, and conducted vegetation surveys in mid-June of 2015 and 2016 to record the relative cover and frequency of invasive species., Variation in invasion rates by soil chemistry and organic matter may indicate that underlying environmental gradients facilitate invasion success.

In this study, the term 'invasive' species refers to species which are both unsown and undesirable in the context of the tallgrass prairie system. While some of these species may be native to the region (i.e. Eastern Redcedar), if they were not members of the desired tallgrass prairie community they were labeled 'invaders'.

To assess the role of biodiversity, disturbance, and nutrient availability in moderating the presence of these unsown and undesired species, I tested the following hypotheses. First, I predicted that as biodiversity increased, invasion density would decrease. Second, I predicted that as resource availability (specifically soil nitrate and organic matter) increased, invasion density would increase. Third, I predicted that when biodiversity and resource availability co-varied, invasion levels would increase with resource availability even given higher levels of biodiversity. Finally, I predicted that the experimental additions of biomass removal and nitrogen addition would lead to increased rates of invasion and invasive species cover by making resources more available for invaders.

II. Methods

STUDY SITE

This study took place at the Platte River Prairies, a restoration managed by the Nature Conservancy in south-central Nebraska. The site, 10km south of Wood River, Nebraska (40°44'37.8"N 98°35'23.9"), is located within the Central Platte River ecosystem, identified as a Biologically Unique Landscape by the Nebraska Game and Parks Commission (NGPC 2011). Soils at the site include Wann loam, rarely flooded; Caruso loam rarely flooded; and Bolent-Clamux complex, occasionally flooded (NRCS).

In 2010, The Nature Conservancy seeded twelve 60m x 60m plots in native tallgrass prairie, with four plots each of Big Bluestem (*Andropogon gerardii*) monoculture, mid-diversity, and high-diversity seed mixes (Nemec 2013). The diversity treatments have established with significant differences between monoculture and high-diversity species richness (34 vs. 73 species, respectively; Price 2015). The 'monoculture' plots have accumulated a number of additional species and are called low-diversity plots throughout this study. Functional groups represented within the whole-plots include C3 (cool-season) grasses, C4 (warm-season) grasses, and both leguminous and non-leguminous forbs. This grouping is a very broad generalization of species groups and was chosen for ease of categorization and notable differences in phenology and nutrient acquisition represented by each group, differences which may influence their response to disturbance (Lavorel et al. 1997).

The site is maintained via burning; the most recent burn was in March of 2015, six weeks before the start of the study, with no additional management during the course of this study. The burn removed all biomass cover at the beginning of the research period, and so large differences in vegetation growth and litter cover were visible by year.

The research site covers an environmental gradient with increasing soil organic matter (% mass lost on ignition) from north to south (Figure 5.1). This gradient correlates with other soil chemical and functional traits, including soil respiration (Solvita CO2 Burst test, ppmC) and pH (Figure 5.2). There is also a significant gradient in nitrate (ppm KCL-extractable NO_3^-) from southwest to northeast (Figure 5.3). These gradients were included as fixed effects in statistical analyses.

Precipitation and temperature during the course of this study varied significantly by year. Precipitation in 2015 was much higher than in 2016, with a major peak in June (25cm total, mostly occurring in a single event). 2016 was more evenly distributed, with the highest rainfall totals in April (16cm) and July (15cm) and low rainfall in other months (Figure 5.4). Average maximum and minimum temperatures were higher in 2016, with sustained higher average temperatures across the spring, summer, and fall (Figure 5.5). The minimum temperatures in January 2015 and 2016 were -19.44ºC and -23.3ºC, respectively, and maximum temperatures in July 2015 and 2016 were 35ºC and 38.3ºC, respectively.

TREATMENTS

In May of 2015, I constructed 2.5 x 5m rainout shelters in the center of each low-diversity and high-diversity research plot. Beneath each shelter, I established four 1m x 2m plots of additional experimental disturbances, with 50cm spacing between each treatment. These disturbance treatments consisted of either no additional treatment, biomass removal (cutting biomass down to 4-8cm height during the first week of July), nitrogen fertilizer addition (30g of inorganic 34-0-0 dry ammonium nitrate fertilizer added twice per summer, first in mid-June and then six weeks following the first

treatment for a rate of 10gNH4NO3/m-1/summer), or a combination of biomass removal and nitrogen addition (Figure 5.5).

Treatments represented current threats to grasslands. Rainout shelters were built to impose water stress to the vegetation, which would potentially lower their threshold of resilience to additional disturbances. Drought is expected to be exacerbated in the mid-latitudes where the majority of grassland systems exist in coming decades (IPCC 2014), and in conjunction with increases in summer temperatures poses an increasingly large threat to grasslands (de Boek et al. 2011). Beneath rainout shelters, four 1m x 2m disturbance plots were established with either no additional treatments, nitrogen addition, biomass removal, or a combination of the two. Nitrogen addition (via inorganic nitrogen ammonia fertilizer) was used to represent the widespread terrestrial eutrophication arising largely from agricultural drift and fossil fuel burning (Suding et al. 2008; Tilman et al. 2008). Biomass removal simulated haying, which is a common management strategy in prairies throughout the United States. Haying is considered an alternative to grazing or fire (Smith et al. 2010), and may alter ecosystem function by removing dominant species at peak growth and reducing the amount of biomass left on the field for subsequent growing seasons.

DATA COLLECTION

Soil collection occurred at two scales across all monoculture and high-diversity whole-plots in early June of 2015 and 2016. Temperatures averaged 67.5^{o}F and 67^{o}F on sampling dates, and sampling occurred no less than 48 hours after any rainfall event. At the 60m x 60m plot scale I collected three composite cores, each made up of three sub-samples dug to 20cm with a hand auger. At the 1m x 2m disturbance treatment scale,

along with two 2m x 1m plots near the rainout shelters with no added disturbance treatments, I collected a single composite core made up of three sub-samples. All soil samples were stored at 2^{o}C pending analysis. Analyses were conducted by Ward Labs, an agricultural testing lab located in Kearney, Nebraska. Whole-plot cores were analyzed for total SOM, KCL-extractable nitrate (ppm NO_3^-). Subplot cores were analyzed for pH, total soil organic matter, total organic carbon, total organic nitrogen, soil nitrate, and microbial biomass carbon in 2015, and total SOM and nitrate in 2016. Soil organic matter was measured as percent lost on ignition via combustion tests; soil inorganic nitrogen as the parts per million KCL-extractable nitrate per sample, and estimated microbial biomass C via a chloroform-extraction method (Figure 5.1).

Soil moisture was recorded via a hand-held moisture meter which recorded moisture in the top 10cm of soils, and via moisture access tubes dug to 30cm and 50cm in the center of each rainout plot. Soil moisture readings were collected four times in June-August 2015 from the edge, 25cm in, 50cm in, and at the center of each subplot as well as from moisture access tubes.

Invasion density was measured in two separate tests; first, an average cover value for invaders within quadrat measurements and second, a count of the numbers of individuals of invasive species within three 2mx60m belt transects randomly located within each whole-plot. Quadrat sampling occurred during mid-June of 2015 and 2016 via 50cmx50cm quadrats, sampled at both the whole-plot and subplot scales. Four north-south transects were evenly spaced across each whole-plot, and eight 0.25m^2 quadrats sampled along each transect using a random start and subsequent eight-meter spacing following the random start (Elzinga et al. 1998). This spacing was used to maximize

distances between each quadrat. Disturbance-addition subplots were also sampled according to a systematic random design. Each subplot within the disturbance-addition subplot (four 2mx1m treatment subplots beneath each rainout shelter, with a 50cm between plots, and two 2mx1m control subplots located 1.5m west of each rainout shelter) was divided into eight $0.25m^2$ quadrats and four of those quadrats randomly selected for measurement. Variables recorded for each quadrat include cover (Daubenmire cover class method, Damgaard 2014), frequency, and the height of each species (the tallest and shortest individuals of each species within each quadrat, cm). Additional measurements collected for each quadrat include percent bare ground, litter cover, and litter depth (Elzinga et al. 1998).

Invasive species included in these surveys represent threats to tallgrass ecosystem structure and function. These species groups include: cool-season (C3-photosynthetic pathway) exotic grasses including Smooth brome (*Bromus inermis*) and Downy brome (*Bromus tectorum*); invasive forbs including Canada and Musk thistles (*Cirsium canadensis* and *vulgare*, Purple loosestrife (*Lythrum salicaria*) and Sweetclover (*Melilotus spp*.); and woody or shrub invaders, including Dogwood (*Cornus sp.*), Ash (*Fraxinus sp.*), Poison Ivy (*Toxicodendron sp.)* and other unidentified tree seedlings. Annual and biennial cool-season grasses displace perennial grass species, reducing the system's nutrient cycling and forage quality and providing fine fuels that encourage widespread fire (D'Antonio and Vitousek 1992). Invasive thistles support non-native pollinators, reduce habitat quality for native fauna, and displace native thistles (Price 2015). Woody species represent an existential threat to the grassland system by

encroaching on open spaces, shading out native grasses and forbs, and leading to regime changes from grassland to forest (Twidwell et al. 2013).

ANALYSIS

All scripts and data files used for analysis (R Studio version 3.4.5; RStudio Team)(Bates et al. 2015) are available in Appendix I. For belt transect measurements, the number of individuals per unsown species per transect was calculated using a Poisson model for individual counts. Quadrat-derived invader measurements used invader cover values to determine invader density. These correlate roughly with measures of invasive species establishment (how many individuals are there?) and success (how big are they?), respectively, as described in previous studies of invader prevalence (Kennedy et al. 2002).

Soil nutrient values were averaged by 60m x 60m plot to provide a general indicator of soil organic matter and nitrate levels per whole-plot. Average values for soil organic matter (avgSOM) and average soil nitrate (avgN) at the whole-plot scale were highly correlated with individual samples (correlations of 0.775 and 0.667, respectively), and are therefore expected to serve as reasonable proxies for resource availability across each whole-plot. This averaging technique flattens variation among sampling sites; however, this tradeoff in precision allows for a test of the resource-availability and biodiversity interaction at a larger scale. Due to relatively low replication and numbers of invaders, assessing variation by continuous soil variables was not feasible, therefore, distance from river categories were used as proxies for average soil nutrient levels in the field, and average soil nitrogen grouped into a four-category variable. Invasion counts and cover values were tested as a function of three main parameters. First, average soil N

(grouped into 4 levels); second, average soil organic matter (grouped into 4 levels); third, plot diversity (low, medium, high).

Soil organic matter is significantly positively correlated with increased distance from the Platte River (r= 0.74), as are soil pH, soil moisture, and microbial biomass, and is grouped into four categories by the distance from the center of each whole-plot to the Platte River. Plot diversity represents the three levels of diversity with which the site was planted in 2010. At the disturbance-addition subplot scale, disturbance treatments were also included as potential sources of variation.

At both whole-plot and sub-plot scales, invader cover was very low and variation among samples too small to model. Only one variable was even a marginally significant predictor of total invader cover at the subplot scale (n addition, estimated increase of 4.11%, p=0.07). Invader cover was not investigated further.

The response variable of invader count was grouped by invader type (cool-season grasses, herbaceous invaders, woody invaders, or all invaders) and a set of sixteen possible models was constructed, from single variables up to the full set of interactions:

[M.1] Invader count ~ N level*SOM category*Diversity

Model selection occurred via AICc model selection, and all models within 2 delta-AIC of the best model were considered sufficiently predictive. The best-fitted model was used to calculate group means and standard errors for each set of invasive species.

III. Results

INVADER COUNTS

The best models across all species groups were generally those containing all three parameters and some of their interactions. The best-fitting model for the count of all invaders was M.1, with positive correlations between each variable and invasion counts, and negative correlations between interactions of the parameters and invasion counts (Table 5.1).

The best-fit model for woody invaders was:

[M.2] Woody count ~ N category + Diversity + SOM category*N category + N category*Diversity,

with significant positive correlations between soil nitrogen and invasion, soil organic matter and invasion, and the interaction of soil organic matter, soil nitrogen, and invasion (Table 5.2). Negative correlations between soil nitrogen, diversity, and invasion were also notable.

The best-fitting model for forb invasion was,

[M.3] Herbaceous forbs count ~ N category + SOM category*diversity,

with significant positive correlations between average nitrogen and invasion and soil organic matter and invasion. The relationship between diversity and invasion varies, and a generally negative correlation between diversity and invasion appears when diversity co-varies with soil organic matter (Table 5.3).

Cool-season grasses had exceptionally low counts in this data set, possibly owing to the measurements taking place after peak growth. The best model for predicting the presence of C3 grasses was the global model, but no parameters were significantly correlated with C3 grass invasion.

To test the effect of soil nitrogen and organic matter independently from plot diversity or location, all species counts were grouped and plotted separately as functions of the variables soil nitrate and soil organic matter. In the cases of both soil nitrate and organic matter levels, invasion counts were highest at the very highest concentrations of these nutrients, with forbs showing a positive association with organic matter and woody invaders showing a positive association with soil nitrate (Figures 5.2 and 5.3).

Invader counts by diversity, including group means and standard error controlling for the parameters of planted row (west to east) and distance from river (site location variables strongly correlated to soil nitrogen, organic matter, and other soil chemistry values), were plotted (Schluter 2016). The apparently large differences in invader counts by diversity appear to be driven by the significant variation in herbaceous forbs and, to a lesser extent, woody invaders, with c3 grass counts too small to show any response to diversity treatments (Figures 5.5, 5.6, 5.7, 5.8).

Sources of significant variation differed depending on the type of survey; among the transect species counts, the most significant predictors of species invasion were soil nutrients and diversity, while quadrat cover surveys were more closely associated with experimental treatment effects, though the overall low invader cover across all quadrats likely confounded these results.

IV. Discussion

This survey of unsown invasive species and soil nutrient levels at the Platte River Prairies research site indicates that both biodiversity and resource availability play a role in controlling the density of biological invasion, though these effects were only notable at the whole-plot scale and varied depending on the measurement used. Variation in

invasion prevalence by invader type provides evidence that invader characteristics are important determinants of invasion frequency and cover; this effect was noticeable in both the transect frequency and quadrat cover surveys.

The study results partially confirm some of my original hypotheses and counter others. First, increased biodiversity was associated with higher invasion densities when considered on its own, countering my initial expectation, but this effect reversed when diversity co-varied with soil nutrients. Second, increased resource availability (soil NO_3- and SOM) was positively correlated with increased invasion density in some cases (Tables 5.1-5.3), but not in others. Contrary to my third hypothesis, when organic matter and whole-plot diversity co-varied they were generally associated with lower invasion densities. This contradicts my original hypothesis that biodiversity would not affect invader cover or frequency at higher resource levels. However, my prediction was partially supported in the case of woody invader frequencies; in transect surveys, as increasing nitrate levels were correlated with higher invasion densities even at higher diversity levels.

The responses of species groups to diversity plantings (including cool-season grasses, herbaceous forbs, and woody invaders) demonstrates a relationship between plant and invasion rates, with soil resource availability, disturbance effects, and invader life traits accounting for much of the variation by plant diversity. In transect counts, woody invaders responded less strongly to increasing biodiversity than forbs, declined with increasing organic matter and diversity, and increased with increasing nitrate levels regardless of planted diversity. Invasive herbaceous forbs, meanwhile, showed a positive

relationship with increasing soil organic matter, declined with increasing soil nitrate, and decreased significantly with increased plant diversity.

Though species traits were not directly measured with this survey, known differences in life histories related to the growth types presented (perennial woody shrubs and trees vs herbaceous annual/biennial forbs), combined with competition for limiting resources, may help to explain the differential responses to diversity between these groups (Tayeh et al. 2015). One trait that may influence the relative density of invasion between forb and shrub species is phenology – many forb species measured in this study begin their growth later in the spring, at the same time as many native prairie forbs and grasses (Laubhan and Shaffer 2006). These invaders are subjected to increased competition for light, space, and nutrients as compared with woody invaders, which can begin growth in early spring before most native prairie species are actively growing and are therefore less limited by competition at a key point in their establishment. Therefore, shrubs and tree seedlings may be less responsive to the plot diversity (Francis 2003). The decline in woody invader counts in response to increasing soil organic matter suggests that the woody invaders may be accessing water deeper in the soil profile and are not limited in their establishment to soils with higher organic matter and moisture levels, and may be less successful in those areas that are more conducive to forb and grass growth.

An interesting finding is that that different responses to increasing organic matter, soil nitrate, and invasion rates emerge when these variables are assessed separately or in combination with species richness. When assessed as separate effects, organic matter and nitrate show positive correlations with forbs and woody invader groups, respectively. When measured as interaction terms with invasion, the response flips and a negative

correlation with invasion density across species groups emerges. This suggests that higher biodiversity plays a mitigating role in biological invasions by outcompeting potential invaders for increased resources (Tilman et al. 2001). An increase in invasion rates across diversity levels in response to increased nitrate levels for woody invaders (Figure 5.4) and in response to increased soil organic matter for forbs (Figure 5.3) suggest that increased resources can lead to increased invader rates, but this effect can be mitigated by increased diversity.

Another key finding, that unsown species had higher cover among the experimental treatments of biomass removal and nitrogen addition, indicates that certain disturbances may aid in species establishment. The removal of light and nitrogen limitations may provide an opportunity for invaders to gain a foothold in an otherwise vigorous and invader-resistant community. While invader cover was still relatively low even in the plots which had biomass removal or nitrogen addition treatments, this survey was conducted following only one season of experimental treatments, suggesting that invasive species may be able to quickly take advantage of resource opportunities.

V. Conclusion

This study fills a persistent gap in biodiversity-ecosystem function (BEF) research by scaling up BEF relationships measured at small experimental scales to a field level (Jentsch et al. 2011; Kreyling et al. 2008). Though the data presented here is low-resolution, it nonetheless demonstrates significant effects of both resource ability and biodiversity on invasion density, indicating that the correlations between diversity, resource availability, and invasion resistance are large enough to appear even at coarse scales of observation. Though experimental evidence of biodiversity's role in limiting

biotic invasion resistance is often limited to more controlled mesocosm experiments (Maron and Marler 2007), this study provides evidence that the relationships between biodiversity on system invasion resistance do scale up to larger settings, and can overcome the environmental variability present in less-controlled systems. This study contradicts previous large-scale studies which indicate that increasing biodiversity can lead to greater invasion densities (Powell et al. 2011) by showing that biodiversity may enhance system resistance to invasion, even when invaders are facilitated by existing resource gradients. Though further research is needed to support these findings, increasing the biodiversity of restoration plantings appears to be a viable strategy for minimizing biological invasion and reducing the need for costly interventions later on to remove invaders from restored systems.

VI. Literature Cited

Bates, D., Maechler, M., Bolker, B., Walker, S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software **67**:1-48.

De Boeck, H. J., Dreesen, F. E., Janssens, I. A., Nijs, I. 2011. Whole-system responses of experimental plant communities to climate extremes imposed in different seasons. New Phytologist **189**: 806–817.

Carter, D., Blair, John M. 2012. High richness and dense seeding enhance grassland restoration establishment but have little effect on drought response. Ecological Applications **22**: 1308–1319.

Clark. M., Tilman, David. 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature Letters **451**:7.

Cotrufo, M. F., Soong, J. L., Horton, A. J., Campbell, E. E., Haddix, M. L., Wall, D. H., Parton, W. J. 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nature Geoscience **8**(10): 776–779.

Damgaard, C. 2014. Estimating mean plant cover from different types of cover data: a coherent statistical framework. Ecosphere **5**(Feb): 1–7.

D'Antonio, C.M., Chambers, J.C. 2006. Chapter 12: Using Ecological Theory to Manage or Restore Ecosystems Affected by Invasive Plant Species. In *Foundations of Restoration Ecology*, Falk, D.A., Palmer, M.A., Zedler, J.B., Eds. Washington, D.C.: Island Press.

Elzinga, C.L., Salzer, D.W., Willoughby, J.W. 1998. *Measuring and monitoring plant populations.* U.S. Bureau of Land Management Papers, Paper 17.

Fargione, J. E., & Tilman, D. 2005. Diversity decreases invasion via both sampling and complementarity effects. Ecology Letters **8**: 604-611.

Francis, J.K., Ed. 2003. *Wildland shrubs of the United States and its territories.* U.S. General Technical Report IITF-WB-1. Department of Agriculture, Forest Service, International Institute of Tropical Forestry [online] (Accessed 3 Feb 2016).

Foster, B., Houseman, G., Hall, D., Hinman, S. 2015. Does tallgrass prairie restoration enhance the invasion resistance of post-agricultural lands? *Biological Invasions* **17**: 3579-3590.

Hautier et al. 2014. Eutrophication weakens stabilizing effects of diversity in natural grasslands. Nature **508**:521-526.

IPCC. 2014. *Climate Change 2014: Impacts, Adaptations and Vulnerability. Summary for Policymakers.* Cambridge: Cambridge University Press.

Jentsch, A., Grant, K., Nagy, L., Schloter, M., Wo, J., Kreyling, J., Hein, R., Otieno, R., Sing, B.K., Elmer, M., Lara, M., Pritsch, K., Stadler, J., Mirzae, H., Rascher, U. 2011. Climate extremes initiate ecosystem regulating functions while maintaining productivity. Journal of Ecology **99**: 689-702.

Kallenbach, C. M., Grandy, A., Frey, S. D. 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nature Communications **7**: 13630.

Kennedy, T.A., Naeem, S., Howe, K.M., Knops, J.M.H., Tilman, D., Reich, P. 2002. Biodiversity as a barrier to ecological invasion. Nature **417**: 636-638.

Kreyling, J., Wenigmann, M., Beierkuhnlein, C., Jentsch, A. 2008. Effects of extreme weather events on plant productivity and tissue die-back are modified by community composition. Ecosystems **11**:752-763.

Laubhan, M.K., Shaffer, T.L. 2006. Seed germination of Cirsium arvense and Lepidium latifolium: implications for management of montane woodlands. Wetlands **26**:1.

Lavorel, S., McIntyre, S., Landsberg, J., Forbes, T.D.A. 1997. Plant functional classifications: from general groups to specific groups based on response to disturbance. Trends in Ecology and Evolution **12**: 474-478.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A., Raffaellil, D., Schmid, B., Tilman, D., Wardle, D.A. 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. Science **294**: 5543.

Maron, J.L., Marler, M. 2007. Native plant diversity resists invasion at both low and high resource levels. Ecology **88**:2651-2661.

Nebraska Game and Parks Commission. 2005. *The Nebraska natural legacy project: a comprehensive wildlife conservation strategy*. Lincoln, Nebraska, USA.

Nemec, Kristine. *The Relationship Between Diversity, Seeding Density, and Ecological Functions in Tallgrass Prairie Restorations*. Dissertation. University of Nebraska-Lincoln.

NRCS Web Soil Survey. USDA. https://websoilsurvey.nrcs.usda.gov/app/. (Accessed 16 Jan. 2017).

Piper, J.K. 2014. Incrementally Rich Seeding Treatments in Tallgrass Prairie Restoration. Ecological Restoration **32**: 396-406.

Price, Katilyn. 2015. Plant diversity affects performance of invasive thistles in restored Nebraska grasslands. Thesis.

Powell, K.I., Chase, J.M., Knight, T.M. 2011. A synthesis of plant invasion effects on biodiversity across spatial scales. American Journal of Botany **98**: 539-548.

Risser, Paul G. 1995. Biodiversity and Ecosystem Function. Conservation Biology **9**: 742-746.

Smith, D., Henderson, K., Houseal, G., Williams, D. 2010. *Tallgrass Prairie Center Guide to Prairie Restoration in the Upper Midwest.* Des Moines: University of Iowa Press.

Suding, K. N., Gross, K. L., Houseman, G. R. 2004. Alternative states and positive feedbacks in restoration ecology. Trends in Ecology and Evolution. **19**: 46–53.

Suding, K. N. 2011. Toward an Era of Restoration in Ecology: Successes, Failures, and Opportunities Ahead. Annual Reviews in Ecology and Evolutionary Systems **42**:465-487.

Tayeh, A., Hufbauer, R.A., Estoup, A., Ravigne, V., Frachone, L., Facon, B. 2015. Biological invasion and biological control select for different life histories. Nature Communications **6**:7268.

Tilman, D., Reich, P. B., Knops, J., Wedin, D., Mielke, T., Lehman, C. 2001. Diversity and Productivity in a Long-Term Grassland Experiment. Science, New Series **294**:5543.

Twidwell, D., W.E. Rogers, S.D. Fuhlendorf, C.L. Wonkka, D.M. Engle, J.R Weir, U.P. Kreuter, C.A. Taylor, Jr. 2013. The rising Great Plains fire campaign: citizens'

response to woody plant encroachment. *Frontiers in Ecology and the Environment* **11**: e64-e71.

Zeiter, M., Stampfli, A. 2012. Positive diversity-invasibility relationship in species-rich semi-natural grassland at the neighborhood scale. Annals of Botany **110**: 1385-1393.

VII. Tables and Figures

TABLES

Table 5.1. Results of a general linear model testing the interaction of all predictive parameters (soil nitrogen, ncat; organic matter, somcat; and diversity, 1, 2, or 3) against the full count of invasive species surveyed in 2-meter wide belt transects in July 2016. Significant parameters marked with an *. 'Estimate' indicates the estimated impact (change in number of invaders, and direction of change).

glm(formula = all ~ N category*diversity*SOM category, family = "poisson", data = invsoil)

Deviance Residuals:

Min	1Q	Median	3Q	Max
-9.1513	-2.5075	-0.3257	1.5398	7.1978

	Estimate	Std.Error	z value	Pr(>|z|)	
(Intercept)	-17.4866	5.2169	-3.352	0.000802	***
ncat	9.9591	2.7745	3.590	0.000331	***
diversity2	0.8028	0.4701	1.708	0.087713	.
diversity3	11.3599	2.7099	4.192	2.77e-05	***
somcat	6.0214	1.4686	4.100	4.13e-05	***
ncat:diversity2	-3.4704	1.0649	-3.259	0.001119	**
ncat:diversity3	-0.6666	0.2698	-2.471	0.013492	*
ncat:somcat	-3.0430	0.8570	-3.551	0.000384	***
diversity2:somcat	3.4387	1.1136	3.088	0.002015	**
diversity3:somcat	-3.1855	0.6788	-4.693	2.69e-06	***

Null deviance: 832.84 on 35 degrees of freedom
Residual deviance: 358.28 on 24 degrees of freedom
AIC: 543.05
Number of Fisher Scoring iterations: 5

Table 5.2. Results of a general linear model describing significant parameters predicting woody species invasion and their impact on measured woody invader counts. 'Estimate' indicates the change in number of invaders and the direction (+/-) of the change. Significant parameters are marked with an *.

glm(formula = woody ~ N category + diversity + N category*SOM category + N category*diversity, family = "poisson", data = invsoil)

Deviance Residuals:

Min	1Q	Median	3Q	Max
-3.9567	-1.9149	-0.0976	1.0160	4.8699

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	4.6528	1.2444	3.739	0.000185	***
ncat	-1.2409	0.5862	-2.117	0.034269	*
diversity2	-0.1972	0.6640	-0.297	0.766433	
diversity3	1.5534	0.5346	2.906	0.003663	**
somcat	-1.4033	0.4140	-3.389	0.000700	***
ncat:somcat	0.6285	0.1902	3.305	0.000951	***
ncat:diversity2	0.2809	0.3024	0.929	0.352988	
ncat:diversity3	-0.8749	0.2272	-3.850	0.000118	***

Null deviance: 262.46 on 35 degrees of freedom

Residual deviance: 162.38 on 28 degrees of freedom

AIC: 295.49

Number of Fisher Scoring iterations: 5

Table 5.3. Results of a general linear model describing variation in herbaceous forbs by significant predictive parameters. 'Estimate' indicates the change in number of invaders and the direction (+/-) of that change. Significant parameters are marked with an *.

glm(formula = herbaceous invaders ~ N category + SOM category*diversity, family = "poisson", data = invsoil)

Deviance Residuals:

Min	1Q	Median	3Q	Max
-7.8890	-2.7926	-0.7604	1.1209	6.5740

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	-6.24665	1.22769	-5.088	3.62e-07	***
ncat	0.35080	0.09593	3.657	0.000255	***
diversity2	6.86543	0.98696	6.956	3.50e-12	***
diversity3	5.38657	1.80623	2.982	0.002862	**
somcat	2.50983	0.28866	8.695	< 2e-16	***
diversity2:somcat	-2.10156	0.30920	-6.797	1.07e-11	***
diversity3:somcat	-1.83154	0.57094	-3.208	0.001337	**

Null deviance: 879.61 on 35 degrees of freedom

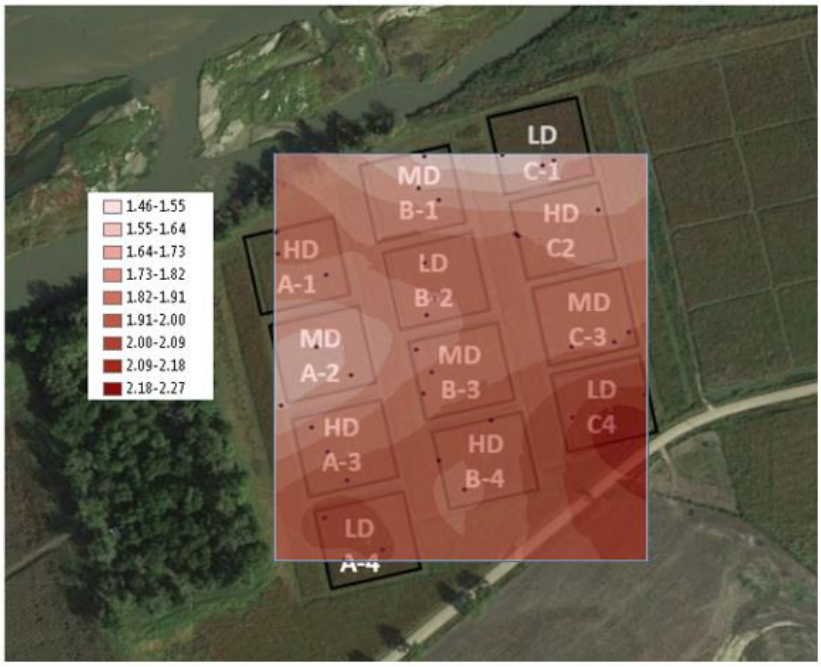
Residual deviance: 354.76 on 29 degrees of freedom

AIC: 460.25

Number of Fisher Scoring iterations: 6

FIGURES

Figure 5.1. Soil organic matter (top) varies north to south, and KCL-extractable N (bottom) varies from west to east. Soil chemistry calculated from composite cores (3 per block) dug to 20cm depth. These soil gradients were significantly correlated with variation in invader frequency.



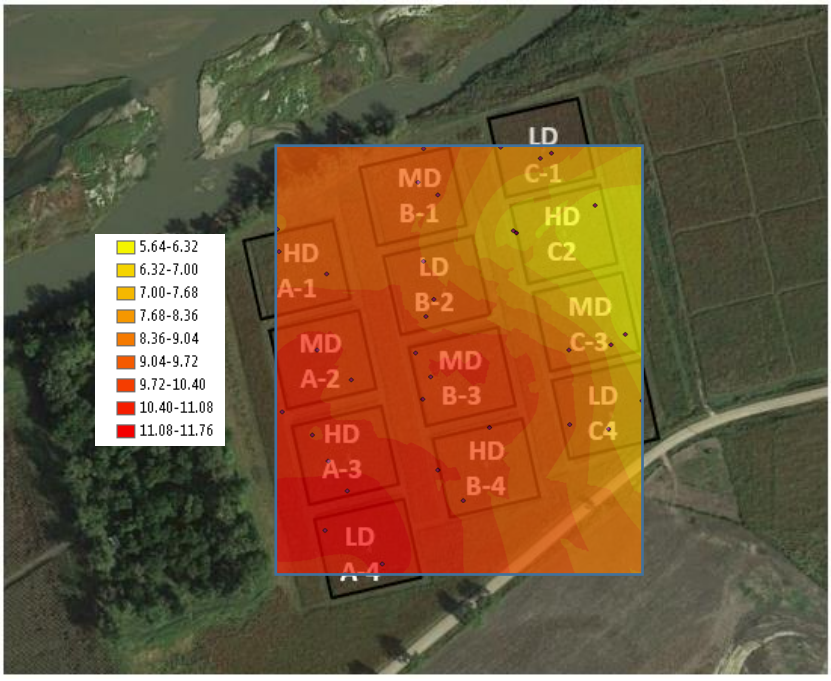


Figure 5.2. Diagram of the experimental disturbance treatments added to the center of each low-diversity and high-diversity research plot in May 2015. Rainout shelters are 5m x 2.5m, and subplot treatments are 2mx1m. Samples were collected 50cm within the border of each of these plots to minimize overlapping treatment effects. 'Control' plots in this diagram indicate plots that were sampled outside the rainout shelters with no additional treatments.

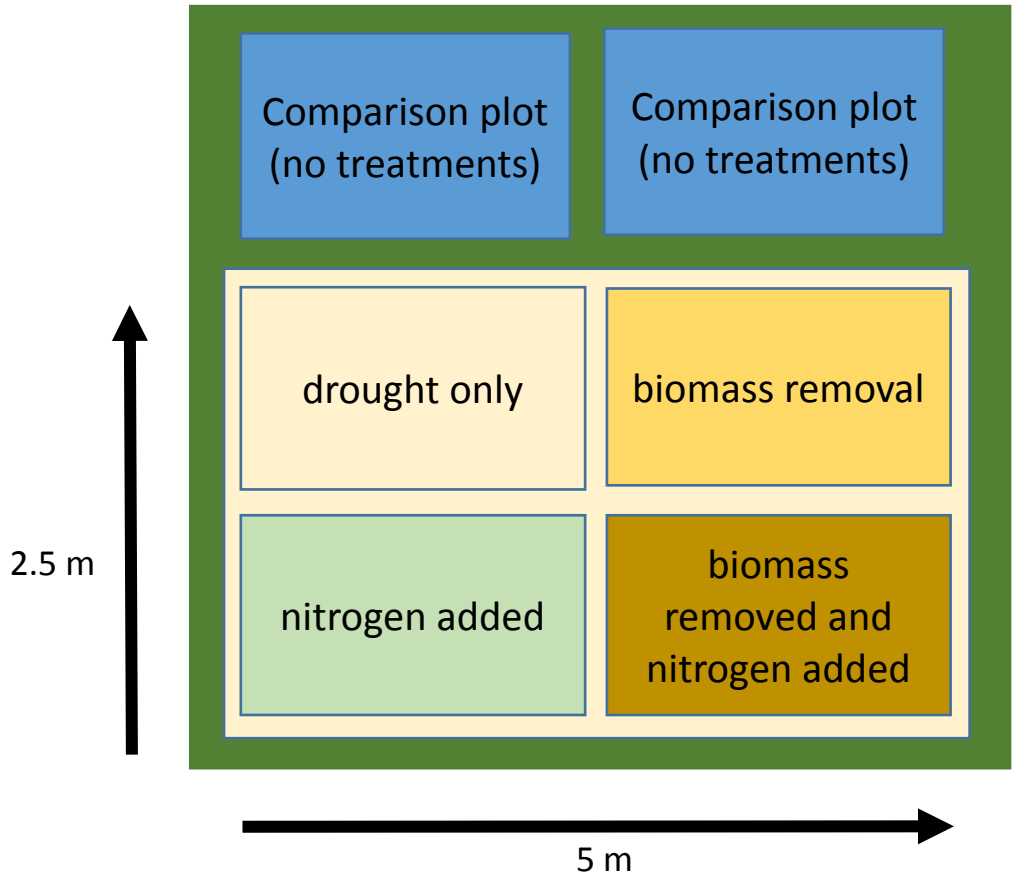


Figure 5.3. Variation in invader counts for 2mx60m transects (number of individuals counted over 36 transects, or 3 transects per 60m x 60m restoration plot) plotted against soil organic matter (somcat). Category 1: SOM<1.6%; 2: SOM=1.6-1.87%; 3: SOM=1.87-2.1%;4: SOM>2.1%. An increase in invasion by SOM is apparent in herbaceous forbs, but not by any other species group.

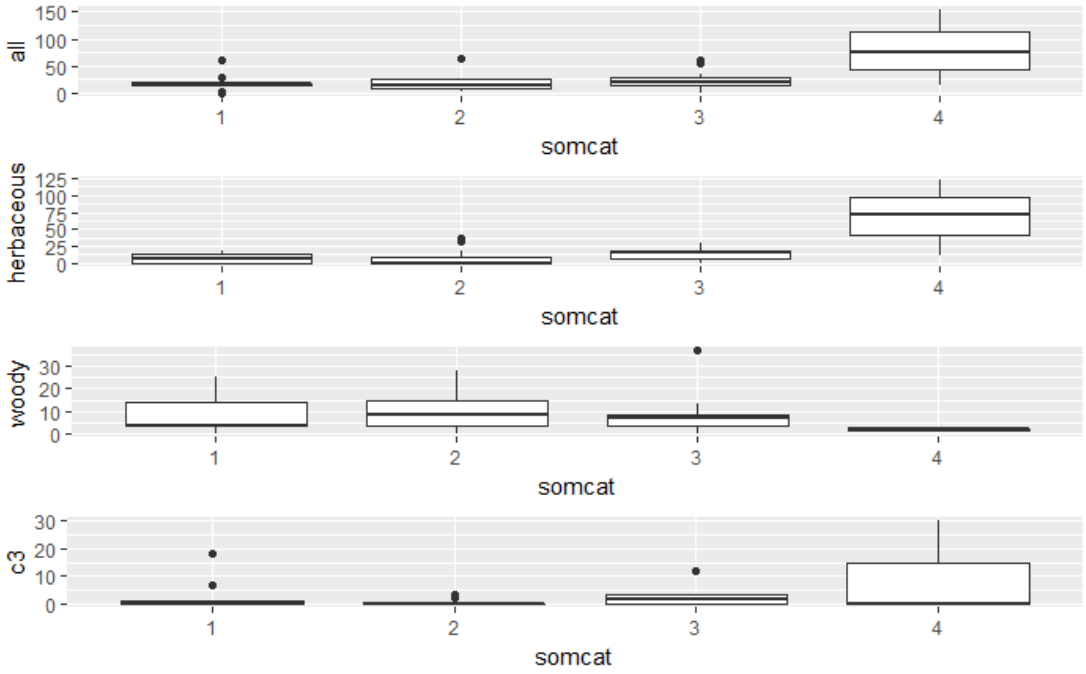


Figure 5.4. Variation in invader counts (number of individuals counted over 36 transects, or 3 transects per 60m x 60m restoration plot) for 2mx60m transects plotted by soil inorganic nitrogen (NO_3-) concentrations (ncat). Category 1:soil N<8.5ppm; 2:soilN=8.5-10ppm; 3:soilN=10-11ppm; 4:soilN>11ppm. An increase in invasion by soil N is apparent in woody invaders, but is less visible among the forb and c3 species groups.

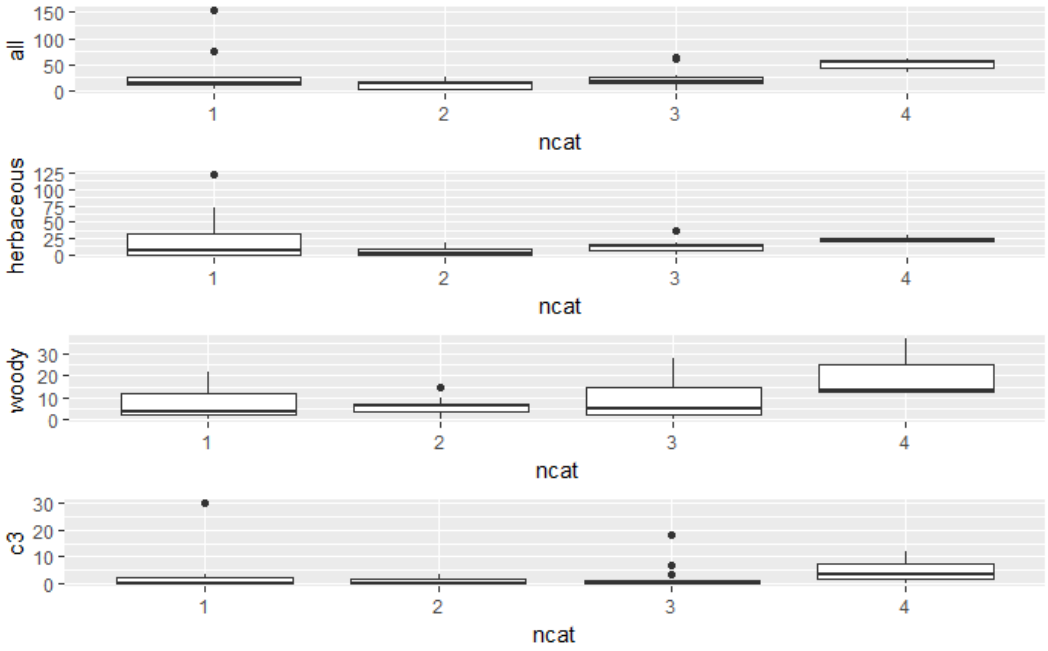


Figure 5.5. Total invasion by diversity, controlling for variation by field location. Group means and standard deviations predicted from the general linear model all~plotID*distance from river*diversity. ‘All’ denotes unsown, non-native species, plotID denotes the sampling location, distance from river refers to distance in meters south of the Platte River, which correlates which correlates strongly with soil organic matter and soil pH, and diversity refers to the planted levels of diversity. Red circles and vertical bars represent group means and +/- one standard error.

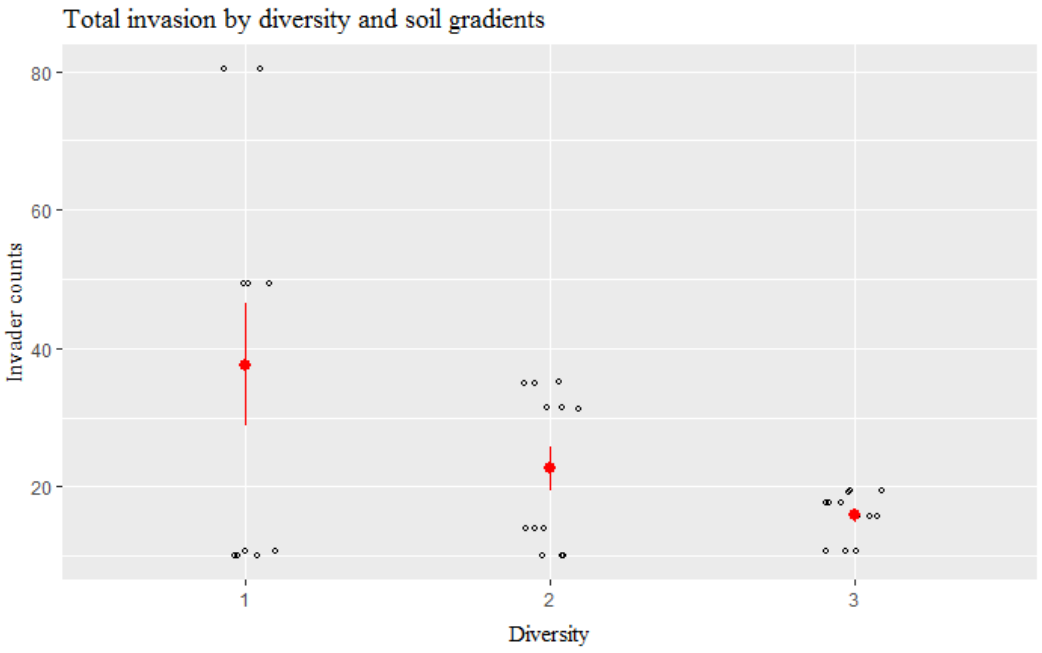


Figure 5.6. Woody invasion by diversity, controlling for variation by field location. Group means and standard deviations predicted from the general linear model all~plotID*distance from river*diversity. 'Woody' denotes unsown shrubs and tree seedlings, plotID denotes the sampling location, distance from river refers to distance in meters south of the Platte River, which correlates which correlates strongly with soil organic matter and soil pH, and diversity refers to the planted levels of diversity. Red circles and vertical bars represent group means and +/- one standard error.

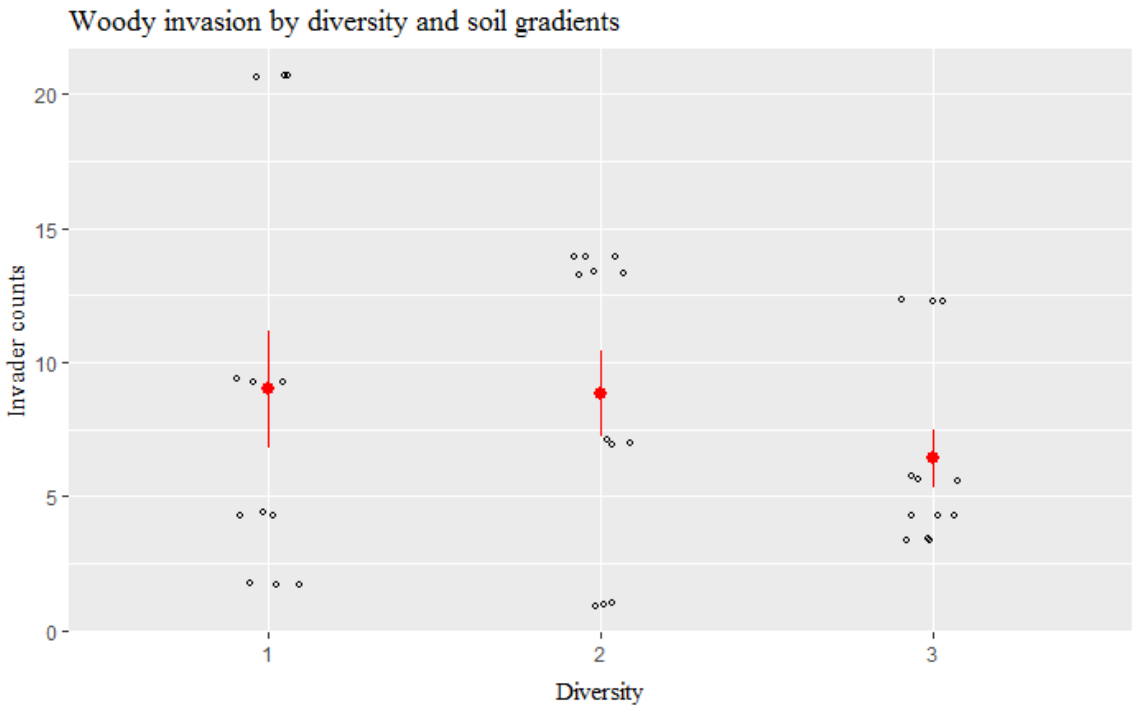


Figure 5.7. C3 (cool-season) grass invasion by diversity, controlling for variation by field location. Group means and standard deviations predicted from the general linear model C3~plotID*distance from river*diversity. ‘C3’ denotes unsown, non-native cool-season grasses, plotID denotes the sampling location, distance from river refers to distance in meters south of the Platte River, which correlates which correlates strongly with soil organic matter and soil pH, and diversity refers to the planted levels of diversity. Red circles and vertical bars represent group means and +/- one standard error.

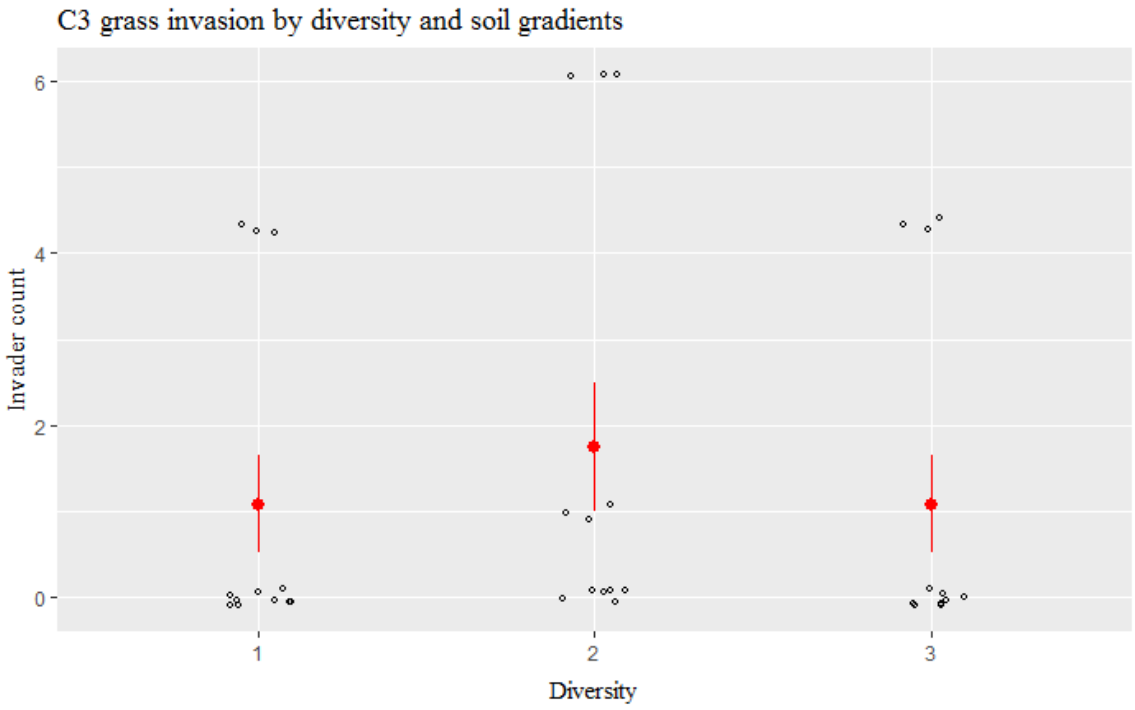
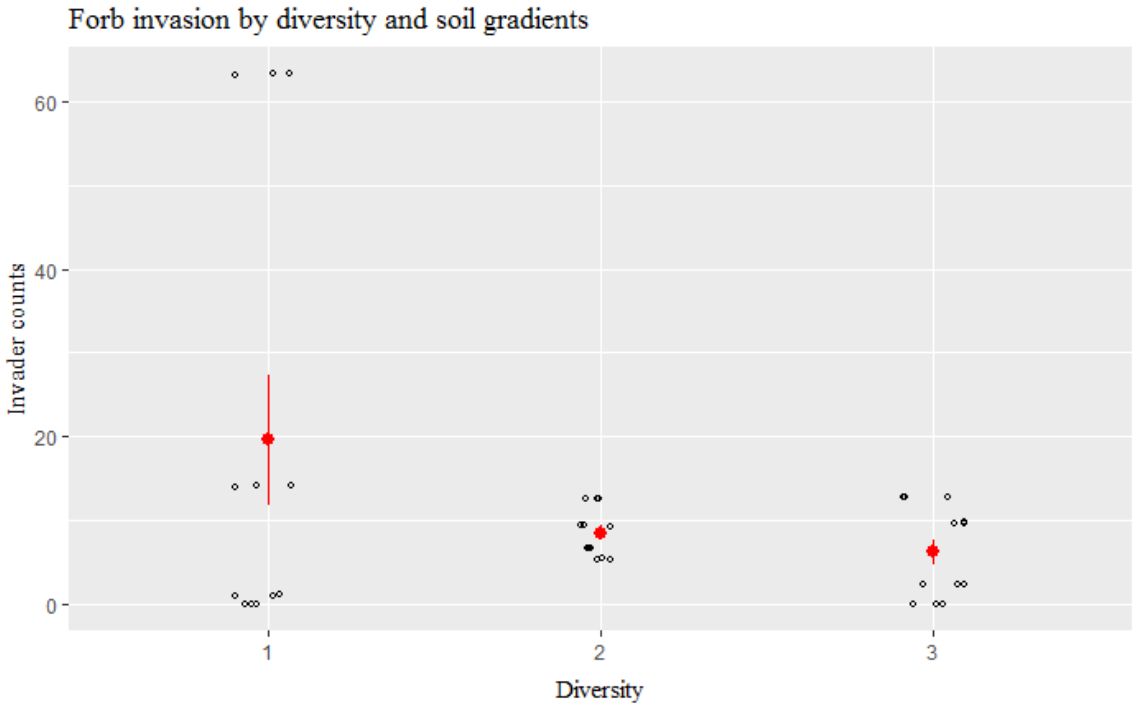


Figure 5.8. Herbaceous forb invasion by diversity, controlling for variation by sampling location. Group means and standard deviations predicted from the general linear model forbs~plotID*distance from river*diversity, where ‘forbs’ denotes unsown, non-native forbs, plotID denotes the sampling location, distance from river refers to distance in meters south of the Platte River, which correlates which correlates strongly with soil organic matter and soil pH, and diversity refers to the planted levels of diversity. Red circles and vertical bars represent group means and +/- one standard error.



CHAPTER 6: CONCLUSION: DIVERSITY AND DISTURBANCE IN A TALLGRASS PRAIRIE RESTORATION, IMPLICATIONS FOR RESEARCH AND MANAGEMENT

I. Introduction

Correlations between biodiversity and ecosystem functioning in grasslands have been well-studied in small-scale, 1m x 1m research plots, but understanding the relevance of relationships found in these studies to larger-scale restorations remains limited. In order to scale up the relationships posited from small-scale experiments, research must be conducted to test those relationships found in tightly-controlled mesocosm studies at larger scales encompassing greater environmental variability. My thesis research is a direct response to this need for larger-scale studies, and applies disturbance treatments to a system planted at low, medium, or high vegetation diversity.

In this study, I tested the relationship between planted diversity and ecosystem functioning by measuring the variation in multiple community functional and structural traits to the planted biodiversity level and to added disturbance treatments. This study falls within the umbrella of biodiversity-ecosystem function research, which posits that greater biodiversity contributes to an increase in the number of functions simultaneously carried out by a single ecosystem (Mason et al. 2005; Lefcheck et al. 2016). While a general interest in biodiversity-ecosystem function relationships has been present throughout ecology, the formal study of the BEF hypothesis began as recently as 1991 when the hypothesis was formally constructed (Ruijven 2013).

My study was designed to test two main hypotheses; first, that biodiversity plays a significant role in shaping ecosystem function, and second, that increased biodiversity can buffer the responses of those ecosystem functions to added disturbances. Evidence in support of the first hypothesis would include a measurable variation in community

composition, functional group composition, ground cover, or vegetation structure by diversity level. Evidence in support of the second would include higher rates of functioning across multiple ecosystem functions at high diversity, and a smaller change in those functions in response to disturbance treatments. In support of my first hypothesis, I found that plot vegetation structure (range in vegetation height, average litter depth and bareground) was driven partially by diversity. In partial support of my second hypothesis, I found that across some functional indices, biodiversity was the single largest factor explaining the measured variation in function.

II. Study Methods and Results

To test my hypotheses directly, I collected measurements related to ecosystem structure as well as three general categories of ecosystem function in response to disturbance treatments. Functions measured included plant physiology and growth strategy, decomposition and nutrient cycling, and resistance to biotic invasion. My results demonstrated that grassland functions respond to a variety of disturbances, and diversity can both buffer and enhance the effects of disturbance on ecosystem function (Table 6.1).

I first measured the relationship between planted diversity, disturbance treatments, and community structure and functional diversity via quadrat surveys in June 2015 and 2016. Response variables included percent bareground, litter cover and depth, the range in vegetation height, and the functional diversity (measured as sown and unsown forbs, C3 grasses, and C4 grasses) of each research plot at the whole-plot (60m x 60m) and the disturbance plot scale (1m x 2m). This survey found that functional richness and evenness varied by planted diversity, as expected, and that at both low and high diversity there was a noticeable decline in functional richness within rainout shelter treatments

compared with non-rainout shelter plots. Species surveys showed that species richness increased with increased distance from river, increased from 2015 to 2016, and increased with increasing soil nitrogen levels. Bareground declined from 2015 to 2016, declined at higher diversity, and increased with increasing distance from the Platte River. Litter cover varied conversely, with greater litter cover in 2016 and greater litter cover in high diversity plots. Litter depth also increased from 2015 to 2016 and declined in biomass removal treatments.

Second, I measured the relationship between planted diversity, disturbance treatments, and vegetation growth strategy in July 2016 via measurements of specific leaf area and leaf chlorophyll content. Community-weighted specific leaf area was higher in high diversity plots and beneath rainout shelters at both low and high diversity; this pattern was matched by individual species sampled. Chlorophyll content was the most rapidly-changing variable measured in this study, and was sampled two weeks following the final biomass removal and nitrogen addition disturbance applications. Community-weighted leaf chlorophyll content was higher in nitrogen-treatment plots at both low and high diversity, but did not vary significantly by diversity or rainout shelter effects. Individual species showed a diverse mix of responses, with one notable trend being a twice as large increase in leaf chlorophyll content for both C4 grasses and forbs in response to nitrogen addition at high diversity compared with low diversity, and a twice-as-large decline in leaf chlorophyll content in response to biomass removal in low-diversity compared with high-diversity plots.

Third, I assessed the role of biodiversity and disturbance treatments in modifying the decomposition of a single-source leaf litter in 2015, and in soil respiration in 2015

and 2016. These tests indicated that litter mass loss was significantly higher beneath rainout shelters compared with plots outside rainout shelters, with no significant differences by diversity or other disturbance treatments. Soil respiration was higher in high-diversity plots than in low-diversity plots, and increased with increased bareground, soil organic matter, and soil nitrogen. The lack of variation in soil respiration in response to treatments indicates that the scale of the disturbance was either too small to be measurable or too ephemeral to be captured by our once-per-year soil measurements.

Finally, I surveyed unsown species in each restoration plot to assess the relationship between biodiversity and resistance to biological invasion. Species chosen for this survey represented various growth strategies, and included species that were unsown and widely considered non-native to the region. My surveys included a transect survey of invader frequency and a separate survey of species cover. Invasive species counts and were generally higher in high-diversity plots, but cover was extremely low at both diversity levels. The woody invader group was unresponsive to diversity treatments, but was responsive to soil organic matter and soil nitrogen with higher counts and cover at higher levels of soil organic matter. C3 grass cover (including Smooth Brome, Foxtail Barley, and Kentucky Bluegrass, among others) increased at higher soil nitrogen levels but declined with diversity. Finally, herbaceous forbs had higher counts at higher soil nitrogen and organic matter contents, but this trend reversed when these variables co-varied with diversity level.

III. Discussion

Across several functional traits, biodiversity was the single largest factor explaining the variation measured in this study. This is most likely a result of one or both

of the following: first, the disturbances implemented to the research plots in this study were too small relative to the overall plot size for the effects of the treatments to be readily apparent due to the mitigating influence of the larger plant community. Second, biodiversity may in fact be a major factor driving the functioning of the study plots, thereby mitigating the effect of added disturbances.

Biodiversity contributed significantly to higher leaf nitrogen content, higher soil respiration rates, and a greater variety in ecosystem structure and functional diversity. Some of the responses to disturbance that were only measurable at high diversity levels may be due to the fact that low-diversity plots simply low levels of functioning to begin with that there wasn't much variation available within the system. In most cases, however, plant physiology was noticeably less variable at the species level in response to disturbance at high-diversity than low-diversity plots, suggesting that the adaptations that allow species to coexist at higher levels of diversity also confer some resilience to disturbance events.

A few of the largest sources of variation in this study were sampling year and soil gradients. These were uncontrolled but measureable sources of variation and were included in analyses. The year variable encompassed two main events which occurred in 2015: first, a prescribed burn in March 2015 removed virtually all litter cover and provided a flush of nutrients for new vegetation growth, and a subsequent major rainfall event in mid-June 2015 led to flooding of nearly the entire field. Standing water was visible in soil moisture access tubes to a depth of 30cm until the end of July, providing sub-irrigation throughout the main growing period for the summer. 2016 was a much warmer year and had lower average rainfall, with no major flooding events. While warm

spring temperatures may have allowed vegetation to begin growing earlier, the deep litter cover (often up to 50cm) left from the previous year maintained cool soil temperatures and limited plant growth through mid-June.

Site environmental gradients encompassed four measured variables: soil pH, which increased with distance from the Platte River; soil organic matter, which increased with distance from the river; soil moisture, which increased with distance from the river, and soil nitrate, which increased from east to west. These variables were slow-changing relative to the two-year duration of this study; therefore, they were used as fixed variables in analyses. While pH was not significantly correlated with any of the response variables I measured, site nitrogen and organic matter were significant predictors of invasive species frequency and cover, soil microbial biomass and respiration, and even influenced functional diversity of the vegetation community.

The traits chosen for this study, and the measurements of their change in response to disturbance, were selected as an attempt to operationalize the concept of ecosystem resilience (Carpenter et al. 2001) by testing a range of system characteristics and their responses to disturbance. The biodiversity-ecosystem-function hypothesis provided a foundational hypothesis for my analysis by predicting that increased biodiversity would contribute to a greater ability to maintain multiple system functions. Stress-testing those functions by adding multiple disturbances to larger restoration plots provided an examination of the limits of ecosystem functioning and allowed me to test my second hypothesis; namely, that increased biodiversity would contribute significantly to ecosystem responses to disturbance.

IV. Implications for Research and Management

Whether the data analyzed in this study support the hypothesis that increased biodiversity supports system resilience is an open question; however, I argue that a broad sampling of system responses to disturbance, at low and high diversity, is a viable method for assessing the resilience of a whole system. Detailed analyses of individual functions may be useful when a certain ecosystem function is at special risk, but to understand the resilience of a given system state it is more important to gain a slightly less precise view of the general structure and functional variability within a system. Because the inherent variability of a given system state can be difficult to assess through straightforward monitoring, stress-testing various ecosystem functions by adding disturbances that are known threats to the system of interest and measuring the resulting variation in ecosystem structure and function can provide one method for determining how flexible or fragile a given system state is. By adding disturbances to a tallgrass prairie at multiple levels of diversity, I was able to compare the variability present at high and low levels of biodiversity and determine whether increased biodiversity led to greater or lesser variation in systemic variability.

Although ecosystem traits may fluctuate widely immediately following disturbance, an area of uncertainty that I did not address in this study is the long-term effects of these disturbances. If a disturbance ceases, will the system resume its pre-disturbance functioning in the following years, or will it have made a directional shift to a new stable state? How long-term or intense must a specific disturbance be to cause a directional shift, rather than a fluctuation, in system functioning? These questions reflect the ongoing difficulty in predicting ecosystem response to stochastic changes in the environment; however, the use of biodiversity-disturbance tests similar to the one

implemented in this study offer a direct, experimental method for testing the mechanisms that shape ecosystem responses to disturbance and make better predictions of system change.

Biodiversity was the central variable manipulated in this study, but there may be other drivers of ecosystem functioning, including vegetation characteristics, faunal interactions, or soil nutrient gradients, where this method of study may produce interesting results. Future studies that manipulate the soil type, faunal interactions, or vegetation functional groups at large scales and add disturbances within those treatments could elucidate the role that each of these potential controlling factors play in mediating ecosystem responses to disturbance.

While this study is in some ways a conventional biodiversity-function study, it differs from previous research by scaling up the size of the diversity manipulations and exerting minimal control over the establishment of the plant communities. In more conventional biodiversity studies (as in Tilman et al. 1997), diversity plots are carefully maintained to preserve specific species groupings. In this study, restorations were established and underwent typical prairie management, and therefore represent a more realistic management scenario with implications that can directly support future grassland management decisions. It also demonstrates that research conducted at small scales can be scaled up to larger fields, as biodiversity effects are large enough to overcome the effects of uncontrolled environmental gradients and stochastic weather events. Finally, this study fits into previous research that supports planting increased biodiversity as a strategy for establishing successful restorations (Nemec 2013; Bach et al. 2012; Price 2015), as investment in a greater diversity of species at the outset may offset more costly

invasive species management or restoration efforts following disturbance events.

V. Literature Cited

Bach, E. M., Baer, S. G., Six, J. 2012. Plant and Soil Responses to High and Low Diversity Grassland Restoration Practices. Environmental Management **49**:412-424.

Carpenter, S., Walker, B., Anderies, J.M., Abel, N. 2001. From metaphor to measurement: resilience of what to what? Ecosystems **4**: 765-781.

Lefcheck, J. S., Byrnes, J. E. K., Isbell, F., Gamfeldt, L., Griffin, J. N., Eisenhauer, N., Duffy, J. E. 2015. Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. Nature Communications **6**: 6936.

Mason, N.W.H., Mouillot, D., Lee, W.G., Wilson, B. 2005. Functional richness, functional evenness and functional divergence: the primary components of functional diversity. Oikos **111**: 112-118.

Price, Katilyn. 2015. Plant diversity affects performance of invasive thistles in restored Nebraska grasslands. Thesis.

Ricotta, C., Bacaro, G., Moretti, M. 2014. A new measure of functional evenness and some of its properties. PLoS ONE **9**: e104060.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., Siemann, E. 1997. The influence of functional diversity and composition on ecosystem processes. Science **277**: 1300-1302.

Van Ruijven, J. 2013. *Biodiversity and Ecosystem Functioning*. Oxford Bibliographies [online].

VI. Tables and Figures

Table 6.1. Ecosystem characteristics and functions measured in this study, metrics used to assess these characteristics, the key sources of variation measured for each metric, and the direction of change ('+': increase, '- ': decrease) for each metric. Characteristics and functions were chosen to cover a range of functions that are performed by grasslands and which may vary measurably by plant diversity. Indicators were chosen as simple representations of the system characteristics. The sources of variation include both general site characteristics and treatments that were measured and included in analyses of variation. The direction of change is a general indicator of either a positive (+) or negative (-) correlation with an increase in the measured value of the source of variation.

Function/ Characteristic	*Response Variable*	*Source of variation*	*Change (+\-)*
Functional diversity	Functional diversity	Plot diversity	+
		Distance from river	+
		Year	+
		Avg. soil nitrogen	+
	Bareground	Diversity	-
		Year	-
		Distance	+
Plant Growth	Leaf chlorophyll	Nitrogen (all)	+
		Diversity (Andropogon)	+
		Rainout (Andr.)	+
		HD&Biomass (Andr.)	+
		Nitrogen (Helianthus)	+
		HD + Rainout (Solidago)	+
		HD + Nitro add. (Soli.)	-
		Biomass (Soli.)	-
		Rainout (Monarda)	+
		Biomass (Mon.)	-
		Nitrogen (Mon.)	+
		Rainout (Glycyrrhiza)	-
		Biomass (Gly.)	-
		Nitrogen (Gly.)	+
	Specific leaf area	Diversity (All)	+
		Rainout (All)	+
		Diversity (Andr.)	+
		Rainout (Soli.)	+
		HD + Rainout (Soli.)	+

Nutrient cycling	Litter decomposition	Rainout	+
	Soil respiration	Diversity	+
		Bare	+
		Soil nitrogen	+
		Soil organic matter	+
		Year	-
Invasion defense	Invasive count	Soil organic matter	+
		Soil nitrogen	+
		HD	-
		HD+Soil organic matter	-

APPENDIX I: R SCRIPTS AND DATA FILES TO REPRODUCE ANALYSES AND FIGURES FOR THE THESIS

Bareground and Litter Summaries for Bevans Thesis

Becca

April 15, 2017

R script to reproduce the correlations and images used in the thesis "PLANT DIVERSITY INFLUENCES THE STRUCTURE AND FUNCTION OF A RESTORED PRAIRIE AND ITS RESPONSES TO ADDED DISTURBANCES"" by Rebecca A Bevans, submitted to the graduate college April 28 2017.

```
knitr::opts_chunk$set(warning=FALSE, message=FALSE, results="hide")
```

1. Load data and packages:

```
library(tidyverse)
library(picante)
library(labdsv)
library(labdsv)
library(ggplot2)
library(pander)
library(lme4)
library(lmerTest)
library(doBy)
library(plyr)

litter=read.csv("~/litter.csv", header=TRUE, row.names=1)
bare<-read.csv("~/bare.csv", header=TRUE, row.names=1)

bare$year=as.factor(bare$year)
bare$diversity=as.factor(bare$diversity)
bare$rainout=as.factor(bare$rainout)
bare$rain=as.factor(bare$rain)
bare$nitro=as.factor(bare$nitro)
bare$biomass=as.factor(bare$biomass)
bare$level=as.factor(bare$level)

litter$year=as.factor(litter$year)
litter$diversity=as.factor(litter$diversity)
litter$rainout=as.factor(litter$rainout)
litter$rain=as.factor(litter$rain)
litter$nitro=as.factor(litter$nitro)
litter$biomass=as.factor(litter$biomass)
litter$level=as.factor(litter$level)
```

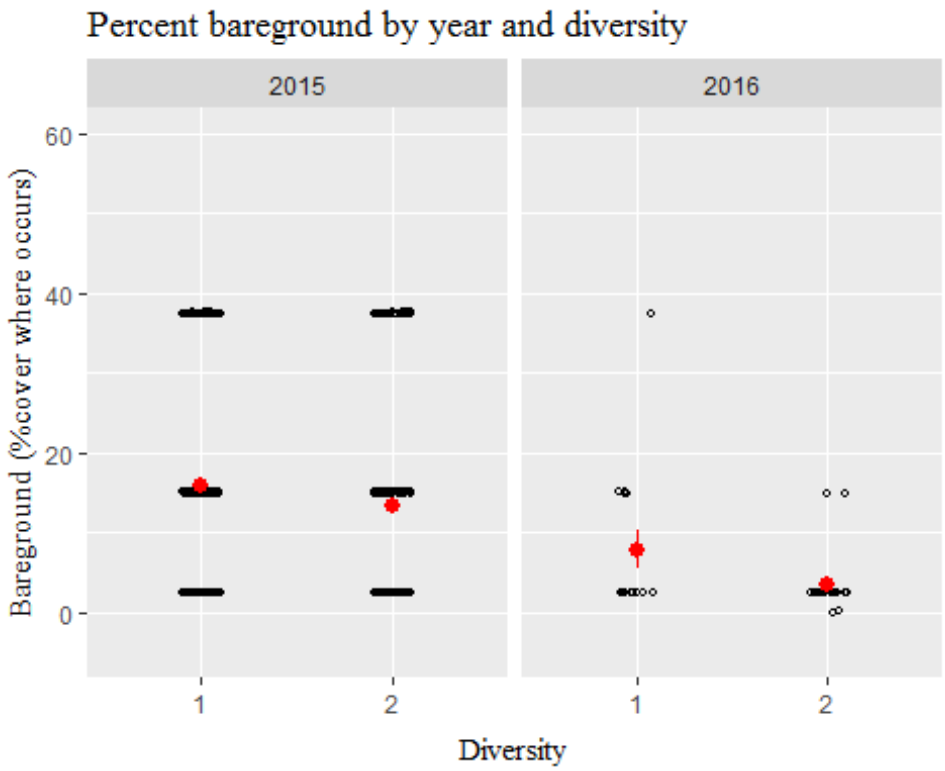
Make tables of changes in bareground and litter:

```
bare.plot<-ddply(bare, c("rep", "year"), summarise,
                 n=sum(pctbare),
                 mean=mean(pctbare, na.rm=FALSE))

pander(bare.plot)

baredata <- ddply(bare, c("diversity", "year"), summarise,
                n = sum(pctbare),
                mean_bare = mean(pctbare, na.rm=FALSE),
                sd=sd(pctbare, na.rm=FALSE),
                se=sd/sqrt(n))

baredata
pander(baredata)


bare.eu.wp<-ddply(bare, c("diversity", "rainout", "year"), summarise,
                n = sum(!is.na(pctbare)),
                mean = mean(pctbare, na.rm=FALSE),
                sd=sd(pctbare, na.rm=FALSE),
                se=sd/sqrt(n))
pander(bare.eu.wp)
bare.mod<-aov(pctbare ~ diversity*rainout*year, data=bare)
summary(bare.mod)
TukeyHSD((bare.mod))
#LSmeans(bare.mod, effect="diversity")


baredist<- ddply(bare, c("dist"), summarise,
                  n=sum(!is.na(pctbare)),
                  mean_bare=mean(pctbare, na.rm=FALSE),
                  sd=sd(pctbare, na.rm=FALSE),
                  se=sd/sqrt(n))
baredist
pander(baredist)
baredist.mod<-lm(mean_bare~dist, data=baredist)

litdepth <- ddply(litter, c("diversity", "year"), summarise,
                n = sum(!is.na(maxht_cm)),
                max_depth = mean(maxht_cm, na.rm=TRUE),
                sd=sd(maxht_cm, na.rm=TRUE),
                se=sd/sqrt(n))
litdepth
pander(litdepth)


pctlit<-ddply(litter, c("diversity", "year"), summarise,
                n = sum(!is.na(pctlit)),
```

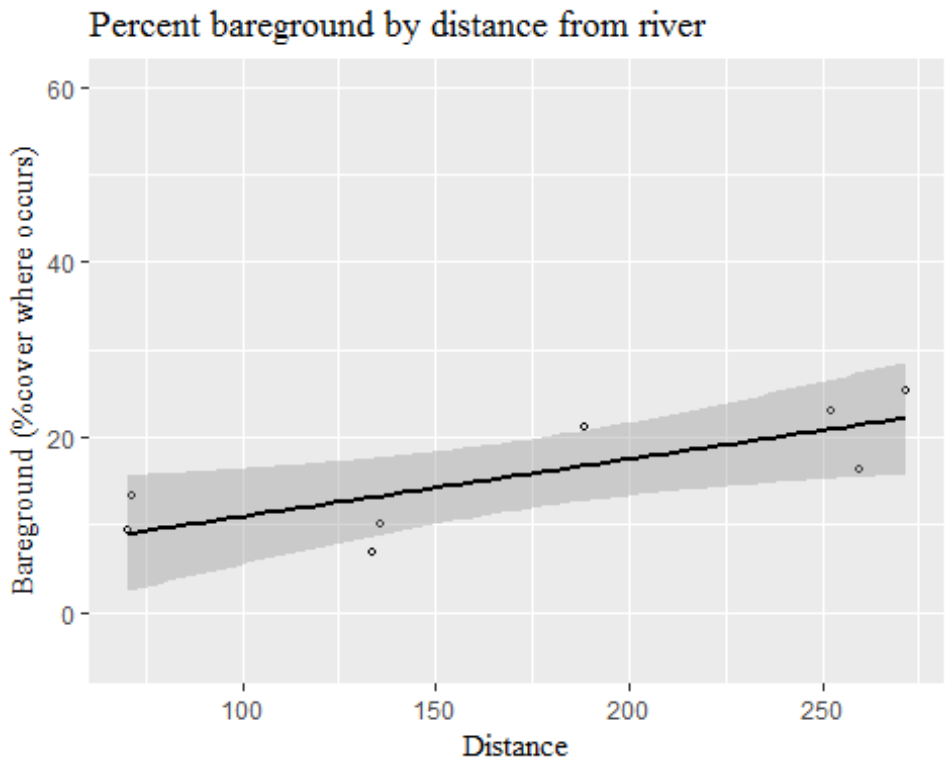
```
                mean_litter = mean(pctlit, na.rm=TRUE),
                sd=sd(pctlit, na.rm=TRUE),
                se=sd/sqrt(224))
pander(pctlit)


litleveldeep<-ddply(litter, c("level", "year"), summarise,
                n = sum(!is.na(maxht_cm)),
                depth = mean(maxht_cm, na.rm=TRUE),
                sd=sd(maxht_cm, na.rm=TRUE),
                se=sd/sqrt(n))

pander(litleveldeep)
```

Plot the data:

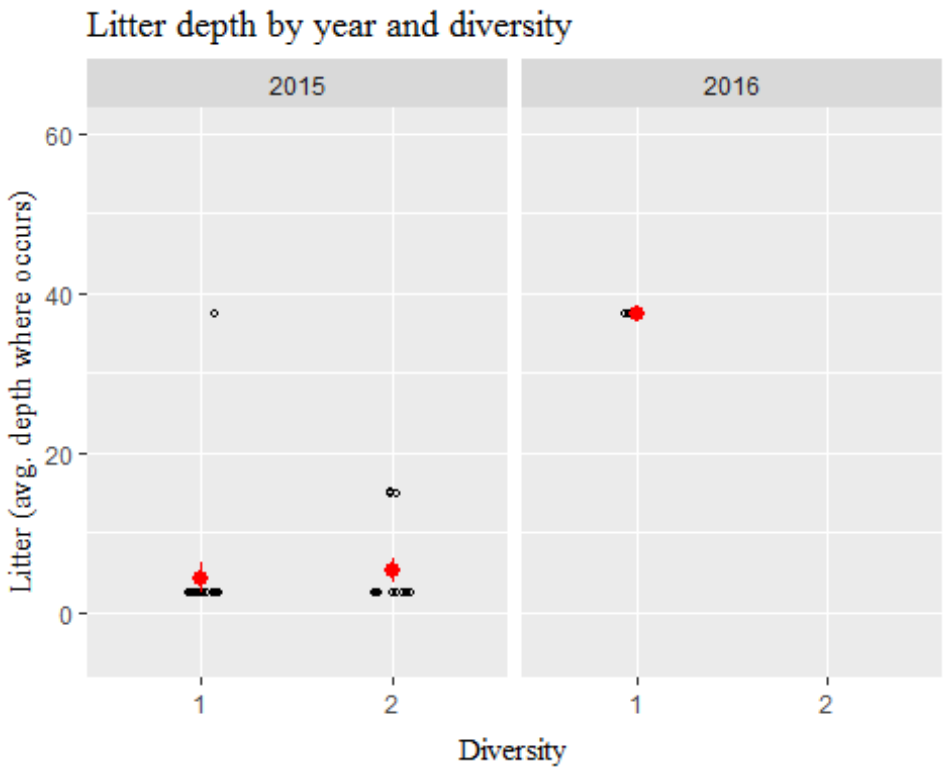
```
bareplot<-ggplot(bare, aes(x=diversity, y=pctbare)) +
  ggtitle("Percent bareground by year and diversity") +
  ylim(ymin = -5, ymax = 60) +
  geom_point(cex=1.0, pch=1.0, position = position_jitter(w = 0.1, h =
0.1)) +
  xlab("Diversity") +
  ylab("Bareground (%cover where occurs)") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))
bareplot + facet_wrap("year") + stat_summary(fun.data=mean_se, fun.args
=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```




```
#distance from river
baredist.mod<-baredist.mod
baredist.mod
summary(baredist.mod)
bareplot<-ggplot(baredist.mod, aes(x=dist, y=mean_bare)) +
  ggtitle("Percent bareground by distance from river") +
  ylim(ymin = -5, ymax = 60) +
  geom_point(cex=1.0, pch=1.0, position = position_jitter(w = 0.1, h =
0.1)) +
  xlab("Distance") +
  ylab("Bareground (%cover where occurs)") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))
bareplot + stat_smooth(method="lm", color="black")
```




```
litplot<-ggplot(litter, aes(x=diversity, y=pctlit)) +
  ggtitle("Litter depth by year and diversity") +
  ylim(ymin = -5, ymax = 60) +
  geom_point(cex=1.0, pch=1.0, position = position_jitter(w = 0.1, h =
0.1)) +
  xlab("Diversity") +
  ylab("Litter (avg. depth where occurs)") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))
litplot + facet_wrap("year") + stat_summary(fun.data=mean_se, fun.args=
list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```



Global models describing bareground and litter cover:

```
#bareground
bare$biomass=as.factor(bare$biomass)
bare$nitro=as.factor(bare$nitro)
bare$site=as.factor(bare$site)
bare.global<-lmer(pctbare ~ dist*diversity*year + diversity*biomass + d
iversity*nitro + (1|block), data=bare)
bare.select<-step(bare.global, data=bare, direction="backward")
bare.select
bare.best<-lmer(pctbare ~ dist + year + nitro + diversity + (1|block),
data=bare)
summary(bare.best)

#litter
litter$biomass=as.factor(litter$biomass)
litter$nitro=as.factor(litter$nitro)
litter$site=paste(litter$block, litter$level)
litter$site=as.factor(litter$site)

litter.global<-lmer(pctlit ~ dist*diversity*year + diversity*biomass +
diversity*nitro + (1|block), data=litter)
litter.select<-step(litter.global, data=litter, direction="backward")
litter.select
```

```
litter.best<-lmer(pctlit ~ dist:diversity:year + (1|block), data=litter
)
summary(litter.best)
```

Community_Thesis

Becca

February 3, 2017

R script to reproduce the correlations and images used in the thesis "PLANT DIVERSITY INFLUENCES THE STRUCTURE AND FUNCTION OF A RESTORED PRAIRIE AND ITS RESPONSES TO ADDED DISTURBANCES"" by Rebecca A Bevans, submitted to the graduate college April 28 2017.

Tests performed to determine variance in community composition and species richness by diversity and treatment type for the thesis.

```
knitr::opts_chunk$set(warning=FALSE, message=FALSE, results="hide")
```

We want to test two main levels of treatment: 1. Effect of diversity on community richness and species/functional composition 2. Effect of treatments on community richness and species/functional composition

Within these levels, there are a few additional sources of variation to take into account: 1. Unknown spatial variation (variation among individual block within the overall diversity level) 2. Known spatial variation (soil nitrogen and organic matter gradients) 3. Known rainout-shelter effects (test this before testing the effect of individual treatments to determine whether or not to group the control plots with the rainout-only plot) 4. Unknown variation by year (there may be large differences between 2015 and 2016 due to variations in weather, sampling accuracy, etc.)

Load libraries and data

```
library(picante)
library(tidyverse)
library(vegan)
library(labdsv)
library(pander)
library(plyr)

#read in the meta2 data.frame
meta<-read.csv("~/meta.csv", header=TRUE, row.names=1)
meta=meta[-1]

row.names(meta)
colnames(meta)
```

Add site, distance, soil OM, and kcl-N to dataset:

```
meta$site<-paste(meta$block, meta$diversity)
meta.dist<-meta[c("site")]
head(meta.dist)
a<-c('a 1', 'a 2', 'a 3', 'a 4','b 1', 'b 2', 'b 3', 'b 4', 'c 1', 'c 2
', 'c 3', 'c 4')
b<-c(70.5, 127.7, 188.6, 252,  64.78,  135.8, 192.66,259.5, 71.5, 133.8
, 195.25, 271.5)
meta.dist<-vegtrans(meta.dist, a, b)
meta$dist<-meta.dist$site

meta.om<-meta[c("site")]
a<-c('a 1', 'a 2', 'a 3', 'a 4','b 1', 'b 2', 'b 3', 'b 4', 'c 1', 'c 2
', 'c 3', 'c 4')
b<-c(1.9,  1.533, 1.9, 2.066, 1.6, 1.833, 1.866, 1.9, 1.5, 1.833, 1.866
, 2.233)
meta.om<-vegtrans(meta.om, a, b)
meta$om<-meta.om$site

meta.n<-meta[c("site")]
a<-c('a 1', 'a 2', 'a 3', 'a 4','b 1', 'b 2', 'b 3', 'b 4', 'c 1', 'c 2
', 'c 3', 'c 4')
b<-c(8.9,  10.93, 10.767, 11.49, 10.186, 9.3, 10.683, 9.766, 8.763, 4.6
3, 7.506, 8.046)
meta.n<-vegtrans(meta.n, a, b)
meta$n<-meta.n$site
```

[[Start here for community composition dataframe]] Read in the community data:

```
comm<-read.csv("~/veg.cover.csv", header=TRUE, row.names="X.1")
comm<-comm[(-1)]
class(comm)
dim(comm)

all.equal(rownames(comm), rownames(meta))
```

Read in the veg.height dataframe (same format as the community composition dataframe):

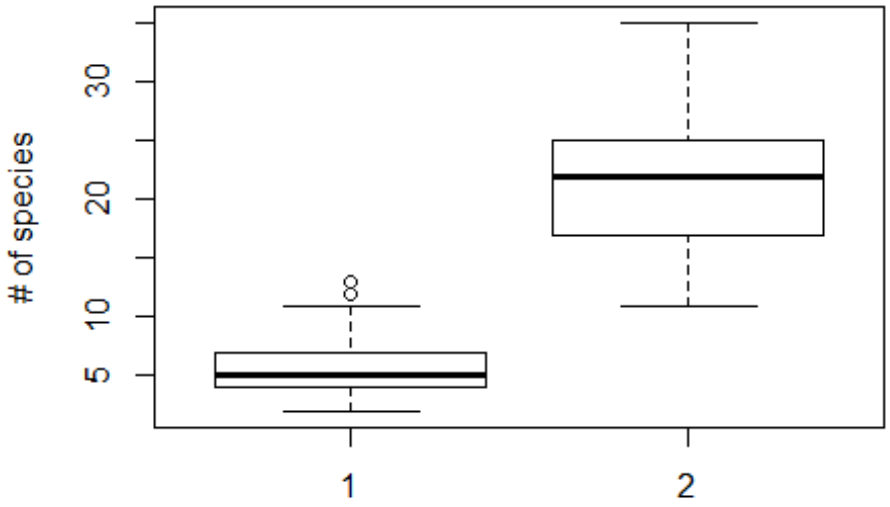
```
height<-read.csv("~/heights.csv", header=TRUE, row.names=1)
rownames(height)
rownames(meta)
```

Explore data - overall species richness by quadrat

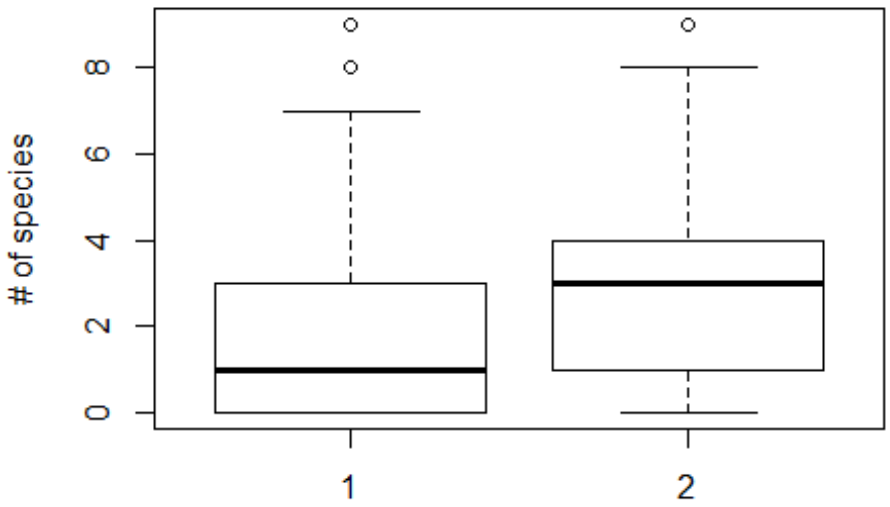
How many plots does each species occur in?

```
#read in the files:
comm.adults<-read.csv("~/comm.adults.csv", header=TRUE, row.names = 1)
comm.seeds<-read.csv("~/comm.seeds.csv", header = TRUE, row.names = 1)
head(comm.adults)
head(comm.seeds)
```

```r
#view differences in species number just with the adults:
meta$diversity<-as.factor(meta$diversity)
boxplot(specnumber(comm.adults)~meta$diversity, ylab = "# of species")
```

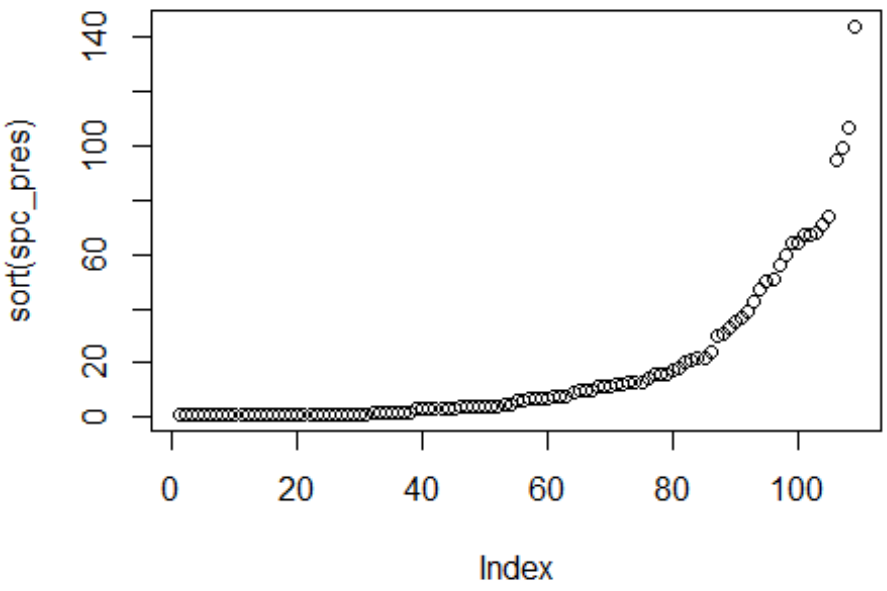



```r
boxplot(specnumber(comm.seeds)~meta$diversity, ylab= '# of species')
```

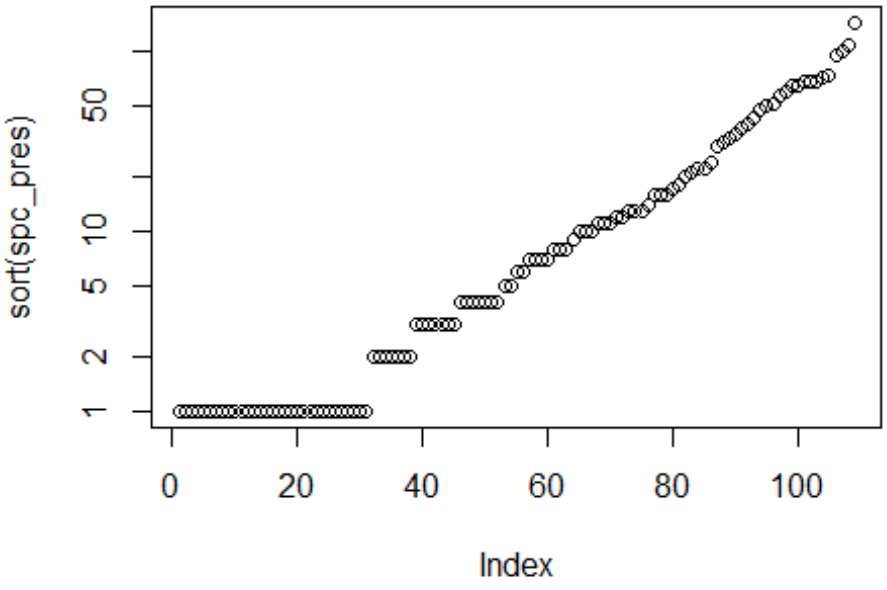


View cover of adult species in plots: translate the daubenmire cover class values to cover midpoints:

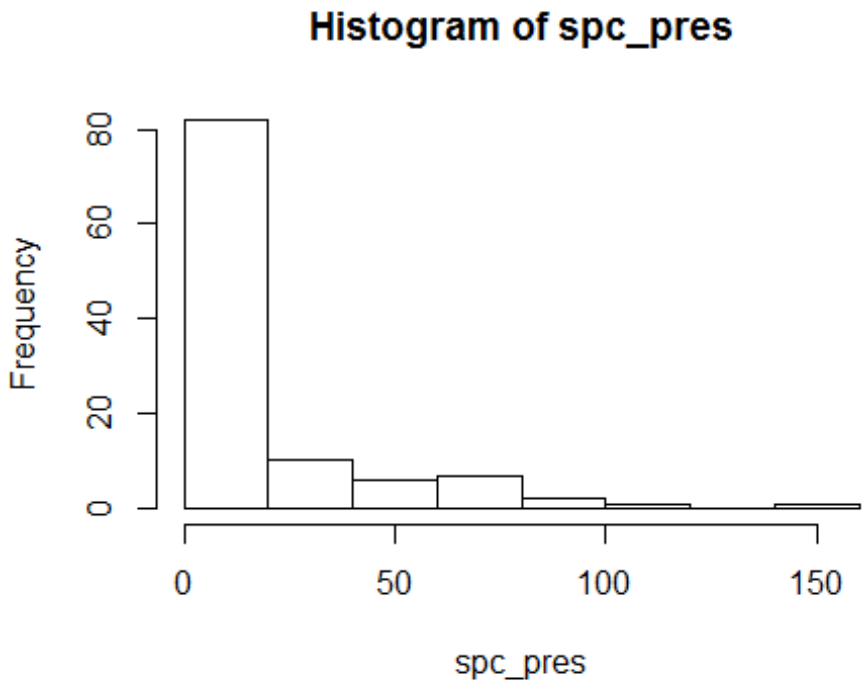
```
comm.cover<-comm.adults
apply(comm.cover, 1, sum)
comm.total<-decostand(comm.cover, method="total")
apply(comm.total, 1, sum)
head(comm.cover)

#list(comm.cover$Ange, comm.cover$Achmil)
#show the presence/abundance curve:
spc_pres<-apply(comm.cover > 0,2, sum)
plot(sort(spc_pres))
```



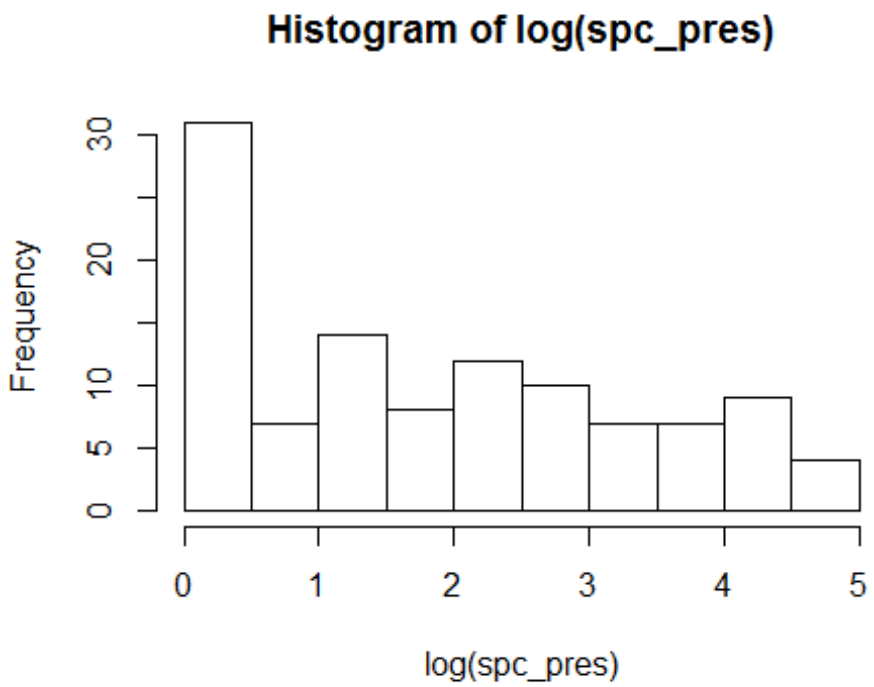

```
#ANGE is overwhelmingly dominant. Most species appear only once.
#log-transform to make it more of a straight line:
plot(sort(spc_pres), log='y')
```



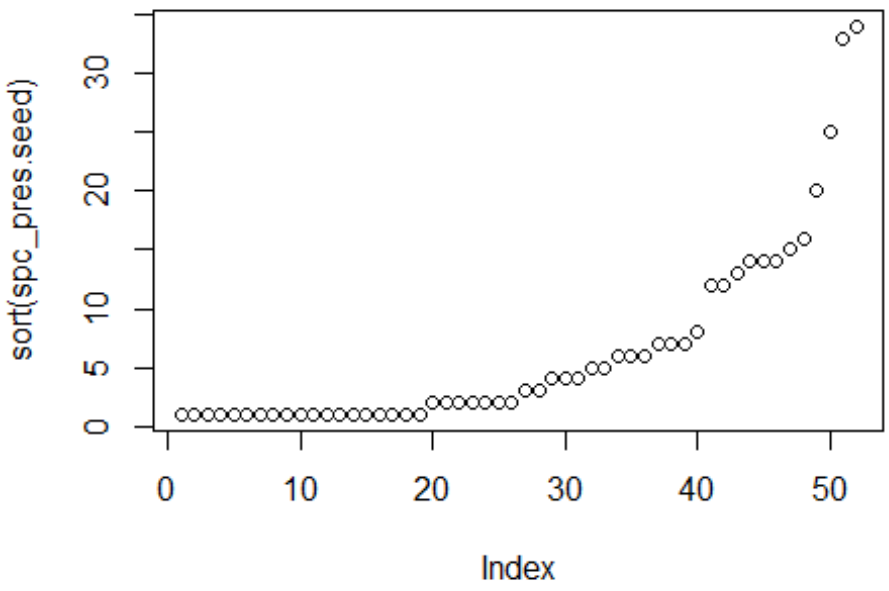
```
species<-sort(spc_pres)
as.matrix(species)
#the next most common species is Achillea millefolium, followed by seta
ria.

hist(spc_pres)
```



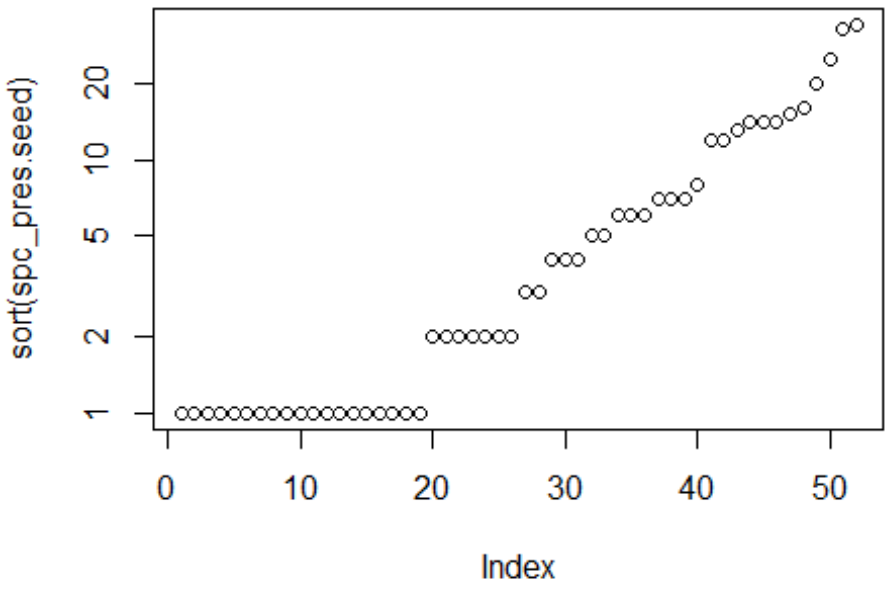

```
hist(log(spc_pres))
```



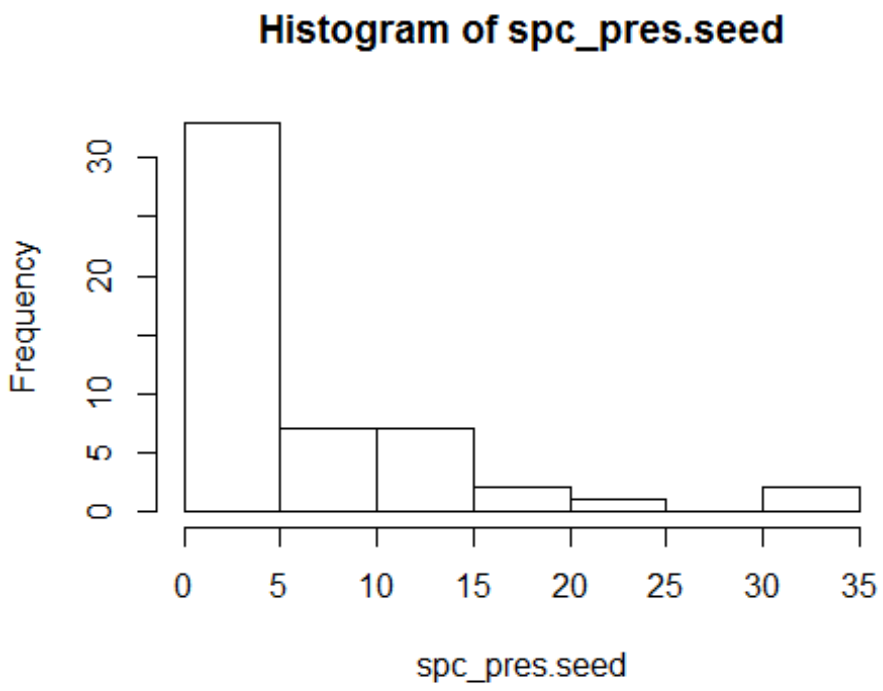

```r
#head(comm.cover.seed)
comm.cover.seed<-comm.seeds
apply(comm.cover.seed, 1, sum)
comm.total.seed<-decostand(comm.cover.seed, method="total")
apply(comm.total.seed, 1, sum)

#list(comm.cover$Ange, comm.cover$Achmil)
#show the presence/abundance curve:
spc_pres.seed<-apply(comm.cover.seed > 0,2, sum)
plot(sort(spc_pres.seed))
```



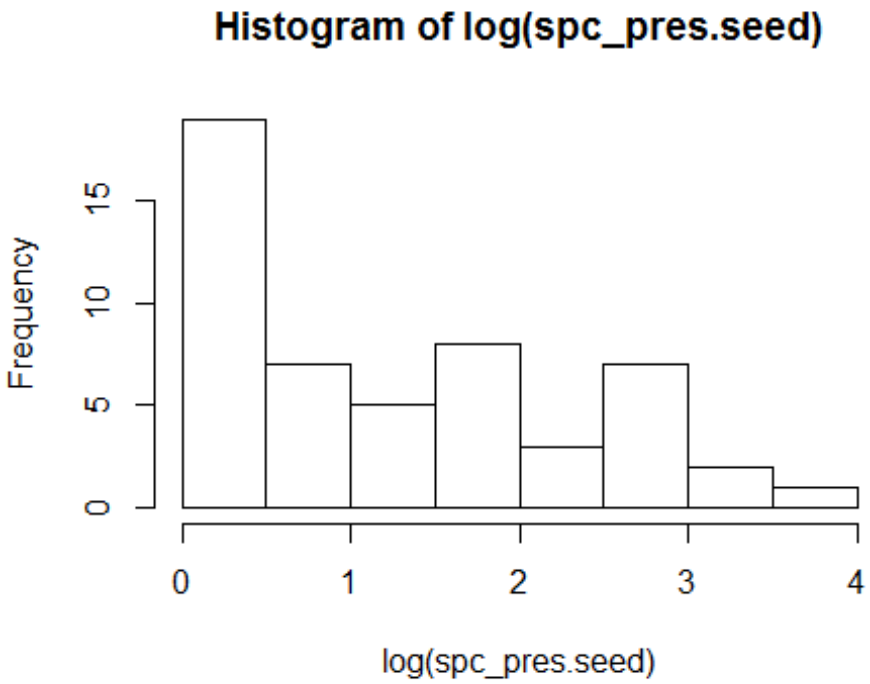

```
#ANGE is overwhelmingly dominant. Most species appear only once.
#log-transform to make it more of a straight line:
plot(sort(spc_pres.seed), log='y')
```



```
species.seed<-sort(spc_pres.seed)
#species.seed
as.matrix(species.seed)
#the next most common species is Achillea millefolium, followed by seta
ria.

hist(spc_pres.seed)
```

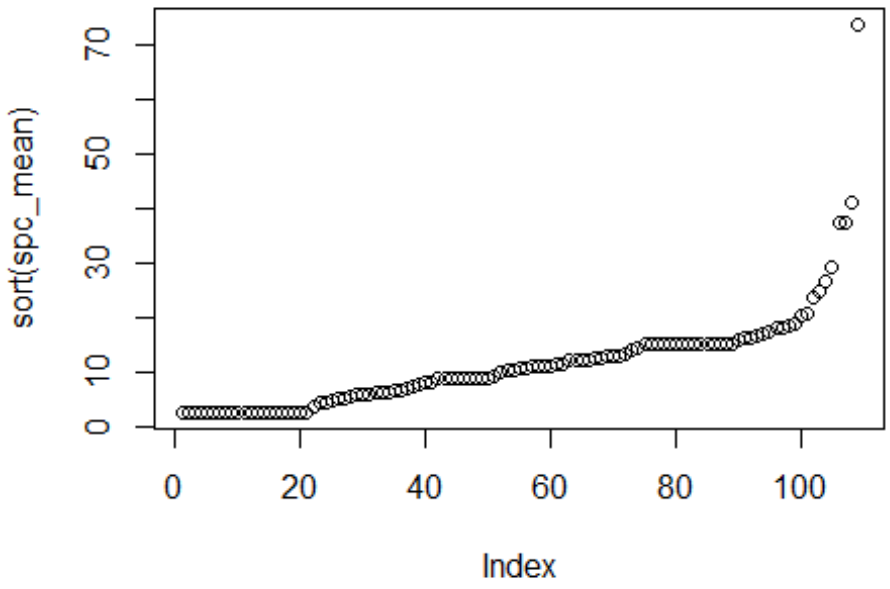



```
hist(log(spc_pres.seed))
```

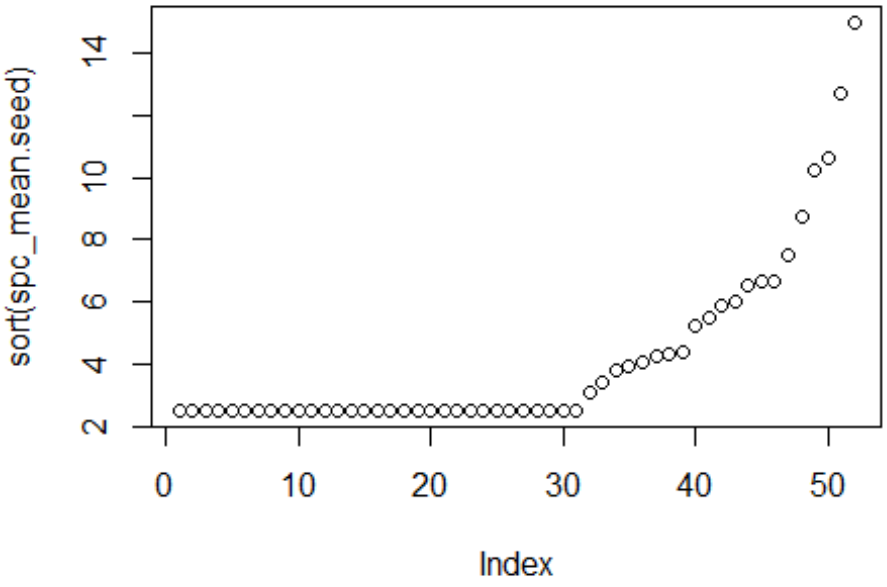


What is the mean cover of each species when it occurs(ignoring zeroes where it is absent)?

```
#adults
pres.cover<-apply(comm.cover, 2, sum)
pres.cover<-as.data.frame(pres.cover)
spc_mean<-pres.cover/spc_pres
spc_mean<-as.matrix(spc_mean)
plot(sort(spc_mean))
```

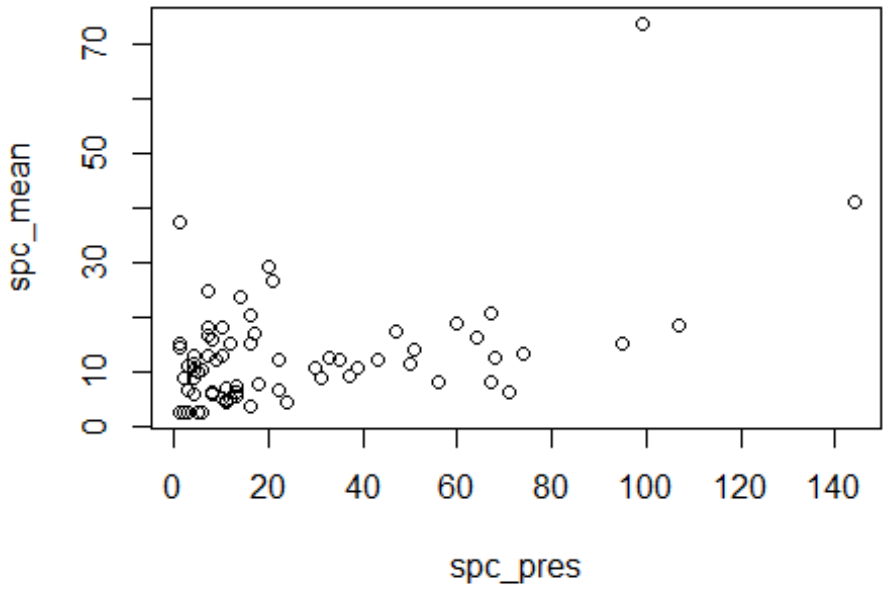



```
#seedlings
pres.cover.seed<-apply(comm.cover.seed, 2, sum)
pres.cover.seed<-as.data.frame(pres.cover.seed)
spc_mean.seed<-pres.cover.seed/spc_pres.seed
spc_mean.seed<-as.matrix(spc_mean.seed)
plot(sort(spc_mean.seed))
```

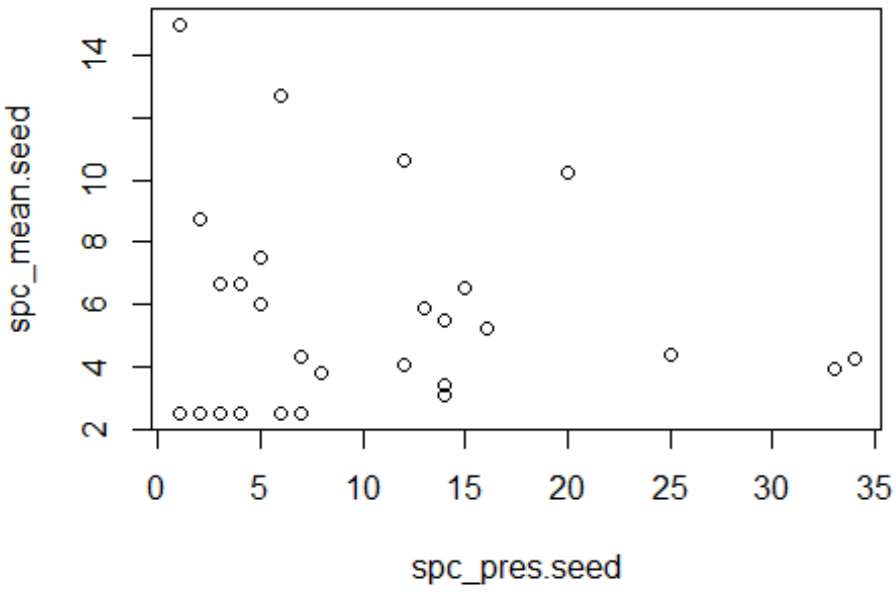


Is the mean abundance of species correlated with the number of plots they occur in?

```
plot(spc_pres, spc_mean)
```



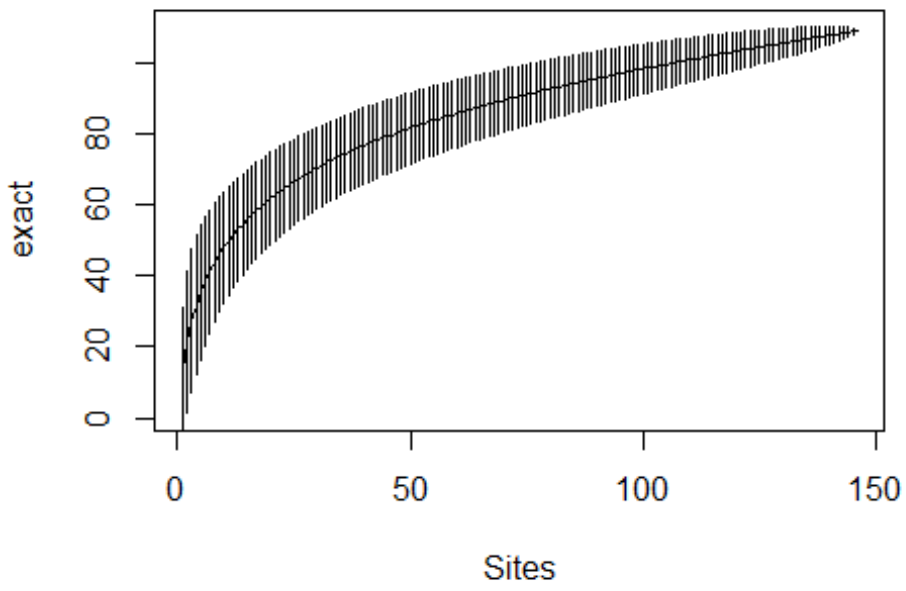
```
plot(spc_pres.seed, spc_mean.seed)
```



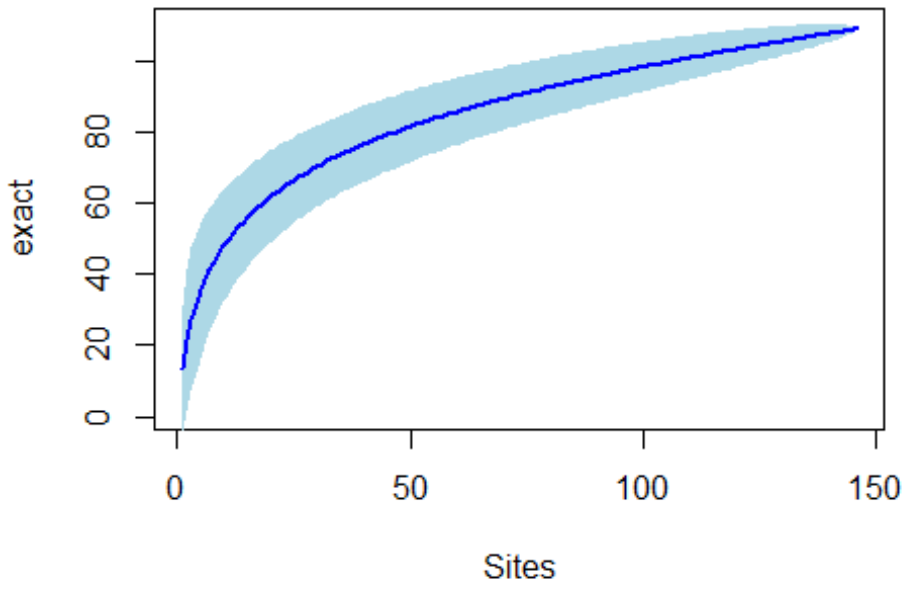

```
#not a strong correlation.
```

Plot species-individual curves:

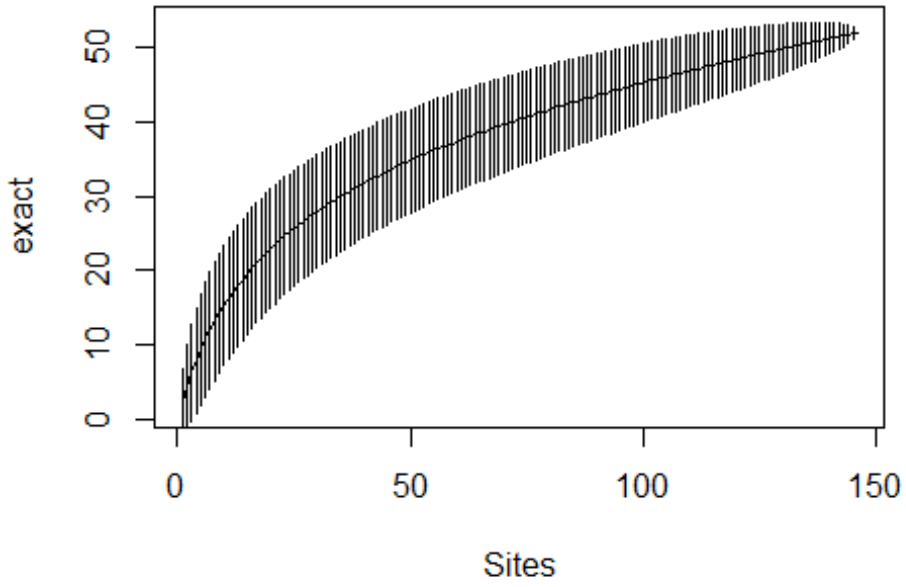
```
#adults
spa<-specaccum(comm.adults)
plot(spa)
```



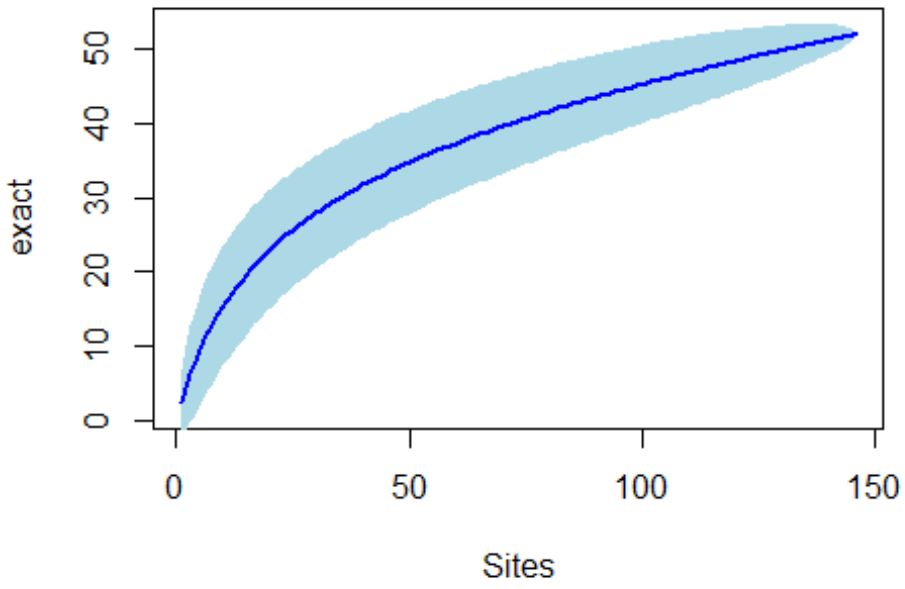

```
plot(spa, ci.type="poly", col="blue", lwd=2, ci.lty=0, ci.col="lightblu
e")
```



```
#seedlings
spc<-specaccum(comm.seeds)
plot(spc)
```



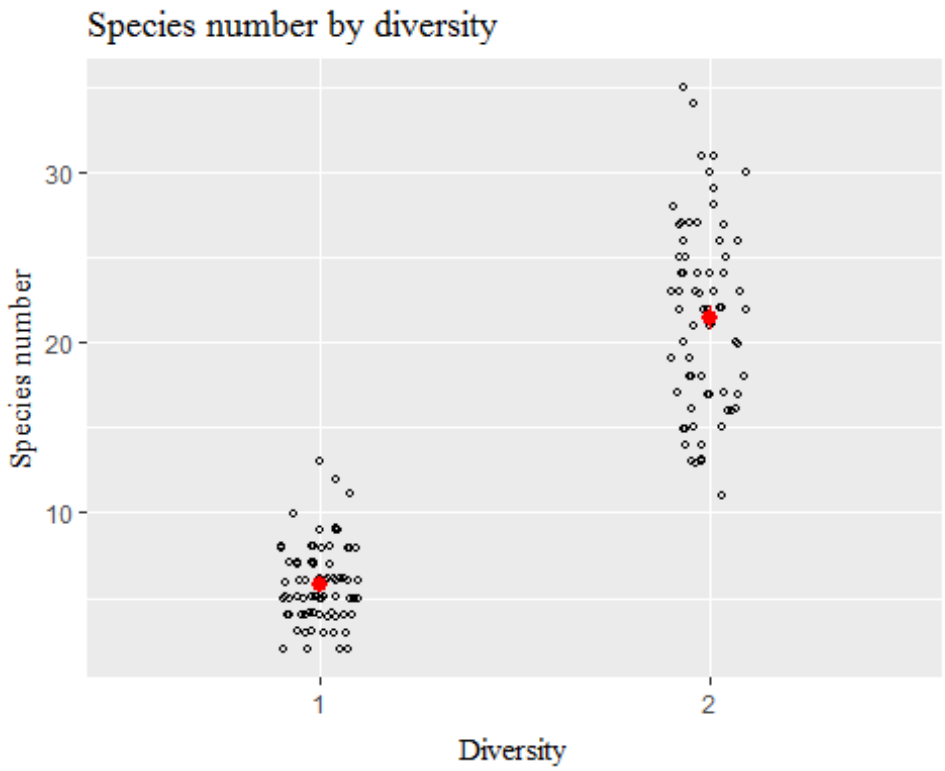

```
plot(spc, ci.type="poly", col="blue", lwd=2, ci.lty=0, ci.col="lightblu
e")
```



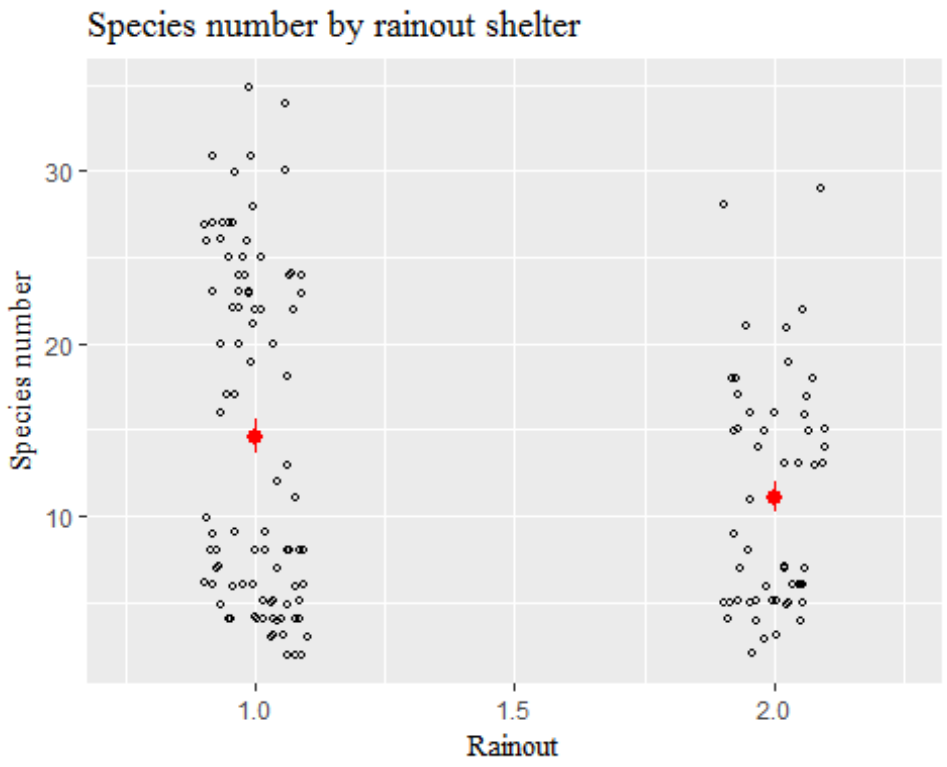
So far we have been viewing the general scope of our data. Now we can starat to test larger-scale differences.

First level analysis: statistical test of difference based on diversity and year:

```
#welch's t-test:
div<-t.test(specnumber(comm.adults)~meta$diversity)
div

divplot<-ggplot(comm.adults, aes(x=meta$diversity, y=specnumber(comm.ad
ults))) +
  ggtitle("Species number by diversity") +
  geom_point(cex=1.0, pch=1.0, position = position_jitter(w = 0.1, h =
0.1)) +
  xlab("Diversity") +
  ylab("Species number") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))
divplot + stat_summary(fun.data=mean_se, fun.args=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```




```
yr<-t.test(specnumber(comm.adults)~meta$year)
yr

trt<-t.test(specnumber(comm.adults)~meta$rainout)
trt

divplot<-ggplot(comm.adults, aes(x=meta$rainout, y=specnumber(comm.adul
ts))) +
  ggtitle("Species number by rainout shelter") +
  geom_point(cex=1.0, pch=1.0, position = position_jitter(w = 0.1, h =
0.1)) +
  xlab("Rainout") +
  ylab("Species number") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))
divplot + stat_summary(fun.data=mean_se, fun.args=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```




```
#year and diversity play large roles in determining species number. Let
's look at composition
```

Both scale and diversity plantings have significant impact on species richness at the level of the individual quadrat. I want to divide up the data set to test for variation within year and diversity. <<<update: Actually, I don't want to do this because then I lose replication. What I really want to do is get the average values for each subplot and transect.>>>

```
comm.adults.16<-subset(comm.adults, meta$year==2016)
comm.adults.15<-subset(comm.adults, meta$year==2015)
```

Load files for 2016 wp and eu community cover:

```
#Everything above does not need to be done again. Start here:
comm.cover.eu.16<-read.csv("~/comm.cover.eu.16.csv", header=TRUE, row.n
ames=1)
head(comm.cover.eu.16)

comm.cover.wp.16<-read.csv("~/comm.cover.wp.16.csv", header=TRUE, row.n
ames=1)
head(comm.cover.wp.16)
```

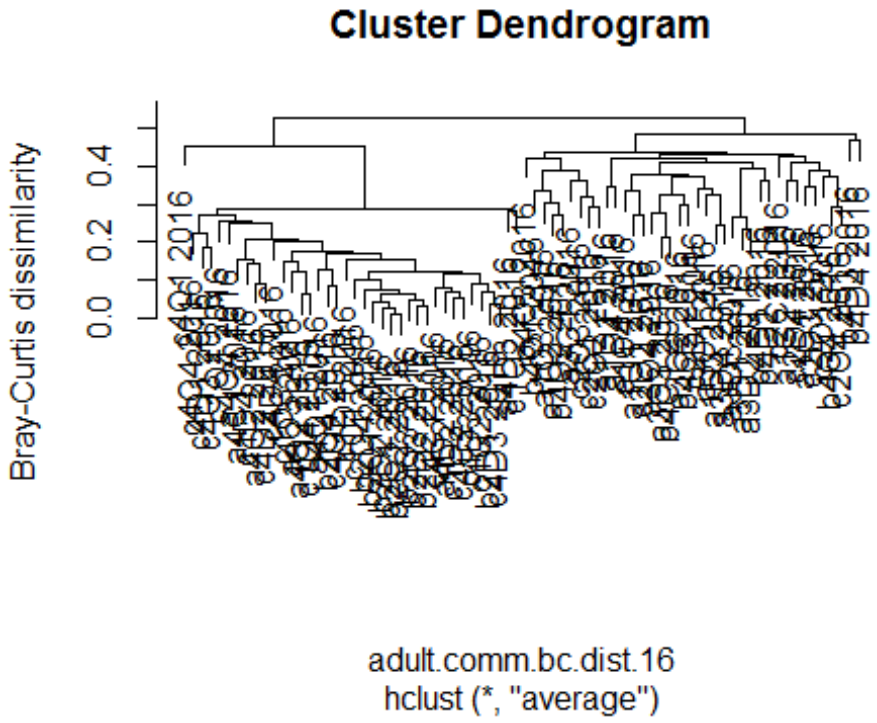
Load files for 2015 wp and eu community cover:

```
comm.cover.eu.15<-read.csv("~/comm.cover.eu.15.csv", header=TRUE, row.n
ames=1)
```

```
head(comm.cover.eu.15)

comm.cover.wp.15<-read.csv("~/comm.cover.wp.15.csv", header=TRUE, row.names=1)
head(comm.cover.wp.15)
```

Cluster by bray-curtis distance:

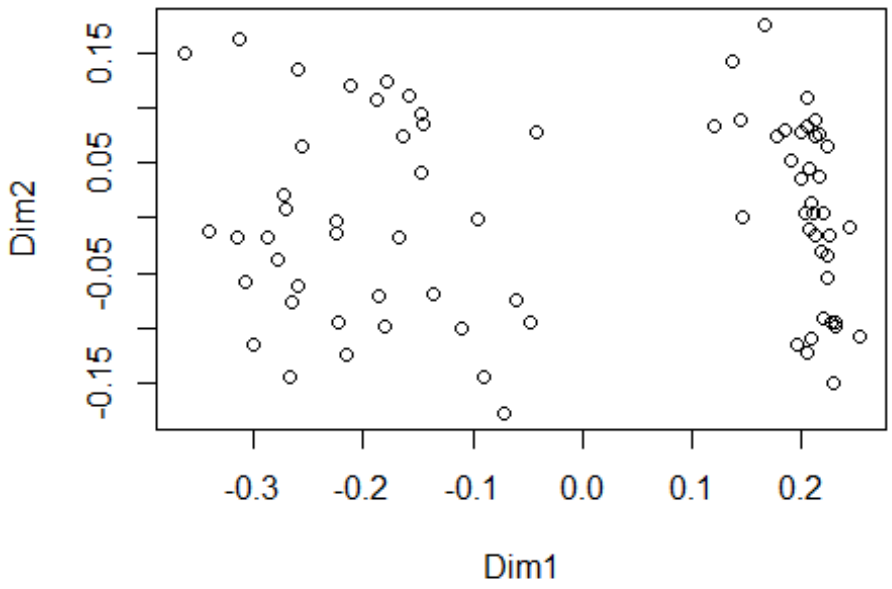
```
#calc. Bray-Curtis distance among samples
adult.comm.bc.dist.16<-vegdist(comm.adults.16, method = "bray")
#cluster communities using average-linkage algorithm
comm.bc.clust<-hclust(adult.comm.bc.dist.16, method = "average")
#plot cluster diagram
plot(comm.bc.clust, ylab = "Bray-Curtis dissimilarity")
```



calculate dissimilarity matrices:

```
library(labdsv)
library(vegan)

colnames(meta)
meta$sub=paste(meta$subplot, meta$year)

colnames(comm.adults)
meta.16<-subset(meta, year==2016)
colnames(meta.16)
```

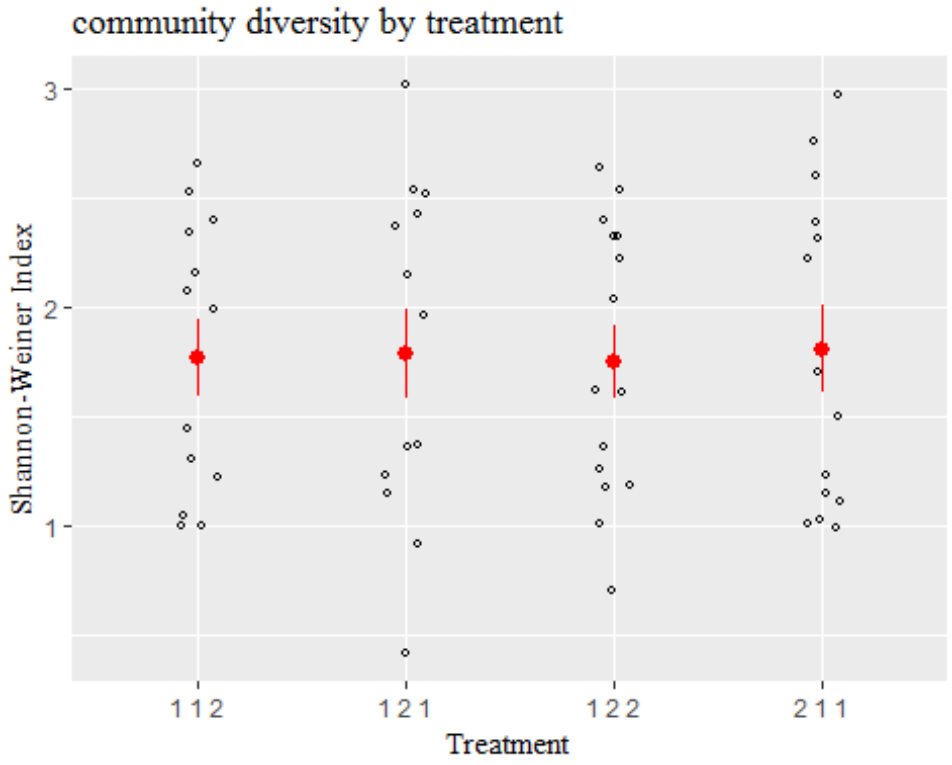
```
all.equal(rownames(meta), rownames(comm.adults))

#calc. Bray-Curtis distance among samples
a.comm.bc<-vegdist(comm.adults, method = "bray")

#run a PERMANOVA to determine key parameters
dist<-adonis(a.comm.bc ~ diversity + year + rainout + biomass + nitro +
 dist + n + om, data=meta, permutations=999, strata=meta$site)
dist

#PCO displays BCDissimilarity along key axes
comm.bc.16.pco<-pco(adult.comm.bc.dist.16, k=5)
plot(scores(comm.bc.16.pco, display="sites"))
```



Calculate the relative evenness and diversity of plant communities.

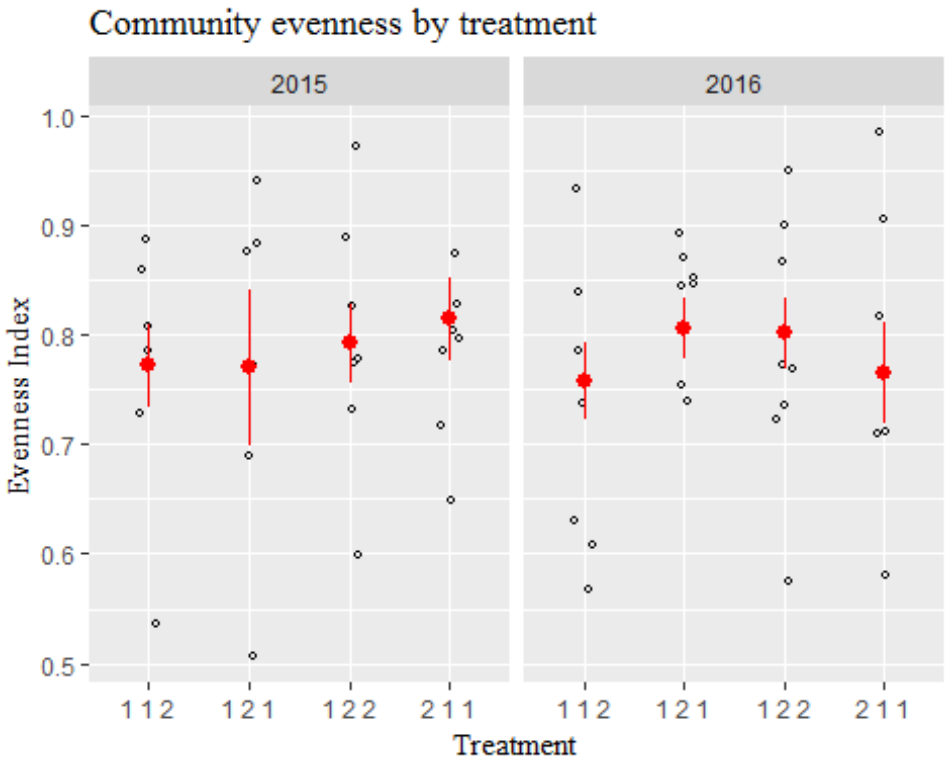
```
library(lme4)
#Subset data frames to view just rainout shelter treatment plots
comm.adults.eu<-subset(comm.adults, meta$rainout==2)
meta.eu<-subset(meta, rainout==2)

#Calculate Shannon diversity and add this variable to the data frame
shannon<-diversity(comm.adults.eu, index = "shannon")
shannon
meta.eu<-cbind(shannon, meta.eu, by="sub")
head(meta.eu)
#Check that the shannon index was added
```

```
colnames(meta.eu)
meta.eu$trt<-paste(meta.eu$rain, meta.eu$nitro, meta.eu$biomass)

#plot the results using ggplot
meta.eu$trt=as.factor(meta.eu$trt)
shannon<-ggplot(meta.eu, aes(x=trt, y=shannon)) +
  ggtitle("community diversity by treatment") +
  geom_point(cex=1.0, pch=1.0, position = position_jitter(w = 0.1, h =
0.1)) +
  xlab("Treatment") +
  ylab("Shannon-Weiner Index") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))
shannon + stat_summary(fun.data=mean_se, fun.args=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```




```
#Now calculate evenness from the diversity index values
evenness.eu.16<-diversity(comm.adults.eu)/log(specnumber(comm.adults.eu
))
evenness.eu.16
meta.eu<-cbind(evenness.eu.16, meta.eu)

#Plot the results using ggplot
even<-ggplot(meta.eu, aes(x=trt, y=evenness.eu.16)) +
  ggtitle("Community evenness by treatment") +
```

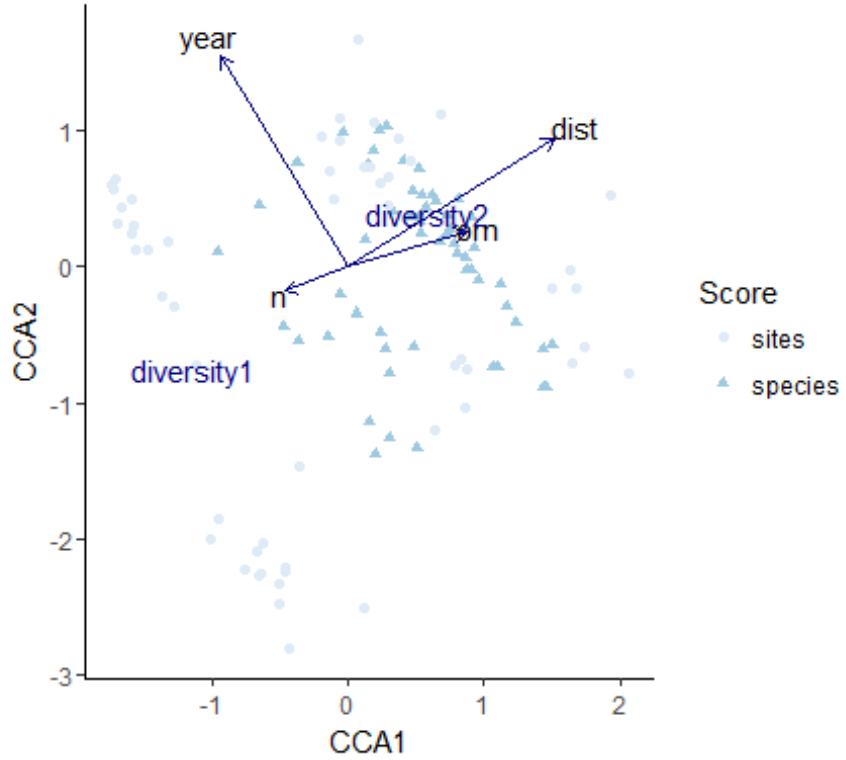
```
  geom_point(cex=1.0, pch=1.0, position = position_jitter(w = 0.1, h =
0.1)) +
  xlab("Treatment") +
  ylab("Evenness Index") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))
even + facet_wrap("year") + stat_summary(fun.data=mean_se, fun.args=lis
t(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```



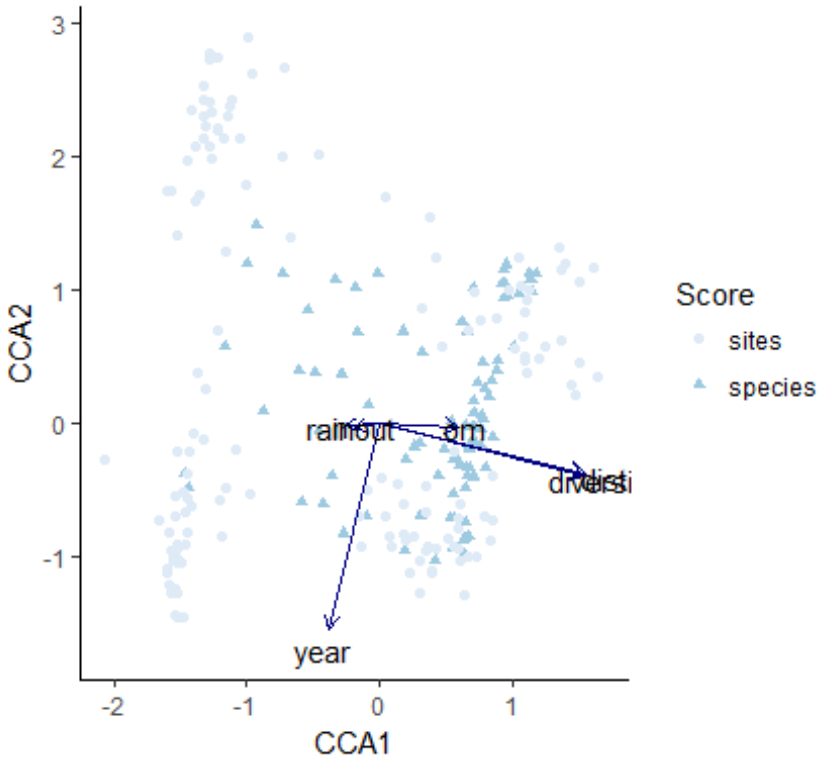

```
#LM describing variation in community evenness by treatmetn and diversi
ty
evenlm<-lm(evenness.eu.16 ~ trt, data=subset(meta.eu, year==2016))
summary(evenlm)
head(meta.eu)

#lmer describing variation in species richness by treatment and diversi
ty
richlm<-lmer(specnumber(comm.adults.eu) ~ trt*diversity + (1|block), da
ta=meta.eu)
summary(richlm)
#diversity is the only strong source of variation (t-value >1)
```

CCA: Plot community composition in multi-dimensional space along known axes of variation.

```
library(ggvegan)
library(labdsv)

#Read in the dataframe
meta.2<-read.csv("~/meta.2.csv", header = TRUE, row.names=1)

#Plot the canonical correspondence plot for just experimental subplots
cca.eu<-cca(comm.adults.eu ~ diversity + year + rainout + dist + n + om
, data = meta.eu)
gg.eu<-autoplot(cca.eu)
gg.eu + theme_classic() + scale_color_brewer()
```



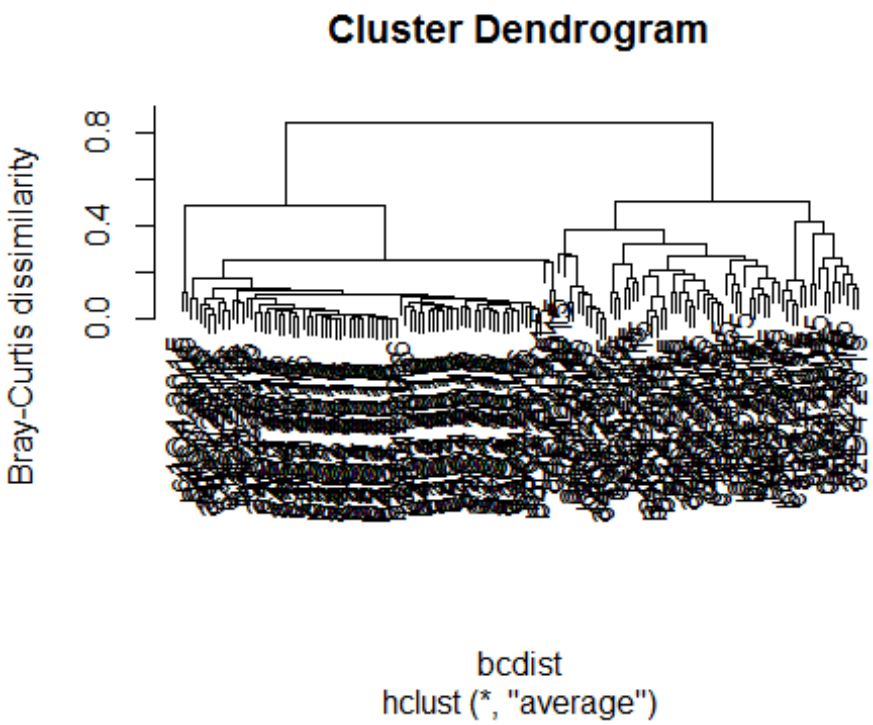

```
#Match the adult cover and metadata rows
comm.adults.2<-comm.adults[rowSums(comm.adults[, -1] > 0) !=0, ]
meta.2[order(match(meta.2[,1],comm.adults.2[,1])),]
all.equal(rownames(meta.2), rownames(comm.adults.2))

#plot the cca for all quadrats including the whole-plot and sub-plot me
asurements
cca.all<-cca(comm.adults.2 ~ diversity + year + rainout + dist + n + om
, data=meta.2)
gg.all<-autoplot(cca.all)
gg.all + theme_classic() + scale_color_brewer()
```



Now do everything again for functional groups.

Cluster by bray-curtis distance:

```
#match the dataframes
comm.categories<-read.csv("~/comm.categories.csv", header=TRUE, row.nam
es=1)
head(comm.categories)
meta<-meta[-148,]

#calc. Bray-Curtis distance among samples
bcdist<-vegdist(comm.categories, method = "bray")
#cluster communities using average-linkage algorithm
comm.bc.clust<-hclust(bcdist, method = "average")
#plot cluster diagram
plot(comm.bc.clust, ylab = "Bray-Curtis dissimilarity")
```



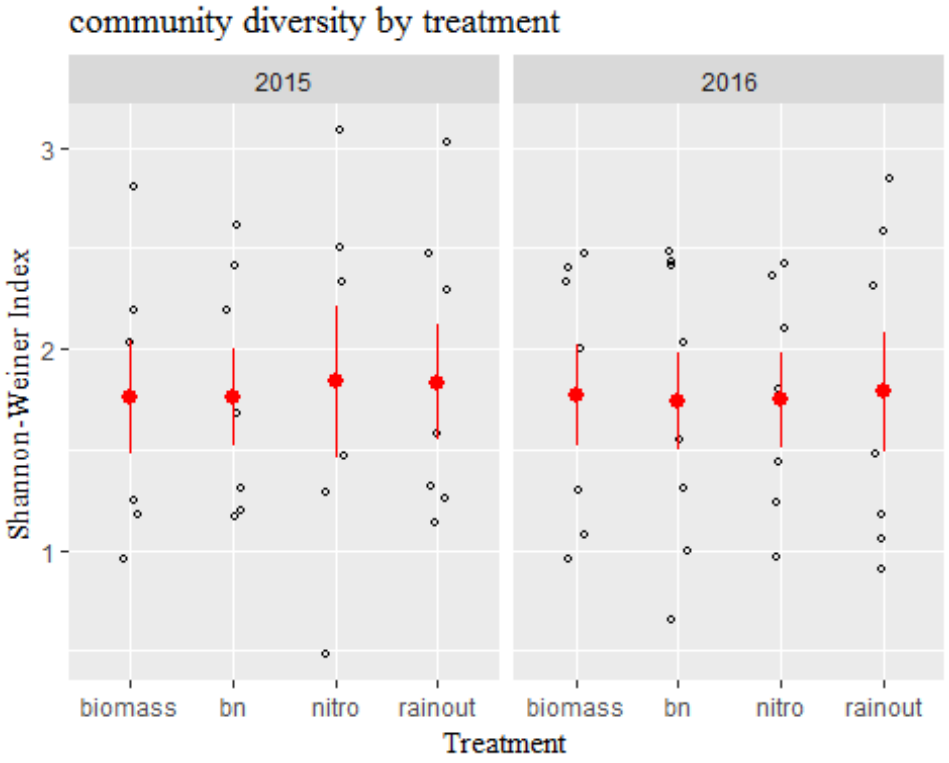
calculate dissimilarity matrices:

```
#Match the dataframes
head(meta)
rownames(meta)
colnames(comm.categories)
all.equal(rownames(meta), rownames(comm.categories))

#calc. Bray-Curtis distance among samples
a.comm.bc<-vegdist(comm.categories, method = "bray")

#use PERMANOVA to determine variation in BCDissimilarity by key sources
 of variation
dist<-adonis(a.comm.bc ~ diversity + year + rainout + biomass + nitro +
 dist + n + om, data=meta, permutations=999, strata=meta$site)
dist
```

Now, create a plot of community composition variation

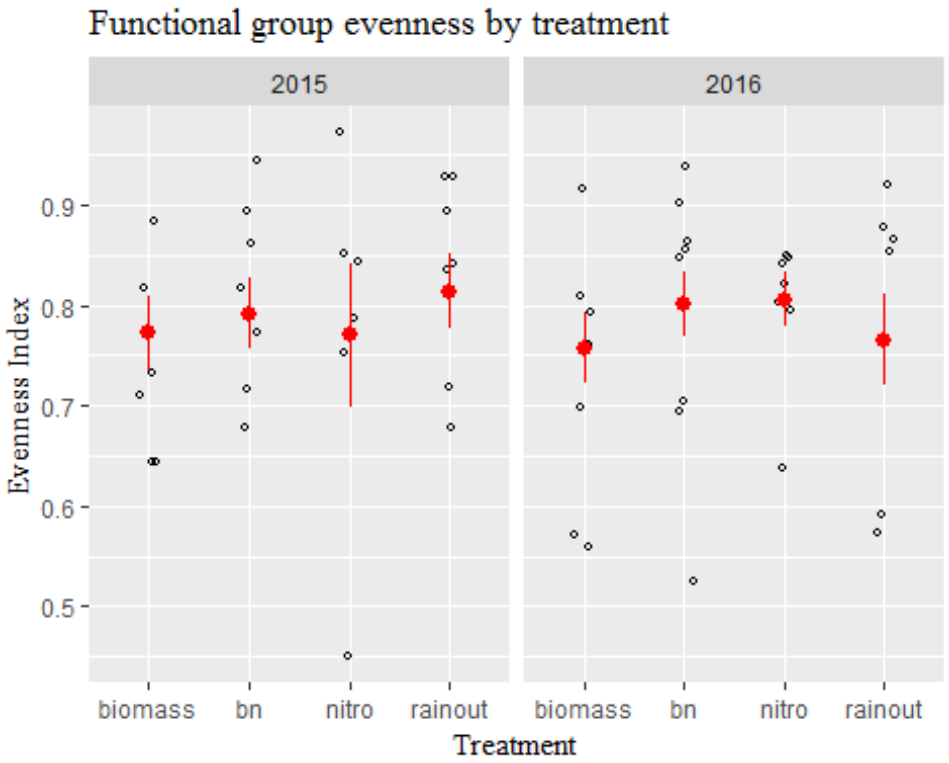
```
comm.cat.eu<-subset(comm.adults, meta$rainout==2)
meta.eu<-subset(meta, rainout==2)

shannon<-diversity(comm.cat.eu, index = "shannon")
shannon
meta.eu<-cbind(shannon, meta.eu)
head(meta.eu)
```

```
#plot shannon weiner diversity by functional group
shannon<-ggplot(meta.eu, aes(x=treat, y=shannon)) +
  ggtitle("community diversity by treatment") +
  geom_point(cex=1.0, pch=1.0, position = position_jitter(w = 0.1, h =
0.1)) +
  xlab("Treatment") +
  ylab("Shannon-Weiner Index") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))
shannon + facet_wrap("year") + stat_summary(fun.data=mean_se, fun.args=
list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```




```
#plot Pileou's evenness by functional group
evenness.eu<-diversity(comm.cat.eu)/log(specnumber(comm.cat.eu))
evenness.eu
meta.eu<-cbind(evenness.eu, meta.eu)

even<-ggplot(meta.eu, aes(x=treat, y=evenness.eu)) +
  ggtitle("Functional group evenness by treatment") +
  geom_point(cex=1.0, pch=1.0, position = position_jitter(w = 0.1, h =
0.1)) +
  xlab("Treatment") +
  ylab("Evenness Index") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
```

```
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))
even + facet_wrap("year") + stat_summary(fun.data=mean_se, fun.args=lis
t(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```

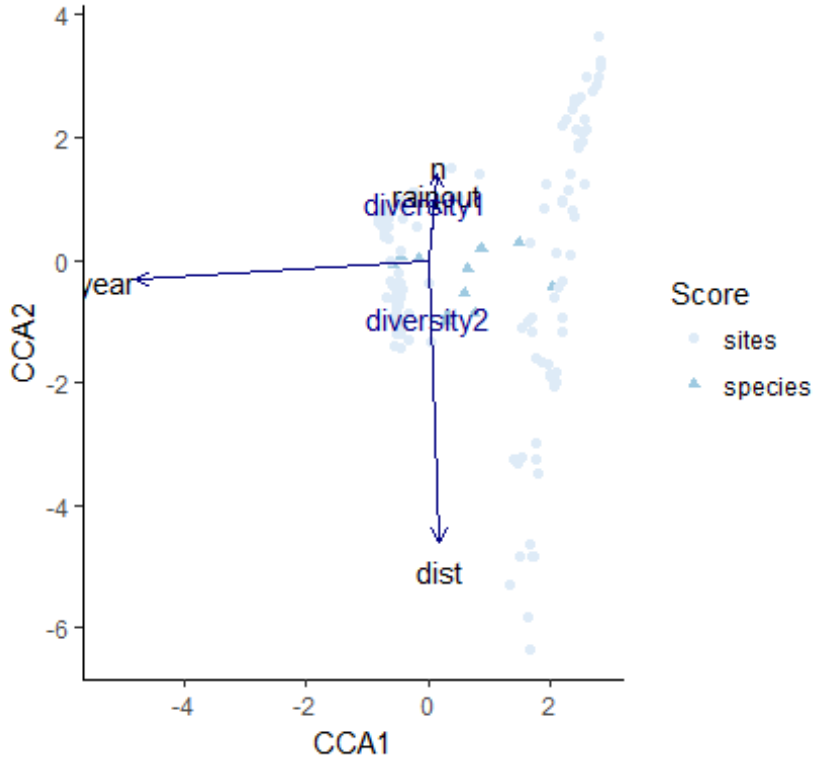



```
#Test the global model to find which parameters are best predictors of
vatiaion in functional group evenness
library(lmerTest)
meta.eu$biomass<-as.factor(meta.eu$biomass)
meta.eu$nitro<-as.factor(meta.eu$nitro)
meta.eu$diversity=as.factor(meta.eu$diversity)
richlm<-lmer(evenness.eu ~ rain + nitro + biomass + (1|block), data=met
a.eu)
summary(richlm)

select<-step(richlm, data=meta.eu, direction="backward")
select

#No parameters are significant - evenness does not vary significantly b
y any treatmeng type.
```

Plot CCA for functional groups

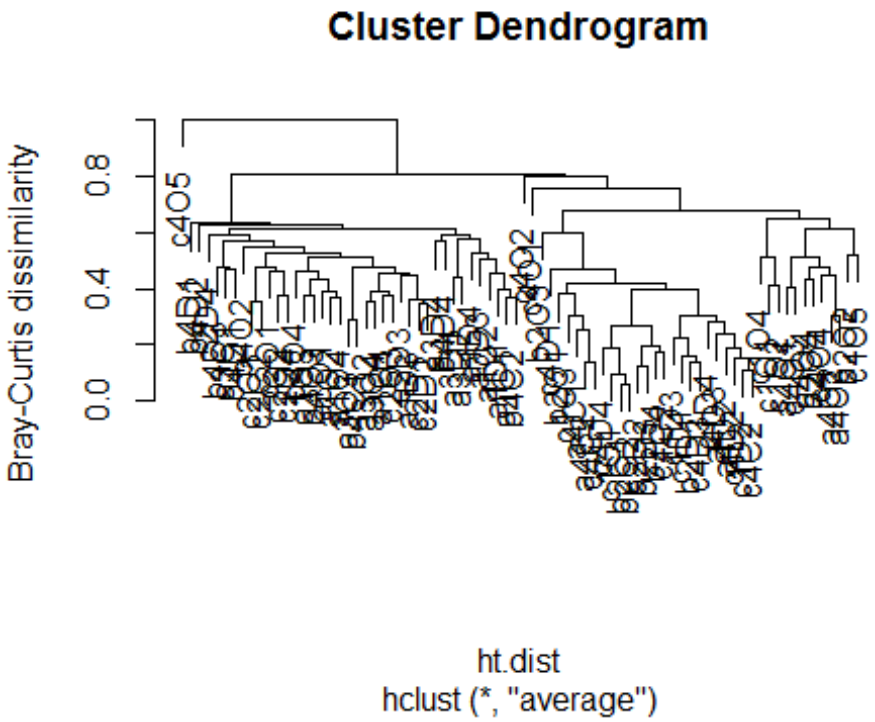
```
library(ggvegan)
library(labdsv)
```

```
cca.cat<-cca(comm.categories ~ diversity + year + rainout + dist + n, d
ata = meta)
gg<-autoplot(cca.cat)
gg + theme_classic() + scale_color_brewer()
```

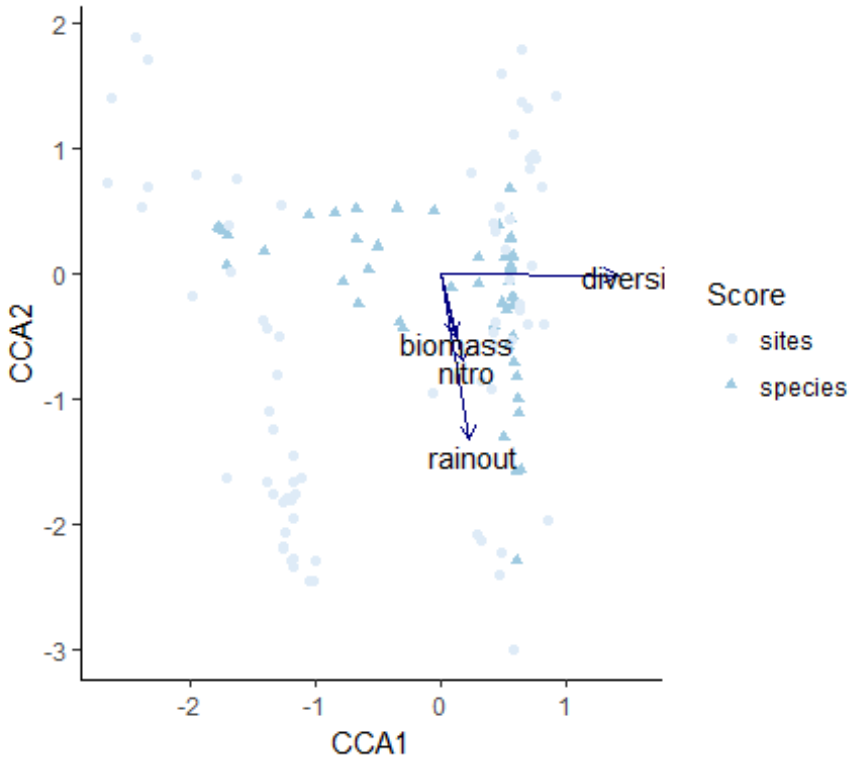


Do it again for heights: Read in the finished matrices.

```
height<-read.csv("~/heights.csv", header=TRUE, row.names=1)
rownames(height)
ht.meta<-read.csv("~/meta.ht.csv", header=TRUE, row.names=1)
rownames(ht.meta)
all.equal(rownames(height), rownames(ht.meta))

#calc. Bray-Curtis distance among samples
ht.dist<-vegdist(height, method = "bray")
##cluster communities using average-linkage algorithm
ht.bc.clust<-hclust(ht.dist, method = "average")
#plot cluster diagram
plot(ht.bc.clust, ylab = "Bray-Curtis dissimilarity")
```

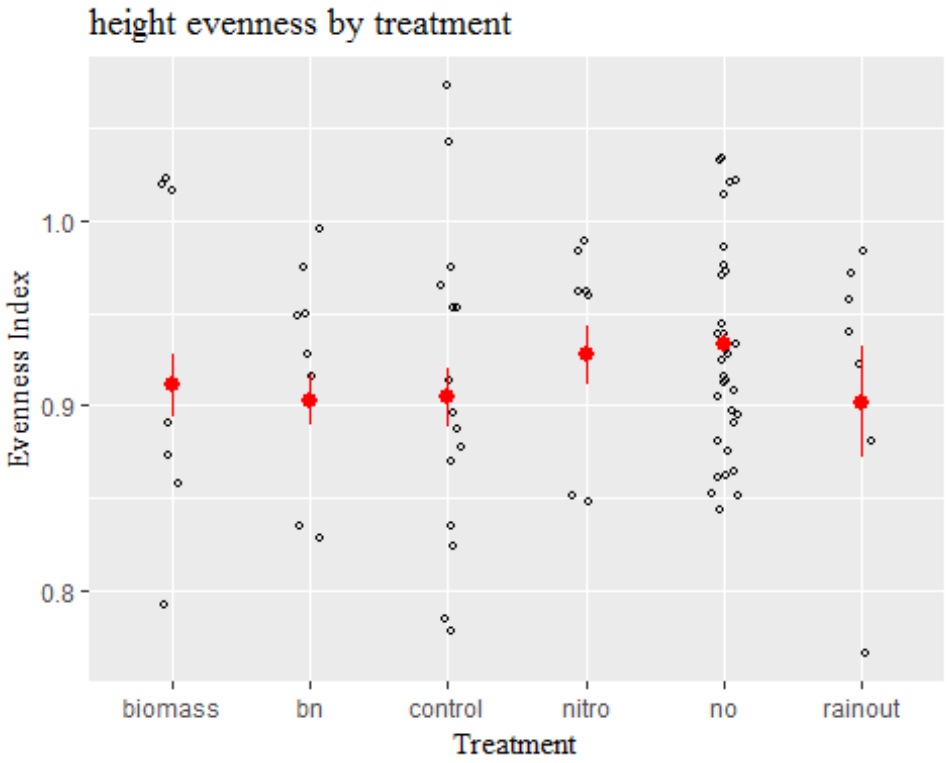



```
library(vegan)
dist<-adonis(ht.dist ~ diversity + rainout + rain + biomass + nitro, da
ta=ht.meta, permutations=999, strata=ht.meta$site)
dist
#diversity and rainout shelters are significant sources of variation
#Rainout effects are much smaller than diversity effects.

#CCA for height variation by rainout and diversity
heights.2<-read.csv("~/heights.2.csv", header=TRUE, row.names=1)
ht.meta.2<-read.csv("~/ht.meta.2.csv", header=TRUE, row.names=1)
all.equal(rownames(ht.meta.2), rownames(heights.2))

cca.ht<-cca(heights.2 ~ diversity + rainout + nitro + biomass, data = h
t.meta.2)
cca.ht<-autoplot(cca.ht)
cca.ht + theme_classic() + scale_color_brewer()
```



How even is the height structure among treatment groups?

```
ht.even<-diversity(heights.2)/log(specnumber(heights.2))
ht.even
meta.ht<-cbind(ht.even, ht.meta.2)
colnames(ht.meta)

even<-ggplot(meta.ht, aes(x=treat, y=ht.even)) +
  ggtitle("height evenness by treatment") +
  geom_point(cex=1.0, pch=1.0, position = position_jitter(w = 0.1, h =
0.1)) +
  xlab("Treatment") +
  ylab("Evenness Index") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))
even + stat_summary(fun.data=mean_se, fun.args=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```




```
#very even structure across treatments

richlm<-lmer(specnumber(heights.2) ~ rainout*diversity + (1|block), dat
a=ht.meta.2)
summary(richlm)
#only diversity is a significant source of variation in vegetation heig
ht.
```

Decomposition for Bevans Thesis

R script to reproduce the correlations and images used in the thesis "PLANT DIVERSITY INFLUENCES THE STRUCTURE AND FUNCTION OF A RESTORED PRAIRIE AND ITS RESPONSES TO ADDED DISTURBANCES"" by Rebecca A Bevans, submitted to the graduate college April 28 2017.

```
knitr::opts_chunk$set(warning=FALSE, message=FALSE, results="hide")
```

Determine the percent weight loss based on treatment, diversity, location, and variables related to distance from river (i.e., som, moisture). Start by looking at the shape of the data.

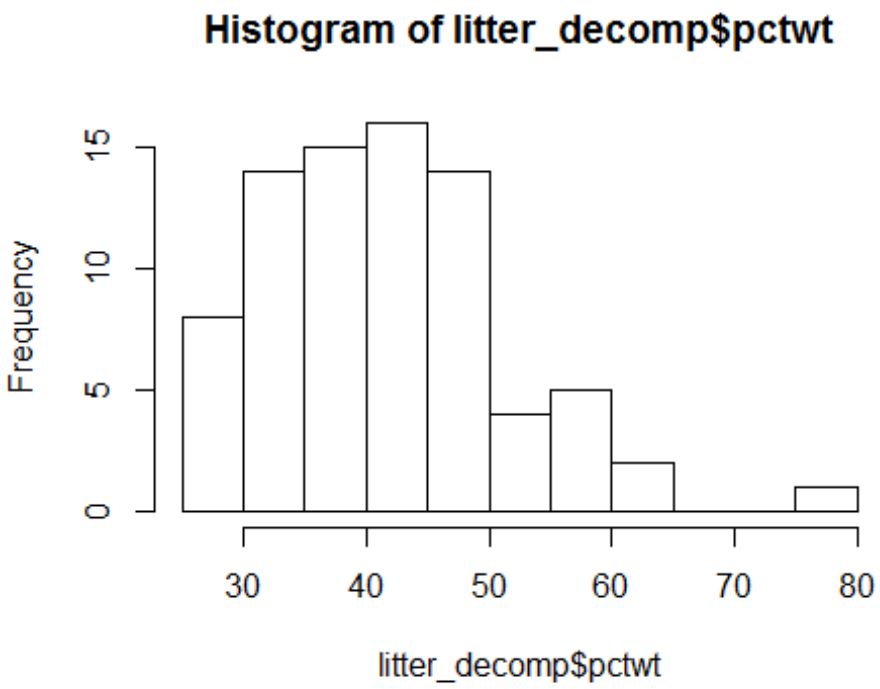
First, load libraries and datasets:

```
library(tidyverse)
library(ggplot2)
library(broom)
```

```
library(lattice)
#library(picante)
library(data.table)
library(labdsv)

litter_decomp<-read.csv("~/litter_decomp.csv", header=TRUE, row.names=1
)
soils<-read.csv("~/soils.csv", header=TRUE, row.names=1)
#View(decomp)
soils$pctwt=soils$pctwtloss*100
litter_decomp$pctwt=litter_decomp$pctwtloss*100
#str(decomp$pctwt)
```

Second, check data distribution:

```
hist(litter_decomp$pctwt)
```



Some skewing to the right in 2015- most samples are betweeen 0.3 and 0.5% weight loss, with some out to 0.8

model selection: Create set of models to choose between, predicting variation in decomp either by site variables, treatment variables, or a mix of the largest-scale site and treatment variables. Finally, a separate rainout shelter model assesses samples from beneath rainout shelters to determine whether significant variation among treatments is apparent.

Site models: pctwt ~ dist + bare + pH + kclN + avg.som + (1|unit) pctwt ~ dist + (1|unit)

Treatment Models: pctwt ~ diversity + rainout + rain + nitro + (1|unit) pctwt ~ diversity + (1|unit)

Site x Treatment Models: pctwt ~ diversity + dist + (1|unit) pctwt ~ rainout + dist + (1|unit) pctwt ~ diversity + dist + bare + pH + kclN + avg.som + (1|unit)

Rainout shelter Model (subset 'rainout==2') pctwt ~ rain + nitro + biomass + (1|unit)

Test the models using MMI:

```
cor(litter_decomp$avg.som, litter_decomp$dist)
library(lme4)
library(lmerTest)
library(AICcmodavg)
library(MuMIn)
library(nlme)
#model list
  m1<-lme(log(pctwt) ~ 1, random = ~1|block, data=litter_decomp)
  m2<-lme(log(pctwt) ~ bare + kclN + avg.som, random = ~ 1|block, data=
litter_decomp)
  m3<-lme(log(pctwt) ~ dist, random = ~ 1|block, data=litter_decomp)
  m4<-lme(log(pctwt) ~ rain + nitro + biomass, random = ~ 1|block, data
=litter_decomp)
  m5<-lme(log(pctwt) ~ diversity, random = ~ 1|block, data=litter_decom
p)
  m6<-lme(log(pctwt) ~ diversity + dist, random = ~ 1|block, data=litte
r_decomp)
  m7<-lme(log(pctwt) ~ rainout + dist, random = ~ 1|block, data=litter_
decomp)
  m8<-lme(log(pctwt) ~ diversity + bare + kclN + avg.som, random = ~ 1|
block, data=litter_decomp)
  m9<-lme(log(pctwt) ~ diversity + rainout, random = ~1|block, data=lit
ter_decomp)
  m10<-lme(log(pctwt) ~ rainout, random = ~1|block, data=litter_decomp)
  m11<-lme(log(pctwt) ~ avg.som, random = ~1|block, data=litter_decomp)
decmod<-list(m1, m2, m3, m4, m5, m6, m7, m8, m9, m10, m11)
aic.table<-aictab(decmod)
aic.table
```

Evaluate the models

```
summary(m10)
litter_decomp$rainout=as.factor(litter_decomp$rainout)
test<-lmer(pctwt ~ rainout + (1|block), data = litter_decomp)
lsmeansLT(test)
```

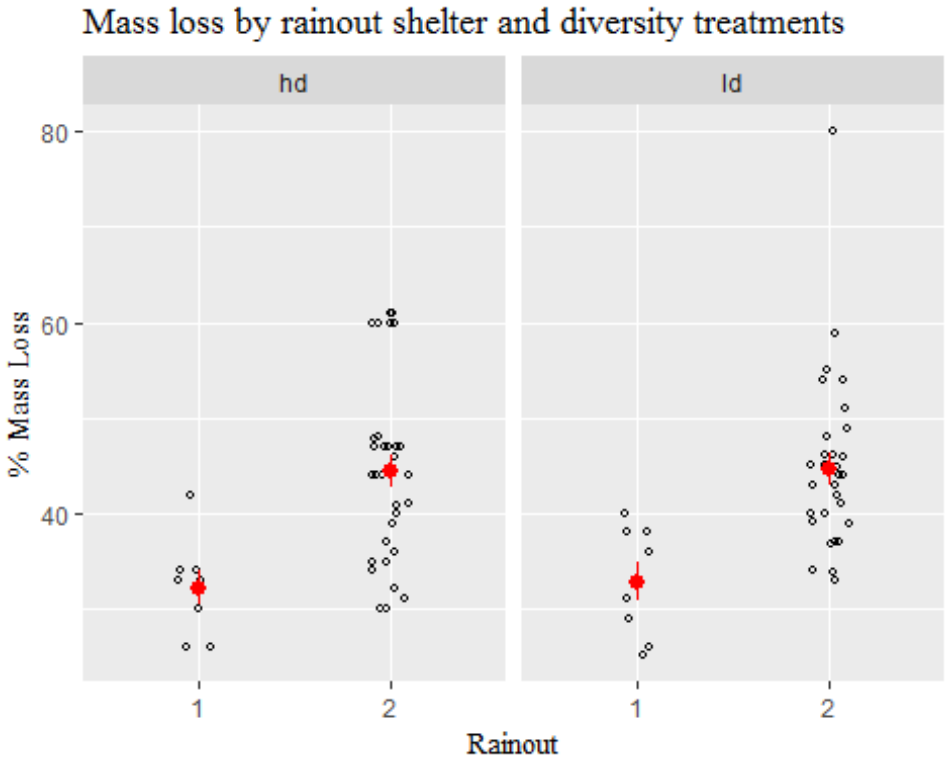
Plot group means and variance

```
library(ggplot2)
litter_decomp$rainout = as.factor(litter_decomp$rainout)
litter_decomp$trt<-paste(litter_decomp$rainout, litter_decomp$nitro, li
```

```
tter_decomp$biomass)
lit.subplot<-ggplot(litter_decomp, aes(x=trt, y=pctwt)) +
  ggtitle("Mass loss by within-shelter treatments and diversity") +
  geom_point(pch=1, cex=1.0, position = position_jitter(w = 0.1, h = 0.
1), color="black") +
  xlab("Treatment") +
  ylab("% Mass Loss") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))

lit.subplot + facet_wrap(~diversity) + stat_summary(fun.data=mean_se, f
un.args=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```


```
litter_decomp$rainout = as.factor(litter_decomp$rainout)
litter_decomp$diversity = as.factor(litter_decomp$diversity)
decomp.div<-ggplot(litter_decomp, aes(x=rainout, y=pctwt)) +
  ggtitle("Mass loss by rainout shelter and diversity treatments") +
  geom_point(pch=1, cex=1.0, position = position_jitter(w = 0.1, h = 0.
1), color="black") +
  xlab("Rainout") +
  ylab("% Mass Loss") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
```

```
        axis.title.y = element_text(family="serif", vjust=0.3))

decomp.div + facet_wrap(~diversity) + stat_summary(fun.data=mean_se, fu
n.args=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```



Soil Respiration

```
soils$diversity = as.factor(soils$diversity)
#model list
  m1<-lme(solvita.ppmC. ~ 1, random = ~1|block, data=soils)
  m2<-lme(solvita.ppmC. ~ year + dist + bare + kclN, random = ~ 1|block
, data=soils)
  m3<-lme(solvita.ppmC. ~ year + dist, random = ~ 1|block, data=soils)
  m4<-lme(solvita.ppmC. ~ year + rain + nitro + biomass, random = ~ 1|b
lock, data=soils)
  m5<-lme(solvita.ppmC. ~ year + diversity, random = ~ 1|block, data=so
ils)
  m6<-lme(solvita.ppmC. ~ year + diversity + dist, random = ~ 1|block,
data=soils)
  m7<-lme(solvita.ppmC. ~ year + rainout + dist, random = ~ 1|block, da
ta=soils)
  m8<-lme(solvita.ppmC. ~ year + diversity + bare + kclN + avg.som, ran
dom = ~ 1|block, data=soils)
  m9<-lme(solvita.ppmC. ~ year + diversity + rainout, random = ~1|block
, data=soils)
  m10<-lme(solvita.ppmC. ~ year + rainout, random = ~1|block, data=soil
```

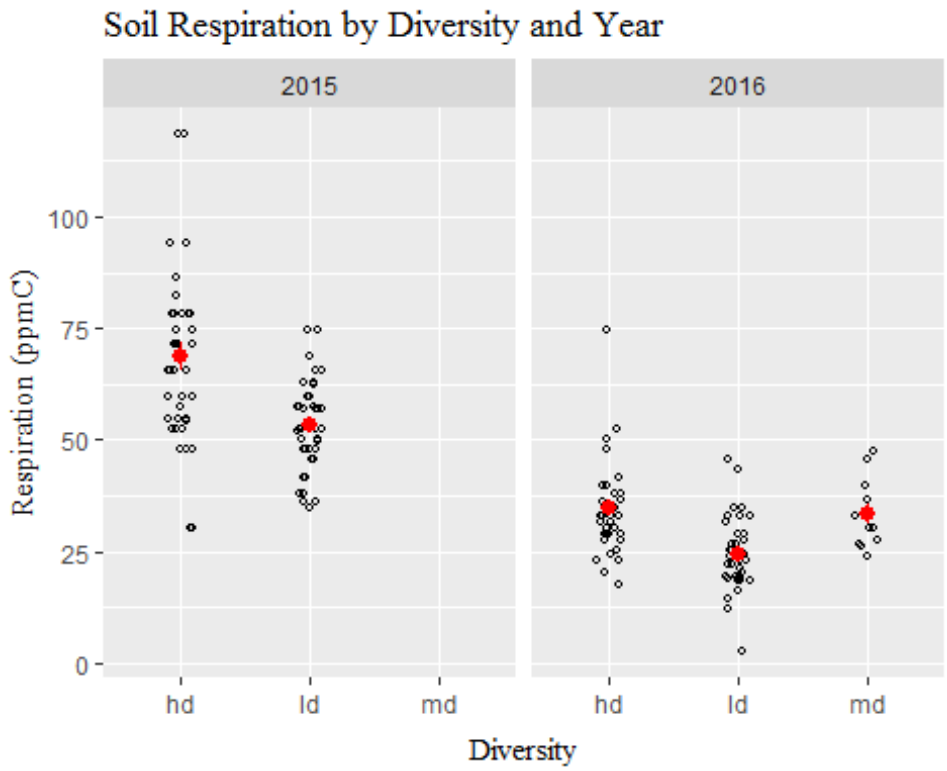
```
s)
  m11<-lme(solvita.ppmC. ~ year + avg.som, random = ~1|block, data=soil
s)

decmod.sol<-list(m1, m2, m3, m4, m5, m6, m7, m8, m9, m10, m11)
aic.tab<-aictab(decmod.sol)
aic.tab

mod2.7<-lmer(solvita.ppmC. ~ year+diversity+bare+kclN+avg.som + (1|bloc
k), data=soils)
summary(mod2.7)
cor(soils$bare, soils$solvita.ppmC.)
#0.46
cor(soils$avg.som, soils$solvita.ppmC.)
#0.08
cor(soils$kclN, soils$solvita.ppmC.)
#0.24
soils$year = as.factor(soils$year)
t<-lmer(solvita.ppmC. ~ year + (1|block), data=soils)
lsmeansLT(t)
#
t1<-lmer(solvita.ppmC. ~ diversity + (1|block), data=soils)
lsmeansLT(t1)
```

Are soil respiration and litter decomposition related?

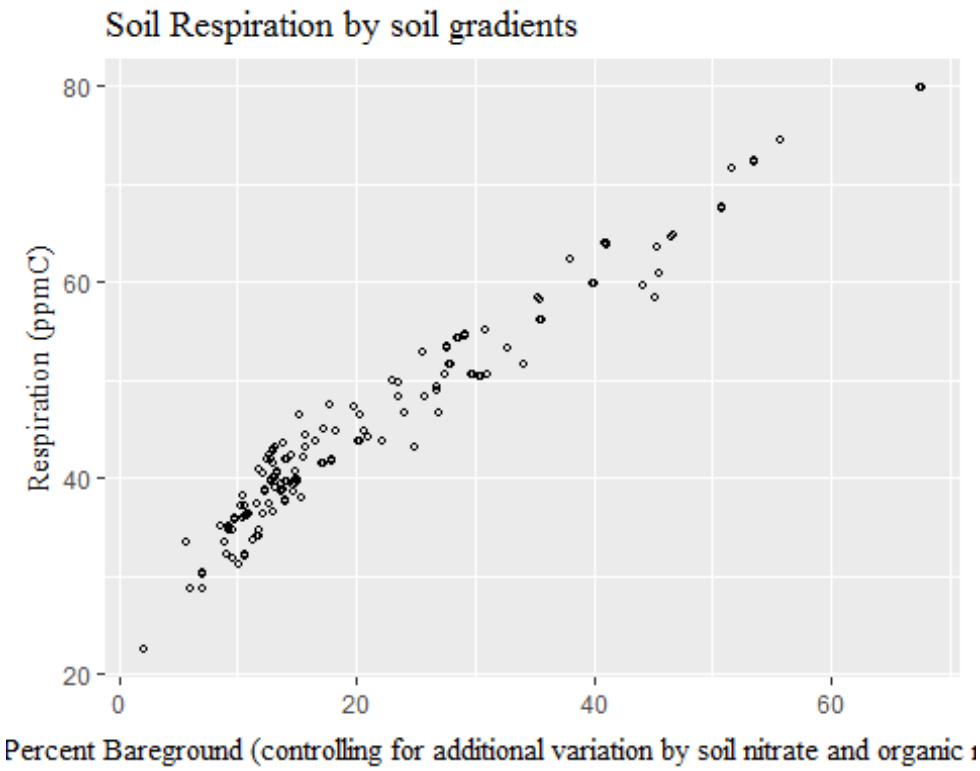
```
lit.sol<-lmer(pctwt ~ solvita.ppmC. + (1|block), data=litter_decomp)
summary(lit.sol)
cor(litter_decomp$pctwt, litter_decomp$solvita.ppmC.)
```

Plot soil respiration

```
soilr.trt<-ggplot(soils, aes(x=diversity, y=solvita.ppmC.)) +
  ggtitle("Soil Respiration by Diversity and Year") +
  geom_point(pch=1, cex=1.0, position = position_jitter(w = 0.1, h = 0.
1), color="black") +
  xlab("Diversity") +
  ylab("Respiration (ppmC)") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))

soilr.trt + facet_wrap(~year) + stat_summary(fun.data=mean_se, fun.args
=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```




```
#regression line
#generate the data:
resp<-lm(solvita.ppmC. ~ bare + kclN + avg.som, data=soils)
dat=soils
dat$pred=predict(resp)

resp<-ggplot(dat, aes(x=bare + kclN + avg.som, y=pred)) +
  ggtitle("Soil Respiration by soil gradients") +
  geom_point(pch=1, cex=1.0, position = position_jitter(w = 0.1, h = 0.
1), color="black") +
  xlab("Percent Bareground (controlling for additional variation by soi
l nitrate and organic matter)") +
  ylab("Respiration (ppmC)") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))

resp
```



SLA for Bevans Thesis

Becca

February 14, 2017

R script to reproduce the correlations and images used in the thesis "PLANT DIVERSITY INFLUENCES THE STRUCTURE AND FUNCTION OF A RESTORED PRAIRIE AND ITS RESPONSES TO ADDED DISTURBANCES"" by Rebecca A Bevans, submitted to the graduate college April 28 2017.

```
knitr::opts_chunk$set(warning=FALSE, message=FALSE, results="hide")
```

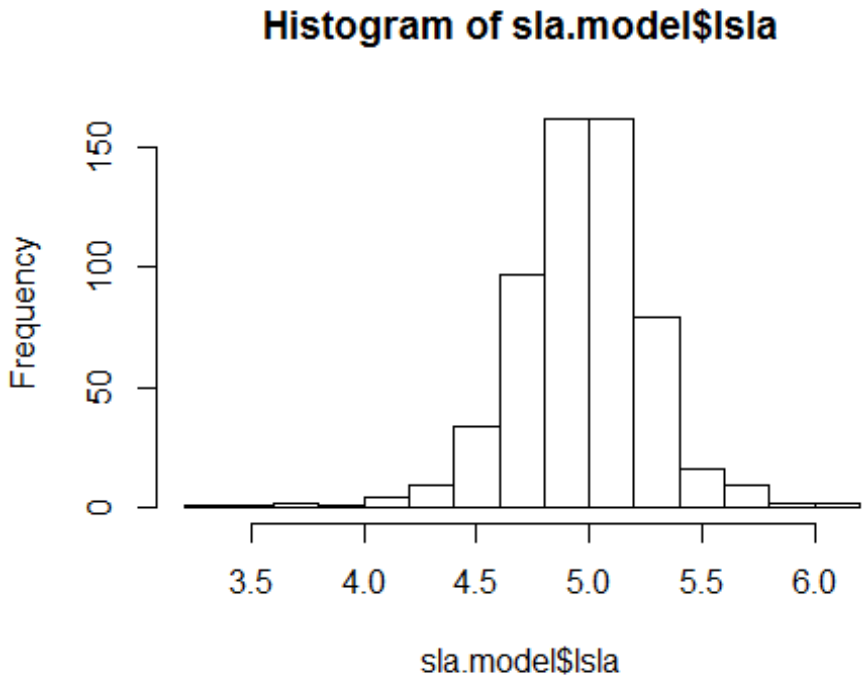
Load data and libraries

```
library(AICcmodavg)
library(lme4)
library(lmerTest)
library(tidyverse)
library(dplyr)
library(broom)
library(stats)
sla.model<-read.csv("~/sla.model.csv", header=TRUE, row.names=1)
sla.cover<-read.csv("~/sla.cover.csv", header=TRUE, row.names=1)

sla.model$rainout<-as.factor(sla.model$rainout)
sla.model$diversity<-as.factor(sla.model$diversity)
```

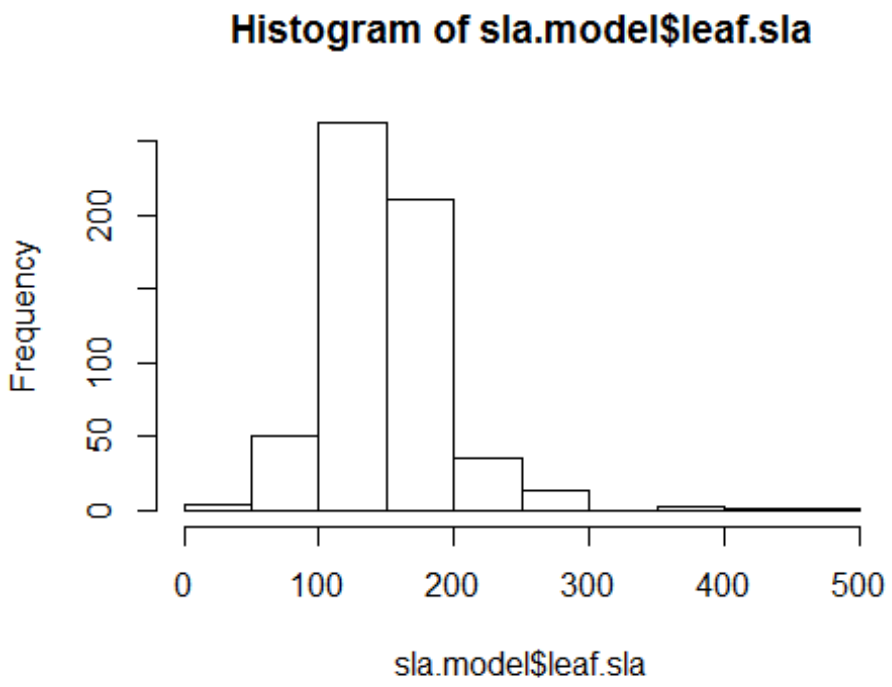
```
sla.model$nitro<-as.factor(sla.model$nitro)
sla.model$biomass<-as.factor(sla.model$biomass)
sla.model$rain<-as.factor(sla.model$rain)
#View(sla.model)
colnames(sla.model)
```

Check data structure:

```
hist(sla.model$lsla)
```




```
hist(sla.model$leaf.sla)
```




```
#fairly skewed either way, but the the lsla is slightly better
```

Test separate effects of rainout, diversity, and site:

```
rainmod<-lm(lsla ~ rainout, data=sla.model)
summary(rainmod)
#rainout causes significant variation

divmod<-lm(lsla ~ diversity, data=sla.model)
summary(divmod)
#diversity causes significant variation, but muh smaller effect than ra
inout shelters

sitemod<-aov(lsla ~ site, data=sla.model)
summary(sitemod)
#significant variation by site
```

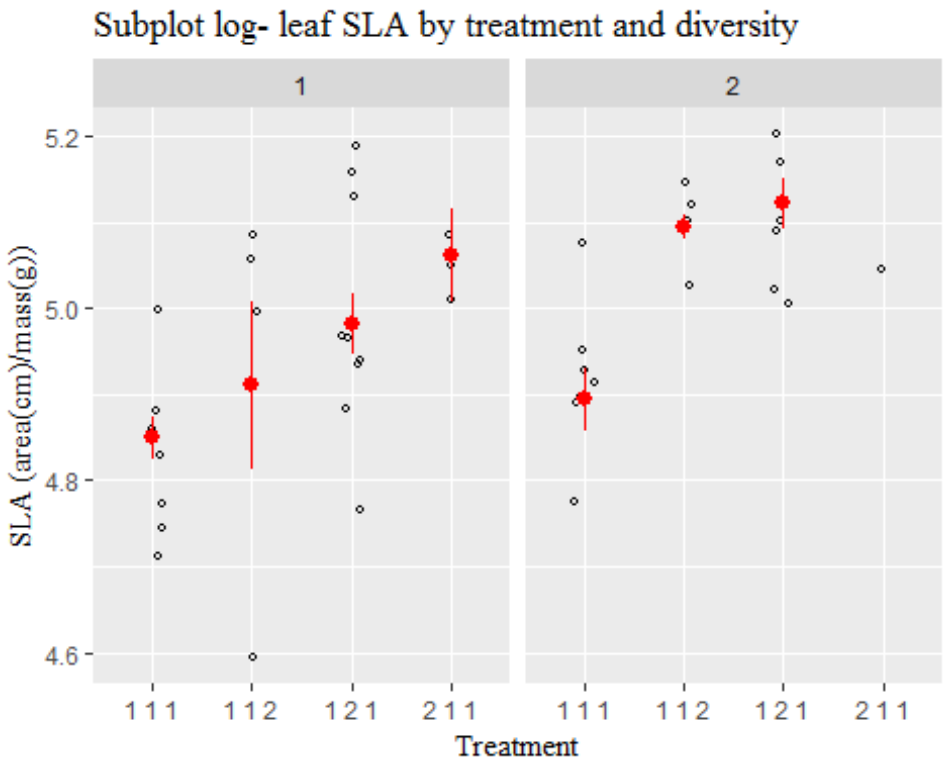
Assess subplot-weighted variation by treatment, diversity, and resource availability.

```
#load the subplot dataframe
sla.sub<-read.csv("~/Bevans_R_Thesis/Data/sla.sub.csv", header=TRUE, ro
w.names=1)
colnames(sla.sub)
sla.sub$diversity=as.factor(sla.sub$diversity)
sla.sub$rainout=as.factor(sla.sub$rainout)
sla.sub$nitro=as.factor(sla.sub$nitro)
sla.sub$biomass=as.factor(sla.sub$biomass)
```

```
#anova to see if differences exist
sub.mod<-aov(lsla.wt ~ diversity*rainout*rain*nitro*biomass, data=sla.s
ub)
summary(sub.mod)
#only significant variation is by diversity and rainout. diversity:rain
out and diversity:rain are the closest to being significant, but still
above .2 probability of randomness
```

Now assess by treat, div, and site gradients.

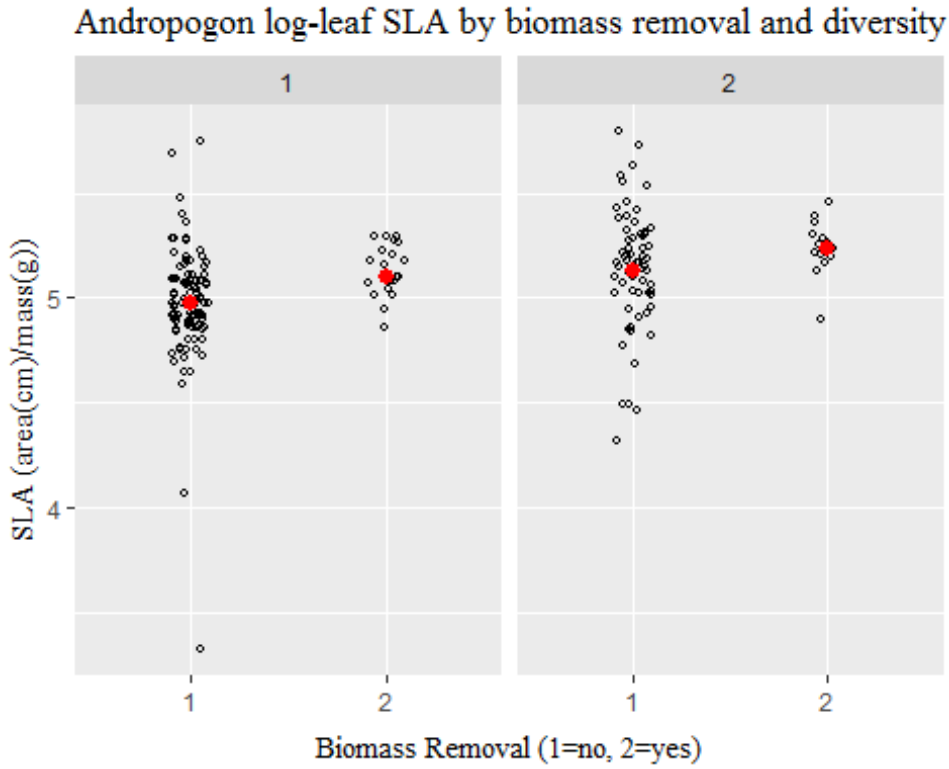
```
#global model
sla.weighted.global<-lmer(lsla.wt ~ diversity*rainout*nitro*biomass*eu.
c*eu.n + (1|site), data=sla.sub)
summary(sla.weighted.global)

#backwards selection
sla.weighted.step<-step(sla.weighted.global, data=sla.sub , direction="
backward")
sla.weighted.step

sla.sub.mod<-aov(lsla.wt ~ diversity*rainout*nitro*biomass, random = ~s
ite, data=sla.sub)
summary(sla.sub.mod)
#nothing even close to significant aside from rainout and diversity
sla.t<-TukeyHSD(sla.sub.mod)

s.plot<-ggplot(sla.sub, aes(x=trt, y=lsla.wt)) +
  ggtitle("Subplot log- leaf SLA by treatment and diversity") +
  geom_point(pch=1, cex=1.0, position = position_jitter(w = 0.1, h = 0.
1), color="black") +
  xlab("Treatment") +
  ylab("SLA (area(cm)/mass(g))") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))

s.plot + facet_wrap(~diversity) + stat_summary(fun.data=mean_se, fun.ar
gs=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```



Now do this for each of my sub-groups (ANGE, Soli, Mofi, Pavi/Sonu).

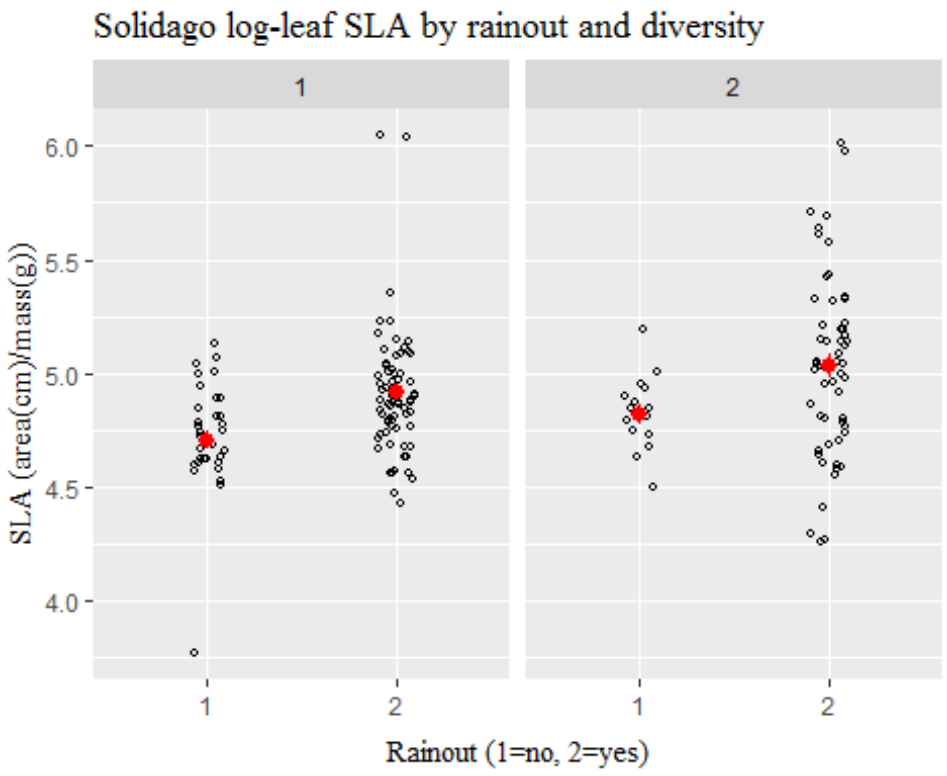
```
#read in the dataframe:
sla.model<-read.csv("~/sla.models.csv", header=TRUE)
sla.model$diversity=as.factor(sla.model$diversity)
sla.model$rainout=as.factor(sla.model$rainout)
sla.model$nitro=as.factor(sla.model$nitro)
sla.model$biomass=as.factor(sla.model$biomass)
###############
#####ANGE#####
ange.sla<-subset(sla.model, taxa=="ANGE")
ange.sla$trt = paste(ange.sla$rain, ange.sla$nitro, ange.sla$biomass)

#global model
ange.global<-lmer(lsla ~ diversity*rainout*nitro*biomass*eu.c*eu.n + (1
|site), data=ange.sla)
summary(ange.global)

#backwards selection
ange.step<-step(ange.global, data=ange.sla , direction="backward")
ange.step

#best model is

ange.mod<-aov(lsla ~ diversity*rainout*nitro*biomass, data=ange.sla)
summary(ange.mod)
#nothing even close to significant aside from rainout and diversity
```

```
TukeyHSD(ange.mod)

a.plot<-ggplot(ange.sla, aes(x=biomass, y=lsla)) +
  ggtitle("Andropogon log-leaf SLA by biomass removal and diversity") +
  geom_point(pch=1, cex=1.0, position = position_jitter(w = 0.1, h = 0.
1), color="black") +
  xlab("Biomass Removal (1=no, 2=yes)") +
  ylab("SLA (area(cm)/mass(g))") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))

a.plot + facet_wrap(~diversity) + stat_summary(fun.data=mean_se, fun.ar
gs=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```




```
#####SOLI########
soli.sla<-subset(sla.model, taxa=="Solidago")

#global model
soli.global<-lmer(lsla ~ diversity*rainout*nitro*biomass*eu.c*eu.n + (1
|site), data=soli.sla)
summary(soli.global)

#backwards selection
```

```
soli.step<-step(soli.global, data=soli.sla , direction="backward")
soli.step

#best model is

library(nlme)
soli.mod<-lm(lsla ~ diversity*rainout*nitro*biomass, data=soli.sla)
summary(soli.mod)
soli.mod<-aov(soli.mod)
#nothing even close to significant aside from rainout and diversity
TukeyHSD(soli.mod)

soli.plot<-ggplot(soli.sla, aes(x=rainout, y=lsla)) +
  ggtitle("Solidago log-leaf SLA by rainout and diversity") +
  geom_point(pch=1, cex=1.0, position = position_jitter(w = 0.1, h = 0.
1), color="black") +
  xlab("Rainout (1=no, 2=yes)") +
  ylab("SLA (area(cm)/mass(g))") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))

soli.plot + facet_wrap(~diversity) + stat_summary(fun.data=mean_se, fun
.args=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```



```
library(pander)
#####HEMAX#####
hemax.sla<-subset(sla.model, taxa==c("HEMAX", "HEPA"))

#global model
hemax.global<-lmer(lsla ~ rainout*nitro*biomass+eu.c+eu.n + (1|site), d
ata=hemax.sla)
summary(hemax.global)

#backwards selection
hemax.step<-step(hemax.global, data=hemax.sla , direction="backward")
hemax.step

#best model is
h.best<-lmer(lsla ~ eu.n + (1|site), data=hemax.sla)
summary(h.best)
lsmeansLT(h.best)

library(nlme)
hemax.mod<-lme(lsla ~ rainout + eu.n, random =~1|site, data=hemax.sla)
anova(hemax.mod)
summary(hemax.mod)
#nothing even close to significant aside from rainout and diversity

#####sonu#####
sonu.sla<-subset(sla.model, taxa==c("SONU", "PAVI"))

#global model
sonu.global<-lmer(lsla ~ rainout+nitro+biomass+eu.c+eu.n + (1|site), da
ta=sonu.sla)

summary(sonu.global)

#backwards selection
sonu.step<-step(sonu.global, data=sonu.sla , direction="backward")
sonu.step

pander(sonu.step)
#best model is

sonu.mod<-lmer(lsla ~ rainout*nitro*biomass +eu.c + eu.n + (1|site), da
ta=sonu.sla)
mod<-anova(sonu.mod)
mod
#site n and om are significant
sonu<-lm(lsla ~ eu.c + eu.n, data=sonu.sla)
summary(sonu)

#####mofi#####
mofi.sla<-subset(sla.model, taxa=="MOFI")
```

```
#global model
mofi.global<-lmer(lsla ~ rainout+nitro+biomass+eu.c+eu.n + (1|site), da
ta=mofi.sla)

summary(mofi.global)

#backwards selection
mofi.step<-step(mofi.global, data=mofi.sla , direction="backward")
mofi.step

pander(mofi.step)
#best model is

mofi.mod<-lmer(lsla ~ rainout*nitro*biomass +eu.c + eu.n + (1|site), da
ta=mofi.sla)
mod<-anova(mofi.mod)
mod
#site n and om are significant
mofi<-lm(lsla ~ eu.c*eu.n, data=mofi.sla)
summary(mofi)
```

CLA_models_thesis

Becca

February 14, 2017

R script to reproduce the correlations and images used in the thesis "PLANT DIVERSITY INFLUENCES THE STRUCTURE AND FUNCTION OF A RESTORED PRAIRIE AND ITS RESPONSES TO ADDED DISTURBANCES"" by Rebecca A Bevans, submitted to the graduate college April 28 2017.

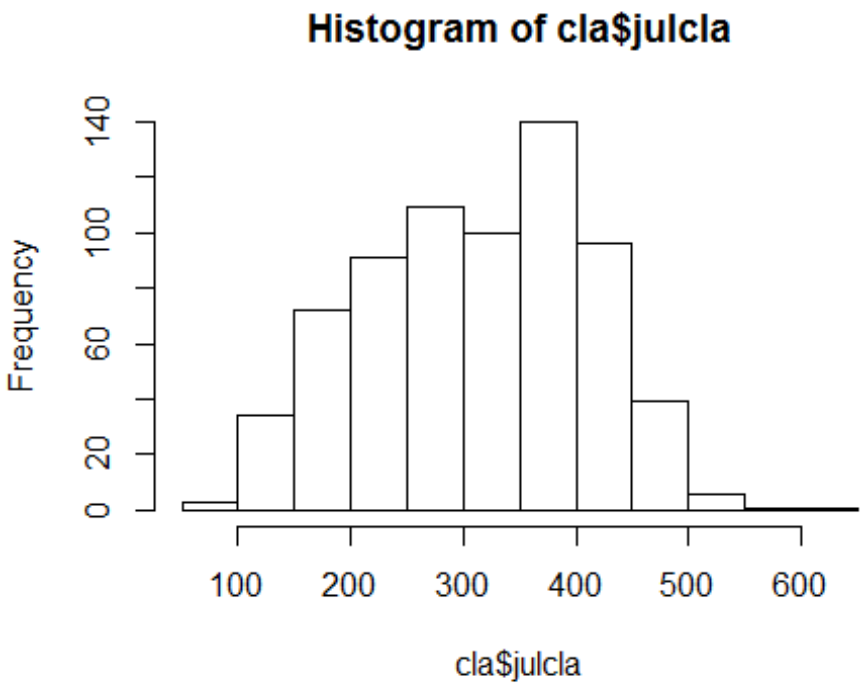
```
knitr::opts_chunk$set(warning=FALSE, message=FALSE, results="hide")
```

load libraries and datasets:

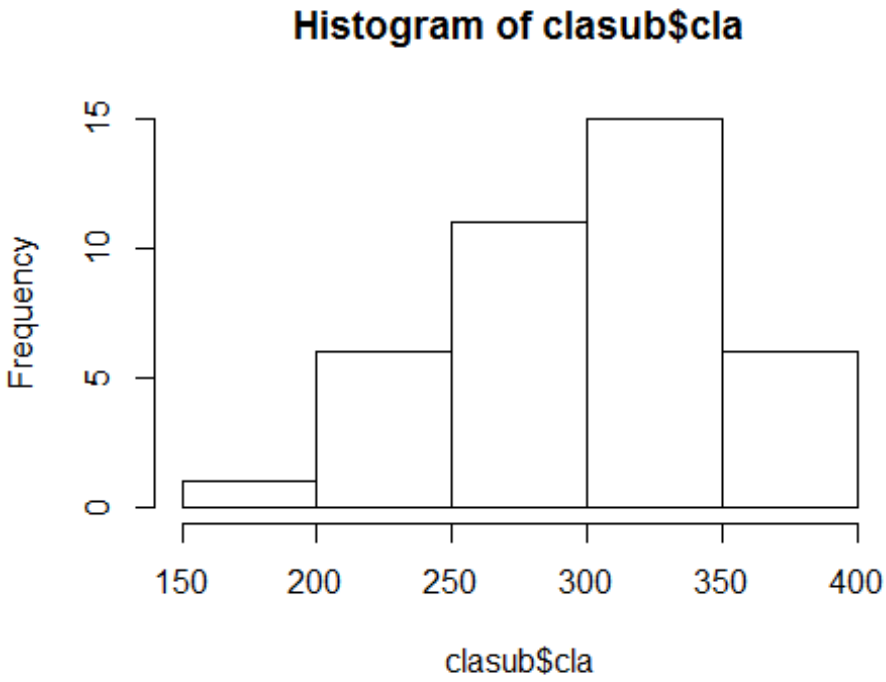
```
library(AICcmodavg)
library(lme4)
library(lmerTest)
library(tidyverse)
library(dplyr)
library(broom)
library(data.table)
library(stats)
library(plyr)
cla<-read.csv("~/cla.csv", header=TRUE, row.names=1)
#this is the data with the site-averaged cla values
clasub<-read.csv("~/clasub.csv", header=TRUE, row.names=1)
```

check if i need to transform my data.

```
hist(cla$julcla)
```




```
hist(clasub$cla)
```



```
#actually, looks pretty good.
```

Test separate effects of rainout, diversity, and site:

```
rain<-lm(julcla ~ rainout, data=cla)
summary(rain)
#rainout causes significant variation

div<-lm(julcla ~ diversity, data=cla)
summary(div)
#diversity causes significant variation, but muh smaller effect than ra
inout shelters

site<-aov(julcla ~ site, data=cla)
summary(site)

block<-aov(julcla ~ block, data=cla)
summary(block)
#use block as random effects variable
#significant variation by site
```

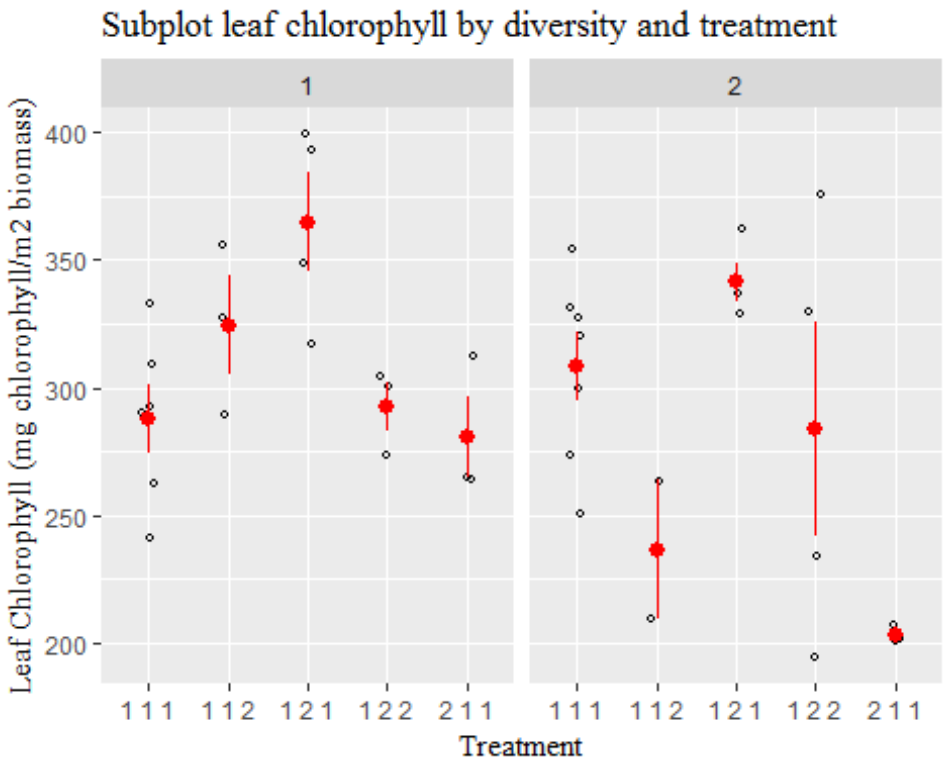
Assess subplot-weighted variation by treatment, diversity, and resource availability.

```
#format the subplot and wholeplot dataframes:
cla$diversity=as.factor(cla$diversity)
cla$rainout=as.factor(cla$rainout)
cla$nitro=as.factor(cla$nitro)
cla$biomass=as.factor(cla$biomass)

clasub$diversity=as.factor(clasub$diversity)
clasub$rainout=as.factor(clasub$rainout)
clasub$nitro=as.factor(clasub$nitro)
clasub$biomass=as.factor(clasub$biomass)

#anova to see if differences exist
cla.mod<-aov(cla ~ diversity*rainout*rain*nitro*biomass, data=clasub)
summary(cla.mod)
#diversity is marginally significant, rainout treatment is significant,
 biomass removal significant, diversity:nitrogen is marginally signific
ant, diversity:rainout treatment is significant (important because each
 of these factors alone is not significant)
```

Now assess by treat, div, and site gradients.

```
#global model
cla.weighted.global<-lmer(cla ~ diversity*rainout*nitro*biomass*eu.c*eu
.n + (1|site), data=clasub)
summary(cla.weighted.global)

#View(clasub)
#backwards selection
```

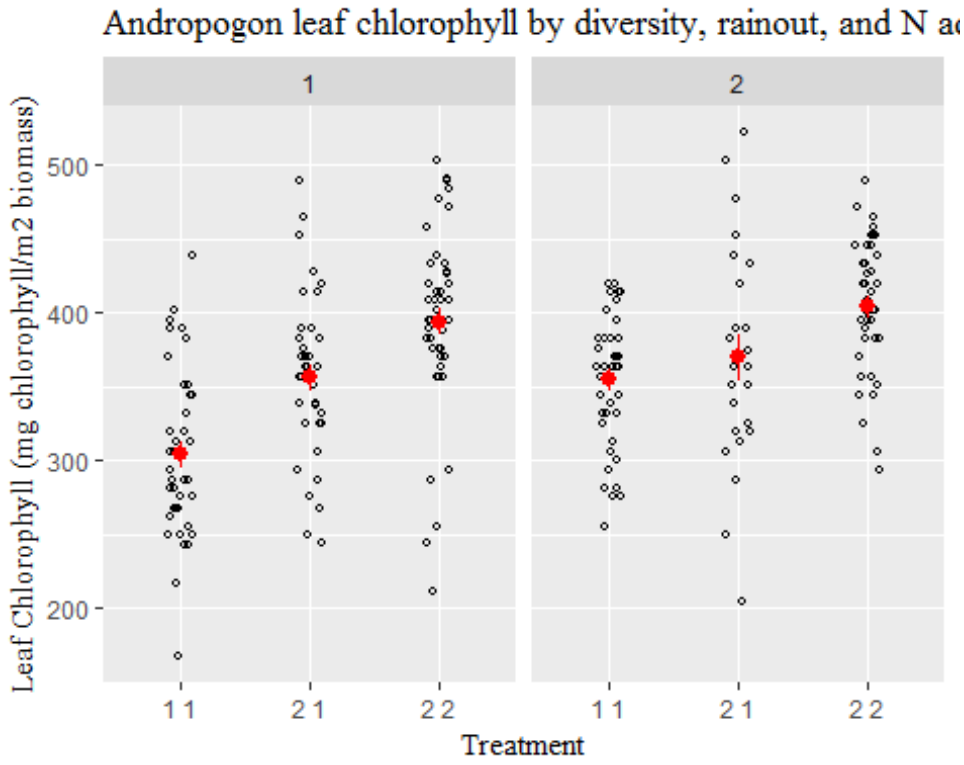
```
cla.weighted.step<-step(cla.weighted.global, data=clasub , direction="b
ackward")
cla.weighted.step

library(nlme)
cla.sub.mod<-lm(cla ~ rainout*diversity*nitro*biomass, data=clasub)
cla.sub<-aov(cla.sub.mod)
#nothing even close to significant aside from rainout and diversity
TukeyHSD(cla.sub)

cla.sub.soil<-lm(cla ~ eu.c + eu.n, data=clasub)
summary(cla.sub.soil)

clasub$trt=paste(clasub$rain, clasub$nitro, clasub$biomass)

cla.subplot<-ggplot(clasub, aes(x=trt, y=cla)) +
  ggtitle("Subplot leaf chlorophyll by diversity and treatment") +
  geom_point(pch=1, cex=1.0, position = position_jitter(w = 0.1, h = 0.
1), color="black") +
  xlab("Treatment") +
  ylab("Leaf Chlorophyll (mg chlorophyll/m2 biomass)") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))

cla.subplot + facet_wrap(~diversity) + stat_summary(fun.data=mean_se, f
un.args=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```



Now do this for each of my sub-groups (ange, solidago, mofi, pavi/sonu, hemax/hepa, hlyle).

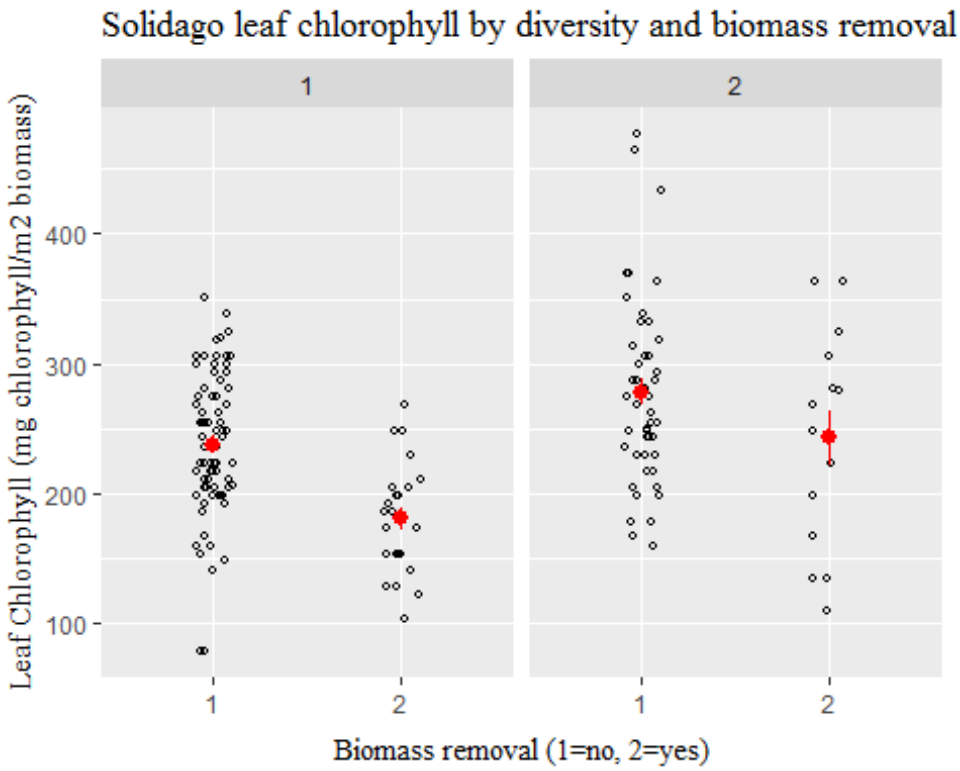
```
###############
#####ANGE#####
ange.cla<-subset(cla, taxa=="ange")

#global model
ange.c.global<-lmer(julcla ~ diversity*rainout*nitro*biomass*eu.c*eu.n
+ (1|site), data=ange.cla)
summary(ange.c.global)

#backwards selection
ange.c.step<-step(ange.c.global, data=ange.cla , direction="backward")
ange.c.step

#best model is all of the variables.

ange.c.mod<-lm(julcla ~ diversity*rainout*nitro*biomass, data=cla )
acm<-aov(ange.c.mod)
summary(ange.c.mod)
#nothing even close to significant aside from rainout and diversity
TukeyHSD(acm)

ange.soil<-lmer(julcla ~ eu.c*eu.n + (1|site), data=cla)
summary(ange.soil)
```

```
ange.cla$diversity=as.factor(ange.cla$diversity)
ange.cla$plt<-paste(ange.cla$rainout, ange.cla$nitro)

ange.cla.plot<-ggplot(ange.cla, aes(x=plt, y=julcla)) +
  ggtitle("Andropogon leaf chlorophyll by diversity, rainout, and N add
ition") +
  geom_point(pch=1, cex=1.0, position = position_jitter(w = 0.1, h = 0.
1), color="black") +
  xlab("Treatment") +
  ylab("Leaf Chlorophyll (mg chlorophyll/m2 biomass)") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))

ange.cla.plot + facet_wrap(~diversity) + stat_summary(fun.data=mean_se,
 fun.args=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```



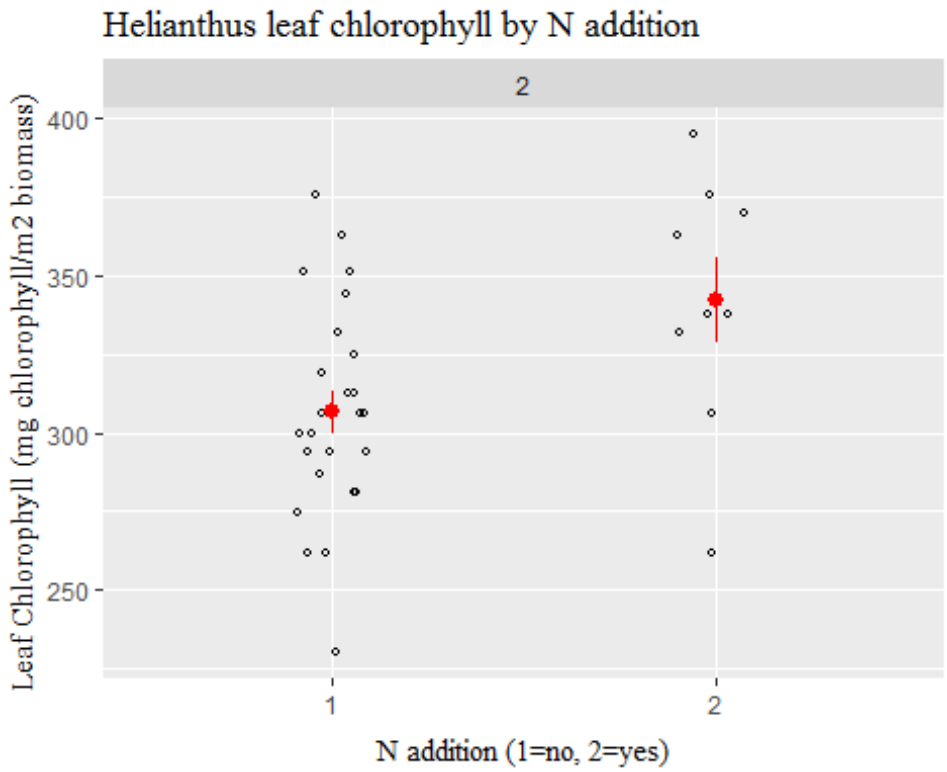

```
#####SOLI########
soli.cla<-subset(cla, taxa=="solidago")

#global model
soli.c.global<-lmer(julcla ~ diversity*rainout*nitro*biomass*eu.c*eu.n
+ (1|site), data=soli.cla)
summary(soli.c.global)
```

```
#backwards selection
soli.step<-step(soli.c.global, data=soli.cla , direction="backward")
soli.step

#best model is all variables

library(nlme)
soli.c.mod<-lm(julcla ~ diversity*rainout*nitro*biomass, data=soli.cla)
soli.c.aov<-aov(soli.c.mod)
summary(soli.c.aov)
#nothing even close to significant aside from rainout and diversity
TukeyHSD(soli.c.aov)

soli.c.soil<-lmer(julcla ~ site.c + site.n + (1|site), data=soli.cla)
summary(soli.c.soil)

soli.plot<-ggplot(soli.cla, aes(x=biomass, y=julcla)) +
  ggtitle("Solidago leaf chlorophyll by diversity and biomass removal")
 +
  geom_point(pch=1, cex=1.0, position = position_jitter(w = 0.1, h = 0.
1), color="black") +
  xlab("Biomass removal (1=no, 2=yes)") +
  ylab("Leaf Chlorophyll (mg chlorophyll/m2 biomass)") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))

soli.plot + facet_wrap(~diversity) + stat_summary(fun.data=mean_se, fun
.args=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```



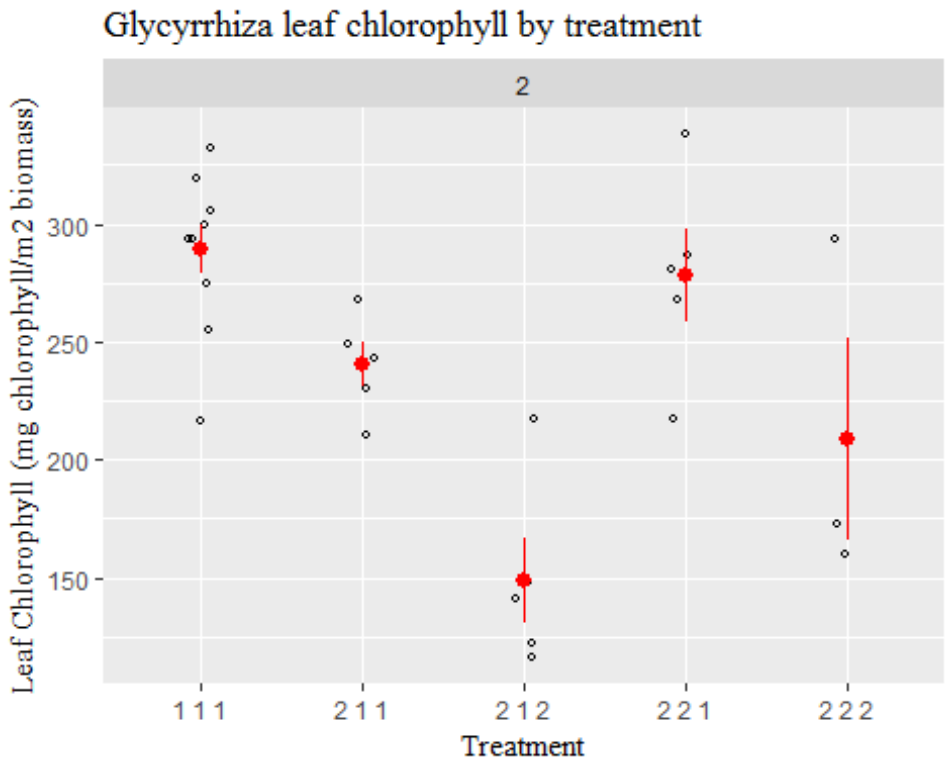

```
#####HEPA/HEMAX########
he.cla<-subset(cla, taxa==c("hemax", "hepa"))
he.cla<-subset(he.cla, diversity==2)
#View(he.cla)
#global model
he.c.global<-lmer(julcla ~ rainout*nitro*biomass*eu.c*eu.n + (1|site),
data=he.cla)
summary(he.c.global)

#backwards selection
he.step<-step(he.c.global, data=he.cla , direction="backward")
he.step

#best model is all variables

he.cla$rain=as.factor(he.cla$rain)
he.cla$nitro=as.factor(he.cla$nitro)
he.c.mod<-lm(julcla ~ rain*nitro, data=he.cla)
he.c.aov<-aov(he.c.mod)
summary(he.c.aov)
#nothing even close to significant aside from rainout and diversity
TukeyHSD(he.c.aov)

he.c.soil<-lmer(julcla ~ site.c + site.n + (1|site), data=he.cla)
summary(he.c.soil)

he.cla.plot<-ggplot(he.cla, aes(x=nitro, y=julcla)) +
```

```
  ggtitle("Helianthus leaf chlorophyll by N addition") +
  geom_point(pch=1, cex=1.0, position = position_jitter(w = 0.1, h = 0.
1), color="black") +
  xlab("N addition (1=no, 2=yes)") +
  ylab("Leaf Chlorophyll (mg chlorophyll/m2 biomass)") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))

he.cla.plot + facet_wrap(~diversity) + stat_summary(fun.data=mean_se, f
un.args=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```



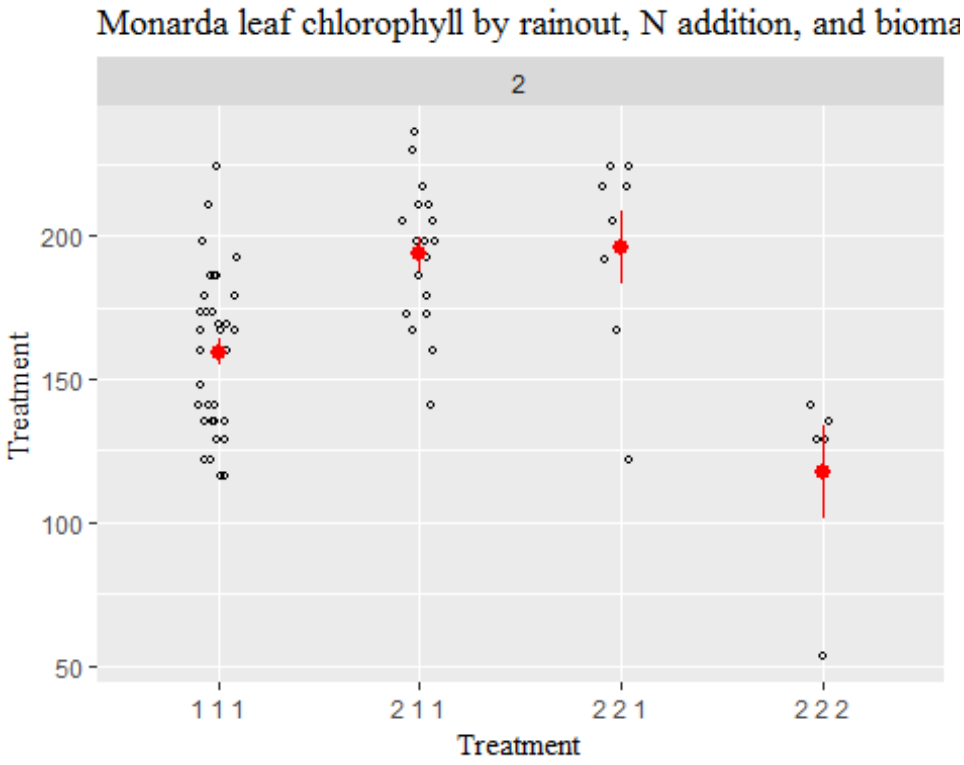

```
#####glyle########
glyle.cla<-subset(cla, taxa=="glyle")
glyle.cla$trt=paste(glyle.cla$rainout, glyle.cla$nitro, glyle.cla$bioma
ss)

#View(glyle.cla)
#global model
glyle.c.global<-lm(julcla ~ rainout*nitro*biomass, data=glyle.cla)
summary(glyle.c.global)

#backwards selection
drop<-drop1(glyle.c.global)
drop
```

```
glyle2<-lm(julcla ~ rainout + nitro + biomass + rainout*nitro + rainout
*biomass, data=glyle.cla)
anova(glyle.c.global, glyle2)
#the model cannot be simplified
#best model is all variables

#glyle.cla$rain=as.factor(glyle.cla$rain)
#glyle.c.mod<-lm(julcla ~ rainout*nitro*biomass, data=glyle.cla)
glyle.c.aov<-aov(glyle.c.global)
summary(glyle.c.aov)
#nothing even close to significant aside from rainout and diversity
TukeyHSD(glyle.c.aov)

glyle.plot<-ggplot(glyle.cla, aes(x=trt, y=julcla)) +
  ggtitle("Glycyrrhiza leaf chlorophyll by treatment") +
  geom_point(pch=1, cex=1.0, position = position_jitter(w = 0.1, h = 0.
1), color="black") +
  xlab("Treatment") +
  ylab("Leaf Chlorophyll (mg chlorophyll/m2 biomass)") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))

glyle.plot + facet_wrap(~diversity) + stat_summary(fun.data=mean_se, fu
n.args=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```




```
#####mofi########
mofi.cla<-subset(cla, taxa=="mofi")
#View(mofi.cla)
mofi.cla$trt=paste(mofi.cla$rainout, mofi.cla$nitro, mofi.cla$biomass)
#global model
mofi.c.global<-lmer(julcla ~ rainout*nitro*biomass*eu.c*eu.n +(1|block)
, data=mofi.cla)
summary(mofi.c.global)

#backwards selection
mofi.step<-step(mofi.c.global, data=mofi.cla , direction="backward")
mofi.step

#the model cannot be simplified
#best model is all variables

#mofi.cla$rain=as.factor(mofi.cla$rain)
#mofi.c.mod<-lm(julcla ~ rainout*nitro*biomass, data=mofi.cla)
mofi.c.aov<-anova(mofi.c.global)

summary(mofi.c.aov)
#nothing even close to significant aside from rainout and diversity

mofi.plot<-ggplot(mofi.cla, aes(x=trt, y=julcla)) +
  ggtitle("Monarda leaf chlorophyll by rainout, N addition, and biomass
 removal") +
  geom_point(pch=1, cex=1.0, position = position_jitter(w = 0.1, h = 0.
```

```
1), color="black") +
  xlab("Treatment") +
  ylab("Treatment") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))

mofi.plot + facet_wrap(~diversity) + stat_summary(fun.data=mean_se, fun
.args=list(mult=1),
  geom="pointrange", color="red", position=position_dodge(w=0.5))
```



Soil Moisture for Bevans Thesis

Becca

April 18, 2017

An R Script to Reproduce the correlations and graphics related to soil moisture for the thesis, " PLANT DIVERSITY INFLUENCES THE STRUCTURE AND FUNCTION OF A RESTORED PRAIRIE AND ITS RESPONSES TO ADDED DISTURBANCES" by Rebecca Bevans, submitted to the UN-L Graduate College April 28 2017.

```
knitr::opts_chunk$set(warning=FALSE, message=FALSE, results="hide")

library(tidyverse)
library(dplyr)
library(plyr)
library(pander)
library(lmerTest)
library(lme4)
#soilm<-read.csv("P:/Research/Thesis/PRP_thesis/Data/AllMoisture2015.csv", header=TRUE)
#View(soilm)
###load corrected data fle:###
################################
soilm<-read.csv("~/soilm.csv", header=TRUE)
```

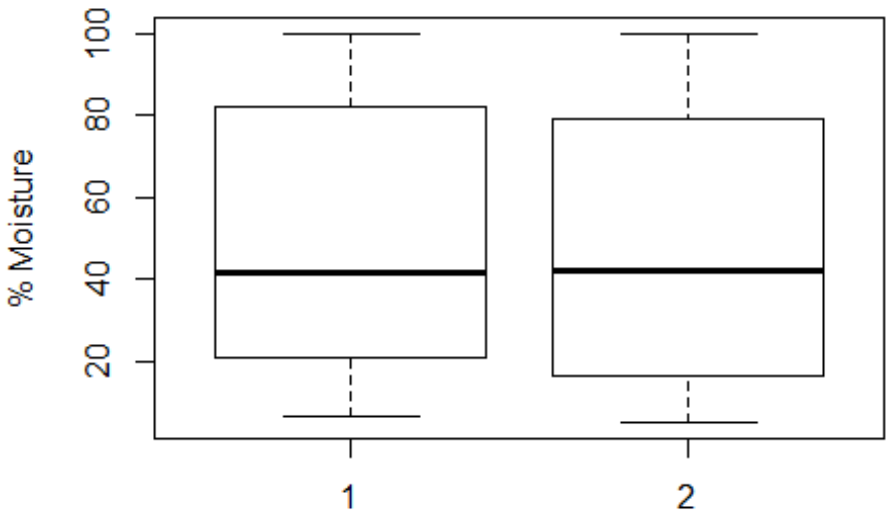
Is there a difference between control and rainout shelter plots?

```
soilm$rainout = as.factor(soilm$rainout)

t.test(soilm$moisture ~ soilm$rainout)
```

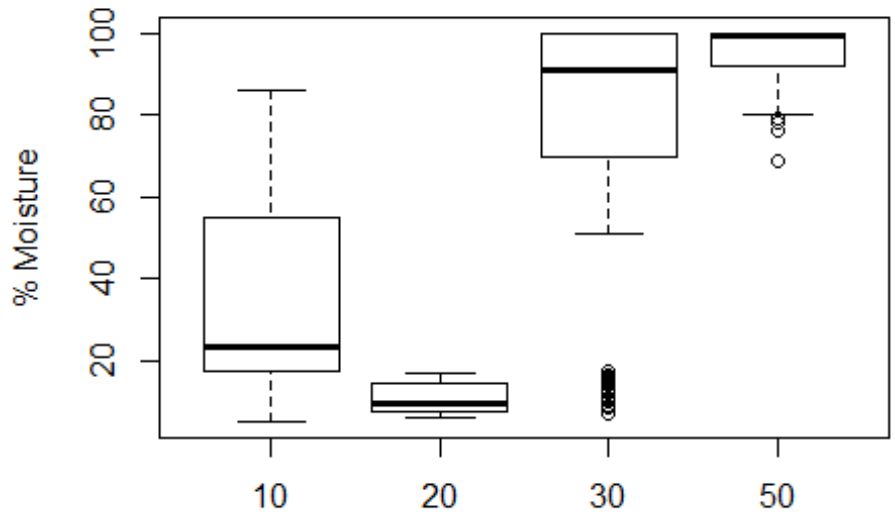
Answer appears to be no: the confidence interval for both groups overlaps.

What are the general trends in soil moisture?

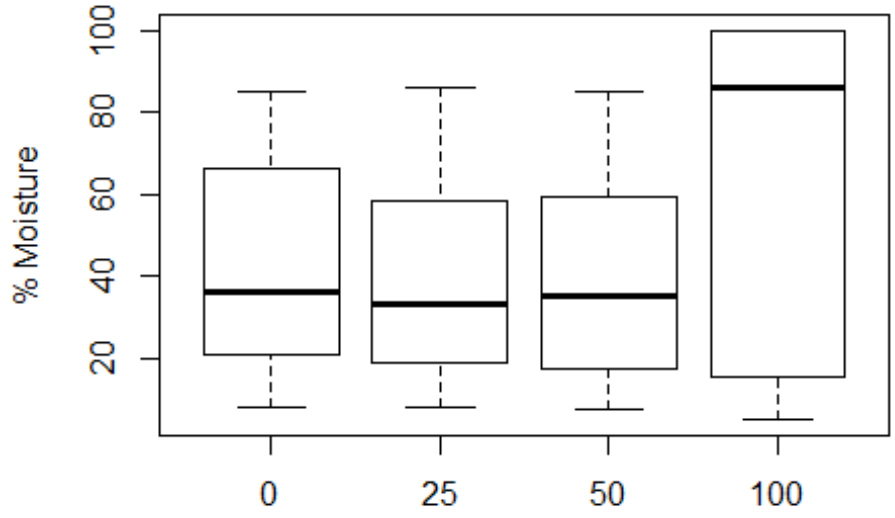
```
boxplot(soilm$moisture~soilm$rainout, ylab= '% Moisture')
```



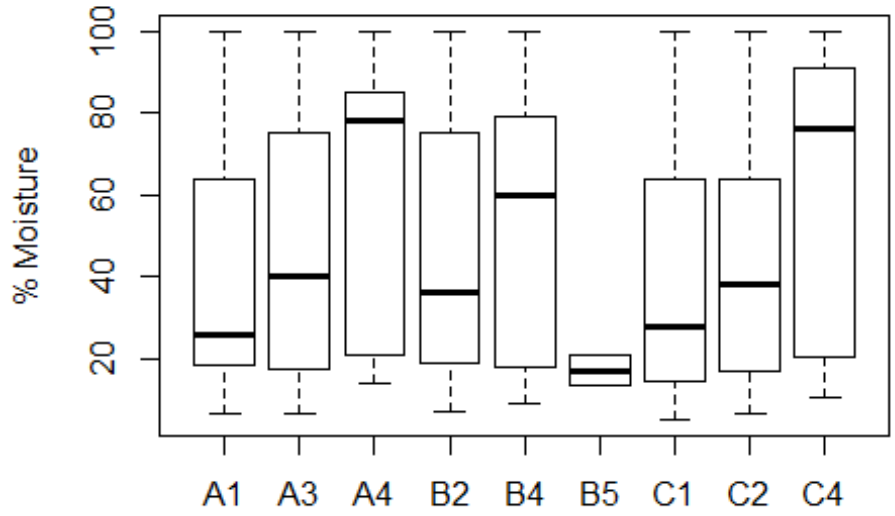

```
boxplot(soilm$moisture~soilm$depth, ylab= "% Moisture")
```



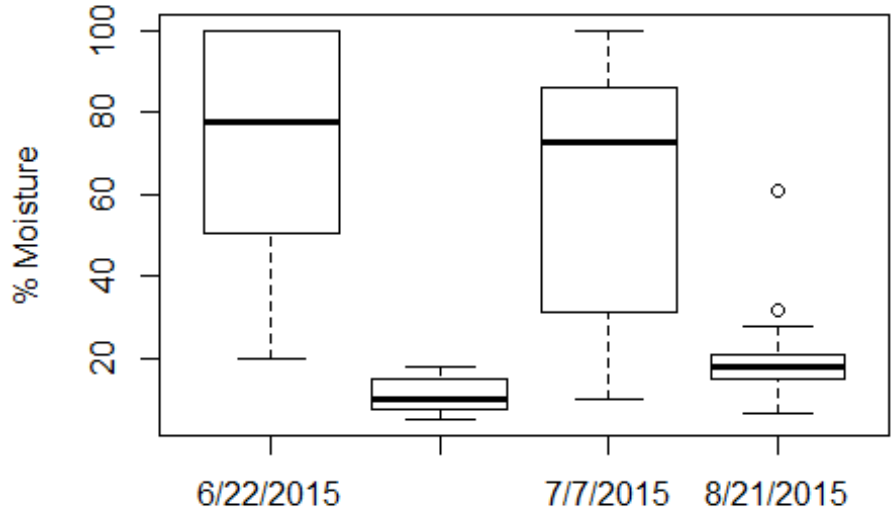

```
boxplot(soilm$moisture ~ soilm$distedge, ylab = "% Moisture")
```



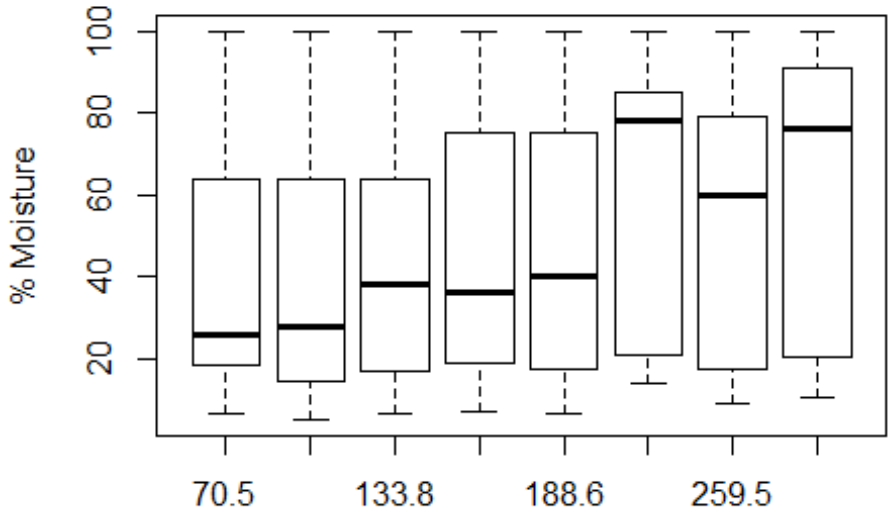

```
boxplot(soilm$moisture ~ soilm$plot, ylab = "% Moisture")
```




```
boxplot(soilm$moisture ~ soilm$date, ylab = "% Moisture")
```




```
boxplot(soilm$moisture ~ soilm$dist, ylab = "% Moisture")
```



Soil moisture increases with increasing distance from river, increases with increasing soil depth, and does not change with distance from edge of plot (except in the middle of the plots, where it is much higher).

Linear model describing variation in soil moisture:

```
moistmod<-lmer(moisture ~ rainout + depth + date + distedge + dist + (1
|plot), data=soilm)
summary(moistmod)

#are differences between categorical variables significant?
library(lmerTest)
lsmeansLT(moistmod)
#yes.

#correlations between continuous variables:
depth<-lm(moisture ~ depth, data=soilm)
summary(depth)

dist<-lm(moisture ~ dist, data=soilm)
summary(dist)
```

Test relationships at the 10cm depth

```
#soilm.10cm=subset(soilm, depth==10)
#write.csv(soilm.10cm, file="P:/Research/Thesis/PRP_thesis/Data/soilm.1
0cm.csv")
```

```
soilm.10cm<-read.csv("P:/Research/Thesis/PRP_thesis/Data/soilm.10cm.csv
")

dist.10cm<-lm(moisture ~ dist + rainout, data=soilm.10cm)
summary(dist.10cm)

cor.test(soilm.10cm$moisture, soilm.10cm$dist)
#there is a significant correlation betweeen soil moisture at 10cm dept
h and distance from the river.

distedge.10cm<-lm(moisture ~ distedge + rainout, data=soilm.10cm)
summary(distedge.10cm)
cor.test(soilm.10cm$moisture, soilm.10cm$distedge)
```

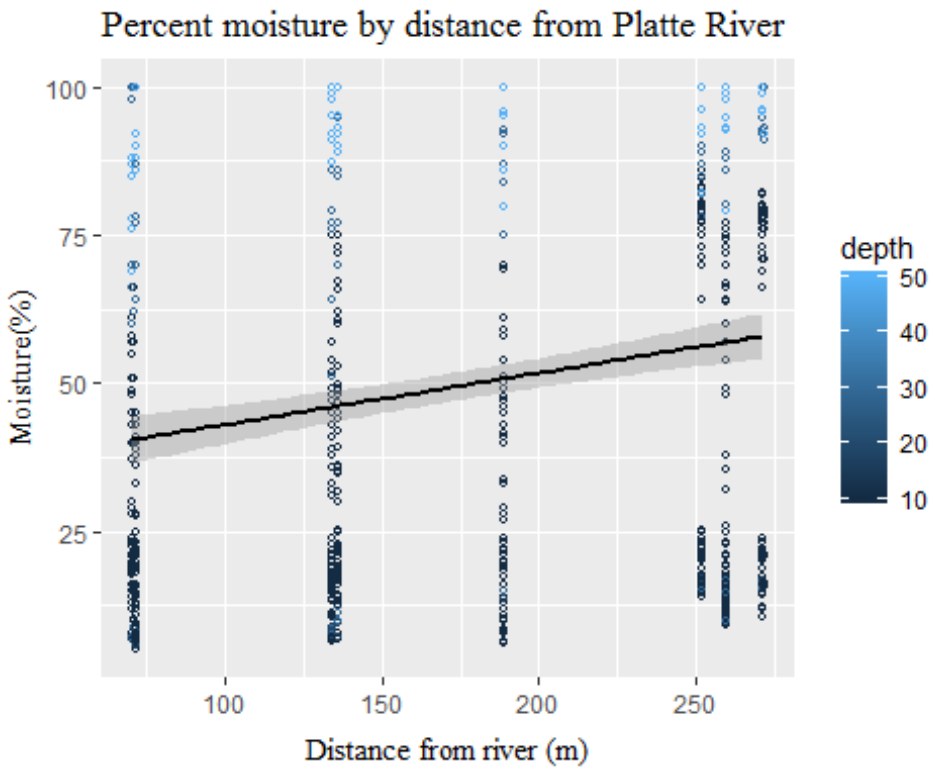
Test relationships at the 30cm depth

```
soilm.30cm<-subset(soilm, depth==30)
rainout.30cm<-lm(moisture ~ dist + rainout, data=soilm.30cm)
summary(rainout.30cm)
```

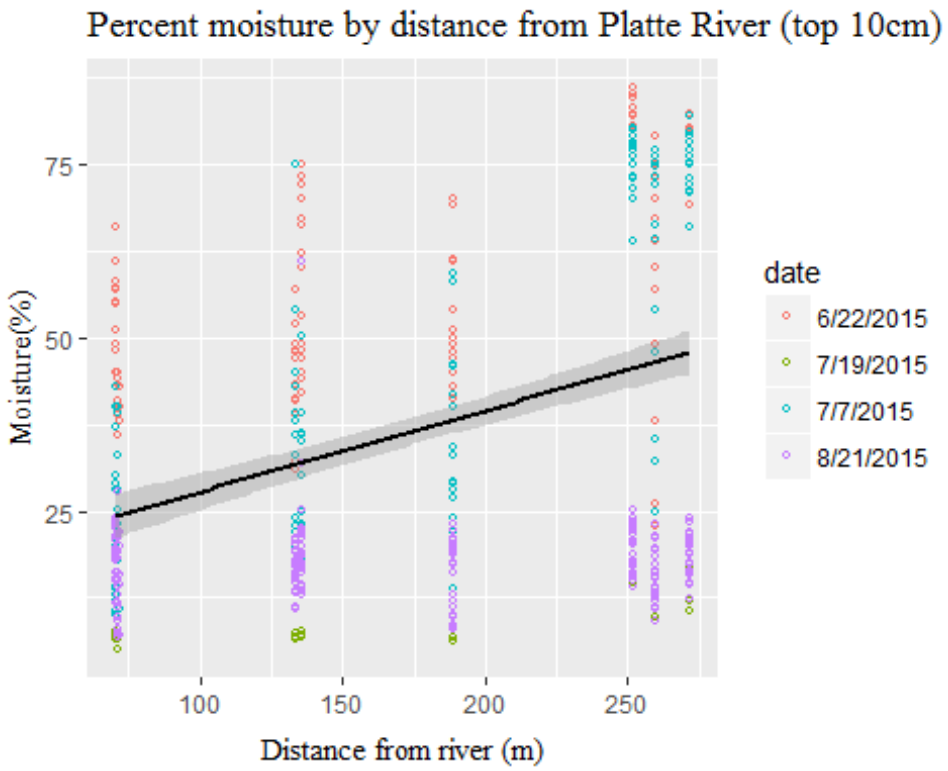
The rainout shelters do affect water availability in the top 10cm, but do not affect moisture at the 30cm depths.

Plot the results:

```
data<-soilm
dat<-data[complete.cases(data),]

moistmod.2<-lmer(moisture ~ rainout + depth + date + distedge + dist +
(1|plot), data=dat)
dat$pred<-predict(moistmod.2)

mmod<-ggplot(moistmod.2, aes(x=dist, y=moisture, color=depth)) +
  ggtitle("Percent moisture by distance from Platte River") +
  geom_point(cex=1.0, pch=1.0, position = position_jitter(w = 0.1, h =
0.1)) +
  xlab("Distance from river (m)") +
  ylab("Moisture(%)") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))
mmod + stat_smooth(method="lm", color="black")
```




```
#try it for just the top 10cm
dat.10<-subset(dat, depth==10)
moistmod.3<-lmer(moisture ~ rainout + date + distedge + dist + (1|plot)
, data=dat.10)
dat.10$pred<-predict(moistmod.3)

mmod<-ggplot(moistmod.3, aes(x=dist, y=moisture, color=date)) +
  ggtitle("Percent moisture by distance from Platte River (top 10cm)")
+
  geom_point(cex=1.0, pch=1.0, position = position_jitter(w = 0.1, h =
0.1)) +
  xlab("Distance from river (m)") +
  ylab("Moisture(%)") +
  theme(plot.title = element_text(lineheight=.8, family="serif", face="
plain", vjust=1),
        axis.title.x = element_text(family="serif", vjust=-0.5),
        axis.title.y = element_text(family="serif", vjust=0.3))
mmod + stat_smooth(method="lm", color="black")
```




```
#for the top 10cm, moisture varies significantly by distance from the r
iver and by the sampling date.
```

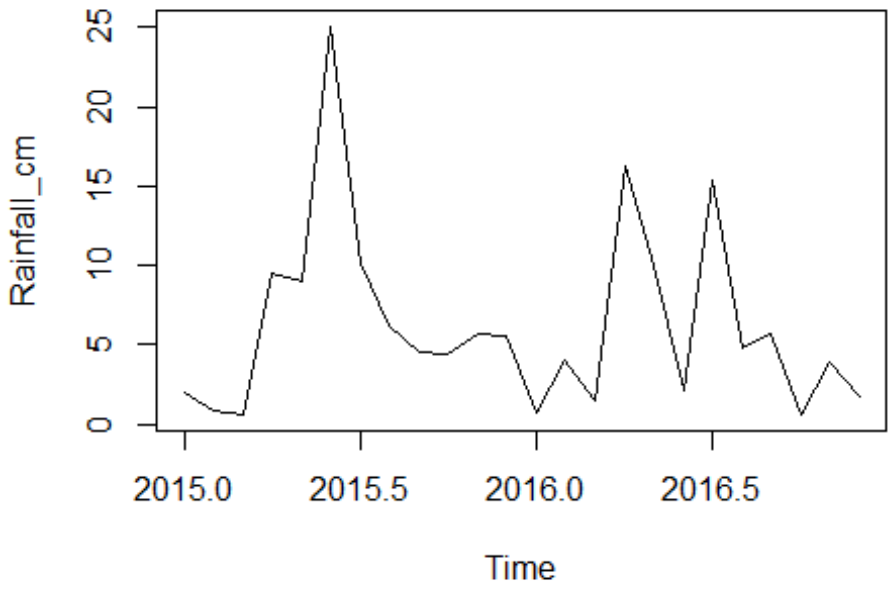
Weather for Bevans Thesis

Becca

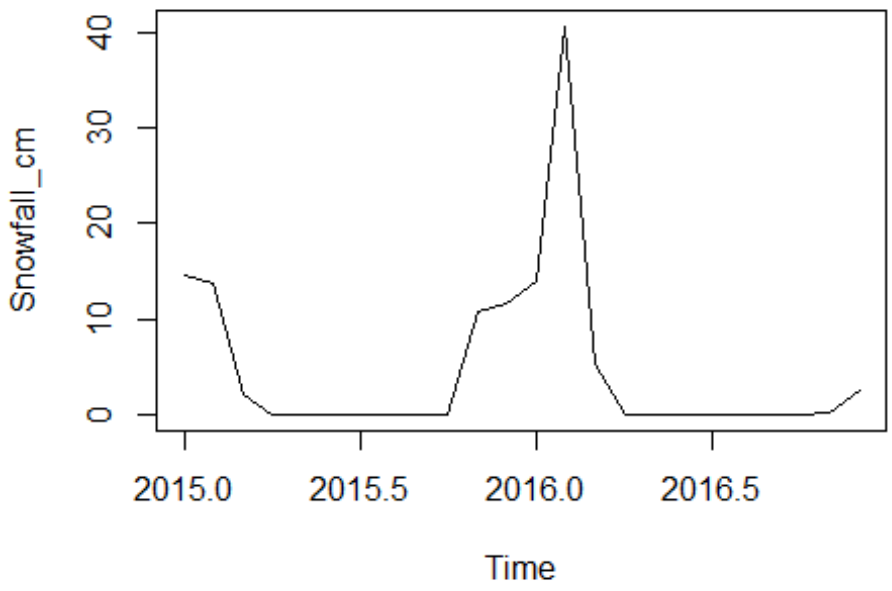
April 13, 2017

R script to reproduce the correlations and images used in the thesis "PLANT DIVERSITY INFLUENCES THE STRUCTURE AND FUNCTION OF A RESTORED PRAIRIE AND ITS RESPONSES TO ADDED DISTURBANCES"" by Rebecca A Bevans, submitted to the graduate college April 28 2017.

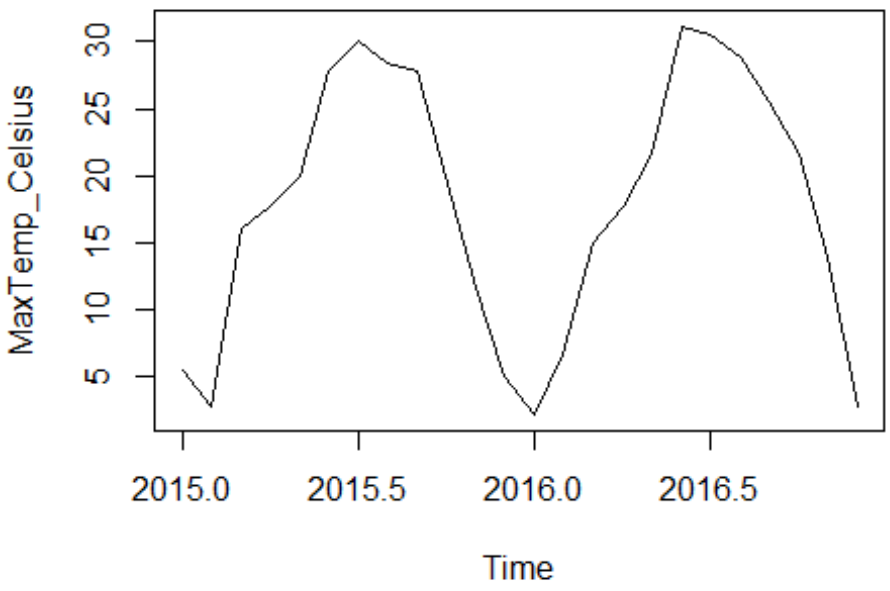
```
knitr::opts_chunk$set(warning=FALSE, message=FALSE, results="hide")

library(tidyverse)
weather<-read.csv("~/Weather.csv", header=TRUE, row.names=1)
weather$date<-paste(weather$year, weather$month)
#View(weather)
rain<-scan("~/rainfall.csv")
rain
Rainfall_cm<-ts(rain, frequency = 12, start=c(2015, 1))
Rainfall_cm
plot.ts(Rainfall_cm)
```



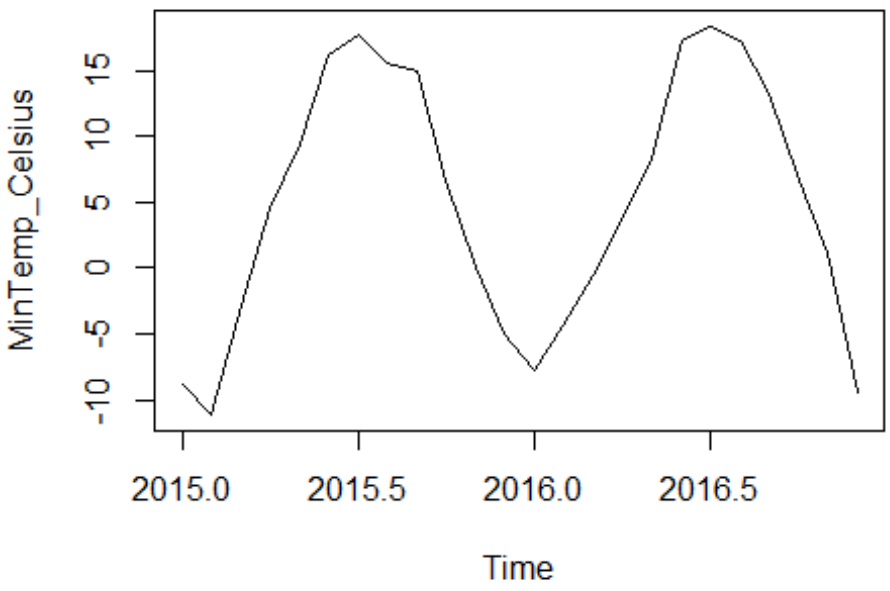

```
snow<-scan("P:/Research/Thesis/PRP_Thesis/Data/snow.csv")
snow
Snowfall_cm<-ts(snow, frequency = 12, start=c(2015, 1))
Snowfall_cm
plot.ts(Snowfall_cm)
```




```
###
avmaxtemp<-scan("P:/Research/Thesis/PRP_Thesis/Data/avmaxtemp.csv")
avmaxtemp
MaxTemp_Celsius<-ts(avmaxtemp, frequency = 12, start=c(2015, 1))
MaxTemp_Celsius
plot.ts(MaxTemp_Celsius)
```




```
###
avmintemp<-scan("P:/Research/Thesis/PRP_thesis/Data/avmintemp.csv")
avmintemp
MinTemp_Celsius<-ts(avmintemp, frequency=12, start=c(2015, 1))
MinTemp_Celsius
plot.ts(MinTemp_Celsius)
```


MinTemp_Celsius
-10
-5
0
5
10
15
2015.0
2015.5
2016.0
2016.5
Time