Human impacts on how savanna plants interact through fire, resources, and microclimate

A Dissertation
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

Peter Douglas Wragg

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Adviser: Dr. David Tilman

August 2015

Acknowledgements

Funding

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

I was supported, in part, by a Scholarship for Doctoral Study Abroad from the South African National Research Foundation. I was also supported by a Graduate School Fellowship and a Doctoral Dissertation Fellowship, both from the University of Minnesota, as well as Teaching Assistantships at the University of Minnesota and Research Assistantships at the University of Minnesota and the Bren School of Environmental Science & Management at the University of California, Santa Barbara.

The work at Cedar Creek Ecosystem Science Reserve reported in chapters 2-4 was supported by major grants from the US National Science Foundation Long-Term Ecological Research Program (LTER) including DEB-0620652 and DEB-1234162. The warming experiment in chapter 4 was also supported by Legislative-Citizen Commission on Minnesota Resources. Grant #048-B1 (Sustainable Biofuels, 2010-2013). Further support for my work in chapters 2-4 was provided by small grants from the University of Minnesota Graduate School in the form of a Carolyn Crosby Research Grant and a Plant Traits Seminar Research Grant (Provost's Award).

Chapter 5 was generated using data from the Nutrient Network (http://www.nutnet.org) experiment, funded at the site-scale by individual researchers. Coordination and data management have been supported by funding to E. Borer and E. Seabloom from the National Science Foundation Research Coordination Network (NSF-DEB-1042132) and Long Term Ecological Research (NSF-DEB-1234162 to Cedar Creek LTER) programs, and the Institute on the Environment (DG-0001-13). I also thank the Minnesota Supercomputer Institute for hosting project data and the Institute on the Environment for hosting Network meetings. Network soil analyses were funded by Sarah Hobbie, Elizabeth Borer, Eric Seabloom, and Noah Fierer.

I acknowledge scholarly travel grants from the University of Minnesota Ecology, Evolution, and Behavior Graduate Program and the University of Minnesota Graduate and Professional Student Assembly.

Direct contributions

Chapters 2-4 build on existing experiments at Cedar Creek Ecosystem Science Reserve. David Tilman established the Big Biodiversity Experiment within which I worked. Troy Mielke, Research Co-ordinator, was instrumental in maintaining and sampling the core experiments in these chapters, and in supporting my work in them. Cedar Creek staff and numerous interns, friends and family also helped extensively with

work at Cedar Creek. Troy Mielke, Kally Worm, and Jim Krueger led the burning and provided total logistical support throughout this work. Dan Bahauddin and Susan Barrott supported automated weighing and other information systems. For chapter 2, Troy Mielke measured fire spread in 1997-1999 and worked with Joe Fargione to measure fire temperature in 2000; Jane Cowles, Mara Sagedahl, Christine Carroll, Laura Jaskiewicz, Jacob Miller and many others helped with later fire temperature measurements. For chapter 3, Cindy Buschena planted the oak trees; Peter Reich guided the 2003-2006 tree surveys; Traci Bartz, Jane Cowles, Jon Anderson, Richelle Caya, Stephanie Wolf, Michael Merriman, Andy Richter, Craig See, Joe Wentzel, Andrew Brandstetter, Whitney Thesing, Andrew Dechaine, Mark Saxhaug, Courtney Jones, Jon Jungers, Margaret Kosmala, and others helped in the field and the lab. For chapter 4, David Tilman and Jennifer Powers established the warming sub-experiment within the Big Biodiversity experiment. Troy Mielke designed the heating apparatus and co-ordinated this experiment; Heather Whittington also contributed to establishing it. Brian Woolums made a major contribution by helping me full-time to establish the seedling and seed additions; many others also helped with the seedling experiment in the field and lab, including Jacob Miller, Jake Thiry, Karen Gerding, Megan Horst, Praseuth Yang, Jane Cowles, Courtney Jones, Samantha Peterson, Whitney Thesing, Megan Potter, Alecia Mienert, Richelle Caya, Nicki Adams, Cedar Schimke, Jon Anderson, Andrew Dechaine, Haley Highmark, Ross Schumacher, Stephanie Erlandson, Ellen Squires, Bridget Steele, Meg Elsen, Joe Wenzel, Kelsey Flathers, Caiti Langer, Craig See, Alex Plattes, Sinafik Mengistu, Krysta Overlander, Jamie Mosel, Jennifer Stogner, James Zahler, Laura Messman, Norris Wang, Nick Voss, Bridget Steele, Ellen Squires, Gerald Schneider, Amanda Galinaux, Amelia Sancilio, Katrina Freund, Michael Lowry, Brittney Larson, Colleen Unsworth, Mike Wragg, Jane Wragg, Belinda Befort, Ben Lowe, Xiao Yi, Mary Spivey, LuAnn Marotte, Chris Buyarski, Cindy Buschena, Xiaojing Wei, and Beth Fallon. Cathleen McFadden analyzed soil nitrate at the University of Nebraska Ecosystems Analysis Laboratory.

For Chapter 5, I established two experiments in the context of the global Nutrient Network collaboration. Ralph Katzwinkel (Summerveld Equine Hospital) and several staff at Mondi Shanduka Newsprint (Gilboa Estate) supported having these experiments on their private land. Jane and Mike Wragg (my parents) applied the nutrient treatments and maintained the fences. Kevin Kirkman, Nicole Hagenah, Terry Everson, Craig Morris, Martin Hampton, Alison Young, and Steven Johnson, all at the University of KwaZulu-Natal, helped with materials, equipment, and lab facilities. Jane and Mike Wragg, Johannes Ncgobo, Sarah Collocott, Seth Hakizimana, Thomas Habanabakize, Kirsten Patrick, Caroline Bell, Lynette Bingham, Stuart Hall, Mark Taylor and others, including volunteers from the Custodians of Rare and Endangered Wildplants Highway group, helped establish and sample the experiments. Trevor Edwards, Christina Curry, Kathleen Gordon-Gray, Kevin Kirkman, and Craig Morris helped identify plant species. These experiments followed the Nutrient Network design established by Elizabeth Borer, Stanley Harpole, Peter Adler, John Orrock, Eric Seabloom and Melinda Smith; I was inspired to establish them by conversations with Kevin Kirkman, Richard Fynn, Melinda Smith, Elizabeth Borer and Eric Seabloom. Standardized Network-wide soil analyses were co-ordinated by Lydia O'Halloran and Missy Rudeen. Eric Lind managed Network data. Elsa Cleland extracted roots from soil samples.

David Tilman, my adviser, provided extensive advice on research design, methods, analyses and writing. David Tilman and my dissertation committee – Jeannine Cavender-Bares, Sarah Hobbie, and Eric Seabloom – gave me hugely helpful comments on this dissertation; Jane Cowles, Amber Eule-Noshoba, Laura Williams, and Joanna Swanson helped improve particular chapters. Adam Clark and Jane Cowles helped with analyses. This work has also been shaped by discussing it with Daniel Nidzgorski, Margaret Kosmala, Sasha Wright, Forest Isbell, Seth Binder, Rachel Putnam, Clare Kazanski, Daniel Stanton, Jane Catford, University of California Santa Barbara Plant Ecology seminar participants, and Carla Staver.

For extensive administrative support during my doctoral program, I thank Lisa Wiggins, Sue Wenker, Julia Knoll, Belinda Befort, and Kate Thompson. Andrew Simons, Sarah Hobbie, Ruth Shaw, and Jacques Finlay supported me during their terms as Director of Graduate Studies in the Ecology, Evolution, and Behavior Graduate Program.

I thank all of these people for their unstinting support, often going far beyond reasonable expectations to help after hours or in challenging conditions.

Indirect contributions

While writing, I have relied particularly for support on Belinda Befort, Ali Swanson, Xiaojing Wei, Jane Cowles, Amber Eule-Nashoba, Adam Clark, Laura Williams, Kate Thompson, Siddharth Iyengar, Mona Ebrish, Joanna Swanson, and Katherine Wheeler.

I thank my teachers, too many to name – everyone who has interacted with me has helped to shape me and thus this work: my doctoral adviser, David Tilman, who points always towards questions that matter and has supported me unreservedly; my final dissertation committee, Jeannine Cavender-Bares, Sarah Hobbie, and Eric Seabloom; additional members of my earlier temporary committee, Ruth Shaw, George Weiblen, and Peter Reich; and many other colleagues who I have talked with as this dissertation formed – among those not yet mentioned are Peter Hawthorne, Christian Balzer, Jessica Savage, Tim Whitfeld, Nick Deacon, Gina Quiram, Emme Bruns, William Eddy, Emily Mohl, Matt Dufort, Nick Fisichelli, Matt Burgess, Charlotte Riggs, Amy Kendig, Nicholas Goldsmith, Kevin Mueller, Jake Grossman, Christine O'Connell, Aaron David, Madhav Thakur, and Elizabeth Kleynhans.

Formative interactions during my undergraduate studies at the University of KwaZulu-Natal (UKZN) included those with Steven Johnson, my BSc (Honours) project adviser; David Ward, who first encouraged me to broaden my horizons beyond my home country of South Africa; and Mike Lawes and Kevin Kirkman, who emphasized the value of brevity (though I'm still working on that). Meeting with Craig

Morris and Richard Fynn was pivotal. I contacted them with questions about grassland management during my high school years: they showed me the long-term burning and mowing experiment at the UKZN Ukulinga Research Farm, encouraged me to study plant ecology to answer my questions, pointed me to David Tilman's books, and engaged me in research projects once I started studying at UKZN. They and Kevin Kirkman introduced me to international collaboration by inviting me to contribute to a project with Melinda Smith, Alan Knapp, and Scott Collins that compared South African and North American grasslands. My interest in grassland plant floristics and conservation, sparked by *Gladiolus inandensis* flowers irrupting from fire-blackened grassland adjacent to my high school home that I learned were narrowly endemic from my mother's wildflower guide, was fanned by Christina Curry and Trevor Edwards of Bews Herbarium at UKZN, Richard Boon, David Styles, Rob Scott-Shaw, and Debbie Jewitt. I could trace such influences more inclusively, or further back in time, but I must stop somewhere; I thank my parents for getting me into nature and all my other teachers, academic and personal.

Dedication

To my teachers.

Abstract

Grasslands and savannas harbor immense plant biodiversity. This biodiversity is threatened by land transformation. Moreover, the biodiversity of even intact ecosystems is affected by widespread human impacts on environmental factors – particularly resources, fire, and climate – that underlie interactions among plant species and structure plant communities. This dissertation examines how plants interact by affecting, and responding to, their environment. Much research has examined how plants interact by depleting shared limiting resources such as nitrogen (i.e., resource competition), and how increasing nitrogen deposition causes biodiversity loss; this research has been mainly north-temperate. To widen understanding of this issue, I examined the roles of multiple nutrients in structuring endemic-rich grasslands of conservation concern on highly weathered soils in South Africa, as part of the globally distributed Nutrient Network experiment. It is less clear how plants interact through fire and microclimate. I investigated how grasses differ from other herbaceous plants (forbs) in their effects on fire behavior and resource availability, and how fire and resources in turn affected savanna tree establishment, in a 16-year biodiversity experiment. I also investigated how plant diversity and composition interacted with experimentally simulated global warming to determine microclimate and resource availability, and how microclimate and resource availability in turn affected seedling establishment of 32 savanna herb and tree species. This research on the multiple ways in which plants interact by influencing their environment sheds light on how human actions alter grassland and savanna biodiversity.

Table of Contents

List of Tables

List of Figures

Chapter 1 : Introduction

Grassland plant biodiversity is threatened by human actions that alter environmental conditions – fire, climate, nutrient availability, herbivore abundance, and others – that determine biodiversity. Tradeoffs in how plant species respond to resource availability and stress cause different species to be favored under contrasting environmental conditions. These tradeoffs in response contribute to large scale differences in vegetation structure, including biome boundaries across climatic gradients (Whittaker 1975), as well as to small-scale variation in species composition and diversity (Tilman 1988, Chesson 2000). Thus, human actions that change environmental conditions underlying tradeoffs that structure plant communities can profoundly alter those communities.

However, species not only *respond* to their environment, but they also *affect* it in ways that can, in turn, alter their abundance and those of other species (Hobbie 1992). This is appreciated in some formulations of the niche concept (Elton 1927, Chase and Leibold 2003) and especially in terms of resource competition, whereby species interact through their effects on, as well as responses to, shared limiting resources (Tilman 1982). Interaction through shared predators (apparent competition) has also been long appreciated (Holt *et al.* 1994). Nonetheless, the different ways in which plants alter their environment and thus affect vegetation dynamics remains an area of expanding research. The role of facilitation by ameliorating microclimate or increasing a limiting resource (e.g., N fixation by legumes) is increasingly appreciated (Bruno *et al.* 2003). The boundaries between tree- and herb-dominated biomes in regions climatically suitable for either are increasingly understood to be partly determined by how trees and herbs differ in their effects on fire behavior, as well as how they respond to fire regime (Grimm 1983, Bond *et al.* 2005, Sankaran *et al.* 2005, Staver and Levin 2012).

Differences in how species impact their environment can result in ecosystem properties -- environmental conditions – different than might be expected from the separate effects of the species in that ecosystem. Biodiversity-ecosystem functioning research (Tilman *et al.* 2014) seeks to understand how the diversity, abundances and characteristics of species in an ecosystem jointly determine ecosystem properties such as net primary productivity. Such research has emphasized the responses of productivity, as well as availability of resources such as nitrate and light. Both competition (e.g., greater resource depletion in more diverse communities suppressing invasion) and facilitation (e.g., N fixation leading to positive complementarity effects of diversity on productivity) feature prominently in this field. However, it is unclear how species diversity and composition jointly influence fire behavior, another environmental factor that can be an important determinant of vegetation structure.

In this dissertation, I investigated how impacts of plants on their environment interact with human impacts on the same environmental factors, to better understand and predict how human-caused environmental change alters plant biodiversity (Fig. 1-1).

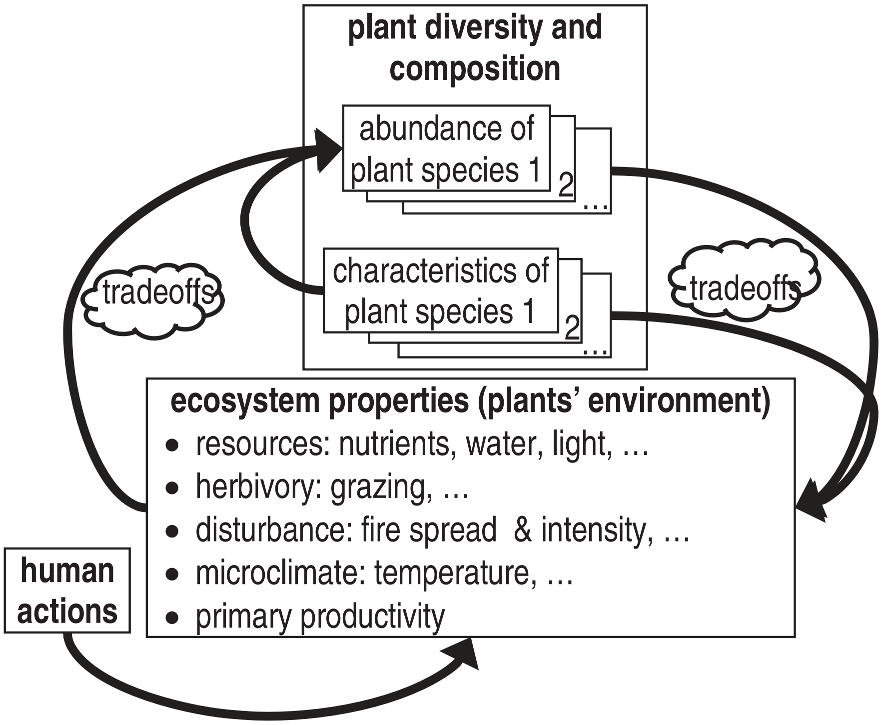
I investigated how herbaceous species diversity and composition influenced fire behavior (chapter 2), and how this combined with resource competition to influence savanna tree abundance (chapter 3). The clearest example of plants interacting through their fire environment is positive feedbacks between herbaceous plant abundance and fire that can lead to flammable grassland or savanna, compared with positive feedbacks between tree abundance and reduced fire that, it has been proposed, can lead to relatively non-flammable forest as an alternative stable state (Grimm 1983, D'Antonio and Vitousek 1992, Staver *et al.* 2011). Grasses and forbs (non-graminoid herbs) have typically not been distinguished in these studies, yet grasslands vary strongly in the relative abundances of grasses and forbs; moreover, there is reason to suspect that grasses are more flammable than forbs but this has apparently not been rigorously tested in the field. Therefore, I tested whether grasses and forbs lead to different fire behavior (chapter 4), and examined how this combines with resource competition to determine the effects of grasses and forbs on savanna tree growth over 16 years (chapter 5). I worked in the longest-running biodiversity experiment, established in 1994 at Cedar Creek Ecosystem Science Reserve, Minnesota. By controlling species diversity and composition at relatively large spatial scales (81 m^2 plots) over 20 years with high replication, this experiment provided a unique opportunity to examine how herbaceous diversity and composition -- and productivity, which they strongly determine -- influenced fire behavior and savanna tree establishment, which have not previously been reported from this experiment.

Interactions among plants that are mediated by resources, fire, or microclimate may be altered if global warming alters these environmental factors, either directly or via changes in species composition, or if species' responses to and impacts on these environmental factors are temperature-dependent (Tilman 2004). In chapter 4, I investigated whether and how warming altered the interactions among plant species, and thus their relative abundances, in warming treatments superimposed on the same biodiversity experiment (Whittington *et al.* 2013). I examined how seedlings responded to the joint effects of plant diversity and composition and warming, and how these effects were mediated by microclimate, resource availability, and fire behavior. This biodiversity experiment, which has spearheaded empirical biodiversity-ecosystem function research, previously yielded insight into how resources mediate interactions among plants. Chapters 2-4 build on this to additionally examine how species interact through fire behavior and microclimate.

Nutrient availability and herbivore abundance are widely impacted by human actions. Agriculture and burning of fossil fuels have more than doubled the availability of nitrogen to plants worldwide (Vitousek *et al.* 1997b); anthropogenic N deposition occurs even on lands not directly touched by humans. Large native grazing animals have been lost from many grasslands, causing grazing intensity to be either reduced or, where domestic grazers such as cattle have replaced them, greatly increased (Mack and Thompson 1982). To investigate how changes in nutrient availability and herbivore abundance alter diversity and the abundances of plant functional types, I manipulated these environmental factors at two sites in South Africa (chapter 5). These experiments both illuminated the roles of multiple soil nutrients in structuring two grassland types that are of conservation concern (the focus of chapter 5) and – with dozens of similar experiments around the world that together form the distributed Nutrient Network experiment (Borer *et al.* 2014a) – shed light on patterns and processes in grasslands globally, including diversity-productivity relationships (Adler *et al.* 2011), exotic plant species' abundance (Seabloom *et al.* 2013) and response to nutrient enrichment and herbivory (Seabloom *et al.* 2015), response of plant diversity to nutrient enrichment and herbivory (Borer *et al.* 2014b), tradeoffs between species' responses to nutrients and herbivory (Lind *et al.* 2013), microbial community structure (Prober *et al.* 2015), and responses of plant productivity to N deposition (Stevens *et al.* 2015) and multiple nutrient additions (Fay *et al.* 2015).

This dissertation contributes to a more nuanced understanding of how plants interact by affecting and responding to multiple environmental factors -- fire behavior and microclimate, as well as resources -- with implications for how human actions that affect these environmental factors will alter grassland biodiversity.

Figure 1-1: Research framework.
Human actions that alter ecosystem properties (environmental factors) can change plant biodiversity both directly and through feedbacks from biodiversity to ecosystem properties.



Chapter 2 : Forbs, grasses, and grassland fire behavior

Summary

Fire is a major determinant of the distribution of the world's herbaceous-dominated biomes, and of species composition and nutrient cycling within those biomes. Fire spread and intensity, and thus impact on ecosystems, depends on the quantity and qualities of fuel formed by the plant species in an ecosystem. Whereas herb-tree differences in plant quality effects on fire are widely appreciated as contributing to positive feedbacks between herbaceous vegetation and fire that could determine the boundaries between grassland, savanna and forest, little is known of how herbaceous plant types differ in their quantity-independent (quality) effects on fire. We asked: Are forbs -- non-grass herbaceous plants, which can be abundant in grasslands and savannas -- equivalent to grasses in their per-unit-biomass influences on fire? Forbs may be less flammable than grasses because forbs tend to be broader- and shorter-leaved, and in some systems drop their leaves earlier to form compact litter on the soil surface, compared with grasses that retain leaves as an aerated, continuous bed of fine fuel. We addressed this question using using 315 annually ignited grassland plots in a 20-year biodiversity experiment, in which plant biomass was dependent on experimentally imposed species diversity and composition treatments.

Grass abundance promoted fire spread area more strongly than did non-grass abundance. For fire to spread through 50% of the plot area required approximately 120 g/m^2 of biomass in grass-only plots versus 210 g/m^2 of biomass in forb-only plots, measured at the time of peak biomass in late summer. Relative to an equivalent abundance of forbs, grasses also resulted in fires that were both more intense, advancing faster and having higher rates of heat release per unit fireline length, and more severe, causing more damage to aboveground plant tissue. Heat release was more upward-distributed in grass-dominated plots: flame lengths were longer and peak temperatures were higher at 50 cm above ground. At this height the temperature was about 160 C in plots with only grasses -- twice the temperature in plots with only forbs, for equal biomass of 300 g/m^2. In contrast, the temperature at ground level per unit biomass and the rate of heat release per unit area were not significantly influenced by composition, apparently because the flaming zone extended over a larger area in grass-dominated than in forb-dominated plots.

Forbs and grasses are not equivalent with respect to their quantity-independent effects on fire: grass promotes fire more strongly per unit mass, so their relative abundance likely alters the effect of fire on plant demography and nutrient fluxes. Considering grasses and forbs separately may improve understanding of shifts in fire-mediated biome boundaries and the dynamics of herbaceous vegetation, including long-term responses to anthropogenic nitrogen deposition that can increase the ratio of grasses to forbs.

Introduction

Fire regime can determine the boundary between grasslands or savannas and forest (Staver *et al.* 2011). In grasslands and savannas, fire regime is frequently a major determinant of species composition (Fynn *et al.* 2005, Cavender-Bares and Reich 2012) and diversity (Uys *et al.* 2004, Kirkman *et al.* 2014), and of nutrient and carbon cycling (Neary *et al.* 1999). Fire regimes are in turn driven by both ignitions and the quantity and characteristics of plant fuel (Archibald *et al.* 2009). Higher fuel loads, i.e., higher quantities of fuel, promote fire spread and intensity (Byram 1959). Finer fuels burn more readily, and thus promote fire spread more strongly per unit fuel quantity. Herbaceous fuels (grasses and forbs) are finer than woody fuels (trees and shrubs), and their distinct impacts on fire spread are pivotal to the distribution of grassland, savannas and forest. However, herbaceous fuels are typically considered homogeneous: either as a fixed state (herbaceous and flammable, versus woody and non-flammable), or as varying in fuel quantity but not quality. Yet, herbaceous vegetation is a heterogeneous mix of species that vary in traits that may influence fire behavior. Our over-arching question is: does variation in fuel characteristics associated with variation in herbaceous species composition, independent of fuel quantity, drive differences in fire behavior that could influence fire-mediated shifts in biome distributions and grassland species composition and nutrient cycling?

The extent of fire in a landscape depends both on ignitions and the area of fire spread resulting from each ignition, which in turn depends on both fuel and weather. In many landscapes, fire extent is limited more strongly by the quantity and characteristics of plant fuel than by the frequency and timing of ignitions (Archibald *et al.* 2009). Whereas fire frequency often refers to the frequency with which a parcel of land is ignited, it is also ecologically relevant to know how frequently any given point within a parcel burns. We refer to this as point fire frequency; it depends both on ignition frequency and subsequent fire spread. Both fire intensity and fire severity also depend both on fuel quantity and characteristics and on weather and ignition type (Cheney *et al.* 1993). For small fires, intensity tends to be higher with line ignitions than point ignitions. Fire intensity is strictly defined as the rates of heat release per unit fireline length or per unit area (fireline intensity and reaction intensity, respectively (Byram 1959)) but also refers to correlated measures such as fire temperature and flame length. Fire severity is the direct impact of fire on ecosystems. Thus, our first step in investigating the influence of plant composition on fire behavior was to characterize the influence of fuel versus ignition and weather.

The primary fuel for grassland and savanna surface fires is aboveground herbaceous vegetation. Increasing quantities of fuel promote increased fire spread, intensity, and severity (Cheney *et al.* 1993, D'Antonio 2000). The quantity of herbaceous biomass, and thus fuel, is influenced by herbaceous productivity and rates of loss to herbivory and decomposition. Herbaceous productivity and loss rates, in turn, depend on external factors including climate, soil parent material, and topography; but also on species richness and composition. Biodiversity experiments in herbaceous systems, such as the one we report here, have shown that biomass also increases strongly with increasing planted species richness (Tilman *et al.* 1996), and with the presence of particular species and functional types. The presence of C4 grasses and legumes strongly increased herbaceous productivity in this experiment. C4 grasses have low tissue N so in N-limited systems like this they can produce more biomass per unit area than can C3 plants with higher tissue N levels (Tilman *et al.* 1997). Similarly, legumes can increase N-limited productivity through N fixation (Knops *et al.* 2000). C4 grasses have low N and high lignin content that inhibits decomposition and herbivory, potentially further increasing fuel load (Ehleringer and Monson 1993, Masubelele *et al.* 2007).

Biomass is our principal measure of plant abundance and fuel quantity, as it is in many studies of fire behavior. We also consider cover, which is often used in studies of fire spread, especially in sparse or patchy landscapes. In general, cover may be the better proxy for fuel connectivity and thus the stronger determinant of whether fire spreads, whereas biomass may be the better proxy for potential energy release and thus be more closely related to fire intensity. However, in the sparse, nutrient-limited grasslands of this experiment, cover rarely saturated and was approximately linearly related to biomass, so we treat them as approximately equivalent measures of plant abundance. Thus, our second step was to characterize the influence of fuel quantity — measured as total herbaceous biomass or cover — on fire behavior.

The effects of fuel quantity on fire behavior provide the context to ask whether characteristics of the herbaceous vegetation — species richness and composition — influence fire behavior in important ways, independent of its quantity.

Herbaceous species richness could influence fire behavior independent of its effect on productivity if structural diversity imparted by different plant species alters the structure of fuel, or if the most flammable species disproportionately influence overall flammability (de Magalhães and Schwilk 2012) and are more likely to be included in higher diversity mixtures by chance (Loreau and Hector 2001). Therefore, we tested for a biomass-independent effect of planted species richness on fire behavior.

Herbaceous species composition could also influence fire behavior independent of effects on productivity. We focus on grasses versus forbs (i.e., non-grass herbaceous plants) because differences in their structure lead us to expect that grasses promote fire more strongly per unit abundance (biomass or cover) than do forbs: most grass species have narrower (finer) leaves which should dry and ignite more readily, and longer leaves that may increase fuel connectivity, compared with leaves of many forb species. Moreover, forbs -- at least in our study system -- tend to drop their leaves in winter, resulting in a litter layer on the soil surface with little aeration that does not promote fire, compared with grasses that tend to retain their leaves and form a well-aerated bed of fine fuel.

Forbs are abundant in many grasslands and savannas (Peterson *et al.* 2007), and even co-dominate or dominate in systems including calcareous grasslands (Willems and van Nieuwstadt 1996), old fields (Tilman 1987), tallgrass prairie (Fuhlendorf and Engle 2004) and montane meadows (Harte and Shaw

1995). Yet, despite their abundance and potentially distinct effects compared with grasses, the impact of forbs on grassland and savanna fire behavior and potentially fire regime has received little explicit attention. If forbs and grass influences on fire are distinct, this could be important in three contexts.

First, grass-fire positive feedback cycles can occur when grasses invade forest and, through increasing fire frequency and intensity, which in turn increases their abundance, displace forest (D'Antonio and Vitousek 1992, D'Antonio 2000, Brooks *et al.* 2004). This feedback can also maintain grassland or savanna as alternate stable states in areas climatically suitable for forest (Bond *et al.* 2005, Staver *et al.* 2011). When this feedback is interrupted, for example through fire suppression, the system can switch to forest and then remain stably in that state as forest inhibits fire (Titshall *et al.* 2000, Hirota *et al.* 2011). Forbs are rarely mentioned in the grass-fire and tree-grass literatures, perhaps being assumed equivalent to grasses in their effects. For example, a review of Dynamic Global Vegetation Models used to predict shifts in the boundaries of grassland, savanna and forest under global climate change, which highlighted the importance of grass-fire feedbacks for these boundary dynamics, lumped grasses and forbs under “grasses”(Baudena *et al.* 2015). But is lumping forbs and grasses justified? Or if forbs have a distinct effect, could distinguishing them improve understanding and prediction of boundary dynamics? For example, basic soils rich in cations and clay promote forb dominance (Tilman 1982, Tilman *et al.* 1994, Willems and van Nieuwstadt 1996); if fire intensity is lower where forbs dominate, could this help explain why such soils are more likely to be forested than more acid, sandy soils that favor grasses? Biome boundary dynamics could be sensitive to such influences of fuel on fire behavior. It has been proposed that fire spread is a percolation process with a threshold abundance of flammable fuel at which the probability of fire spread switches sharply between zero and one (Schertzer *et al.* 2014), and that this can make alternate stable states more likely and switches between states more abrupt and less easily reversed. If the relative abundance of grasses versus forbs alters such a percolation threshold this could influence biome boundaries.

Second, fire regime can profoundly alter the herbaceous species composition of grasslands and savannas. If the species favored by frequent fire in turn promote fire, this could lead to positive feedbacks analogous to those between trees and herbaceous plants. For example, if grasses promote fire and fire in turn promotes grasses, relative to forbs, this could create grass-dominated and forb-dominated alternative stable states. Fire does not appear to favor grasses over forbs in general (Kirkman *et al.* 2014), but in some systems it does (Collins 1987, Gibson and Hulbert 1987). Thus, understanding whether grasses and forbs have substantially distinct effects on fire would shed light on the possibility of such feedbacks.

Third, fire can volatilize nutrients and also alter other nutrient fluxes to and from vegetation and soil through changes in species composition and structure (Peterson and Reich 2001, Reich *et al.* 2001). Distinct influences of forbs and grasses on fire would have implications for understanding these ecosystem processes.

Therefore, we tested the hypothesis that grasses promote fire spread and intensity more strongly than forbs.

In addition, we asked whether finer subdivisions of plant types, beyond grasses and forbs, further influence fuel characteristics and explain additional variation in fire behavior. It is most often C4 grasses, rather than C3 grasses, that are implicated in contemporary fire-grass cycles through which grassland invades forest (D'Antonio and Vitousek 1992, Brooks *et al.* 2004) and in the expansion of grassland into forest in the Miocene (Scheiter *et al.* 2012). However, it is not clear whether this is because C4 grasses result in higher fuel loads through increased productivity and lower rates of decomposition and herbivory, or because they differ in the per-mass characteristics of the fuel they produce. C4 grasses may promote spring fire more strongly than C3 grasses per unit biomass if C4 grasses are killed aboveground by cold, and therefore have lower moisture content during spring burns, while C3 grasses retain living, green tissue overwinter (Ripley *et al.* 2010). Legumes may promote fire more than non-legume forbs per unit of aboveground biomass, if the typically compound, dissected leaves of legumes more readily dry and heat to ignition point. More finely still, do individual species differ in how strongly they promote fire per unit biomass, and if so are their effects associated with their traits? We present species' effects on fire in relation to the ratio of leaf length to width, a readily measured trait that may capture one aspect of fuel fineness and flammability, but we do not aim to elucidate which traits drive differences in flammability among species.

Here we assess the influence of herbaceous fuel quantity and quality by measuring fire behavior in a long-running, highly replicated grassland biodiversity experiment, where species diversity and composition treatments created profound gradients in plant abundance and species composition (including the ratio of grasses to forbs). We measured three complementary aspects of fire behavior in replicate plots. a) Fire spread area, the extent of area burned following one ignition, which is inversely related to point fire frequency, and fire spread distance. b) Fire intensity, both in the strict sense of rates of heat release per unit fireline length or per unit area (fireline intensity and reaction intensity) and associated measures, temperature at two heights, flame length, and rate of advance. c) Fire severity, the direct impact of fire on ecosystems, which we assessed using fire damage to plants.

We ask 1) What is the role of fuel (i.e., the properties of a plot, principally fuel quantity and characteristics) versus other factors such as weather or ignition pattern in determining fire behavior? 2) How does the quantity of herbaceous fuel, i.e., total herbaceous biomass or cover, influence fire behavior? 3) Does herbaceous species richness influence fire behavior, independent of the quantity of herbaceous biomass? 4) Do grasses and forbs influence fire behavior differently, per unit abundance? We also ask, with less power due to the distribution of species' abundances in this experiment (see Discussion), 5) Do C4 versus C3 grasses, or legumes versus non-legume forbs, influence fire behavior differently per unit abundance? 6) Within the grass and forb categories, do herbaceous species differ in how they influence fire behavior per unit abundance?

Independently-burned plots in this experiment provided extraordinary replication through space and time with which to assess the influence of plant communities on fire behavior while holding constant ignition (annual) and randomizing across both the limited environmental variation that existed when the experiment was established and minute-to-minute variation in weather between the times that different plots were burned.

Methods

Experimental design

We addressed our questions in the Big Biodiversity experiment (experiment number E120) at Cedar Creek Ecosystem Science Reserve, Minnesota, U.S.A. (Tilman *et al.* 1997). This experiment was established in 1994 using dominant and common perennial species of nearby remnant tallgrass prairie oak savanna, the predominant vegetation of this region before European settlement. Fire scar records indicate that fire was approximately annual before European settlement ((Pierce 1954), cited by (Tilman *et al.* 2000)); a long-term experiment at this site indicates that burning less than every 3 years allows the tree canopy to close (Peterson and Reich 2001). Lightning starts fires most often late in the summer growing season in this region, but most fires in a nearby oak savanna occurred when trees were dormant over the past 200 years, even before European settlement, suggesting that ignitions by people have long been important (Wolf 2004). It is not clear whether these dormant season fires occurred in the late fall, winter, or early spring.

342 plots were established in a grid, each 9 x 9 m, separated by mown aisles at least 4 m wide. Of these plots, 154 core plots were assigned random draws of 1, 2, 4, 8 or 16 species from a pool of 18 species (listed in Fig. 2-6) (Tilman *et al.* 2001) and maintained by weeding through 2014. We focused on these plots; the only two woody species (*Quercus* spp.) were always rare in these plots and were removed in 2010, prior to collecting most of the data used in the present study. Additional plots were planted with 1, 2, 4, 8, 16 or 32 species from an expanded pool of 32 species (Tilman *et al.* 1997); for the years in which they were also sampled (until 2000), we combined these plots with the core plots for a total of up to 315 plots in our analyses.

Fire conditions

The experiment was burned every spring, as soon as possible after the snow that blankets the ground most winters melted and while vegetation was still dormant; burn dates ranged from 25 March until 28 April in the years that fire behavior was measured, 1997-2000 and 2010-2014.

Until 2006, the experiment was burned as one block by first establishing blacklines along the downwind edges and then igniting headfires in the upwind plots and allowing fire to jump from plot to plot. Burning the whole experiment as a block took about two hours. From 2007 onwards, each plot was ignited independently along its upwind edge using a driptorch: rows of plots were ignited sequentially, starting with the down-wind row and working up-wind. Burning the full grid of plots independently in this way took about four hours.

The plots could have been correlated in their fire behavior because fire sometimes spread from one plot to its neighbors and, for safety, plots at the edge of the grid were sometimes ignited around all edges instead of only the upwind edge. However, the spatial positions of plots did not significantly influence the results, which suggests that the plots were effectively independent experimental units. There was no evident spatial pattern in the fire behavior response measures when plotted against their spatial co-ordinates. Nor was there significant spatial autocorrelation in the residuals from our central multiple regressions of fire behavior response measures against grass and forb biomass (Mantel tests for correlation between differences in residuals and distances in space between plots: $P > 0.15$, mostly much larger). Moreover, the diversity and composition treatments were randomly allocated to plots, so any correlation among plots in fire behavior would not lead to spurious associations with the treatments.

Mean air temperatures, recorded hourly and averaged over the duration of each burn, ranged from 8.5-18.4 °C in 1997-1999, and 0.4-24.3 °C in 2010-2014. Similarly-calculated mean relative humidity ranged from 21-32% in 2010-2014; mean wind speeds ranged from 2.7-4.3 m/s in 1997-1999, and 1.5-3.9 m/s in 2010-2014. We mostly use averages for these two sets of years, but we also present year-specific data for 2000, 2011 and 2013; in these burns, respective mean air temperatures were 14.7 °C, 18.0 °C and 24.3 °C, and mean wind speeds were 1.9, 2.9 and 2.8 m/s. Mean relative humidity in 2011 was 32%, and in 2013 was about 34%. Weather was measured about 1 km from the experiment (Cedar Creek dataset E080), except that in 2013 humidity was measured at a comparable weather station 8 km away.

Fire behavior

We characterized fire behavior in two broad ways: 1) the areal and linear extent of fire spread; and 2) the characteristics of the fire's behavior within the area that it burned.

First, we visually estimated fire spread area as the percent of each plot that burned following a single ignition in 2010, 2011, 2013 and 2014. Each year, plots that had not burned completely following one ignition were re-ignited; this modestly increased the proportion burned in some plots, though many remained incompletely burned. Fire spread area was estimated following any re-ignitions each year 1997-1999 and 2010-2014. (I.e., in 2010, 2011, 2013 and 2014 fire spread area was estimated both following one ignition and again after any re-ignitions.) We supplemented areal fire spread with estimates of linear fire spread. Each year 2010-2014, we inferred from post-burn photographs whether fire had spread a distance of at least 7 m following one ignition, from each plot's upwind ignited edge to a line 7 m downwind. We used a 7 m criterion to avoid interference from back-burning that occasionally occurred in the downwind 2 m of the 9 m square plots when fire back-burned across aisles. We validated our photographic inferences using video of fire spread through subsets of 23 plots in 2010, 13 plots in 2011, and 69 plots in 2013; photographic inferences were correct in all cases.

Second, we measured fire temperature as our main metric of fire behavior within burned areas. Fire temperature was estimated using metal tags — pyrometers — with Omega Laq paints (Omega Engineering, Stamford, CT) of varying melting points placed in each plot before burning (Wally *et al.* 2006). In 2000, 9 paints with melting points spanning 70-650 °C were spotted on aluminum tags wrapped in aluminum foil and clipped 10 cm above the ground to reinforcing bars at each corner of a 5 x 5 m square centered in each of 176 plots. In 2011, 13 paints spanning 79-788 °C were spotted on copper plant tags (National Band and Metal, Newport, KY), covered by a second tag, and placed at ground level and 50 cm above ground at three of the four locations used in 2000 in each of the 154 core plots. We used identical tags in subsets of 100 plots in 2010 and 90 plots in 2014. We verified paints' rated melting points by placing sample tags in a calibrated muffle furnace for one minute at increasing temperatures, and scored paints by comparing them with reference images of melted and unmelted paints using a dissecting microscope. Tags were assigned the melting temperature of the highest melted paint; when no paints were melted, they were assigned 20 °C to approximate ambient temperature. We calculated the median fire temperature at each height for each plot in each year.

In addition to the fire temperature measurements, we measured other characteristics of flame fronts known to influence plants. As fire spread through the marked central 5 x 5 m of each plot, we measured flame height and angle, flaming zone depth, and the time required for the flame front to advance 5 m. The locations and heights of these bars provided dimensional references for the measurements, which we made on digital video recordings of 63 plots in 2013. We used these measurements to calculate flame length and forward rate of spread. We calculated fireline intensity, the rate of energy release per unit length of fire front (Byram 1959), by multiplying the forward rate of spread by the fuel load (approximated as biomass per unit area late the previous summer) and the approximate heat of combustion 20 MJ/kg (Williams *et al.* 1998, van Wagtendonk 2006). We assumed the heat of combustion to be constant across species compositions because it varies very little among herbaceous species (Byram 1959, Kidnie 2009). We calculated reaction intensity per unit area by dividing fireline intensity by the flaming zone depth (i.e., the distance from the flame front to the back of the flame zone, measured perpendicular to the flame front).

Fire severity

As a common biotic index of fire severity, we measured fire damage of similarly sized individuals of two plant species also used for other studies in a subset of plots that spanned the diversity gradient. First, we planted and marked 12 seeds of *Quercus macrocarpa*, the dominant tree in bur oak savanna at this site, at systematic locations in each of 32 plots after the spring 2010 burn, and assessed how the 372 resulting seedlings were damaged by the spring 2011 burn. Second, we marked 99 haphazardly selected reproductive plants of the forb *Liatris aspera* in 37 plots in fall 2013, and assessed how they were damaged by the spring 2014 burn.

Plant abundance and fuel load

The climate is continental, and most growth is during the summer from May until August. Cold winters, largely below freezing, inhibit decomposition and herbivory, so we used plant cover and biomass measured in late summer to represent the fuel for spring fires. Each July from 1996-1999, percent cover of each species was estimated using four 1 m^2 quadrats in each plot. These estimates indicate absolute (not relative) abundance: plants, bare ground and litter together summed to 100%. Total dry plant biomass, excluding litter from previous years, was measured simultaneously by clipping strips (Tilman *et al.* 2006); in addition to herbaceous biomass, this included biomass of *Quercus* spp. trees, but woody biomass was negligible (the mean and maximum relative woody cover was 0.22% and 17%, respectively). Woody plants were removed from the experiment in 2010. Each July from 2010-2014, dry herbaceous biomass was measured using clip strips and sorted to species and previous years' litter. Litter from previous years was minimal; it was excluded from analyses, but including it did not change the results or conclusions (not shown).

To assess how well summer (July) biomass represented fuel loads in the following spring, we clipped biomass immediately before burning in spring (March) 2015 in strips adjacent to those clipped the previous summer in 30 plots ranging from grass-dominated to forb-dominated. We did not separate previous-season biomass from older litter in the spring samples. Spring herbaceous biomass, i.e. fuel load, was closely related to herbaceous biomass the previous summer (correlation coefficient $r = 0.87$). Moreover, a multiple regression of spring biomass versus biomass the previous summer and its interaction with the proportion grass the previous summer showed that forb-dominated plots lost slightly but not significantly more biomass (interaction $p = 0.131$). This regression, in which the intercept was set at zero, estimated that in exclusively forb plots spring biomass (i.e., fuel load) was 59 % of previous summer biomass whereas in exclusively grass plots spring biomass (fuel load) was 72 % of previous summer biomass (Fig. 2-S1).

Plant traits

To represent one aspect of fuel fineness and potentially flammability, we calculated species' leaf length:width ratios using leaf dimensions obtained at the same study site (Willis *et al.* 2010). Length and width were measured for the leaf as a whole, not leaflets or lobes.

Analyses

We assessed the effects of different plant types (grass/forbs, functional groups, species) on measures of fire behavior and severity using multiple regressions with each plant type as an independent variable. To perform two-tailed tests for whether two plant types differed in their effects on fire, we used 10,000 bootstrap samples (each drawn randomly with replacement from the data rows) to estimate the sampling distribution of the difference between the two types' coefficients. We also used these bootstrap samples to estimate 95% confidence intervals of the coefficients. Fire spread area, a percentage, was logit-transformed when used as a dependent variable to linearize its relationship with independent variables (Warton and Hui 2010). The logit transformation, $\log(p/1-p)$ where p is a proportion, is not defined for $p = 0$ (0%) or $p = 1$

(100%), so we added 0.1% to values below 50% and subtracted 0.1% from values above 50% before transformation.

Plots without fuel have zero fire spread and intensity, so we fit these regressions through the origin by omitting intercepts. For logit-transformed dependent variables, and for logistic regressions, omitting an intercept fits the regression through 0 on the logit scale, or 0.5 on the probability scale, when the independent variables are 0; to instead fit the regression through approximately 0 on the probability scale, we used the logit of 0.1% as an offset (Gelman and Hill 2007). (The logit transformation is undefined for 0; our conclusions were not sensitive to using 0.1% versus another arbitrarily small value.) For the fire temperature dependent variables, we fit lines through the ambient temperature when the independent variables were 0 by subtracting the ambient temperature from the dependent variables before fitting regressions and adding the ambient temperature to fitted values. We did not test whether plant type coefficients differed from zero because, without intercepts, non-zero coefficients could indicate that the mean of the dependent variable was non-zero rather than that the plant type's abundance explained variation in that dependent variable. Instead we tested whether coefficients differed from each other, using bootstrapping as described above.

We estimated variance components and mixed-effects regression models using reduced maximum likelihood (REML) with the R package lme4 (Bates *et al.* 2014). We performed bootstrapping using the R package boot (Canty and Ripley 2015) and calculated other statistics using standard R functions.

Results

Control of fire behavior by fuel

Relatively stable plot characteristics, principally the abundances of the (perennial) plants which changed slowly, accounted for most of the variance in fire spread area and fire temperature according to variance components analyses using random effects regressions (with only an intercept as a fixed effect). A plot random effect, representing persistent plot effects, accounted for most of the variance in fire spread area in 1997-1999 (79%) and in fire spread area following a single ignition in 2010-2014 (59%), as well as in fire temperature in 2010-2014 (62% at ground level, 61% at 50 cm above ground). Only 2-6% of the variance in these measures was attributed to a year random effect, which represents variation among years (averaging across plots) due to weather leading up to and during each burn or mean productivity the previous season. The remaining 19-35% of variance in fire spread area and temperature arose from a combination of measurement error and variation in plot effects between years, which could be due to minute-to-minute variation in weather, or year-specific variation among plots in ignition pattern or productivity.

Diversity-driven herbaceous plant abundance: Fuel quantity effects on fire behavior

Herbaceous biomass increased linearly with increasing log planted species richness treatments (Fig. 2-1C, $F_{1,152} = 156.6$, $P < 0.001$), as previously reported for this experiment (Tilman *et al.* 1997, Tilman *et al.* 2001, Reich *et al.* 2012). Accordingly, fire spread area and fire temperature and other fire response variables reported in later sections all increased similarly and significantly ($P < 0.001$) with herbaceous biomass, cover, and planted species richness. These results are from regressions with herbaceous biomass, cover, or species richness as the sole independent variable, an estimated intercept, and responses transformed as described in figure captions. Fire also responded similarly to effective species richness, e^H, where H is Shannon diversity estimated from species' relative biomass (not shown).

Following single ignitions along their upwind edges, fire spread through a larger percentage of the area of plots that had more plant biomass late the preceding summer (Fig. 2-2A, B). Plots with more than about 300 g/m^2 of biomass consistently burned completely whereas, in plots with less biomass, fire spread area varied from near zero to near complete, both in a representative year (2011, Fig. 2-2A) and averaged across multiple years (2010-2014, Fig. 2-2B). Increased fire spread area is equivalent to increased effective fire frequency at each point, or fire return interval: a given point within a plot would be expected to burn every 1/(proportion of plot that burned) years. Thus, a fire spread area of 20% is equivalent to a point fire frequency of every 5 years; a fire spread area of 100% is equivalent to an annual point fire frequency.

Fire spread area showed a similar saturating increase with total plant cover as with total biomass, averaging over the years that both abundance measures were estimated (1997-1999, Fig. 2-3A, B), consistent with an approximately linear relation between these abundance measures (Pearson's correlation coefficient $r = 0.79$). However, more plots had low biomass (< 50 g/m^2) in these earlier years (3-5 years after the experiment was established in 1994) than in 2010-2014 (Fig. 2-2B). Most of these low-biomass plots had low fire spread, and formed a lower tail of a sigmoid curve consistent with fire spread probability suddenly increasing at a threshold plant abundance (inflection point) of about 200 g/m^2 (Fig. 2-3A) or 40% cover (Fig. 2-3B).

In 1997-1999 fire spread area was estimated after incompletely burned plots had been re-ignited, so fire spread may have been modestly over-estimated at low cover compared with 2010-2014 when we report fire spread area after a single ignition of each plot. However, re-ignition increased fire spread by, on average, only 5.4% of the plot area in 2010, 2011, 2013 and 2014 when spread was estimated both before and after any re-ignitions.

As for biomass and cover, fire spread through a larger fraction of plots' areas following one ignition as planted species numbers increased from 1 to 16 (Fig. 2-1A). Averaged over four years, fire spread in monocultures ranged from less than one fifth of plot area to complete, but in 16-species plots was consistently near-complete. Mean fire spread approached completeness even in an average 4-species plot, but became more consistently complete with further increases in richness.

Whereas fire spread area saturated at 100%, fire temperature increased approximately linearly with increasing total plant biomass (Fig. 2-4A, B, Fig. 2-3C) and cover (Fig. 2-3D) late the preceding summer, and with log planted species richness (Fig. 2-1B). Fire temperatures ranged well above 300 C near ground level. Plot-level median fire temperatures were about 300 C at ground level in plots with 400 g/m^2 of biomass the previous year, near the upper limit of above-ground productivity in this experiment, in 2011 (Fig. 2-4A). Similarly, median temperatures of about 300 C were measured 10 cm above ground in plots with 400 g/m^2 of biomass (Fig. 2-3C) or a mean plant cover of about 80% (Fig. 2-3D) in 2000. Every 16-species plot had a higher median fire temperature than any monoculture.

In subsequent sections, we examine how diversity and composition of plant fuel may influence fire behavior independent of the quantity — the total abundance — of fuel. We focus on fire spread area and temperature, but also report how other measures of fire behavior are influenced by the grass-forb distinction that is our focus.

Plant diversity: Fuel quantity-independent effects on fire behavior

Planted species richness did not explain much variation in fire spread area or temperature independent of that explained by herbaceous biomass, so it does not appear that species richness influenced fuel characteristics in a major way other than by its effects on biomass. However, adding log-transformed planted species richness as an independent variable to regressions already containing herbaceous biomass as an independent variable did increase fit slightly for logit-transformed fire spread area ($P = 0.048$, adjusted R^2 increased from 0.61 to 0.62) and for fire temperature ($P < 0.001$, adjusted R^2 increased from 0.84 to 0.88). In both cases, the log-transformed number of planted species had a positive effect, controlling for biomass the previous summer.

Grass and forb relative abundance: Fuel quantity-independent effects on fire spread

Fire spread area following one ignition increased more strongly with grass biomass than with forb biomass in the 154 core plots, in multiple regressions forced through the origin (bootstrap test of equality of the coefficients of the grass biomass and forb biomass independent variables: $P = 0.036$ in 2011, $P = 0.012$ for 2010-2014 mean values). Fig. 2-2A,B represent these multiple regressions by plotting predicted values for plots containing only grasses (red lines) or only forbs (blue lines): a given fire spread was attained at a lower total biomass if that biomass was entirely grasses than if it was entirely forbs. Plots with both grasses and forbs should fall between these two extreme (grass-only or forb-only) compositions. Fig. 2-6A shows the coefficients of the same multiple regression, for 2010-2014. Fire spread area was logit-transformed and regressions were forced through the origin, so larger coefficients indicate both steeper slopes of fitted lines at their inflection points (where the slopes are steepest, at a fire spread area of 50%) and that the inflection point occurs at a lower value of the independent variable. Fire spread area increased by up to 1.3% or 0.8% with each gram of grass or forb biomass, respectively (Fig. 2-6A). Accordingly, fire spread through 50% of the area was predicted at approximately 120 g/m^2 of exclusively grass biomass versus 210 g/m^2 of exclusively forb biomass (Fig. 2-2B). These estimates of the differences between grass and forb effects are conservative: the grass-dominated points in Fig. 2-2A,B, and more flexible curves (not shown), indicate that fire spread rose even more steeply with grass cover, reaching 50% at approximately 50 g/m^2 of grass biomass. Nonetheless, we present this simple regression structure because it leads to qualitatively similar conclusions as more complex regressions and is readily extended to functional group and species effects.

The difference in the effects of grass versus forb abundance on fire spread area was even stronger from 1997-1999, when species' relative abundances were assessed using their percent cover instead of their biomass and more plots (315, assembled from a larger pool of 32 species) were sampled. Plots containing only grass had up to a 5.8% increase in fire spread area per percent of grass cover, compared with up to a 2% increase in fire spread per percent of non-grass cover ($P < 0.001$, Fig. 2-6G); the multiple regression fit a fire spread of 50% in plots with 30% grass-only cover, or 80% non-grass-only cover (Fig. 2-3B).

We also analyzed the effect of grasses versus forbs on fire spread using the randomized experimental composition and diversity treatments in the 154 core plots, which strengthened the inference that the grass/forb distinction causes fire spread instead of the reverse. We used ANCOVA-style multiple regressions of logit-transformed fire spread area against independent variables indicating whether at least one grass species was planted, log planted species richness (to account for more diverse plots having higher fuel quantity, and being more likely to include a grass by chance), and their interaction (interaction never significant). Plots with at least one grass species planted had significantly higher fire spread area, in 1997-1999 (following all ignitions; adding whether a grass was planted to a regression containing log planted species richness significantly improved fit, $P < 0.001$) and in 2010-2014 (following one ignition; adding whether a grass was planted to a regression significantly improved fit, $P < 0.001$).

Another measure of a plot's propensity to carry fire is whether it carried fire a certain distance following one ignition. Much like fire spread area, with which it was tightly correlated ($\rho = 0.85$, 2010-2014 means), the probability of carrying fire at least 7 m increased more rapidly with grass biomass than with forb biomass, both in 2011 ($P = 0.001$, Fig. 2-2D) and averaged over 2010-2014 ($P < 0.001$, Fig. 2-2E). The multiple regression fitted to 2010-2014 means indicates that a 50% probability of carrying fire requires about 70 g/m^2 of exclusively grass biomass, or about 270 g/m^2 of exclusively forb biomass (Fig. 2-2E).

Consistent with a dominant influence of grass biomass, during 2010-2014 both fire spread area and probability of carrying fire at least 7 m were more tightly related to grass biomass than to total biomass (Fig. 2-2B,C,E,F).

The forb species dropped their leaves during winter (pers. obs.), resulting in a compact and un-aerated litter layer on the soil surface that likely had low flammability. The leafless forb stems remaining upright were coarser than other herbaceous fuels and disconnected from one another. In contrast, the grass species retained their senesced leaves over winter and thus formed a well aerated, fine, connected fuel bed. This may have contributed to plant types' distinct effects on fire spread.

Grass and forb relative abundance: Fuel quantity-independent effects on fire intensity

A multiple regression showed that fire temperature at ground level in 2011 increased similarly with previous-season grass biomass and forb biomass (P = 0.912 for test of equality of grass and forb coefficients, Fig. 2-4A), after adjusting biomass for the mean proportion of the area within 20 cm of each pyrometer that burned. (Measured fire temperature would have depended on whether the fuel around a pyrometer burned, as well as how hot it burned; this adjustment accounted for whether the fuel burned, to reveal effects on fire temperature independent of effects on fire spread.) A similar multiple regression showed that fire temperature at 10 cm above ground in 2000 increased marginally non-significantly more strongly with grass cover than with non-grass cover (P = 0.054, Fig. 2-3D); especially given the small difference in slopes between grass- and non-grass-dominated plots in 2000 and that we were unable to adjust for the proportion of the area around each pyrometer that burned that year (an adjustment that reduced the difference between grass and forb effects in 2011), we infer the per-biomass effects of grasses and non-grasses on fire temperature within 10 cm of the ground were equivalent.

In contrast to fire temperature within 10 cm of the ground, at 50 cm above ground in 2011 fire temperature depended strongly on species composition even after adjusting biomass for the proportion burned around the pyrometers (P = 0.011, Fig. 2-4B). For a biomass of 300 g/m^2, the fitted temperature was about 160 C for plots with only grasses, double that of plots with only forbs. An analysis using the experimental composition and diversity treatments supported a causal influence of the grass/forb distinction: adding whether grass was planted to a regression of temperature at 50 cm above ground against log planted species richness significantly improved fit (P = 0.019).

Aspects of fire behavior related to spread rate also increased most strongly with grass biomass, and were correlated with fire temperature. In 2013, multiple regressions indicated that flame length (Fig. 2-4C) and forward rate of spread (Fig. 2-4D) both increased more strongly with grass biomass than with forb biomass (P = 0.006 and P = 0.002, respectively). At a biomass of 300 g/m^2, plots with only grass were estimated to have flames 2.8 m long advancing at 0.8 m/s, about three times greater than plots with only forbs. Both flame length and rate of spread were more strongly correlated with fire temperature 50 cm above ground (Pearson's r = 0.86 and 0.64, respectively) than with fire temperature at ground level (r = 0.70 and r = 0.55, respectively), across 15 plots with suitable measurements in 2010 or 2011. The difference in flame length between grass- and forb-dominated plots arose from differences in flame height, not angle (not shown). Fireline intensity, the rate of heat release per unit time per unit length of fire front estimated from rate of spread and biomass, increased more strongly with grass biomass than with forb biomass (Fig. 2-4E, P < 0.001). Using a multiple regression, plots with 300 g/m^2 of grass-only biomass had fitted values of approximately 4 500 kW/m, more than double that of forb-only plots with similar biomass. Fireline intensity was correlated similarly with fire temperature at ground level (r = 0.75) or 50 cm above ground (r = 0.76).

In contrast, reaction intensity, the rate of heat release per unit time per unit area, tended to increase slightly more strongly with forb than with grass biomass, though this difference was not significant (P = 0.157). This was because the flaming zone extended farther behind the fire front in grass-dominated than forb-dominated plots (Fig. 2-4F, P < 0.001): for a biomass of 300 g/m^2, the fitted flame zone depth was about 3.5 m for plots with only grass and about 0.8 m for plots with only forbs. Thus, the greater intensity per length of fireline in grass-dominated plots was spread over a larger area, compared with forb-dominated plots of similar biomass. Also in contrast to the other measures, reaction intensity was more tightly correlated with ground level temperature (r = 0.65) than with temperature 50 cm above ground (r = 0.40).

All six measures of fire intensity shown in Fig. 2-4 increased non-linearly with fire spread area (Fig. 2-S2): in incompletely burned plots (fire spread area < 95%), fire intensity measures were low to moderate whereas in completely burned plots, fire intensity measures ranged from low to high. Thus, though only completely burned plots had high fire intensity and all plots with high intensity burned completely, fire spread area and fire intensity measures are not redundant.

Grass and forb relative abundance: Fuel quantity-independent effects on fire severity

Two plant species, each with pre-marked individuals of similar size, showed a stronger increase in fire damage with increasing grass biomass than with forb biomass in multiple regressions (both P < 0.001, Fig. 2-5). Seedling *Quercus macrocarpa* trees in 2011 and adult *Liatris aspera* forbs in 2014 were fitted as reaching half of their maximum damage scores in plots with about 200 g/m^2 of grass biomass, versus about 300-350 g/m^2 of forb biomass. Pre-fire plant size varied relatively little within species (height mean ± s.d.: *Quercus* = 6.55 ± 0.22 cm, *Liatris* = 78.2 ± 15.2 cm), and did not significantly influence damage score when added as a predictor to multiple regressions (not shown). Fire damage scores were correlated with ground level fire temperature (Spearman's rank correlation rho = 0.79-0.89) and fire spread area (rho = 0.67-0.82); these fire behavior measures predicted fire damage as well as biomass did (not shown).

Relative abundances of functional types within grasses and forbs: Fuel quantity-independent effects on fire behavior

We investigated whether fire spread was influenced differently by C4 grasses versus C3 grasses, or by legumes versus non-legume forbs, using multiple regressions with the biomass or cover of each of these functional groups as independent variables (Fig. 2-6B,H).

In the 154 core plots from 2010-2014, fire spread did not increase more with C4 grass biomass than with C3 grass biomass, overall (P = 0.339, Fig. 2-6B). One common C3 grass, *Koeleria macrantha*, had a more positive effect than any of the C4 grasses (*Schizachyrium scoparium*, *Sorghastrum nutans*, *Andropogon gerardii*, and *Panicum virgatum*), whereas the other common C3 grass, *Poa pratensis*, had a less positive effect than any of the C4 grasses (Fig. 2-6C). C3 grasses were much less abundant than C4 grasses, which limits the power of this comparison. In a similar multiple regression analysis using percent cover of functional groups as independent variables, fire spread area from 1997-1999 did increase significantly more strongly with C4 grass cover than with C3 grass cover (P = 0.003, Fig. 2-6H); this analysis included more plots (315) and additional species, but the difference was also significant using only the 154 core plots. Again, though, individual C4 grass species did not differ consistently from C3 grass species in their effects (Fig. 2-6I).

Legume biomass increased fire spread more than did non-legume forb biomass, from 2010-2014 (P = 0.033, Fig. 2-6B). However, legume cover and non-legume forb cover did not differ significantly in their effects, from 1997-1999 (P = 0.742, Fig. 2-6H). For both datasets, the species within these groups overlapped in their effects, though estimates for most non-legume forbs were highly uncertain due to their low abundances (Fig. 2-6C,I).

From 1996-1999, non-grass cover was overwhelmingly dominated by forbs but also included rare small oak trees (*Quercus* spp.: mean absolute cover 0.04%, maximum absolute cover 2%). A multiple regression estimate of the effect of tree cover on fire spread area was wildly imprecise, perhaps due to their rarity, when tree cover was included as an independent variable alongside the covers of the herbaceous functional groups (Fig. 2-6H).

Though fire temperature at 50 cm above ground level increased more quickly with grass biomass than with forb biomass (Fig. 2-4B, Fig. 2-6D), a multiple regression with the biomasses of four functional groups as independent variables showed that C3 grasses and C4 grasses did not differ significantly in their influences (P = 0.372), and nor did legumes and non-legume forbs (P = 0.063, Fig. 2-6E). Non-legume forbs tended to increase fire temperature less per unit biomass than did legumes (Fig. 2-6E), but species in these groups overlapped in their coefficients, most of which were imprecisely estimated (Fig. 2-6F).

Relative abundances of individual species: Fuel quantity-independent effects on fire behavior

Species within the grass and forb categories had distinct influences on fire behavior, but these differences were weak compared to the distinction between grasses and forbs. We investigated whether species have distinct influences on fire behavior using multiple regressions with each species' biomass or cover as an independent variable (Fig. 2-6C,F,I). These regressions against species' abundances explained a little more variation — adjusted R^2 was higher, and AIC was lower — than did the above regressions capturing the grass/forb distinction. Fire spread area was explained by species' biomasses with adjusted $R^2 = 0.74$ (Fig. 2-6C) versus by grass & forb biomasses with adjusted $R^2 = 0.71$ (Fig. 2-6A), with a difference in AIC of 25.1; AIC differences greater than 2 suggest that the increased explanatory power of the more complex per-species regression outweighs the increased complexity. Fire spread area was explained somewhat better by species' covers with adjusted $R^2 = 0.81$ (Fig. 2-6I) versus by grass and forb covers with adjusted $R^2 = 0.70$

(Fig. 2-6G), difference in AIC = 687.5. Fire temperature was explained by species' biomass with adjusted $R^2 = 0.81$ (Fig. 2-6F) versus by grass/forb biomass with adjusted $R^2 = 0.78$ (Fig. 2-6D), difference in AIC = 8.1. It is not clear whether the grass/forb distinction dwarfed differences between species within these groups because the within-grass and within-forb species' differences are small or because most species were too rare to estimate their effects with high precision.

We present the estimates of species' effects on fire behavior in relation to the ratio of leaf length to width (Fig. 2-6C,F,I) because this is a readily measured trait that may capture an aspect of fuel fineness and flammability. Species with longer, thinner leaves tended to increase fire spread more, in a simple linear regression of species' effects on fire spread (coefficients from multiple regressions of fire spread against each species' abundances, reported in the previous paragraph and Fig. 2-6 caption) against their trait values. This was not significant for the effects of species' biomass on fire spread in 2010-2014 (Fig. 2-6C) but was significant and strong for the effects of species' cover on fire spread in 1997-1999 ($P < 0.001$, $R^2 = 0.65$, Fig. 2-6I), and was also significant for the effect of species' biomasses on fire temperature at 50 cm above ground ($P = 0.035$, $R^2 = 0.30$, Fig. 2-6F). The two rarest herbaceous species, which had mean biomass < 0.2 g/m^2 and wildly imprecise estimates of their effects on fire, were excluded from these analyses. The grass species all had longer, narrower leaves than any of the forb species. Regressions including both leaf length:width and a grass/forb dummy variable showed that these positive effects of leaf length:width on flammability can partly explain the grass/forb influence on flammability, but do not explain additional variation among species within grasses or forbs (not shown).

Discussion

Species richness and composition treatments established profound gradients in total plant biomass and cover, and in functional composition, that were independent enough to reveal that grass abundance promoted fire spread area -- or equivalently increased point fire frequency, or decreased point fire return interval -- more strongly than did forb abundance with total abundance held constant. Relative to an equivalent abundance of forbs, grasses also resulted in fires that advanced faster, had higher rates of heat release per unit fireline length, and caused more damage to marked plants. Heat was released higher in grass-dominated plots -- flame lengths were longer and temperatures were higher at 50 cm above ground (but not at ground level). However, the flaming zone was deeper in grass-dominated plots, spreading heat release over a larger area so that the rate of heat release per unit area was not significantly influenced by composition.

Neither C4 versus C3 grasses, legume versus non-legume forbs, nor individual species consistently differed substantially in their effects on fire behavior per unit abundance, after accounting for whether they were grasses or forbs. However, the power of these comparisons was limited because C3 grasses and non-legume forbs, and most species, rarely reached high abundance.

Plant fuel controls fire behavior

There were no unburned plots to indicate definitively whether plant abundance influenced fire behavior or the reverse. However, we strongly infer that total plant abundance influenced fire behavior because plant fuel quantity and characteristics are known from first principles to influence fire behavior (Byram 1959), and no other persistent properties of plots could explain the dominant influence of plot on variation in fire behavior, overriding the influence of year or year-by-plot interactions. Our finding that year explained only 2-6% of variation in fire spread area and fire temperature implies that variation in fire weather or experiment-averaged productivity between years had little influence on fire behavior. Variation in weather between years may have had little influence because most burns were conducted under a limited range of low-humidity and windy conditions conducive to fire, following dry weather that allowed fuels to dry. We infer that temporally consistent differences in fuel between plots were the major controls of fire spread rather than differences between plots in ignition pattern or weather at the moment of burning, because a) year * plot interactions and measurement error combined contributed much less than plot to plot variation in fire spread area after a single ignition, and b) re-igniting incompletely burned plots only modestly increased the percent area burned.

Fuel quantity (diversity-driven herbaceous plant abundance) promotes fire

Planted species richness treatments were randomly assigned to plots, allowing strong inference that increasing species richness (or, similarly, effective number of species in biomass samples) caused increased fire spread area and temperature, as well as increased intensity (not shown). This was largely explained by the increase in biomass with increasing diversity previously reported from this experiment (Tilman *et al.* 1997, Tilman *et al.* 2006, Reich *et al.* 2012), which arose primarily from mechanisms such as complementary resource use and positive feedbacks with soil fertility that strengthened through time (Fargione *et al.* 2007, Fornara and Tilman 2009, Reich *et al.* 2012).

Our measurements are broadly consistent with measurements of fireline intensity in relation to total fuel load from African, South American, and Australian savannas (Trollope *et al.* 1996, Williams *et al.* 1998, Govender *et al.* 2006); with measured flame heights in South African savannas (Trollope *et al.* 1996); and, for our grass-dominated plots, with rates of forward spread in grass-dominated African savannas (Trollope *et al.* 1996) and Australian grasslands (Cheney *et al.* 1993). Nonetheless, there are some potential influences to consider in interpreting the absolute magnitudes of our measures of fire behavior. Biomass and cover late the previous summer imperfectly represented the fuel loads for our spring fires because growth and decomposition occurred after abundance was sampled. However, the intervening time was mostly too cold for growth or rapid decomposition so this discrepancy was likely small. Our estimates of rates of heat release (intensity per length of fireline, and per area of fire) are upper bounds because they assume that fuel was entirely consumed (Stronach and McNaughton 1989). This overestimate is likely modest because fuel consumption typically appeared fairly complete, consistent with fuel consumption >99% in early spring fires in tallgrass prairie elsewhere (Bragg 1982); further, another method of estimating fireline intensity (a power law regression of grassland fireline intensity versus flame length (Alexander and Cruz 2012)) yielded median values 19% *larger* than those presented. Fires might have been more intense had they occurred in fall (autumn) before fuel was compressed by winter snowpack and before most forbs dropped their leaves, which likely reduced aeration and convective and conductive heat transfer similarly to how cutting grass before burning it reduced fire spread rate by 18% in an Australian grassland (Cheney *et al.* 1993). Conversely, the severe winter cold cured herbaceous fuels, leading them to dry rapidly between snowmelt and burning, which may have promoted fire in spring relative to autumn. Fire intensity may have been higher in more extensive burns, though rapidly igniting the entire edge of each 9 x 9 m plot from 2007 onwards maximized fireline length and thus intensity (Byram 1959). Lastly, this experiment was essentially flat; fire spread would be faster uphill and slower downhill for a given fuel condition.

In this experiment in 1997-1999 plant cover and biomass were approximately linearly related, so they were similarly related to fire behavior. In more productive systems in which plant cover saturates at 100%, increases in biomass beyond the point at which cover saturates might result in further increases in fire temperature (which increased linearly with biomass within the range of our data) but not in fire spread area (which saturated at well below 100% cover).

Weak effects of plant diversity, independent of fuel quantity, on fire behavior

Planted species richness further increased fire spread area and temperature over and above the effects of biomass (fuel quantity); this weak additional diversity effect could be due to a) additional complementarity effects, such as more evenly dispersed vertical and horizontal distribution of fuel in a diverse mixture; b) higher likelihood of the most flammable species being included in higher diversity mixtures (selection effect, (Loreau and Hector 2001)), a plausible explanation because the most flammable species disproportionately determined flammability of mixtures in a lab experiment with tree fuels (de Magalhães and Schwilk 2012); or c) greater loss of biomass in lower diversity plots between late summer biomass sampling and spring burning, but this is unlikely because decomposition rates of common substrates were similar across the diversity gradient (Knops *et al.* 2001). Regardless of mechanism, the variation uniquely explained by diversity (and not total biomass) was small.

Effects of forbs and grasses on fire behavior, independent of fuel quantity: Plant structure

Differences between grasses and forbs in leaf retention as well as leaf and stem morphology may help explain why fires in grass-dominated plots tended to carry over larger areas, spread faster, and be more intense per unit fireline length than fires in forb-dominated plots with similar total plant abundance.

Grasses tended to retain their senesced leaves over winter, so that -- even though most leaves attached near the ground on most species -- grasses formed a well aerated fuel bed in the spring, at least following winters without an exceptionally heavy snowpack. Aeration promotes fire by promoting drying, oxygen supply, and heat transfer during burning. In contrast, the forb species in this experiment dropped their leaves during winter (pers. obs.; within a growing season at this site, forbs as a group dropped their leaves a third more quickly than did grasses, though that difference was not significant (Craine *et al.* 1999)). These dropped forb leaves settled into a compact, poorly aerated and relatively non-flammable mat flat on the soil.

After leaf fall, forb stems form fuel that is relatively coarse and less flammable compared with grasses. Stems of several of the forb species in this experiment are somewhat woody, and thus effectively coarser as fuels and slower to dry than grass stems of the same diameter. Forb leaves also formed coarser fuels than grass leaves: all grass species have both longer and narrower leaves, and hence higher length:width ratios, than all of the forb species (all dicotyledons) in this experiment. Finer fuels burn more readily, promoting both fire spread area and a quicker increase in fire intensity following ignition, for several reasons (Byram 1959). Finer fuels have less boundary layer resistance to air flow (wind), and thus have higher oxygen availability during combustion. Finer fuels may also heat to ignition temperature more rapidly through convective heat transfer ahead of the flame front, though conversely radiative heat received from the approaching fire front may be more easily lost and solar pre-heating may be weaker (Murray *et al.* 2013). Finer fuels typically also have a higher surface area:volume ratio, increasing efficiency of radiative heat transfer which penetrates only the surface of fuel, though leaf thickness and surface area:volume ratio did not differ consistently between grasses and forbs. (Following Vile *et al.* (2005), leaf thickness was approximated as the reciprocal of the product of leaf dry matter content, LDMC, measured for this study, and specific leaf area, SLA, measured by Cadotte *et al.* (2009).) SLA, and particularly LDMC which can influence fuel moisture, have been associated with flammability elsewhere (Lavorel and Garnier 2002, Murray *et al.* 2013) but did not differ consistently between the grasses and forbs in this experiment.

Grass-dominated fuels may also be better connected to one another than forb-dominated ones, which would also promote fire spread area and intensity (D'Antonio 2000, Brooks *et al.* 2004). Leafless forb stems are poorly connected to one another, whereas the longer leaves of grasses may increase physical continuity of fuel by bridging fuel patches. Whole plant structure, and the spatial arrangement of plants, also influences vertical and horizontal fuel structure and thus -- perhaps -- fire behavior. However, grasses and forbs did not differ consistently in height (Cadotte *et al.* 2009), and did not appear to differ consistently in the degree to which stems were clustered (some species have discrete individuals, whereas others spread by rhizomes or stolons) or in the dispersion of clusters (pers. obs.).

Evaluating these hypotheses for why grasses and forbs influence fire behavior differently is challenging, for several reasons. First, leaf length:width and leaf drop are correlated with each other and with other traits that may be at least as important. Second, the major difference appears to be between a single lineage -- the grasses -- and other plants; grasses are distinct in many ways, making it difficult to infer the role of particular traits. Third, associations between traits and fire behavior could indicate either that the trait influences fire behavior or that fire promotes species with certain traits. Therefore, we propose hypotheses rather than drawing inference about traits underlying our results.

The more upward distribution of heat in grass- than forb-dominated plots, indicated by higher temperature at 50 cm above ground (but not at ground level) and longer flame lengths for a given total biomass, may have arisen through the same processes that lead to faster spread and thus higher intensity, discussed above (Byram 1959). That flames were taller in grass-dominated plots need not have been due to differences in fuel height structure. Indeed, there was no consistent difference in measured live height between grass and forb species, though fuel height distributions at the time of burning were not measured and greater leaf drop by forbs may have resulted in a lower fuel height distribution for forbs. Fire temperature at ground level may have been insensitive to functional composition, despite temperatures being higher in grass-dominated plots at 50 cm height, because the increased fireline intensity in grass-dominated plots was offset by a combination of a deeper flaming zone (i.e., flames extending further behind the fire front) and more upward distribution of heat in those plots. This is consistent with findings that peak soil temperature need not be related to fireline intensity (Bradstock and Auld 1995).

Effects of forbs and grasses on fire behavior, independent of fuel quantity: Other explanations

Differences in heat released per unit of fuel burned, and the completeness with which fuel burned, are unlikely to explain why each unit of grass biomass increased fire intensity considerably more strongly than did each unit of forb biomass. We assumed a constant heat yield per unit biomass because heat of combustion for various grass and forb species had a coefficient of variation of only 5.3% in a Canadian tallgrass prairie (Kidnie 2009), and 1.2% in a South American grassland (Britton *et al.* 1976). If plant types differed in how completely they were consumed by fire, this would likely make the difference in effect of grasses and forbs on fireline intensity (Fig. 2-4E) conservative, rather than reverse our conclusions, because (coarser) forbs would likely be less completely consumed than (finer) grasses (Kauffman *et al.* 1994), in which case intensity would be over-estimated for forbs relative to grasses because we assumed that combustion was complete.

A larger difference between spring fuel loads and biomass (or cover) the previous summer for forbs than for grasses, due to forbs having lower autumn growth, higher decomposition, or higher herbivory, could have contributed to the different effects of grasses and forbs on fire. Plant abundance was measured in late July, by which time live plant cover and total biomass had approached their maxima, and grasses and forbs did not differ consistently in their phenology (Whittington *et al.* in press). However, C4 grasses -- particularly in the tribe Andropogoneae, which includes three of our four C4 grass species -- tend to have relatively slow decomposition and herbivory rates due to high tissue C:N and lignin:N ratios (Taylor *et al.*

1989, Hobbie 1992, Cornwell *et al.* 2008), which could partly explain why they promote fire (Bond *et al.* 2003). In a decomposition study in another biodiversity experiment in the same field, after one year more than 80% of biomass of the C4 grass *S. scoparium* remained compared with about 50% of mixed-species biomass (Knops *et al.* 2001), implying that other species had lower decomposition rates. Large-scale multi-site analyses have found that forbs decompose faster that graminoids (Reich *et al.* 2007, Cornwell *et al.* 2008). However, this finding contrasts with the strong overlap among functional groups in decomposition-related traits found by (Craine *et al.* 2002) in prairie species at this site; functional group could have been confounded with differences in habitat in the cross-site analyses, if a larger fraction of graminoids in those analyses were from grasslands and a larger fraction of herbs were from forest. There could have been greater loss of forb than grass tissue to insect herbivory during autumn, which would be consistent with a tendency for seedlings of some of the grass species in this experiment to be less palatable to crickets than seedlings of some of the forb species (Burt-Smith *et al.* 2003). Herbivores larger than mice were excluded from our experiment by a fence and trapping, so would not have influenced fuel loads. However, the reduction in biomass from one summer to the following spring was only 13% greater in forb-dominated than in grass-dominated plots, so these effects are unlikely to fully account for grasses' much stronger impact on fire per unit abundance the previous summer.

The dormant-season timing of burns in this experiment accords with most historic and recent pre-historic fires occurring in the dormant season in this region (Wolf 2004). The effect of grass proportion may be somewhat weaker in late fall fires than in the spring fires in this experiment, given less time for differential decomposition and leaf drop.

A lower fire spread percolation threshold for grass-dominated plots

The sigmoidal increase in proportional fire spread area with increasing plant cover (Fig. 2-3A,B) is consistent with fire spread as a percolation process, and grasses appeared to result in a lower percolation threshold than forbs. Percolation processes exhibit thresholds in occupancy -- in this case, the fraction of the landscape that is flammable -- at which the probability of fire spreading from one patch to at least one other patch rapidly increases from zero to one (Cox and Durrett 1988). Here, flammable means having flammable fuel that ignites readily under the conditions of the burn. The apparent threshold in our data was about 40% total plant cover irrespective of composition, or 30% for exclusively grass plots (Fig. 2-3B), suggesting that the threshold is lower for grasses than for forbs. Indeed, the fitted line for forb-only plots suggested a radically higher threshold of about 80% cover, though no forb-only plots burned completely so this is an extrapolation that we treat with caution. The lower cover threshold for percolation in grass-dominated than forb-dominated plots may be due to a higher probability of fire spreading between grasses than forbs because grasses are finer fuels and thus ignite more readily.

Our results inform observations that invasive grasses have radically increased fire spread area in arid shrublands in the Mediterranean (Grigulis *et al.* 2005) and the North American Great Basin (Balch *et al.*

2013). Invasive grasses are understood to have made the landscape more connected in terms of fire spread. Our results suggest this could be due to either an increase in total fuel above a percolation threshold or a lower percolation threshold with more flammable fuel. Our results are consistent with this potential for profound effects of increasing grass biomass on fire behavior when productivity is low, holding constant ignition regime and environmental conditions.

Steep declines in burn area as tree cover increases above 40% (Archibald *et al.* 2009), presumed to correspond to flammable grass cover falling below 60%, have been interpreted as consistent with a threshold of 59% found in physical systems and predicted by theory (Cox and Durrett 1988, Abades *et al.* 2014, Schertzer *et al.* 2014). There are at least 4 possible reasons for why the apparent threshold in our data was lower than the theoretical 59%, except for the most forb-rich plots (Fig. 2-3B). First, the visual estimates of percent plant cover may be imperfectly calibrated, or cover may have increased after the late summer estimates due to further growth or flattening by snowpack, but from personal observations this is unlikely to explain such a large (up to two-fold) discrepancy. Second, fire burning with wind is a biased form of percolation, which may have a lower threshold for spread (Ohtsuki and Keyes 1986). Third, the threshold of 59% is predicted for well mixed landscapes; clumping of flammable patches allows fire to spread at lower cover (O'Neill *et al.* 1992), which could partly explain our results because flammable plant matter in sparsely covered plots was clumped into individual plants. Fourth, our estimates of percent cover may have been at a finer scale than the scale of fire spread. Models in which fire can jump over unoccupied patches predict a lower percolation threshold, and are equivalent to models using a coarser resolution (O'Neill *et al.* 1988). Cover was estimated in 0.5 x 1 m quadrats with a grain size as fine as 1 cm^2. Except in the sparsest plots, much of the measured bare ground was composed of numerous small bare ground patches (for example, on the order of 1 cm^2 each) interspersed within plant matter, rather than extensive bare patches (personal observations); fire likely jumped readily across these small bare patches. Therefore, our fine-resolution measures of plant cover would likely equate to higher cover values, resulting in a threshold estimate closer to the theoretical 59% value, at a coarser resolution that accounts for the ability of fire to jump readily over small gaps.

Forbs, tree-grass interactions, and biome shifts

Higher flammability of grasses than trees is key to our understanding of tree-grass interactions (Scholes and Archer 1997, Bond 2008), including the hypothesis that feedbacks between vegetation and fire can lead to grassland, savanna, or forest biomes as alternate stable states at intermediate rainfall (Grimm 1983, Bond *et al.* 2005, Hirota *et al.* 2011, Staver *et al.* 2011). Theoretical treatments of tree-grass interactions underlying this biome shift typically omit forbs. In such treatments, grasses promote and are promoted by fire, and trees suppress and are suppressed by fire (Staver and Levin 2012, Abades *et al.* 2014, Schertzer *et al.* 2014). Forbs are implictly assumed to be 1) negligible, either in abundance or per-abundance impact, or 2) equivalent to grasses. Forbs may indeed be negligible in abundance in some C4 grass-dominated tropical savannas and other ecosystems. However, our results suggest that forbs have substantial per-abundance impacts on fire behavior and that these differ from the effects of grasses. Therefore, where forbs are a large proportion of herbaceous plant biomass and cover they should neither be ignored nor lumped with grasses without due consideration.

Where forbs are common, considering their abundance separately from grasses could improve understanding of tree demography and fire behavior, and thus of fire-mediated shifts between herbaceous and woody dominance. Our results show that grass-dominated fuels produce more extensive fires that are more intense and release heat higher above ground and are thus more likely to topkill trees by damaging their aerial buds or other tissues (Trollope *et al.* 2002, van Wagtendonk 2006). Increased fire spread with increased cover and biomass of plants, especially of grasses, profoundly inhibited the establishment of oak trees (*Quercus macrocarpa* and *Q. ellipsoidalis*) in this experiment (chapter 3): fire limited trees' height to about 60 cm, holding them in a "fire trap," by repeatedly top-killing them before they could grow large enough for their stems to survive fire (Bond and van Wilgen 1996). A tree cover threshold, below which a system switches from non-flammable to flammable, is hypothesized to make shifts between grassland or savanna and forest more sudden, assuming that grass forms the remaining cover (Schertzer *et al.* 2014). Forbs could alter such dynamics if they are a substantial part of the non-tree cover, because our results suggest the percolation threshold may be steeper and occur at lower herbaceous cover for grass- than forb-dominated systems (Fig. 2-3B). Therefore, caution may be needed in transferring quantitative findings between systems with different ratios of grass to forb abundance.

Distinguishing the different effects of grasses and forbs on fire could change the qualitative dynamics of a fire-vegetation system, compared with averaging the effects of grasses and forbs, only if grasses and forbs respond differently to fire. There is no evidence that grasses and forbs differ generally in their response to fire. For example, in some other tallgrass prairies and mesic grasslands forbs did not respond to fire consistently differently than did grasses (Engle and Bidwell 2001, Kirkman *et al.* 2014). However, high fire frequency did promote grasses over forbs in one tallgrass prairie, Konza Prairie (Collins 1987, Gibson and Hulbert 1987), so distinguishing grasses from forbs may help understand the qualitative dynamics of some systems.

A stronger positive influence of grasses than of forbs on fire spread and thus tree suppression could help explain why acid, sandy soils that favor grasses over forbs tend to be grassland or savanna whereas more basic, clay-rich soils that favor forbs over grasses tend to be forested, in some regions. Soil texture only weakly predicts tree cover globally (Sankaran *et al.* 2005, Lehmann *et al.* 2014), perhaps because its effects can reverse depending on context. For example, high clay content may be associated with low tree cover in some Australian and African savannas where clay suppresses trees through water-logging or high grass productivity and fire intensity (Williams *et al.* 1996, Bond 2008), whereas high clay content may be associated with high tree cover (forest) in South America (Ruggiero *et al.* 2002). High clay content may favor trees through high water holding capacity and nutrient availability that increase tree growth rate and thus the likelihood that trees avoid top-kill by fire (Murphy and Bowman 2012). Our findings raise another possibility. In European grasslands and perhaps other regions, soils with high clay content are often also rich in cations and relatively basic, and catio-rich and basic soils promote forb dominance (Tilman 1982, Tilman *et al.* 1994, Willems and van Nieuwstadt 1996). Thus, if soils that are rich in clay and basic promote forbs over grasses, and if this reduces fire intensity as it did in our experiment, this could help explain why such soils are more likely to be forested than sandier, more acid soils. N deposition favors grasses over forbs in European and other grasslands (Xia and Wan 2008, Stevens *et al.* 2009, Xia *et al.* 2009), which may increase flammability and thus favor grassy biomes over forest, in regions where fire remains important. (Fire is suppressed in many heavily industrialized regions receiving the highest levels of N deposition.)

Feedbacks between plant species abundance and fire behavior: Are fire impact and response related?

If species that promote fire (relative to other species) also respond positively to it (relative to other species), and species that inhibit fire are relatively inhibited by it, this could cause positive feedbacks between species' abundances and fire. Such positive feedbacks could lead to alternative stable state dynamics analogous to those involving grasses, trees and fire, which could inhibit local coexistence of species or plant types that differ in their effects on fire and responses to fire. This could also reduce diversity at larger scales if there is a lack of patchiness, or it could increase species or plant type turnover across the landscape and thus increase larger-scale (gamma) diversity if it creates patches differing in flammability and composition.

There is evidence that C4 grasses can both promote, and increase in response to, fire; indeed, it has been suggested that grasses of the Andropogoneae tribe may have been selected for high flammability, in turn intensifying selection for fire tolerance and competitive ability under high fire frequency (Bond and Midgley 1995, Bond *et al.* 2003, Schwilk 2003). The abundances of C4 grass species had relatively large coefficients in regressions with fire spread area or fire temperature as the dependent variable, which could indicate that these species promoted fire, but these species also increased in abundance in response to increasing experimentally controlled fire frequency in other tallgrass prairie experiments (*S. scoparium* at this site, not significant (Li *et al.* 2013); *S. scoparium*, *Panicum virgatum* and *Sorghastrum nutans* at Konza Prairie (Forrestel *et al.* 2014)). Their higher abundance with frequent fire may be due to their intolerance of the litter that builds up when fire is infrequent (Knapp and Seastedt 1986). Thus, the apparent positive effects of these species on fire in our experiment could have partly reflected an increase in their abundance in response to fire (which in turn may have been influenced by their abundance or by the abundance of co-occurring species).

However, it is not clear that C4 grasses differ from other herbaceous groups or species in the same way that grasses differ from trees, which -- through their woodiness -- typically both inhibit initial fire spread when in a dense stand and are inhibited by fire relative to grasses. Woodiness presents a tradeoff between increased ability to compete for light and inhibit fire, and increased vulnerability to fire, at least when young; this imposes some consistency on how grasses versus trees interact with fire (though exceptional fire-adapted trees do promote fire by retaining dead branches and having flammable leaves, and depend on fire for serotinous seed release). But no such tradeoff is evident between different species of herbaceous plants, or between broader herbaceous categories such as grasses and forbs. For example, the C3 grass *Poa pratensis* declined in abundance at high fire frequency in another experiment at this site (Li *et al.* 2013) and at Konza Prairie (Forrestel *et al.* 2014), but did not appear to inhibit fire spread in our analyses. In general, traits that determine species' impacts on fire are largely different than, and not necessarily strongly correlated with, traits that determine their response to fire, suggesting that species' impacts on and responses to fire may not be related (Lavorel and Garnier 2002). On the other hand, relationships between traits and species' responses to and impacts on fire are understood mainly at the level of qualitative traits, such as seeding versus re-sprouting after fire (Lavorel and Garnier 2002); elucidating how species' responses to and impacts on fire are related, and the functional basis of this, *within* these broad qualitative categories (all of our species may be described as "perennial resprouting herbs") would indicate the potential for such positive feedbacks.

Conversely, if species that promote fire decrease in abundance when fire is frequent and vice versa, this could cause negative feedbacks between species' abundances and fire. Such negative feedbacks could stabilize community composition and promote local coexistence of species with contrasting effects on fire and responses to fire. This could contribute to increased spatial (Collins and Smith 2006) or temporal (Bowles and Jones 2012) homogeneity in species composition at higher fire frequency in other tallgrass prairies, alongside other explanations such as environmental filtering with fewer species adapted to high than to low fire frequency (Forrestel *et al.* 2014). Such negative feedbacks could, like positive feedbacks, either increase or reduce diversity at larger scales depending on how patchy composition is.

Implications of grass versus forb effects on fire for nutrient losses

Fire temperatures hot enough to volatilize N, P, and S occurred in some plots, especially those dominated by grasses, with implications for ecosystem management. Increased fire spread in grass- versus forb-dominated plots of a given biomass could also influence nutrient cycling through changes in surface albedo, microclimate, and reduced plant demand for water and nutrients (Wan *et al.* 2001, Snyman 2002), but these processes are less easily predicted than losses to volatilization.

Plot median temperatures ranged up to approximately 300 C at ground level (in 2011), 400 C at 10 cm above ground (in 2000), and 250 C at 50 cm above ground (in 2011). These fire temperature measures are comparable with results from similar pyrometers in Kansas tallgrass prairie (Gibson *et al.* 1990), but are nonetheless conservative estimates of maximum fire temperature. Temperatures from similar pyrometers under-estimated maximum (Iverson *et al.* 2004, Kennard *et al.* 2005) or peak 1-minute mean (Wally *et al.*

2006) temperatures from continuously-logged thermocouples, though these measures were closely correlated. Neither pyrometers nor thermocouples measure actual flame temperatures, which can be higher, on the order of 870 C for wood burning (Martin *et al.* 1969). Some of our pyrometers recorded temperatures higher than the medians that we report, up to 610 C. Thus, temperatures higher than those we measured may have occurred.

Nitrogen (N) was likely volatilized at a higher rate in grass-dominated plots than in forb-dominated plots with equivalent biomass, due to 1) higher fire spread and 2) higher canopy fire temperatures. First, higher fire spread in grass-dominated plots would have caused higher N volatilization because fire temperatures near the ground were high enough to volatilize N (above 70 C (Knops *et al.* 2002)) in all but the barest plots (> 65 g/m^2, Fig. 2-3C and Fig. 2-4A; > 15% cover, Fig. 2-3D). This is consistent with findings that most N in other burned grassland biomass is volatilized (Raison *et al.* 1985, Hobbs *et al.* 1991). Second, within areas that burned, grasses likely promoted nutrient volatilization more strongly than did forbs per unit biomass through higher canopy fire temperatures: temperatures at 50 cm aboveground were high enough to volatilize nitrogen for grass biomass of 100 g/m^2 but for forb biomass of more than 200 g/m^2. However, a large fraction of biomass fuel was nearer the surface (pers. obs.), where temperatures were similar for grasses and forbs, the effect on fire spread was likely more important.

Effects of grass versus forb biomass on soil N could influence ecosystem dynamics, because soil N availability limits plant growth at this and many other grassland sites (Tilman 1987, Vitousek and Howarth 1991, LeBauer and Treseder 2008). In this experiment, total soil N increased with the abundance of C4 grasses, indicating that increased N loss through volatilization with higher grass abundance was more than offset by other processes (Fornara and Tilman 2008). Processes by which C4 grass abundance has been found to increase soil N include a high root:shoot ratio resulting in high root litter inputs per unit aboveground biomass (Fornara and Tilman 2008), and litter with a high C:N ratio that that decomposes slowly (Fornara *et al.* 2009, Pellegrini *et al.* 2014) and can increase N immobilization and slow N mineralization (Ojima *et al.* 1994, Blair 1997, Fynn *et al.* 2003), reducing N loss to leaching. This lack of a reduction in total soil N with increased fire is consistent with a trend across other sites for fire not to significantly affect total soil N in the short term (Wan *et al.* 2001). Nonetheless, sustained multi-decade increases in fire spread and intensity have reduced total N in surface soil (< 6 cm depth) in some systems (Fynn *et al.* 2003). We need to better understand how compositional impacts on fire behavior interact with other processes -- including large mammal herbivory and N deposition -- to influence N dynamics. Fires that volatilize nutrients and increase surface light availability may be important for avoiding species loss with anthropogenic N deposition, in nutrient-poor prairies (Hautier *et al.* 2009, McLauchlan *et al.* 2014). Where N deposition increases the ratio of grasses to forbs, this may help to maintain vigorous fire and thus reduce the extent of species loss.

Phosphorus (P) and sulphur (S) may also have been volatilized more from grass-dominated plots, with higher fire spread and canopy fire temperatures, than from forb-dominated plots with similar biomass. P may have been volatilized in the hottest-burning plots, at 281 C (Haynes *et al.* 2014). However, P loss to volatilization is typically much lower than P return to soil as ash (Raison *et al.* 1985). S is the final element that possibly would have been volatilized, at 444 C (Kauffman *et al.* 1994, Haynes *et al.* 2014). Temperatures above 444 C were measured only in the 2000 burn, but volatilization temperatures can be lower for organic forms of elements than those cited (Raison *et al.* 1985). P and S do not limit productivity in this system (Tilman 1984, Tilman 1987), but P and perhaps S can be limiting in other grasslands (Fay *et al.* 2015). Other plant nutrients are unlikely to have been volatilized at the temperatures we measured (Haynes *et al.* 2014).

Conclusion

Fire spread area, point fire frequency and fire intensity all increased more strongly with grass than with forb abundance, holding total fuel quantity constant, in this long-term, replicated field experiment in which ignitions were annual and diversity and composition treatments were randomized across variation in other environmental conditions. The profound variation we observed in fire behavior — and hence the effective frequency and intensity of fire at any point — despite annual ignitions highlights that fuel quality and quantity, not just ignition regime, determine effective fire regime.

In particular, our results suggest that for systems where forbs are abundant, explicitly considering them, distinct from grasses, will improve understanding of fire-mediated interactions between herbaceous and woody vegetation that influence biome boundaries. Better understanding how differences in the impacts of herbaceous functional groups and species on fire relate to differences in their response to fire will indicate whether analogous fire-vegetation feedbacks influence composition within herbaceous communities. In promoting fire, grasses also promote nutrient loss through volatilization relative to forbs.

The difference between grasses' and forbs' per-abundance impacts on fire behavior may be stronger in less intense fires, because fuel structure is more influential at lower fire intensity (Byram 1959). Accordingly, we found that the influence of composition (holding total abundance fixed) on fire spread area was most pronounced at low fire spread area. As grassland and savanna fires decrease in intensity as people change ignition regimes and suppress fire, and alter fuel arrangement through land management (Archibald *et al.* 2013), the differential influences of grasses and forbs on fire behavior may become increasingly important for understanding ecosystem dynamics.

Figure 2-1: Effects of planted species richness on fire behavior and biomass.

Among 154 grassland plots spanning a diversity gradient established by seeding in 1994 and maintained by weeding and annual burning, plots with higher planted species richness had fire spread through a larger percentage of the area following one ignition (A), higher fire temperature at ground level (B), and higher herbaceous plant biomass (C). Fire spread area (A) was averaged for each plot over 2010, 2011, 2013 and 2014; late summer biomass (C) was averaged over available preceding years (2010, 2012, 2013). Fire temperatures for each plot are medians of three measurements. Lines are linear fits against log planted species richness; fire spread area is logit-transformed. Planted species richness is jittered for clarity.

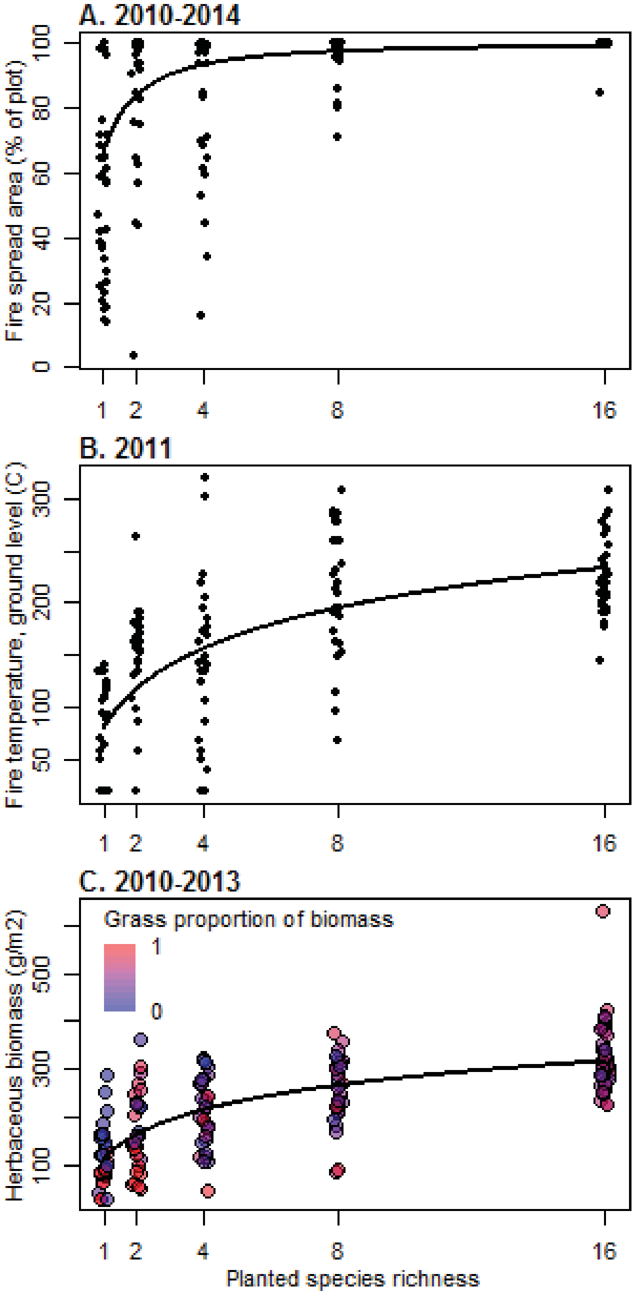


Figure 2-2: Effects of herbaceous biomass and composition on fire spread.

In 154 grassland biodiversity experiment plots, fire spread area (A-C) and probability of carrying fire at least 7 m (D-F) following single ignitions increased more strongly with grass biomass than with forb biomass. Fires occurred in spring, after biomass was measured late the preceding summer. Curves are fitted values for plots with only grass biomass (red) or only forb biomass (blue), from multiple regressions with grass and forb biomass as independent variables. These were either linear regressions with logit-transformed fire spread area as response (A-B) or logistic regressions with whether or not fire was carried as response (D-E). Regressions were forced through the origin. P-values are bootstrap tests of the equality of grass and forb biomass effects. Both fire spread area and probability of carrying fire were more tightly correlated with grass biomass (C, F) than with total herbaceous biomass (B, E: ρ is Spearman's rank correlation coefficient). In B-C and E-F, fire spread and biomass in preceding summers were averaged as in Fig. 2-1. E-F show the proportion of years 2010-2014 in which each plot carried fire. Y axis values in D-F are jittered for clarity.

Figure 2-2 (caption on previous page).

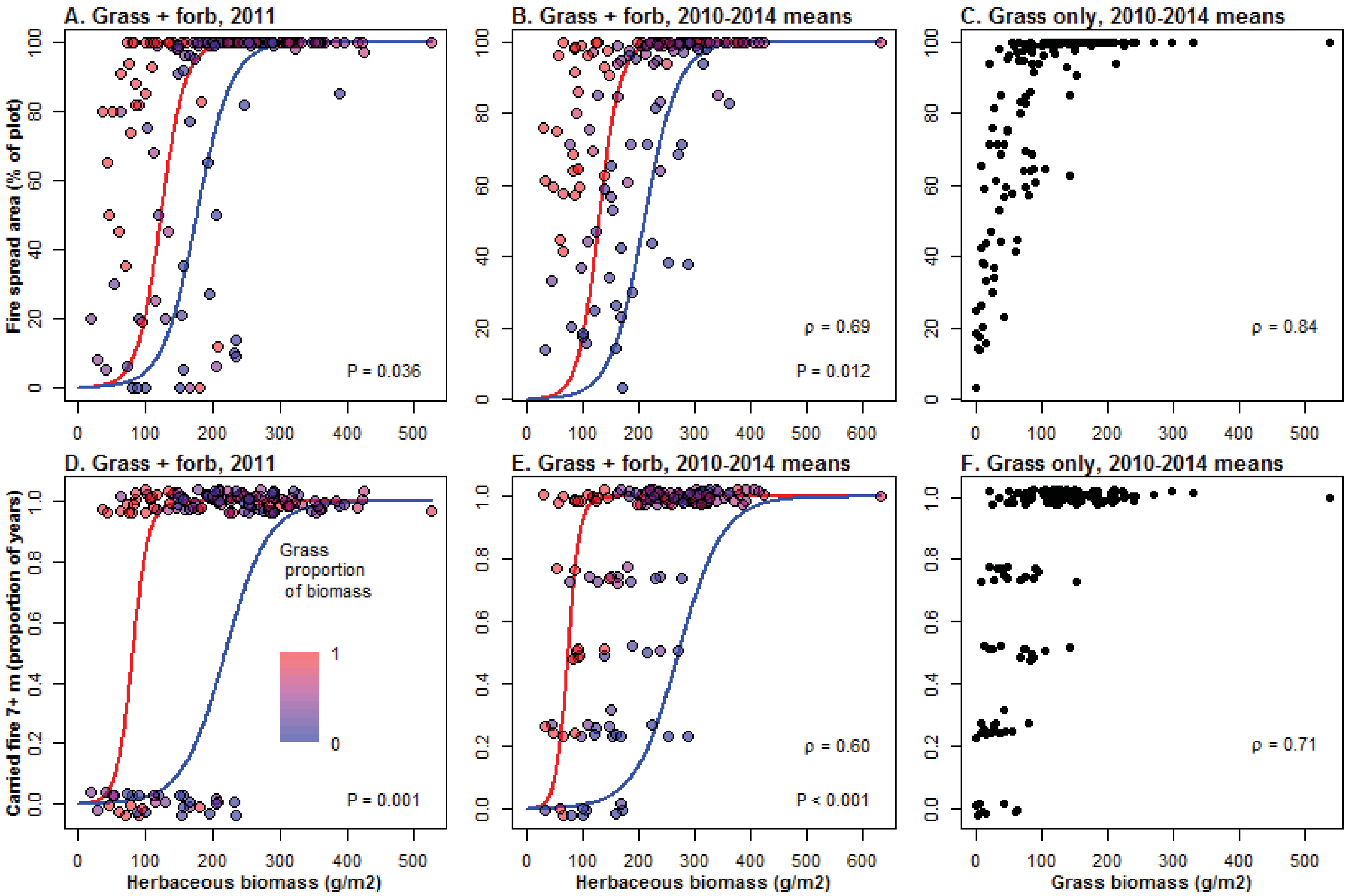


Figure 2-3: Effects of herbaceous biomass, cover, and composition on fire spread and temperature.
In grassland biodiversity experiment plots, total plant biomass (A,C) and cover (B, D) late the previous summer influenced both fire spread area (A-B) and fire temperature 10 cm above ground (C-D). Black curves are fitted values from linear regressions against total biomass or cover forced through the origin, with fire spread area logit-transformed. Fitted values from multiple regressions with grass and non-grass biomass as independent variables are shown for plots with only grass biomass (red curves) or only non-grass biomass (blue curves). P-values are bootstrap tests of the equality of grass and non-grass biomass effects. Non-grasses were primarily forbs, but included up to 2 % of trees (*Quercus* spp.). Fire spread area (A-B) was averaged for each of 315 plots over spring of 1997-1999; late summer biomass or cover was averaged over preceding years (1996-1998). Fire temperature (C-D) was measured in a subset of 176 plots.

Figure 2-3 (caption on previous page).

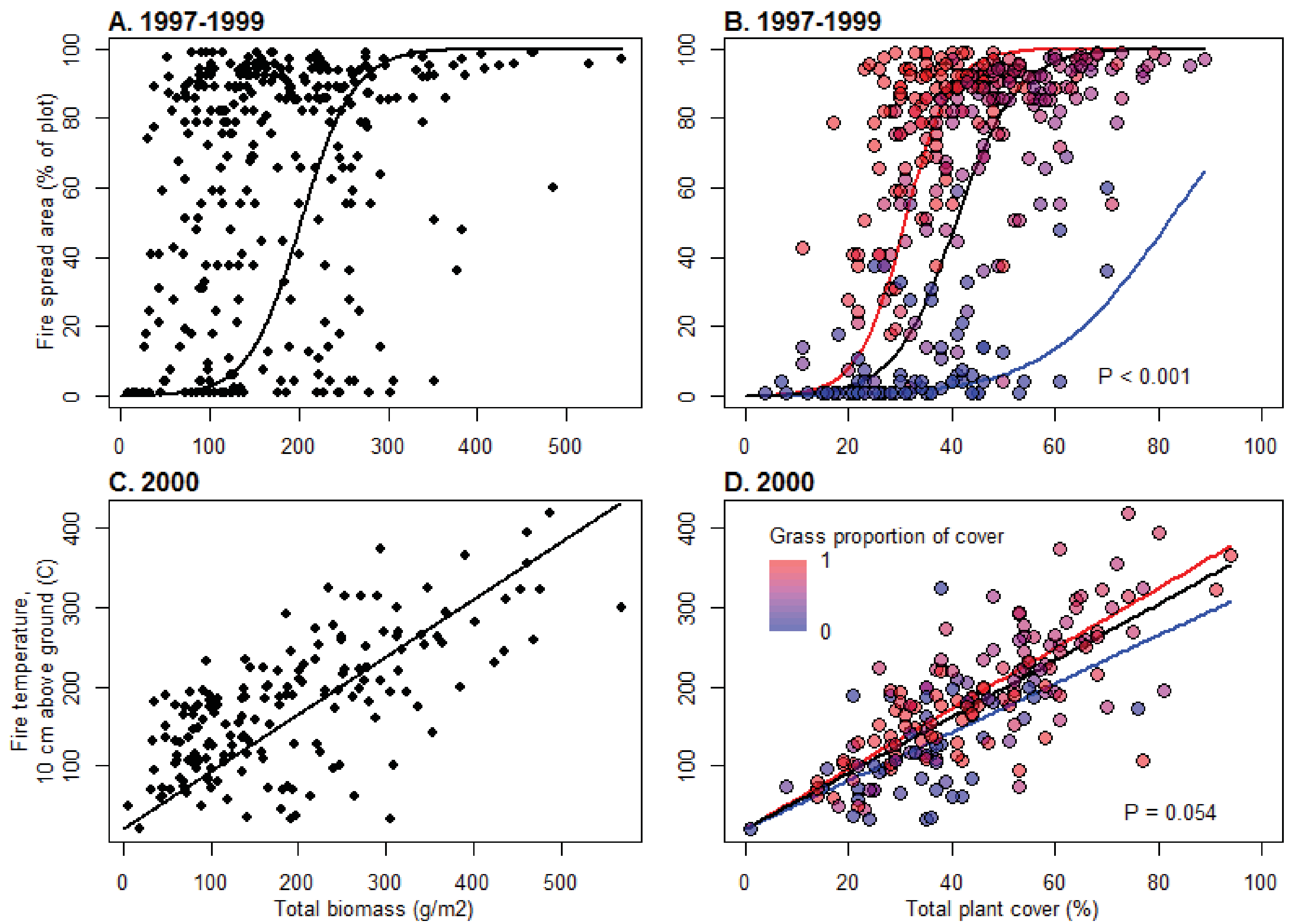


Figure 2-4: Effects of herbaceous biomass and composition on fire intensity.

In grassland biodiversity experiment plots, fire temperature at ground level (A) and 50 cm above ground (B), flame length (C), rate of forward spread (D), fireline intensity (rate of energy release per length of fire front, E), and flaming zone depth (F) all increased more strongly with grass biomass than with forb biomass, except for fire temperature at ground level (A). Lines are fitted values for plots with only grass biomass (red) or only forb biomass (blue), from multiple regressions with grass and forb biomass as independent variables. Regressions were forced through the origin. P-values are bootstrap tests of the equality of grass and forb biomass effects. For A and B, low measured temperatures can reflect either low fire temperature or lack of fire around a pyrometer, so regressions fitted to the unadjusted grass and forb biomass shown by the points (dotted lines, statistics not shown) conflate fire temperature and fire spread. To isolate the effects of *burned* biomass on fire temperature, we fit regressions to grass and forb biomass independent variables that were multiplied by the mean proportion of area burned in a 20 cm radius around the pyrometers in a plot (solid lines and P-values). We measured temperature in 154 plots at ground level (A), in 135 plots at 50 cm above ground (B), and other variables in an opportunistically determined subset of 63 plots.

Figure 2-4 (caption on previous page).

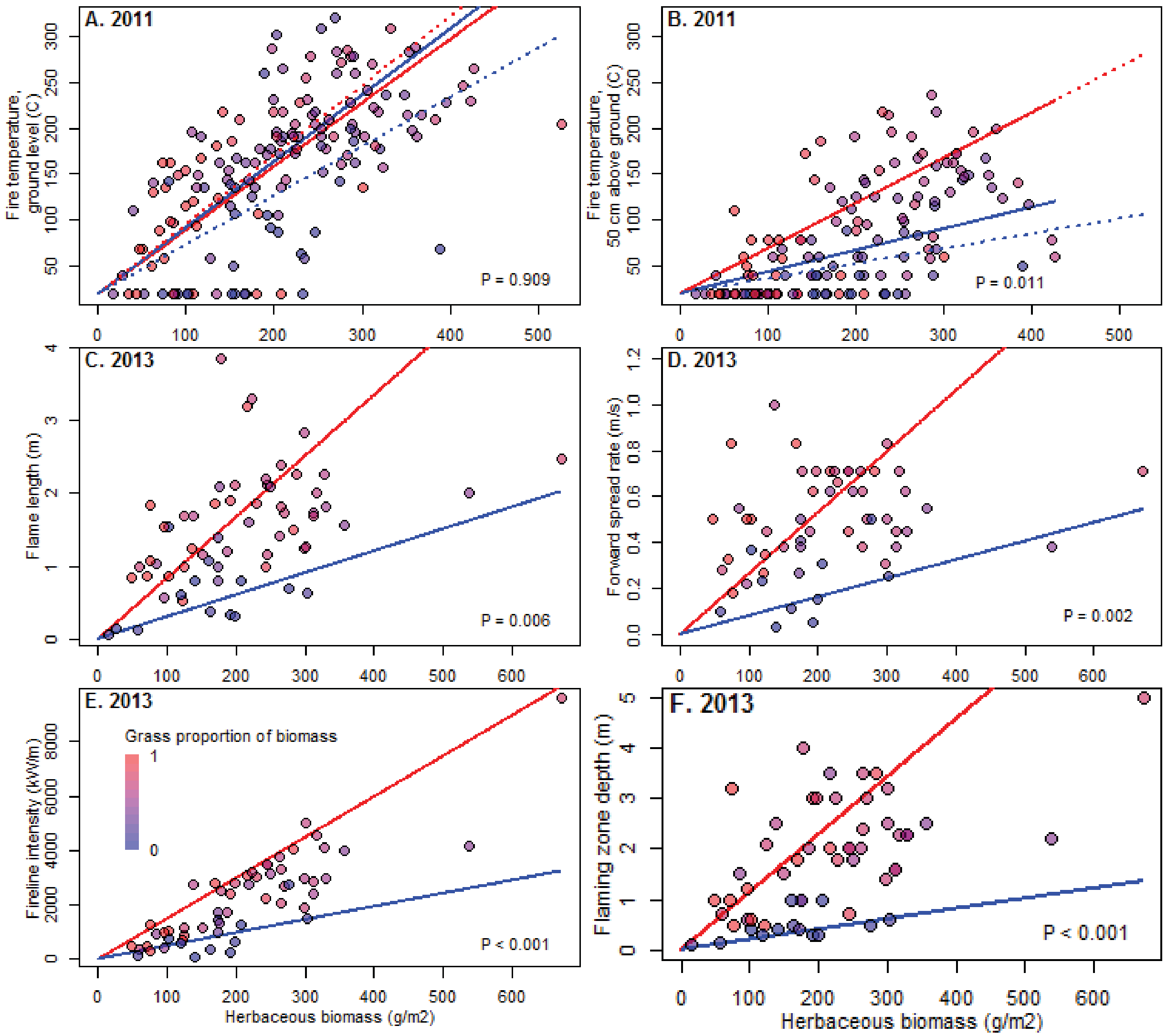


Figure 2-5: Effects of herbaceous biomass and composition on fire severity.

Severity of fire effects on plants increased more strongly with grass biomass than with forb biomass, measured the preceding late summer. Severity of fire damage to similarly-sized pre-marked individuals was scored visually: 0 = unburned, 1 = scorched (darkened), 2 = severely damaged (black; stem fallen for *Liatris*), or 3 = consumed; points are medians of 12 one-year-old *Quercus* seedlings in each of 32 plots (A) or of 2-5 reproductive *Liatris* plants in each of 37 plots (B). Curves are fitted values for plots with only grass biomass (red) or only forb biomass (blue), from mixed effects multiple regressions forced through the origin with grass and forb biomass as independent variables, logit-transformed severity score of each plant as response, and plot as a random intercept. P-values are bootstrap tests of the equality of grass and forb biomass effects. Color indicates the proportion of biomass in a plot that is grass (red, 1) or forb (blue, 0).

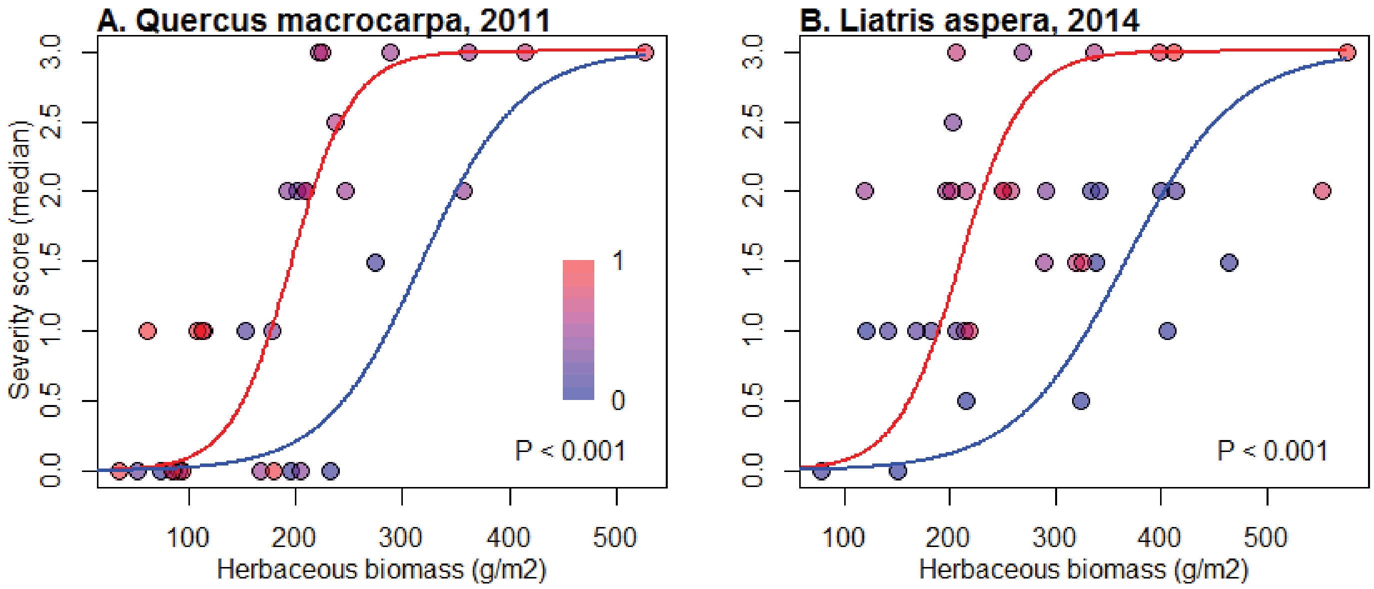


Figure 2-6: Effects of herbaceous functional groups and species on fire spread and temperature.

Functional groups and species differed in how their biomass or cover the preceding late summer influenced fire spread area and temperature 50 cm above ground. Coefficients of multiple regressions of fire spread area (A-C and G-I, logit transformed) or temperature (D-F) against the biomasses of grass and non-grass (A, D, G), functional groups (B, E, H), and species (C, F, I) are shown with bootstrap 95 % confidence intervals. These regressions were forced through the origin. The back-transformed coefficients in A-C and G-I indicate the maximum rate of increase in fire spread area per gram of biomass or percent of cover, i.e., the slope at the inflection point on a sigmoid curve for plots occupied exclusively by a given plant type. The grass and forb coefficients in A, D, G correspond to the red and blue curves in Figures 2-2B, 2-4B and 2-3B, respectively. To isolate the effects of *burned* biomass on fire temperature (D-F), we multiplied the biomass independent variables by the mean proportion of area burned in a 20 cm radius around the pyrometers in a plot before fitting the regressions, as in Fig. 2-4A,B. For grass-forb and functional group comparisons, P-values are bootstrap tests for equality of the indicated pair of coefficients. Species' coefficients are plotted against the ratio of leaf length to width ($\log_{10}$ transformed); fit lines and statistics are from simple linear regressions of species' coefficients against their values of this trait, with coefficients weighted by the inverse of their bootstrap variances. All panels use the 154 plots planted with the core pool of 16 species, except G-H which use an expanded set of 315 plots planted with a larger species pool. Species abbreviations: Am = *Achillea millefolium*, Ac = *Amorpha canescens*, Ag = *Andropogon gerardii*, At = *Asclepias tuberosa*, Dp = *Dalea purpurea* (syn. *Petalostemum purpureum*), Dv = *Dalea villosa* (syn. *Petalostemum villosum*), Kc = *Koeleria macrantha* (syn. *K. cristata*), Lc = *Lespedeza capitata*, La = *Liatris aspera*, Lp = *Lupinus perennis*, Mf = *Monarda fistulosa*, Pp = *Poa pratensis*, Pv = *Panicum virgatum*, Sr = *Solidago rigida*, Sn = *Sorghastrum nutans*, Ss = *Schizachyrium scoparium.* (Additional planted species that were extremely rare in these plots and thus omitted from species regressions: *Agropyron smithii, Elymus canadensis, Quercus macrocarpa, Quercus ellipsoidalis.*)

Figure 2-6 (caption on previous page).

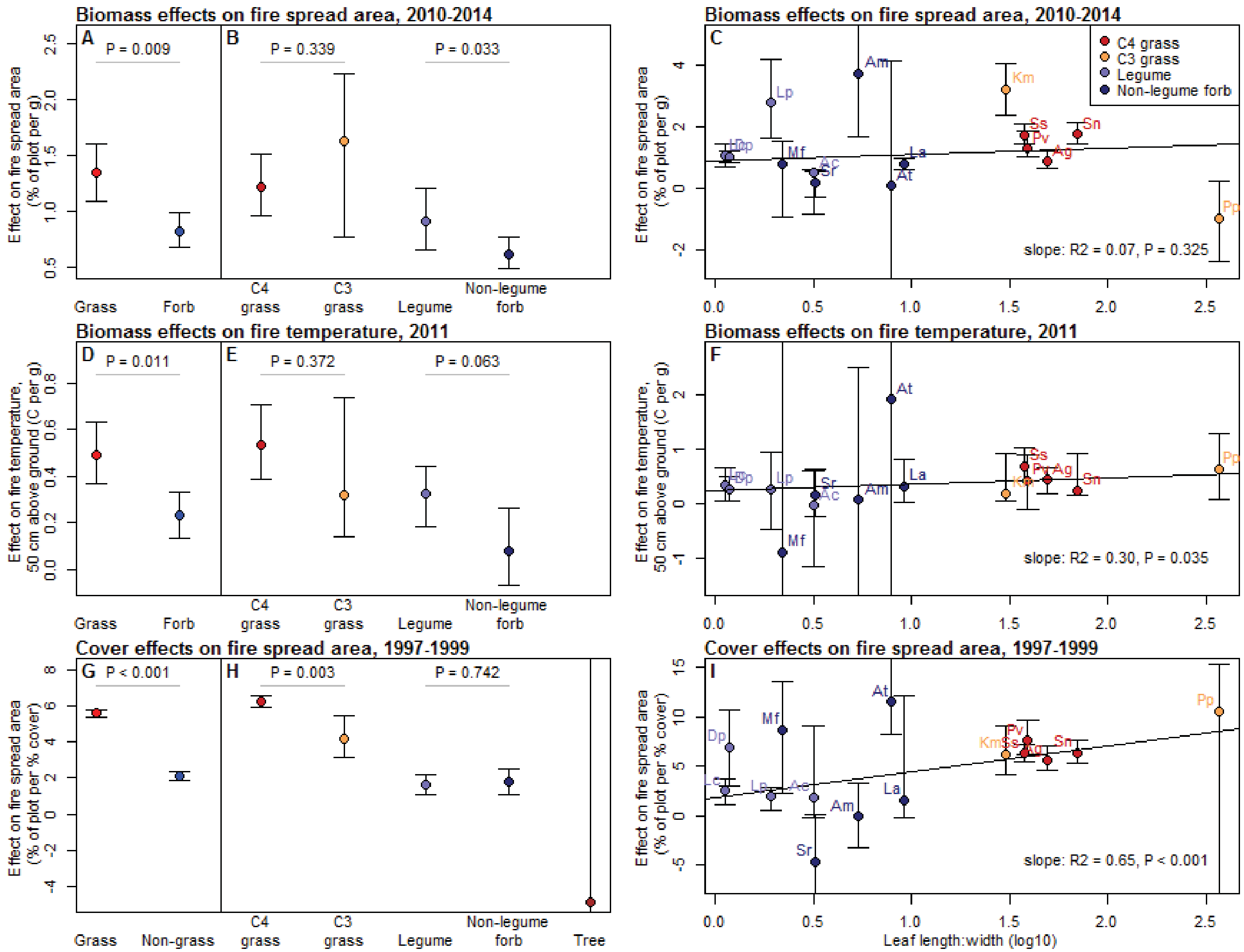


Figure 2-S1: Herbaceous biomass in spring versus late the previous summer.

In grassland biodiversity experiment plots, total pre-burn biomass including litter in spring 2015 was slightly lower in plots with only forbs (blue line) than in plots with only grasses (red line), for a given biomass excluding litter late the previous summer. Lines are fitted values from a multiple regression, forced through the origin, with biomass the previous summer and its interaction with the proportion grass as independent variables. The dashed line is the 1:1 line.

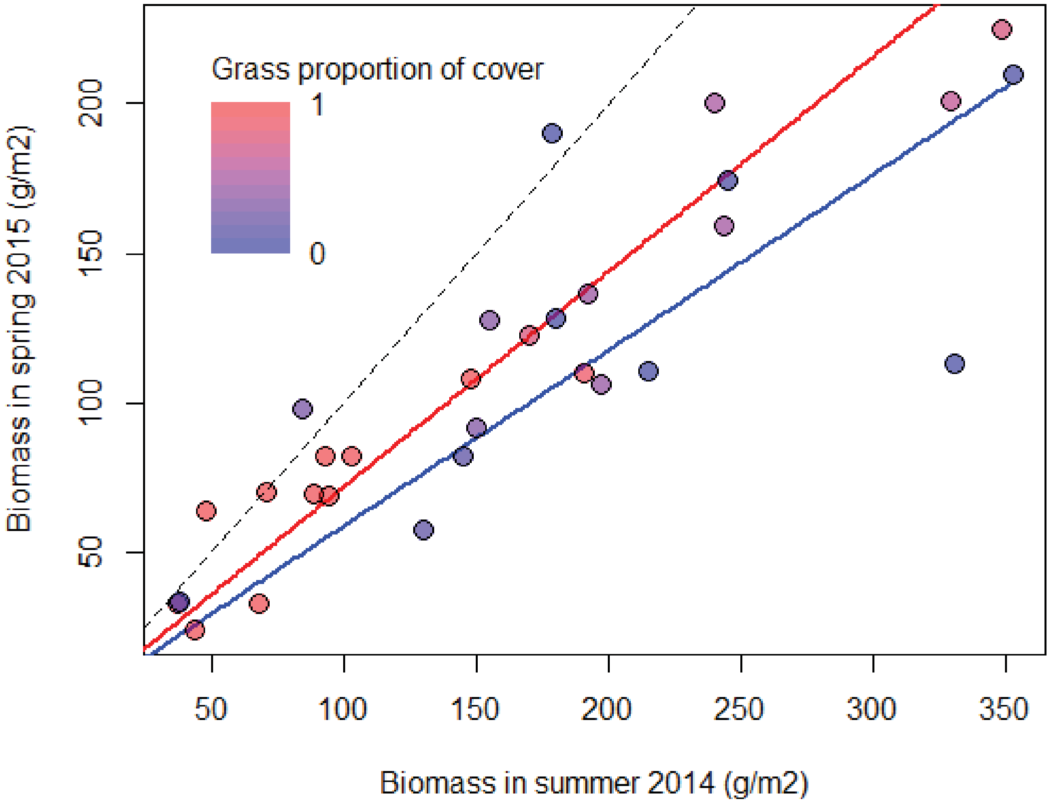
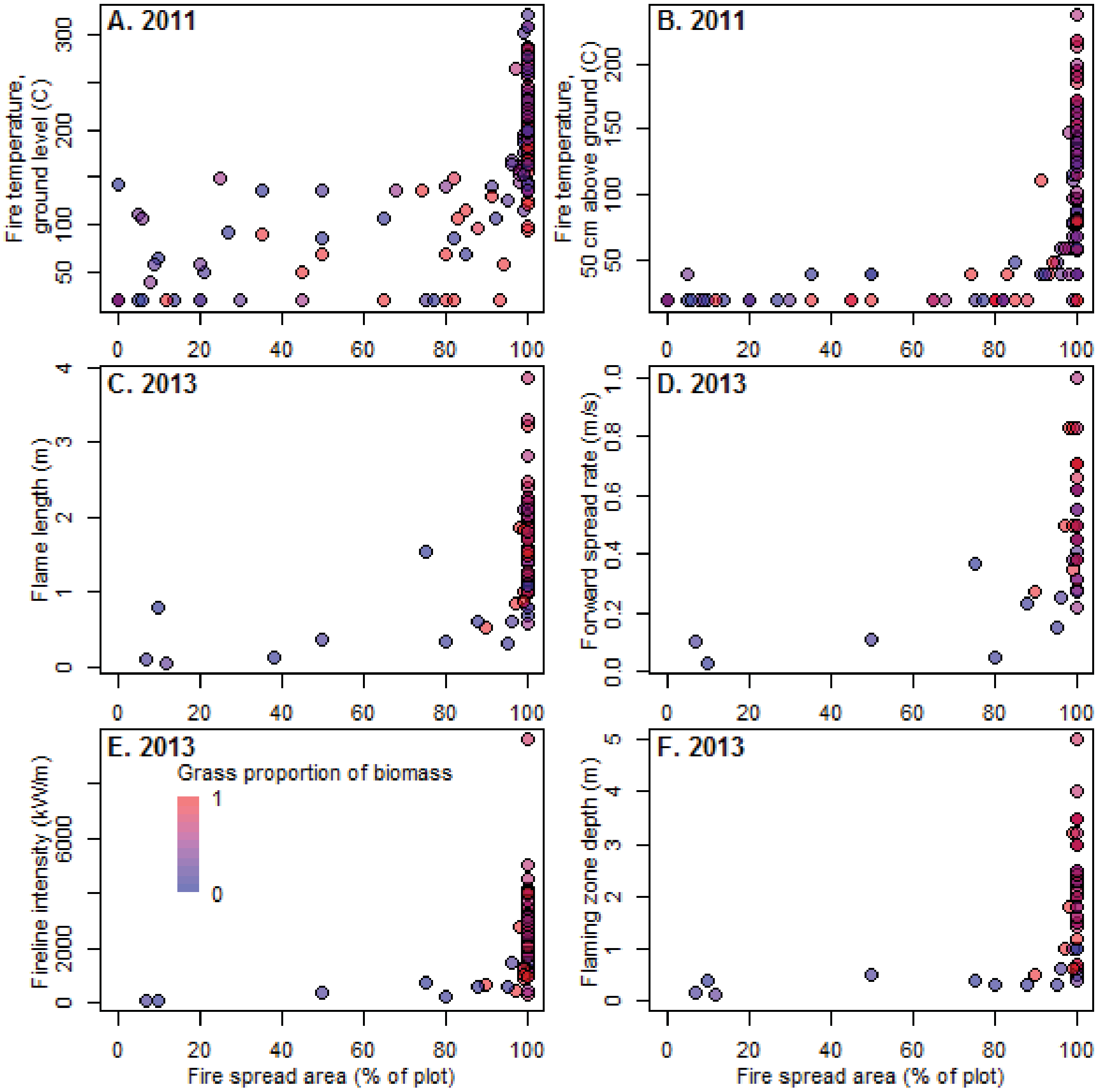


Figure 2-S2: Relationships between fire behavior measures.

All six measures of fire intensity were low in plots with low fire spread area following a single ignition. Fire intensity was highly variable in plots with high fire spread area: fire intensity increased with biomass, even above the biomass at which fire spread was complete (compare Figures 2-3A,C). Fire spread completely through all plots in which fire intensity was high.



Chapter 3 : Herbs influence savanna tree abundance through size-dependent fire damage and resource competition

Summary

Grasslands, savannas, and forests have been hypothesized to be alternate stable states in regions with intermediate rainfall. It has been proposed that fire-dependent feedback effects of trees and of herbaceous plants maintain each of their alternative states and that such feedback effects are also impacted by resource availability and climate. However, the roles of fire and resource availability in such feedbacks – and how these are altered by changes in the biomass, diversity, and functional composition of herbaceous vegetation – remain unclear. We present results of experimental studies of the effects of herbaceous vegetation and its combustion on survival and growth of savanna trees. Herbaceous biomass, especially C4 grass biomass, promoted fire and competed for resources which led to a profound suppression of savanna oak tree establishment.

We tested for effects of herbaceous plant biomass and functional composition on the survival and aboveground growth of fire-adapted bur oak (*Quercus macrocarpa*) and pin oak (*Quercus ellipsoidalis*) trees seeded in 77 annually-ignited plots of a biodiversity experiment at Cedar Creek Ecosystem Science Reserve, Minnesota, USA. Planted species richness treatments created an 11-fold gradient in total (aboveground + belowground) herbaceous biomass, from monocultures to diverse 16-species plots. After 16 years, plots with the highest herbaceous biomass had aboveground tree biomass per planted tree seed (i.e., the summed biomass of all trees in a plot divided by the number of seeds planted, a fitness measure that integrates survival and growth) that was 50-fold lower (bur oak) or 1000-fold lower (pin oak) than in plots with the lowest herbaceous biomass, mainly due to differences in growth rather than survival. Lower savanna tree survival and growth in plots with higher herbaceous biomass was associated with increased fire spread area and reduced availability of light and nitrate. Herbaceous plants competitively depleted available soil nitrate despite increasing total soil nitrogen. Fire spread prevented trees from maturing by top-killing small trees; top-killed trees were unable to regrow large enough to escape repeat top-kill under annual burning and thus remained in a "fire trap" at high herbaceous biomass. Herbaceous biomass was tightly associated with planted species richness, and these two variables each explained savanna tree performance similarly. The species richness treatments were drawn from a pool of species including equal numbers of C4 and C4 grasses, legumes, and non-legume forbs, resulting in plots varying in functional composition. C4 grasses inhibited savanna tree establishment more strongly than other herbaceous functional types, both by promoting fire spread and depleting soil nitrate. Conversely, legumes increased soil nitrate availability but promoted fire spread, perhaps by facilitating C4 grasses, and thus had little net effect on savanna tree establishment.

High herbaceous plant abundance associated with the plant diversity typical of regional prairies and savannas profoundly suppressed savanna tree abundance by increasing both fire spread and competitive depletion of resources. Reduced savanna tree abundance could in turn promote fire spread and reduce nitrate availability, and thus further reduce tree abundance in a positive feedback. Conversely, a reduction in herbaceous plant abundance or diversity could release savanna trees to grow to maturity and increase in abundance, which could in turn reduce fire spread and increase resource availability, and thus further increase tree abundance. Therefore – together with variation in climate, ignition regime, soil parent material, herbivory, and tree species' characteristics – the biomass, diversity, and functional composition of herbaceous plants may influence the shifting distributions of grassland, savanna, and forest.

Introduction

Though tree abundance tends to increase with rainfall, extensive tropical, subtropical and temperate regions with intermediate rainfall can exist as seemingly alternative stable states of grassland, savanna, or closed-canopy forest (Whittaker 1975, Grimm 1983, Sankaran *et al.* 2005, Staver *et al.* 2011). Some of this variation may be explained by positive feedbacks that lead to alternative stable states of a) grassland dominated by herbaceous vegetation that promotes and is resilient to fire, in which trees tend to be either absent or small and top-killed by fire; b) savanna, similar to grassland except with intermediate tree cover, typically with some individuals of fire-adapted tree species that are large enough to escape being top-killed by fire and reproduce by seed; or c) forest, dominated by woody vegetation that can suppress and be sensitive to fire (Bond *et al.* 2005, Hirota *et al.* 2011, Staver *et al.* 2011, Ratajczak and Nippert 2012). However, the boundaries of these biomes remain enigmatic; in particular, soil resource availability may also limit trees (Bond 2008), and the relative roles of fire disturbance and edaphic conditions remain unclear.

Both fire behavior and resource availability depend on herbaceous plants as well as extrinsic factors such as ignition regimes, soil parent material, and climate. Herbaceous plants have been found to influence savanna trees through both fire spread and intensity (Govender *et al.* 2006, Higgins *et al.* 2007, Onodi *et al.* 2008) and resource competition (Davis *et al.* 1998, Ward 2005), but few if any experiments have examined how fire and resource availability may jointly mediate how herbaceous plants influence savanna trees. Here we investigate how herbaceous plants influence savanna tree performance via effects on fire behavior and resource availability.

A variety of studies suggest that shifts from grassland and savanna to forest, and from forest to savanna and grassland, are associated with shifts in fire frequency (Grimm 1984, Peterson and Reich 2001, Bond *et al.* 2005, Cavender-Bares and Reich 2012), increased nutrient availability (Ward 2005), changes in climate (Grimm 1983), increasing atmospheric CO_2 concentration (Kgope *et al.* 2010, Moncrieff *et al.* 2013), changes in herbivory (Staver *et al.* 2009), and exotic species introductions (D'Antonio and Vitousek 1992). Many of these factors are thought to influence trees by altering their interactions with herbaceous vegetation. Therefore, understanding how herbaceous vegetation influences trees could contribute to a more mechanistic and predictive understanding of the determinants of tree abundance and shifting biome distributions.

We hypothesize that the biomass of herbaceous vegetation may be an important determinant of savanna tree establishment via 1) fire-dependent feedback effects and 2) availability of resources that can limit survival and growth of savanna trees. First, higher herbaceous biomass should increase fire intensity (Cheney *et al.* 1993, D'Antonio 2000) and, if it increases horizontal continuity of fuel, may also increase fire spread and point fire frequency (Brooks *et al.* 2004). Fire frequency often refers to the frequency with which a parcel of land is ignited, but a plant is most affected by the frequency with which the patch immediately surrounding it burns. This depends on fire spread as well as ignition frequency; we refer to it as point fire frequency. These expectations were supported in this experiment, reported in detail elsewhere (chapter 2). We hypothesize that high point fire frequency and intensity at high herbaceous biomass results in a fire-dependent feedback effect proposed by Bond and van Wilgen (1996), a “fire trap” that prevents savanna trees from becoming established. Frequent fire in savannas has selected – both evolutionarily and in terms of community assembly – for fire tolerance. In addition to thick bark that confers fire resistance to stems (Pausas 2014), most savanna tree species can resprout from the base using reserves stored belowground if they are top-killed by fire, i.e., if all aboveground stems are killed (Bellingham and Sparrow 2000, Bond and Midgley 2003, Clarke *et al.* 2013). The probability that a fire-adapted, resprouting savanna tree is top-killed by a fire often declines nonlinearly with size, being very high for small trees, decreasing sharply at an intermediate escape size, and being very low for large trees. A top-killed tree that resprouts but does not grow larger than the escape size before the next fire can be caught in a "fire trap" (Bond and van Wilgen 1996, Hoffmann and Solbrig 2003). A savanna tree cannot escape the fire trap unless the time interval between fires become sufficiently long for it to grow to its escape size. Second, higher herbaceous biomass may increase supply and retention of limiting soil resources such as N and moisture by increasing soil organic matter, yet may more strongly compete for these resources, reducing resource availability to savanna trees (Tilman *et al.* 1996).

Herbaceous species richness may influence savanna tree abundance both because herbaceous species richness increases herbaceous biomass when other influences are held constant (Tilman *et al.* 1996), and because herbaceous species richness *per se* may affect fire and resources through other mechanisms even after accounting for its effects on the quantity of herbaceous biomass. For example, it has been proposed that more diverse fuel loads may burn better (de Magalhães and Schwilk 2012).

We also hypothesize that different functional types of herbaceous plant species differ in their effects on savanna tree establishment due to both distinct effects on total herbaceous biomass and distinct effects per unit mass on resource availability and fire behavior. C4 grasses are reported to promote fire particularly strongly through their high productivity and retention of unpalatable, decay-resistant, flammable leaf fuel (D'Antonio and Vitousek 1992, D'Antonio 2000, Brooks *et al.* 2004). It is not well known how herbaceous plants of other functional groups affect fire spread and intensity, though a positive interaction between C4 grasses and legumes -- whereby legumes increase C4 grass biomass through N fixation (Tilman *et al.* 1997) – implies that legumes could promote fire indirectly. By increasing litter quantity through increased productivity, C4 grasses and legumes can increase soil organic matter and thus the supply of inorganic nitrogen and water retention. Legumes can further increase nitrate availability through their high tissue N. Conversely, C4 grass tissue, low in N, can slow N mineralization; moreover, C4 grasses deplete nitrate to particularly low equilibrium levels in monoculture (Wedin and Tilman 1993). C3 grasses and non-legume forbs have not been reported to have such strong effects on fire or nitrate. Thus, different herbaceous functional types could have opposing effects on resource availability.

Using 16 years of data from an annually-burned savanna biodiversity experiment in which planted species richness and composition treatments established profound gradients in herbaceous biomass and functional composition, we ask (1) how the quantity of herbaceous biomass influenced savanna tree survival and growth, and (2) whether and how these influences were mediated by fire or resource availability or both; (3) whether herbaceous species richness influenced savanna tree survival and growth through mechanisms independent of herbaceous biomass; (4) whether and how the functional composition of herbaceous vegetation influenced savanna tree survival and growth, both through their contributions to total herbaceous biomass and through distinct effects per unit biomass; and (5) whether and how tree size-dependent top-kill and subsequent regrowth underlie fire-mediated effects of herbaceous biomass and functional composition on savanna tree performance. We examine these questions for two fire-adapted, resprouting savanna oak tree species with contrasting life history strategies: a slower-growing, more fire-tolerant and resource-conservative species (bur oak, *Quercus macrocarpa*) and a faster-growing, less fire-tolerant and more resource-demanding species (pin oak, *Quercus ellipsoidalis*).

Methods

Experimental design

We report results from a biodiversity experiment (experiment number E120) at Cedar Creek Ecosystem Science Reserve, Minnesota, USA (Tilman *et al.* 1997). Plots were seeded using random draws of 1, 2, 4, 8, or 16 perennial species from among those abundant in nearby oak savanna, one of few remnants of this habitat neither replaced by forest due to fire suppression since European settlement (Cottam 1949, Grimm 1984, Tester 1989) nor transformed by agriculture or urbanization. These species represented five functional groups: the dominant trees of nearby savanna, bur oak (*Quercus macrocarpa*) and pin oak (*Q.*

ellipsoidalis), which can grow single stems 20+ m tall when undamaged but can repeatedly resprout multiple stems when damaged; C4 grasses (*Andropogon gerardii*, *Panicum virgatum*, *Schizachyrium scoparium* and *Sorghastrum nutans*); C3 grasses (*Elymus canadensis*, *Koeleria macrantha, Pascopyron smithii* [syn. *Agropyron smithii*] and *Poa pratensis*); herbaceous legumes (*Amorpha canescens*, *Dalea purpurea* [syn. *Petalostemum purpureum*], *D. villosa* [syn. *P. villosum*], *D. candida* [syn. *P. candidum*], *Lespedeza capitata*, and *Lupinus perennis*); and other forbs (*Achillea millefolium*, *Asclepias tuberosa*, *Liatris aspera*, *Monarda fistulosa*, *Oligoneuron rigidum* [syn. *Solidago rigida*]) (Tilman *et al.* 2001). Herbaceous species were seeded in 1994 and 1995; trees were seeded in April 1995. Seeding rates for each species were inversely proportional to plot diversity: plots planted with 1, 2, 4, 8 or 16 species were given 170, 85, 43, 21, or 11 seeds of each allocated tree species, i.e., 1, 0.5, 0.25, 0.12, or 0.07 seeds m^{-2} (initial plot area 13 × 13 m; plots were reduced to 9 × 9 m in 1998). Bur oak seeds (mean mass 2.05 g, estimated viability 99-100%) were from Itasca Co., MN (240 km to the N). Pin oak seeds (mean mass 1.38 g, estimated viability 95%) were from Floyd Co., IA (250 km to the S). Of 168 plots in this experiment, we examine the 77 plots planted with at least one tree species, including 52 with pin oaks and 61 with bur oaks (37 had both).

The experiment was maintained by burning annually in spring as soon as possible after snow-melt (March-May). Fire scars show that annual burning was common in this region before European settlement ((Pierce 1954), cited by (Tilman *et al.* 2000)), and many savanna regions in Africa, South America and Asia currently burn approximately annually (Archibald *et al.* 2013). Non-planted species were manually weeded out three times each growing season. Selective herbicides were occasionally used for weed control.

Savanna tree abundance after 16 years

In September (autumn) 2010, we measured the size and weighed or estimated the aboveground biomass of each stem of the 656 surviving trees. We measured each stem's height and diameter 5 cm above ground. We harvested each stem (with its leaves), dried it at 60 °C to constant mass, and weighed it, with two exceptions. First, in four plots in which trees 1.1 – 2 m tall were especially numerous, we harvested only a random sample of trees in this size class. We estimated non-harvested trees' stem biomasses from their height (h, cm) and diameter 5 cm above ground (d, mm) using regressions from the harvested stems: $\log_e$ (bur oak dry biomass, g) = $-2.7338 + 0.7645 \log_e(d^2 \times h)$, and $\log_e$ (pin oak dry biomass, g) = $-1.18352 + 1.42089 \log_e(d) + 0.07204 \log_e(d)^2 + 0.07901 \log_e(h)^2$, $R^2 = 0.97$ for both species. Second, we estimated the oven-dry biomasses of a sample of large harvested stems (from 69 trees taller than 1 m) by adjusting their air-dry biomasses using the air-dry moisture content of comparably sized stems that were oven-dried.

Several lines of evidence indicate that the oaks we measured were the cohort planted in 1995. Subsequent seed inputs were negligible because no trees grew large enough to produce seeds, acorn-dispersing birds (jays) rarely flew over the experiment, and acorn-dispersing mammals (squirrels) were never observed. Accordingly, no oaks were found in 127 unweeded plots in which oaks had not been planted, interspersed among our experimental plots and established at the same time, in a species composition survey in 2007. Further, annual growth rings dating back to 1995 were evident in lignotubers of even the smallest trees.

Herbaceous biomass

We measured herbaceous biomass as the sum of aboveground and belowground components. Peak aboveground dry herbaceous biomass was estimated in the late growing season (July-August) each year from 1996 until 2006 by clipping at the ground surface an area of 12 m x 0.1 m in each plot (Tilman *et al.* 2006). Litter from previous years was excluded (mean 4 % of total aboveground biomass, 2001-2006). Root dry biomass to 30 cm depth was estimated using soil cores in 1997-2004 and 2006 (Fornara and Tilman 2009). Both aboveground and root biomass of herbaceous plants can influence savanna tree establishment, yet they were correlated ($r = 0.77$), so we focus on their sum and refer to this throughout as herbaceous biomass. To measure herbaceous species' relative abundances, each herbaceous species' aboveground biomass was estimated from 1996-2000 by multiplying its proportional cover by total aboveground herbaceous biomass, and from 2001-2006 by sorting and weighing aboveground biomass by species.

Fire behavior and resource availability

We characterized fire behavior and resource availability to investigate their influence on savanna tree survival and growth. Plots varied in how completely and intensely they burned. Plots that did not burn completely were re-ignited, but this only modestly increased fire spread area and many plots with low herbaceous biomass nonetheless burned incompletely (chapter 2). The percentage of each plot's area through which fire spread was estimated visually following spring burns (after any re-ignitions) in 1997-1999 and 2010-2011. Maximum fire temperatures were estimated in 2010 and 2011 using 13 Omega Laq (Omega Engineering, Stamford, CT) paints with melting points spanning 79-788 °C on copper plant tags (National Band and Metal, Newport, KY). In each plot, we averaged fire temperature measured at ground level and 50 cm above ground at each of three positions (2 m from plot edges) (chapter 2).

Soil nitrogen and moisture availability often limit plant growth in the sandy soils at this site (Tilman 1987, Davis *et al.* 1999). NO_3^- and NH_4^+ concentrations were measured in each plot using 0.01 M KCl extractions, to estimate the N readily available to plants, during the growing season between late June and August once in each year 1996-1999, 2002, and 2006, and 6 times in 2001 (Tilman *et al.* 1997, Fornara and Tilman 2009). Gravimetric soil moisture was measured simultaneously. Percent of light (photosynthetically active radiation) penetrating to the soil surface was measured in each plot once in each year 1996-2000.

Savanna tree fire damage and annual growth

To investigate top-kill as a mechanism for the effects of fire on tree growth, we observed fire damage to 406 individually-marked trees during the 2010 spring fire and measured their change in size from before the fire until the end of the succeeding growing season. Immediately before the 5 April 2010 burn, we estimated the biomass of each tree's tallest live stem using its height and diameter and the above equations from the 2010 harvest, for up to 12 systematically selected trees (all if fewer than 12) per species per plot. Around each tree, we estimated the proportion of a circle 20 cm in radius that burned. In July, we determined whether each tree had been top-killed by comparison with a pre-burn photograph.

We calculated the annual relative rate of change in biomass of each tree's tallest live stem by subtracting its pre-fire estimated $\log_e$ biomass from its post-growing season (September 2010) $\log_e$ biomass. This approximates annual change because it includes most of the growing season in this temperate continental climate: for both species in 2010 bud break was in late April, after our spring survey, and leaf fall began in mid October, less than a month after our autumn survey (C. Buyarski, unpublished data). This rate reflects fire damage as well as growth: if a tree's tallest live stem in spring was killed by fire, the autumn measurement was of a different stem. This largest-stem rate of change in biomass represented the whole-tree rate of change in biomass: relative changes in largest-stem biomass from 2006 to 2010 were correlated with relative changes in whole-tree aboveground biomass over that period (not shown).

Analyses

We analyzed the responses of tree survival and growth to herbaceous biomass, species richness, functional composition, fire behavior, and resource availability using (generalized) linear mixed models. For each plot, we averaged each independent variable over all available samples. To linearize relationships, we $\log_e$-transformed tree biomass, tree biomass per planted seed, herbaceous biomass, planted species number and soil NO_3^- concentration, and logit-transformed fire spread area (expressed as a proportion of plot area) when it was a dependent variable (Warton and Hui 2010).

Tree biomass per planted seed (the total tree biomass in a plot divided by the number of tree seeds planted in the plot, equivalent to the product of proportional survival and mean tree biomass) included zeroes for the many plots with no surviving trees, which prevented log-transformation (the log of zero is undefined) and violated the assumptions of standard error distributions. Therefore, equivalent to using biomass per planted seed (which we graphed), in regressions we used total biomass per plot as the response with number of seeds planted as an offset and a compound Poisson error distribution (Zhang 2013). This error distribution allows a large number of zeros: it is suitable when there are a Poisson-distributed number of individuals surviving (an empirically reasonable approximation to the binomial survival process in this case), and each of those survivors has a gamma-distributed biomass (also empirically reasonable), and the biomasses of the individuals are summed. Results from these compound Poisson regressions were in all cases qualitatively similar to linear regressions using log-transformed mean biomass per planted seed (with half the minimum non-zero value added to all values to allow the zeros to be log-transformed).

We used R packages lme4 (Bates *et al.* 2014) and cplm (Zhang 2013) to fit generalized linear mixed models and compound Poisson mixed models. We report tests of individual coefficients using Wald z scores, and joint tests of multiple coefficients (such as a test for the effect of herbaceous biomass on tree survival across both tree species) using Wald χ^2 statistics from Type II ANOVAs that assess effects after accounting for all other non-interaction effects, using R package car (Fox and Weisberg 2011). We also used likelihood ratio comparisons of pairs of regression models to assess whether adding a variable to a regression significantly improved fit.

We obtained similar results to those that we present if we 1) excluded the eight plots with more than 100 g/m^2 of tree biomass (including the five plots without any herbaceous species planted), 2) used herbaceous biomass, fire spread, and resource availability data from the subset of years in which all were measured (1997-1999), or 3) used the number of herbaceous species planted in place of the total number of species (including trees) planted.

Results

Herbaceous biomass influences savanna tree performance

Increasing herbaceous biomass (measured as the sum of aboveground and belowground biomass) strongly reduced savanna tree survival and growth, from planting until harvest 16 years later. Tree survival decreased with increasing herbaceous biomass (Fig. 3-1A,B), weakly and non-significantly in bur oaks but three-fold in pin oaks (significantly more strongly in pin oaks: tree species x herbaceous biomass interaction $z = -2.17$, $P = 0.030$, in a logistic regression with the number of trees surviving in the final year as a proportion of the number of seeds planted in a plot as the dependent variable; log herbaceous biomass [continuous], tree species, and their interaction as fixed effects; and plot as a random intercept to account for plots with both tree species being represented twice). The mean final aboveground dry biomass of surviving trees decreased 100-fold (bur oaks) or 300-fold (pin oaks) as herbaceous biomass increased across its range (Fig. 3-1C,D; overall herbaceous biomass effect $\chi^2_1 = 23.64$, $P < 0.001$ in a similar regression with $\log_e$ aboveground biomass of trees in a plot as the dependent variable and a Normal error distribution; interaction with species not significant). These concordant effects of herbaceous biomass on survival and mean biomass compounded, according to a similar regression with summed aboveground biomass of all trees in a plot in 2010 as the dependent variable and a compound Poisson error distribution. Number of seeds planted was an offset, so this can be interpreted as biomass per planted seed. As herbaceous biomass increased across its range, aboveground tree biomass per planted seed decreased 50-fold for bur oaks (Fig. 3-1E, $z = -5.92$, $P < 0.001$) and 1000-fold for pin oaks (Fig. 3-1F; effect significantly stronger in pin oaks than in bur oaks: interaction $z = -3.85$, $P < 0.001$). Five earlier tree surveys, spanning years 9-15, showed similar effects of herbaceous biomass on tree size (Fig. 3-S1).

Other measures of tree size correlated tightly with whole-tree aboveground biomass, and responded similarly to herbaceous biomass: the biomass, diameter and height of a tree's largest stem; the mean biomass and diameter of a tree's stems; and tree cross-sectional lignotuber area, a potential proxy for tree belowground biomass (not shown). For both species, the mean diameter and height of the largest stem per tree declined from greater than 20 mm diameter and 100 cm height in monocultures to less than 5 mm diameter and 25 cm height in the most diverse plots.

Fire behavior and resource availability influence savanna tree performance

Herbaceous biomass was also associated with increased fire spread area, and decreased NO_3^- and light availability, which in turn were associated with lower savanna tree performance (survival and growth). Separate simple linear regressions showed that increasing log herbaceous biomass increased fire spread area (Fig. 3-2A, $z = 17.18$, $P < 0.001$, $R^2 = 0.85$) and soil moisture (Fig. 3-2C, $z = 2.05$, $P = 0.044$, $R^2 = 0.05$), and reduced light penetration (Fig. 3-2D, $z = -13.04$, $P < 0.001$, $R^2 = 0.69$) and NO_3^- concentration (Fig. 3-2B, $z = -2.40$, $P = 0.019$, $R^2 = 0.07$). Log herbaceous root biomass was slightly more strongly associated with reduced NO_3^- concentration ($z = -2.89$, $P = 0.005$, $R^2 = 0.10$). NH_4^+ was less clearly related to diversity than was NO_3^- (not shown). Increasing herbaceous biomass also increased fire temperature, which was collinear with fire spread area (chapter 2); we chose to use fire spread area because it was available for more years and explained more variation in tree performance.

Plots with higher fire spread area and lower NO_3^- concentration had lower savanna tree survival and biomass (Fig. 3-3). We used separate regressions with each of survival, tree mean biomass, and tree biomass per seed as dependent variables; fire spread area and the three resource availabilities, plus their interactions with tree species and a tree species main effect, as joint independent variables; and plot as a random intercept. Higher mean fire spread area was associated with lower tree survival ($\chi^2_1 = 9.53$, $P = 0.002$, Fig. 3-3A), tree mean biomass ($\chi^2_1 = 3.89$, $P = 0.049$, Fig. 3-3B), and tree biomass per seed ($\chi^2_1 = 8.50$, $P = 0.004$, Fig. 3-3C). Higher NO_3^- was associated with higher tree survival (not significant, $\chi^2_1 = 3.04$, $P = 0.081$, Fig. 3-3A), mean tree biomass ($\chi^2_1 = 9.72$, $P = 0.002$, Fig. 3-3B), and tree biomass per seed ($\chi^2_1 = 23.8$, $P < 0.001$, Fig. 3-3C). NO_3^- was more strongly positively associated with tree biomass and tree biomass per seed in pin oaks than bur oaks ($\chi^2_1 \geq 8.12$, $P \leq 0.004$). Higher light availability was associated with significantly lower tree survival ($\chi^2_1 = 5.20$, $P = 0.023$, Fig. 3-3A) but conversely with (non-significantly) higher mean tree biomass ($\chi^2_1 = 1.44$, $P = 0.229$, Fig. 3-3B), and higher tree biomass per seed ($\chi^2_1 = 4.23$, $P = 0.040$, Fig. 3-3C). In high herbaceous biomass plots, trees were typically dwarfed beneath the dense herbaceous canopy (pers. obs.), so it is plausible that herbaceous plants inhibited tree growth by intercepting light. Higher soil moisture was non-significantly associated with higher survival ($\chi^2_1 = 0.012$, $P = 0.913$, Fig. 3-3A), mean tree biomass ($\chi^2_1 = 0.91$, $P = 0.341$, Fig. 3-3A) and tree biomass per seed ($\chi^2_1 = 3.17$, $P = 0.075$, Fig. 3-3A). The two tree species did not respond significantly differently to any of these environmental variables except for NO_3^-, which was more strongly positively associated with increased growth in pin oaks than in bur oaks.

Fire spread and resource availabilities correlated weakly enough to allow the above regression estimates of their independent effects on savanna tree establishment: no more than 50% of the variance in each independent variable could be explained by a linear combination of the other independent variables, based on variance inflation factors. We did not find significant pairwise interaction effects among these independent variables using backwards or forwards stepwise regression.

Fire spread area, NO_3^-, and light jointly explained the effects of herbaceous biomass on savanna tree performance: adding herbaceous biomass to regressions already containing fire spread area, NO_3^-, and light did not explain significantly more variation in tree survival, biomass, or biomass per seed (likelihood ratio model comparisons: $P > 0.224$).

Herbaceous diversity influences savanna tree performance through herbaceous biomass

The herbaceous biomass gradient was caused primarily by planted species richness treatments: herbaceous biomass increased strongly with species richness (Fig. 3-S2A and as reported previously, (Tilman *et al.* 1997, Tilman *et al.* 2001, Reich *et al.* 2012)). Accordingly, the effects of herbaceous biomass on savanna tree establishment reported above can also be interpreted as effects of the herbaceous species richness treatments. Higher species richness inhibited savanna tree establishment (Fig. 3-S3), apparently by increasing fire spread (Fig. 3-S4A), increasing light interception (Fig. 3-S4D), and reducing soil NO_3^- (Fig. 3-S4B).

We did not find statistically significant evidence that planted species richness had an additional influence on savanna tree establishment independent of its influence on the amount of herbaceous biomass. Log herbaceous biomass -- which refers throughout to the sum of aboveground and belowground components -- explained significantly though slightly less variation in tree biomass per seed than did log planted species richness (not shown). However, herbaceous biomass did explain the effect of species richness on tree biomass per seed when it was split into aboveground and belowground components, apparently because NO_3^- increased slightly more strongly with belowground herbaceous biomass ($R^2 = 0.19$) than with total herbaceous biomass ($R^2 = 0.15$). Adding planted species richness to regressions already including aboveground and belowground herbaceous biomass as joint independent variables did not explain significantly more variation in tree survival, biomass, or biomass per seed ($P >= 0.079$). Nor did species richness explain significant additional variation in fire spread, NO_3^- availability, or light availability after accounting for herbaceous biomass.

Both fire and resources were needed to explain the effect of planted species richness on savanna tree establishment (using regressions not including herbaceous biomass). Adding species richness as an additional independent variable to regressions already containing fire spread area explained significantly more variation in tree biomass per planted seed ($\chi^2_2 = 9.187$, $P = 0.010$), suggesting that species richness influences trees through an additional mechanism such as resource competition. Similarly, adding species richness as an independent variable to regressions already containing NO_3^- and light resources explained significantly more variation in tree biomass per planted seed ($\chi^2_2 = 10.38$, P = 0.005), suggesting a role for an additional mechanism such as fire. Together, fire spread area, NO_3^-, and light availability could account for the influence of species richness on tree establishment, just as they accounted for the influence of herbaceous biomass: adding species richness to a regression containing fire, nitrate and light availability did not explain significantly more variation in tree biomass per planted seed ($\chi^2_2 = 0.45$, p = 0.798).

Herbaceous functional composition effects on savanna tree performance

Herbaceous functional composition influenced savanna tree performance through both a) influences on the total quantity of herbaceous biomass, and b) biomass-specific effects on trees of herbaceous biomass. We parse these two influences of herbaceous functional composition on trees by characterizing it in two ways. First, we characterized the planted species composition treatments by whether at least one species in a functional group was represented in the plot (functional group presence, Fig. 3-4 left column); the numbers of species planted in each functional group had similar effects, not shown. These treatments influenced both the quantity of herbaceous biomass and its biomass-specific effects. Second, we examined functional group effects per unit aboveground biomass, using the log of the summed aboveground biomass of all species in each functional group averaged over all available years; differences in the effects of functional group biomasses on trees indicate functional group effects that were independent of their contribution to total aboveground herbaceous biomass (Fig. 3-4 right column). We used aboveground biomass instead of the sum of aboveground and belowground biomass as our measure of functional groups' abundances because roots were not sorted to species or functional group, but aboveground and aboveground+belowground herbaceous biomass were highly correlated (r = 0.86) and can be interpreted similarly except as noted below. We synthesize below results of multiple regressions (Fig. 3-4) of tree biomass, herbaceous biomass, fire spread and resource availability (dependent variables) against either the planted presences of all functional groups or the aboveground biomasses of all functional groups (independent variables). For tree biomass, species and its interaction with the functional groups was also an independent variable, and there was a random intercept for plot.

C4 grasses inhibited savanna trees more strongly than did other herbaceous functional groups, both because they were the most productive and because they promoted fire spread and depleted soil NO_3^- and light the most strongly per unit abundance. Tree biomass per seed was significantly lower in plots with at least one C4 grass planted than in plots without any herbaceous functional groups planted, and this effect was stronger for pin oaks than for bur oaks (Fig. 3-4A, z = -2.205, P = 0.027 for bur oaks; pin oak * C4 presence interaction z = -3.17, P = 0.002). C4 grasses were influential partly because their presence most strongly increased herbaceous biomass (Fig. 3-4C). Accordingly, C4 grasses had the highest mean abundance across the experiment (Fig. 3-S2B) even though all functional groups were similarly represented in the seeding design. The high productivity of C4 grasses partly explains why their planted presence increased fire spread (Fig. 3-4E) and depleted light (Fig. 3-4I) and soil NO_3^- (Fig. 3-4G) more strongly than did other functional groups. However, C4 grasses also had stronger effects *per unit aboveground biomass* on all of these variables than did other functional groups (Fig. 3-4B,D,F,H,J). C4 grasses may have reduced NO_3^- most strongly per unit aboveground biomass partly because they have a higher ratio of root biomass to shoot biomass than other functional groups, evidenced by their higher total biomass per unit aboveground biomass (Fig. 3-4D), combined with NO_3^- being more tightly related to herbaceous root biomass ($R^2 = 0.19$) than to herbaceous aboveground biomass ($R^2 = 0.04$).

C3 grass presence was also associated with reduced biomass per seed of both savanna tree species (Fig. 3-4A, $\chi^2_1 = 7.74$, $P = 0.005$). Discordantly, the presence of C3 grasses did not significantly influence total biomass (Fig. 3-4C) – indeed, their mean biomass across the experiment was less than a tenth that of C4 grasses (Fig. 3-S2C) – nor fire spread (Fig. 3-4E) nor resource availability (Fig.4G,I). The presence of non-legume forbs did not significantly affect tree performance (Fig. 3-4A, $P = 0.161$), fire spread (Fig. 3-4E), or resource availability (Fig. 3-4G,I).

Legume presence did not significantly affect savanna tree performance (Fig. 3-4A, $P = 0.279$); this reflected strong but counter-acting influences of legumes on NO_3^-, fire, and light availability. The presence of legumes (N fixers) strongly increased the availability of NO_3^- (Fig. 3-4G), in contrast to the other functional groups which all tended to reduce it, and legumes also had the most positive effect on NO_3^- per unit biomass (Fig. 3-4H). Higher NO_3^- availability tended to increase tree performance (Fig. 3-3). Conversely, legume presence may have hindered tree growth by strongly increasing herbaceous biomass (Fig. 3-4C), and accordingly increasing fire spread (Fig. 3-4E) and reducing light availability (Fig. 3-4I). Legumes may have increased total biomass through their own productivity: their mean abundance across the experiment approached that of C4 grasses. Legumes may also have increased the productivity of other functional groups, by fixing N: legume effects on herbaceous biomass, fire spread and light were all relatively weaker when the biomasses of the other functional groups were statistically controlled (in regressions against functional group biomasses) than when the biomasses of the other functional groups were not controlled (in the regressions against functional group presences).

Thus, herbaceous functional groups influenced savanna tree establishment in distinct ways, both through their productivity and through additional biomass-specific effects on fire spread and resource availability (Fig. 3-7B). No pairwise interactive effects of functional groups (presence or biomass) on tree performance, examined using backwards and forwards stepwise regression, were robust to the alternative data subsets described in Methods.

Herbaceous biomass influences savanna tree annual growth rate through fire damage (top-kill)

Trees were more likely to be top-killed by fire at higher herbaceous biomass, and top-killed trees grew relatively less than non-top-killed trees; this could explain why trees grew more slowly at higher herbaceous biomass (Fig. 3-5). Probability of top-kill increased from less than 20 % at low herbaceous biomass (< 250 g/m^2) to near 100 % at the highest herbaceous biomass ($> 1\,500$ g/m^2, Fig. 3-5A,B, logistic regressions on individual trees with plot as a random intercept for each tree species separately: for both species, $z > 4.35$, $P < 0.001$).

We use "annual growth rate" to refer to the mean annual relative rate of change in biomass of trees' largest stems during the year leading up to the harvest (Fig. 3-5C,D), acknowledging that this includes both growth and loss to fire. Annual growth rate depended on whether a tree was top-killed, but, after accounting for top-kill, not on herbaceous biomass. Growth rate did not depend on herbaceous biomass within either top-killed or non-top-killed trees ($P > 0.299$), in separate linear regressions for each species' top-killed and non-top-killed trees with plot as a random intercept. Mean growth rates of top-killed trees were not significantly different than zero (mean +- standard error 0.03 +- 0.06 yr^{-1} for bur oaks, -0.08 +- 0.10 yr^{-1}for pin oaks, black lines in Fig. 3-5C,D), whereas trees that escaped top-kill had significantly higher and non-zero growth rates (0.40 +- 0.09 yr^{-1} for bur oaks, 0.72 +- 0.10 yr^{-1} for pin oaks, green lines in Fig. 3-5C,D). These mean growth rate estimates are from separate regressions for each species with top-kill as the independent variable and a plot random intercept.

To combine the higher likelihood of top-kill at higher herbaceous biomass with the slower growth of top-killed trees, we used a weighted average of the fitted growth rates of top-killed and non-top-killed trees weighting by the fitted probability of top-kill given herbaceous biomass. This weighted average provides a reasonable fit to the (noisy) decrease in growth rate with increasing herbaceous biomass (gray lines in Fig. 3-5C,D).

These annual growth responses to herbaceous biomass (Fig. 3-5C,D) are consistent with longer-term growth responses (Fig. 3-1B; Fig. 3-S1), so the mechanism they suggest – that herbaceous biomass increases top-kill, and that top-killed trees grow more slowly – may also operate in other years. In high herbaceous biomass plots, mean annual growth rates indistinguishable from zero are consistent with static tree sizes over seven years (Fig. 3-S1). The mean annual growth rates for non-top-killed trees of 0.4 – 0.7 yr^{-1} (Fig. 3-5C,D; higher in pin oaks than in bur oaks) corresponded with relative rates of increase in whole tree biomass over all 16 years (from mean seed biomass to final aboveground biomass) of approximately 0.5 yr^{-1} in plots with the lowest herbaceous biomass and largest trees. Thus, top-kill appears to be an important mechanism by which herbaceous biomass inhibited tree growth. But why did herbaceous biomass promote top-kill?

Caught in a fire trap: Savanna tree size influences top-kill and growth rate

Trees were more likely to be top-killed in plots with higher herbaceous biomass both because fire spread area was higher (Fig. 3-2A) and because trees were smaller (Fig. 3-1C,D) and thus more vulnerable to fire damage (Fig. 3-6A,B). Top-kill, in turn, slowed trees' growth, creating a feedback that prevented trees from growing large enough to escape top-kill and holding them in a "fire trap." Moreover, lower mean growth rates among top-killed trees approaching the escape size may have further inhibited escape from the fire trap.

The probability that a tree was top-killed decreased steeply with increasing tree size (Fig. 3-6A,B, separate logistic regressions for each tree species with plot as a random intercept: for bur, $z = -5.57$, $P < 0.001$; for pin, $z = -3.37$, $P < 0.001$). The probability of top-kill also increased with the proportion of area in a 20 cm radius around a tree that burned ($z = 0.009$, $P = 4.74$) in bur oaks (the only tree species with sufficient sample size and variation to include per-tree fire spread area and its interaction with tree size as additional independent variables). In bur oaks, top-kill was unlikely even in small trees where fire spread area was low, whereas top-kill increased steeply with decreasing tree size where fire spread area was high, consistent with top-kill being due to fire (Fig. 3-6A, negative tree size x fire spread interaction, $-z = 2.07$, $P = 0.038$). Mortality (i.e., death of the individual, in contrast with top-kill that leaves the rootstock alive to resprout) was negligible in the harvest year: of 657 trees surveyed before the burn, only one (a small pin oak) failed to resprout after being top-killed.

Trees' pre-fire size did influence their annual growth rates even after accounting for whether they were top-killed (Fig. 3-6C,D). We fit separate regressions for top-killed and non-top-killed trees of each species, with annual growth rate as the dependent variable and diameter of the largest stem as the independent variable, and plot as a random intercept. In both species, trees that escaped top-kill had positive mean growth rates that did not vary significantly with size (Fig. 3-6C,D green lines, slope $P > 0.465$). In contrast, among top-killed trees the mean growth rate decreased significantly with increasing size (Fig. 3-6C,D black lines: slope significantly negative for bur oak, $z = -1.97$, $P = 0.048$; slope not significantly negative for pin oak, though its sample size was smaller: $z = -0.20$, $P = 0.84$). The largest top-killed trees tended not to recover to their pre-burn size. Replacing stem diameter with tree height or estimated tree biomass lead to similar conclusions (Fig. 3-S5, Fig. 3-S6).

What is the net effect of these size-dependent top-kill and growth rates on tree growth? We characterized the overall mean growth rate for trees of a given size as a weighted average of the regression-fitted growth rates for top-killed and non-top-killed trees of that size, weighting by the fitted probability of top-kill at that size (Fig. 3-6C,D). For bur oaks, we did this separately for trees with 0 % and 100 % fire spread area to illustrate the full parameter space. In bur oaks, at 0 % fire spread area the rate of change of tree biomass was consistently positive (Fig. 3-5C blue line). However, at 100 % fire spread area (typical of plots with high herbaceous biomass), the growth rate dipped below zero at an intermediate diameter (7 mm, Fig. 3-5C orange line). This diameter may be a stable equilibrium size towards which trees would tend to converge if they were narrower than about 12 mm; trees wider than 12 mm had positive mean growth rates. In pin oaks, there was a similar dip in growth rate at a diameter of 6 mm, potentially a stable equilibrium size (Fig. 3-5D gray line). An alternative analysis, plotting size at the end of the growing season against pre-burn size, led to similar conclusions (Fig. 3-S7). The modal sizes of the trees were broadly consistent with these potential equilibrium sizes in both the harvest year (Fig. 3-6E,F) and earlier years (Fig. 3-S1), at least in bur oaks. Pin oaks had fewer trees and a less distinct mode. Thus, a feedback between top-kill by fire and tree size may have constrained tree size in plots with high herbaceous biomass (Fig. 3-7A): small trees were likely to be top-killed, and top-killed trees were unlikely to grow large enough to escape being top-killed again. The decline in growth rate with increasing size among top-killed bur oaks may have strengthened the grip of this feedback, holding trees more firmly in the fire trap.

Discussion

In experimental communities with higher herbaceous plant abundance resulting from higher planted species richness, individuals of two fire-adapted, resprouting savanna tree species were profoundly smaller, and one species (pin oaks) had somewhat lower survival, after 16 years. This is consistent with earlier findings that herbaceous biomass can inhibit savanna tree establishment (Bond 2008), including at this site (Inouye *et al.* 1994). High herbaceous biomass appears to have inhibited savanna tree establishment by two mechanisms: first, by profoundly increasing fire spread, resulting in increased point fire frequency and intensity despite annual ignitions in all plots, and second, by reducing the availability of nitrate and light resources. Below, we discuss the importance of these two mechanisms.

Herbaceous biomass inhibits savanna tree establishment through fire

The positive effect of herbaceous biomass on fire spread and fire temperature, and consequent suppression of tree establishment, is consistent with earlier studies of savanna dynamics and tree invasions (Bond 2008). Increased herbaceous biomass was associated with increased herbaceous cover and thus greater fuel continuity, which promotes fire spread (chapter 2, \D'Antonio, 2000 #8735}. Increased fire spread in higher-biomass plots resulted in higher point fire frequency and suppressed tree establishment by repeatedly top-killing trees smaller than a threshold "escape" size, holding them in a fire trap and delaying or preventing them from reaching a size large enough to escape top-kill and produce seeds (Bond 2008). This top-kill dynamic, combined with no effect of tree cover on fire spread when excluding the few highest-tree plots (chapter 2), shows that the associations we present between fire spread and tree abundance reflect fire spread suppressing tree abundance far more strongly than the reverse.

The fire trap may have been reinforced by a decrease in mean growth rate (annual relative rate of change in size, reflecting growth *per se* as well as loss to fire and other causes) with increasing pre-burn size among top-killed trees. Top-killed trees above a potential equilibrium size – which was below the escape size at which they were likely to escape top-kill – tended to regrow to a size smaller than their pre-burn size, i.e., to have negative rates of change in size. This could have reduced trees' chances of escaping the fire trap at high herbaceous biomass. Grady & Hoffman (2012) found similar size-dependent regrowth dynamics of top-killed trees. Combining the effect of tree size and fire spread area on top-kill with the effect of top-kill and tree size on growth rate (Fig. 3-6) extends Grady & Hoffman's (2012) graphical approach to include the growth dynamics of non-top-killed trees. We applied Grady & Hoffman's (2012) approach to our data in Fig. 3-S7, with the same implications as Fig. 3-6. These relationships imply equilibrium sizes for fire-trapped savanna trees (Grady and Hoffmann 2012) that are consistent with modes in the tree size distributions, at least of bur oaks (Fig. 3-5E).

However, stochastic variation in top-kill and growth rates could allow some savanna trees to escape the fire trap even when mean rates of increase in biomass imply this is unlikely. Variation can arise a) among trees within a plot (Wakeling *et al.* 2011), due to differences in herbaceous biomass, burn and herbivore history, access to resources, or other factors, and b) among years, due to variation in climate (Higgins *et al.* 2000, Ziegler *et al.* 2008) or other factors. Escaping the fire trap through such stochastic variation seems likely in our system because for both tree species the potential equilibrium appears to be only a weak dynamical attractor. The potential equilibrium size is only a little smaller than the escape size, so if a tree near the potential equilibrium size escaped top-kill for just two years and grew at the mean growth rate for non-top-killed trees, it would be more than likely (probability > 0.5) to escape top-kill in the next burn. This is consistent with bur oaks' high abundance and recruitment even in relatively frequently burned savannas in a larger scale, longer-term experiment at this site. In plots that had been burned 11 or more times during 32 years, bur oaks had numerous trees in the smallest recorded size class (5-10 cm diameter) (Peterson and Reich 2001), suggesting they may be able to recruit into this size class even with frequent fire, though even those small trees could have grown above escape size while fire was suppressed before that experiment started.

Herbaceous biomass affects savanna tree establishment through resource availability

Soil NO_3^- is a strongly limiting resource in this system; other nutrients do not limit herbaceous productivity (Tilman 1987). High-biomass, diverse plots accumulated more soil organic matter and total N through increased litter inputs and other mechanisms, and had higher soil N mineralization rates (Zak *et al.* 2003, Dybzinski *et al.* 2008, Fornara and Tilman 2008). Soil NO_3^- concentrations could have been reduced in higher herbaceous biomass plots by higher fire spread and fire temperature, which may have volatilized more N during burning and increased N leaching after burning (Knops *et al.* 2002), but such losses must have been strongly outweighed by increased N inputs. Despite having higher N mineralization (supply) rates, available soil NO_3^- concentrations were reduced in plots with higher herbaceous biomass, apparently due to greater uptake of NO_3^- by higher herbaceous root mass (Fargione and Tilman 2005b, Mueller *et al.* 2013). NH_4^+ concentration may not have been strongly associated with herbaceous biomass because stronger uptake at higher biomass was offset not only by increased mineralization rates but also by increased adsorption of this cation to increased soil organic matter (Fornara and Tilman 2009, Mueller *et al.* 2013).

The reduced savanna tree growth in high herbaceous biomass plots apparently due, in part, to lower soil NO_3^- concentrations despite higher total soil N is consistent with experimental removals of herbaceous plants that increased soil resource availability and released trees from competitive suppression (Davis *et al.* 1998, Davis *et al.* 1999, Porensky and Veblen 2012). We infer that the lower growing-season NO_3^- concentrations in higher herbaceous biomass plots indicate high uptake by herbaceous plants and low NO_3^- availability to trees, rather than high uptake by trees, because the aboveground live biomass of herbaceous plants per unit area was approximately 3,000 times greater than that of trees. Thus, we infer that herbaceous plants competitively suppressed tree growth by depleting NO_3^-. *Quercus* spp. vary in their preferences for NO_3^- versus NH_4^+ (Kuster *et al.* 2013, BassiriRad *et al.* 2015). N preferences have apparently not been studied in bur oak or pin oak but NO_3^- was the only form of N that was significantly associated with tree performance in this study, and in another study of bur oak in an adjacent experiment (Wright *et al.* 2013). In the sandy soils at this site, higher NH_4^+ reflects higher cation exchange capacity due to increased organic matter in higher-diversity plots whereas lower NO_3^- reflects competitive resource uptake.

Light interception by herbaceous biomass was associated with increased savanna oak tree survival but reduced growth in our multiple regressions that also accounted for fire and NO_3^-. The survival result is surprising because these tree species are associated with high-light environments, and are unable to regenerate in deep shade. Nonetheless, this result is consistent with increased survival of savanna oak tree seedlings in shade treatments at this site (Davis *et al.* 1999); increased survival of some tree species' seedlings in the presence of herbaceous vegetation in old fields (Gill and Marks 1991, Berkowitz *et al.* 1995); and increased survival and growth of small tree seedlings at high herbaceous biomass in high diversity plots due to higher humidity and less extreme temperatures within denser canopies in an adjacent experiment (Wright *et al.* 2013, Wright *et al.* 2014). Thus, a facilitative effect of herbaceous biomass on survival of small savanna trees through shading is plausible but surprising and requires further investigation. The contrasting higher growth at higher light availability of the trees that did survive – and thus higher tree biomass per planted seed at higher light availability – is consistent with a shift from facilitative to competitive effects of high herbaceous biomass as tree seedlings grow (Wright *et al.* 2013, Wright *et al.* 2014). While the tallest trees' canopies were above the herbaceous canopy and thus unaffected by light interception by herbaceous plants, most trees were less than 1 m tall (Fig. 3-S5E,F), short enough to be overshadowed by herbaceous plants.

Soil moisture did not explain variation in savanna tree survival or growth despite strong effects of experimentally manipulated soil moisture on oak tree establishment at this site (Davis *et al.* 1999, Davis *et al.* 2007) and elsewhere (Myster 1993). This may be because there was little variation in soil moisture across our plots. The trend towards higher soil moisture at higher herbaceous biomass (Fig. 3-2C) is consistent with higher soil organic matter, and thus water-holding capacity, at high biomass and diversity

(Hudson 1994, Fornara and Tilman 2008); this trend may have been weakened by a counteracting increase in water uptake by higher herbaceous biomass.

Herbaceous diversity and biomass are parallel explanations for savanna tree establishment

Planted species richness did not clearly influence savanna tree establishment over and above the effects of herbaceous biomass, with which it co-varied strongly (Fig. 3-S2A, (Tilman *et al.* 1997, Tilman *et al.* 2001, Reich *et al.* 2012)). This may be because herbaceous biomass captures the mechanisms by which diversity inhibited plant establishment. For example, complementary resource uptake in space and time at high diversity in this experiment lead to high resource uptake that resulted in both high herbaceous biomass and, because low resource levels remained available, low invasibility by other herbaceous species (Kennedy *et al.* 2002, Fargione and Tilman 2005b). Thus, the oak responses are consistent with biotic resistance to invasion through more complete resource use by more diverse communities (Elton 1958, Levine *et al.* 2004). Moreover, they suggest increased fire spread in more diverse herbaceous communities as another mechanism for biotic resistance to invasion by species vulnerable to fire.

The profound effect of planted species richness on savanna tree establishment (Fig. 3-S3) is no less striking because it was mediated by herbaceous biomass. Of these two tightly associated factors (herbaceous species richness and biomass), we emphasize herbaceous biomass, because it is a more generally applicable driver in savannas. Herbaceous biomass can be profoundly influenced by diversity, as it is here, but it can also be influenced by other factors such as climate and soil parent material. The influences of herbaceous biomass that we found may also apply when such other factors more strongly determine herbaceous biomass.

Tree planting density was confounded with planted species richness: more trees were planted in less diverse plots. Satiation of seed predators could have contributed to the modestly higher survival at lower planted species richness and herbaceous biomass, where tree planting density was higher (Poulsen *et al.* 2007). However, it is unlikely that consumer satiation caused the vastly higher tree growth per surviving individual that we observed at low planted species richness and herbaceous biomass. There was little evidence of herbivory on the trees (pers. obs.), and indeed community-wide insect herbivore abundance and pathogen loads were higher at low diversity (high tree density) in this experiment (Mitchell *et al.* 2002, Haddad *et al.* 2009). We know of no other mechanism of positive density dependence likely to have influenced our results.

C4 grasses inhibit savanna tree establishment more strongly than do other herbaceous plants

Herbaceous functional groups differed in how they influenced savanna trees, both through differences in productivity and biomass that influenced trees as discussed above and through differences in their effects per unit biomass (abundance). For C4 grasses, a strong positive effect on productivity (Tilman *et al.* 1997) accorded with strong positive per-unit-abundance effects on fire spread, NO_3^- depletion, and light interception, to significantly inhibit savanna tree establishment. Previous work found that C4 grasses turned a given amount of N acquired into a larger amount of biomass than other functional groups (Tilman *et al.* 1997). Low tissue N concentration may make C4 grasses' tissue less subject to herbivory and decomposition, which would combine with high productivity to result in high fuel loads; in addition to their morphology which creates well aerated fine fuel beds, this could explain why C4 grasses promote fire particularly strongly (chapter 2, D'Antonio and Vitousek 1992, Bond and Midgley 1995, Masubelele *et al.* 2007, Bond 2008).

C4 grasses may also reduce resource availability more effectively per unit biomass than other functional groups. C4 grasses can grow at soil N concentrations below those required by other groups, allowing them to deplete NO_3^- more strongly than other plants and making them superior competitors for nitrogen (Kennedy *et al.* 2002, Fargione and Tilman 2006). C4 grasses may have intercepted more light per unit biomass than other functional groups because their leaves are presented with less overlap than those of forbs (pers. obs.), but the reason for this result remains unclear.

Our finding that C3 grasses inhibited savanna tree establishment despite their low abundance and weak impacts on fire spread and resource availability is puzzling, but accords with an earlier finding at this site of C3 grasses strongly inhibiting savanna trees even without the presence of fire and without depleting NO_3^-, light or water resources (Davis *et al.* 2005).

Legume presence increased productivity, as reported previously (Tilman *et al.* 1997), which would have tended to inhibit tree establishment through fire spread and light interception, but this was offset by their positive effect on NO_3^- so their net effect on tree establishment was weak. However, in other contexts herbaceous legumes could influence tree establishment. For example, in systems without fire legumes might have a more positive net effect on tree establishment.

Because herbaceous functional groups differ considerably in their influence on savanna tree establishment, considering their abundances separately is likely to offer additional insight compared with total herbaceous biomass, especially where herbaceous biomass is not strongly dominated by a single functional group. Even where herbaceous biomass is dominated by one herbaceous functional group, caution should be used in applying results from a system dominated by a different herbaceous functional group.

Savanna tree species' strategies

Of the two savanna tree species in this experiment, herbaceous biomass more strongly inhibited pin oak –a species in the "red oak" group, considered to have faster growth, lower fire tolerance, and likely higher tissue N concentrations and requirements – compared with bur oak, in the "white oak" group. Accordingly, reduced NO_3^- availability reduced growth more strongly in pin oaks than in bur oaks in this experiment (Fig. 3-3B). Pin oaks, with higher maximum relative growth rates (indicated by larger maximum tree sizes after 16 years, Fig. 3-1b) and higher allocation to stems and leaves (Reich *et al.* 2003), are better able to compete for light in high soil resource conditions. Conversely, bur oaks are better able to tolerate low soil resource availability through higher resource use efficiency and higher allocation belowground, promoting resource acquisition. Higher belowground allocation in bur oaks may also promote resprouting; bur oaks also have thicker bark, and are thus considered more fire-tolerant (Cavender-Bares *et al.* 2004, Pausas 2014). In a size-representative sample of the trees that we harvested, bur oaks had thicker bark 2 cm above ground level than did pin oaks, for a given stem diameter or aboveground biomass (J. Budiac & P.D. Wragg, unpublished). Pin oaks were reduced in abundance relative to bur oaks by high burn frequency treatments in a large fire frequency experiment also at Cedar Creek Ecosystem Science Reserve, where pin oaks appeared unable to escape the fire trap to recruit into the 5 – 10 cm diameter size class (Peterson and Reich 2001). Therefore, it is surprising that fire did not affect pin oaks significantly more negatively than bur oaks (Fig. 3-3A). Reduced N availability associated with high fire frequency treatments in the large fire frequency experiment (Reich *et al.* 2001) may play a large role in the differential response of pin oaks and bur oaks to fire frequency. It is also possible that the duration of our experiment was too short to reveal a differential impact of fire spread on these oak species. Bur oaks' longer life span allows even rare recruitment into large fire-invulnerable size classes to be "stored" during long periods unfavorable to recruitment, making bur oaks more likely than pin oaks to coexist with grasses via a storage effect (Warner and Chesson 1985, Higgins *et al.* 2000).

Our results and others (Peterson and Reich 2001, Cavender-Bares and Reich 2012) suggest that the tree recruitment dynamics driving vegetation structure in oak savannas of the Midwestern USA are broadly similar to the dynamics in other mesic savannas around the world. Therefore, it would be profitable to include these temperate savannas in emerging functional comparative research investigating links between different fire response strategies (involving bark and other tree traits) that prevail in different subtropical and tropical regions and the biogeographic and environmental histories of these regions (Pausas 2014).

Implications for biome distributions

Our results imply that environmental changes that directly reduce fire or increase resource availability, such as fire suppression or N deposition, can promote tree encroachment in grasslands and savannas. Fire suppression has indeed been widely found to lead to conversion of grassland or savanna to forest (e.g., (Tilman *et al.* 2000, Titshall *et al.* 2000)). No such generality has emerged for the influence of N deposition on savanna tree establishment; our results suggest that the net effect of N deposition will depend on its effects on herbaceous biomass – and consequent effects on fire behavior and resource dynamics – as well as its more direct effect on N supply rate.

Herbaceous biomass may influence the distribution in space and time of grassland, savanna, and forest through fire-driven positive feedback effects, in two related ways. First, greater herbaceous biomass, especially of C4 grasses, can influence the feedback between tree size and top-kill by increasing fire spread and reducing NO_3^- availability and thus tree growth, making it less likely that trees escape top-kill. An ecosystem with all trees fire-trapped and sufficiently small is considered a grassland; reductions in herbaceous biomass, and especially of C4 grasses, could allow fire-trapped trees to mature (Myster 1993, Ganguli *et al.* 2008) to form savanna. Herbaceous biomass may be reduced in various ways. Increased grazing reduces herbaceous biomass, which has been reported to weaken fire and promote savanna tree establishment (Roques *et al.* 2001, van Langevelde *et al.* 2003), though grazing can also directly suppress tree establishment so the net effect of grazing is not easily predicted. Loss of herbaceous plant diversity may also reduce herbaceous biomass (Tilman *et al.* 1996, Cardinale *et al.* 2011, Tilman *et al.* 2014), though the role of these experimentally demonstrated biodiversity-productivity relationships in natural systems remains controversial (Vellend *et al.* 2013)). Particular functional groups may change in abundance due to various global changes. For example, global warming may increase abundance of C4 grasses and legumes (Cowles *et al.* in revision); especially if herbivory holds legumes in check and results in a stronger increase in C4 grasses with warming, this could strengthen savanna tree suppression. If grazers disproportionately remove grasses by selecting against unpalatable forbs then savanna trees may increase, whereas if grazers selectively consume legumes and thus decrease ecosystem N (Knops *et al.* 2000) then savanna trees may decrease.

Second, increased savanna tree survival and growth (and ultimately, presumably, increased seed production and density) at lower herbaceous biomass can increase tree cover, which could feed back to reduce fire spread and intensity by shading out more flammable herbaceous plants, increasing relative humidity and thus fuel moisture, and reducing wind speed (Brooks *et al.* 2004). Based on their large leaf size, litter of our oak tree species was likely relatively flammable compared with that of other oak trees (Engber and Varner 2012), but trees were not abundant enough to reliably estimate their effects on fire spread (chapter 2). A weakened fire regime could, in turn, allow more trees to escape the fire trap, reinforcing a shift from fire-promoting, low tree cover grassland or savanna to fire-retarding, high tree cover forest (Hirota *et al.* 2011, Staver *et al.* 2011). Increased tree cover may also feed back to further affect tree cover through its effects on resource availability (Reich *et al.* 2001), herbivory (Scholes and Archer 1997), and potentially herbaceous species diversity (Briggs *et al.* 2002, Peterson and Reich 2008, Price and Morgan 2008, Ratajczak *et al.* 2011) and composition (Scholes and Archer 1997, Peterson *et al.* 2007), each of which may affect trees as discussed above. These positive feedbacks can sharpen boundaries in space between grassland, savanna, and forest, and make temporal transitions between them rapid (Titshall *et al.* 2000, Briggs *et al.* 2002) and not easily reversed (Grimm 1983, Staver *et al.* 2011).

Conclusions

These results show that the biomass, diversity and functional composition of herbaceous vegetation -- in particular the abundance of C4 grasses and legumes -- can profoundly influence savanna tree survival and growth through both fire spread and resource competition, holding constant other drivers of tree abundance: climate, ignition regime, initial soil conditions, large herbivore absence, and the characteristics of regional tree species. Further work is needed to understand how spatial and temporal variation in herbaceous biomass and functional composition may interact with these other drivers to influence the enigmatic distributions of grassland, savanna and forest.

Figure 3-1: Associations between herbaceous biomass and tree performance.

Increasing herbaceous biomass (averaged over all years) was associated with reduced tree survival **(A-B)**, mean biomass per surviving tree **(C-D)**, and biomass per planted seed **(E-F)**, all 16 years after planting. Fit lines are logistic (A-B), linear with the dependent variable log transformed (C-D), or compound Poisson (E-F), as described in the text; herbaceous biomass is log-transformed in all cases. Points represent plots.

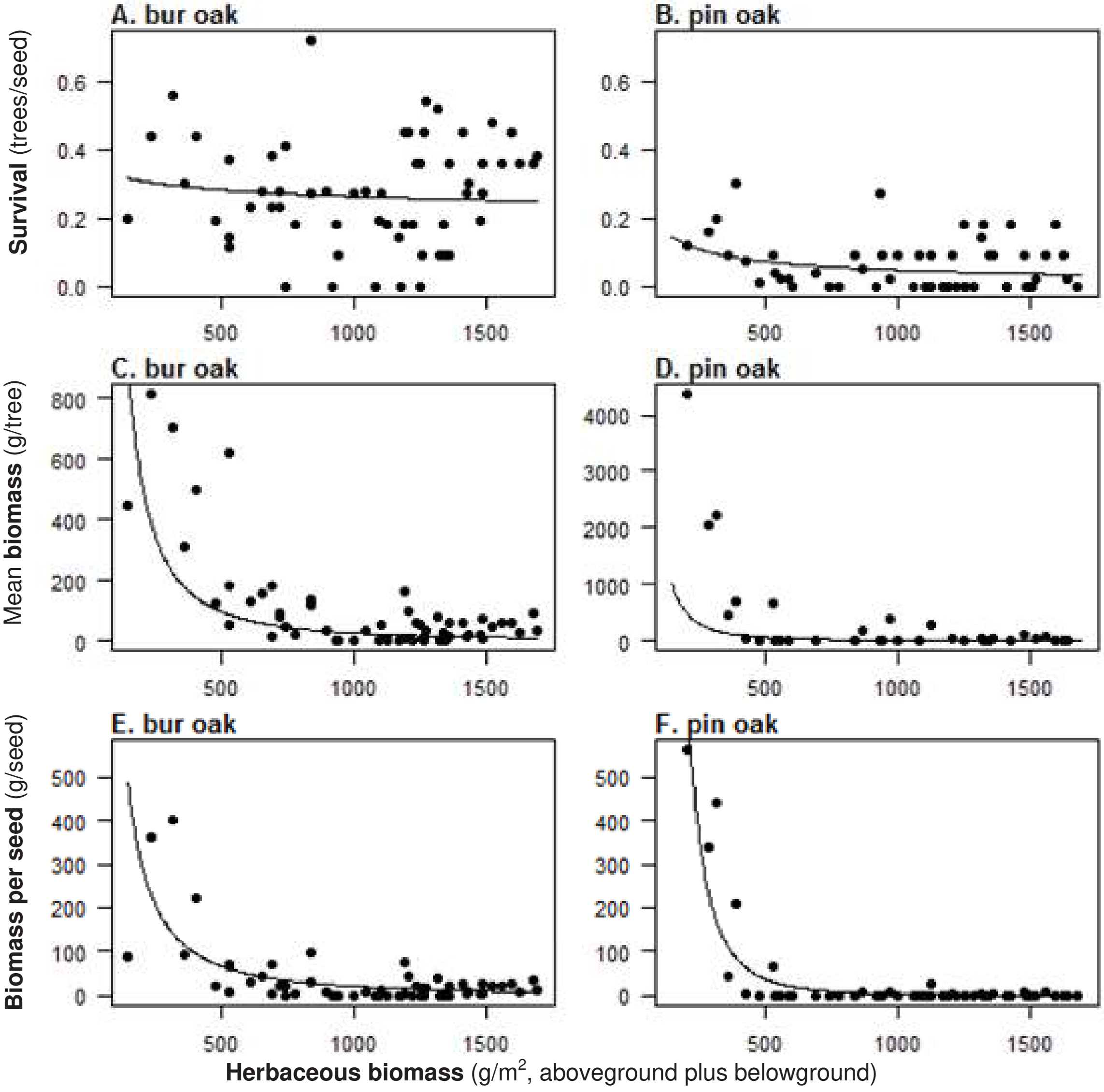


Figure 3-2: Associations between herbaceous biomass and environmental factors. Increasing herbaceous biomass was associated with increased fire spread area (**A**), reduced NO_3 concentration (**B**), increased soil moisture (**C**), and reduced light penetration (**D**). Values are averaged over all years. Fit lines are linear, though fire spread area was logit-transformed and NO_3^- and herbaceous biomass were log-transformed.

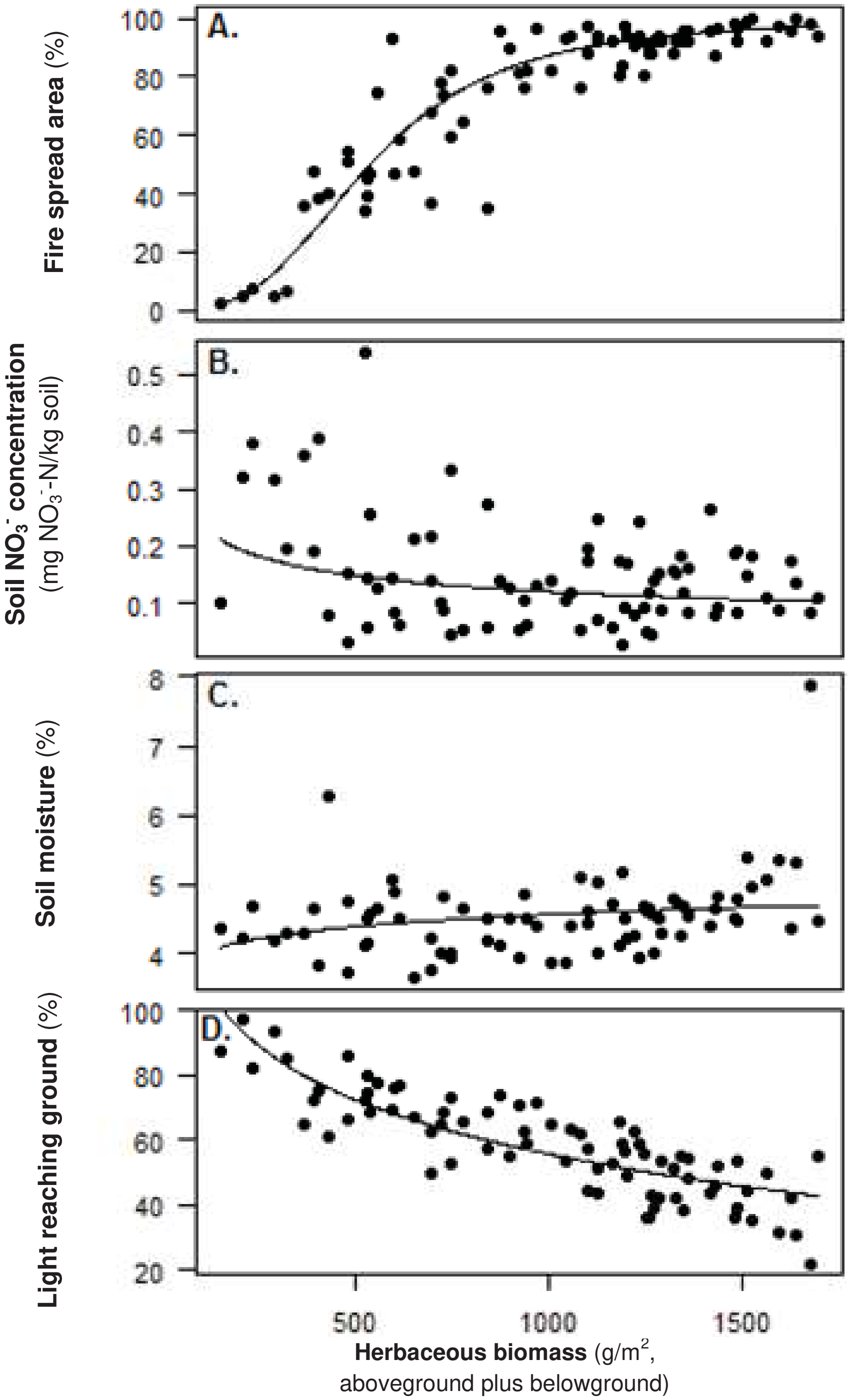


Figure 3-3: Associations between environmental factors and tree performance.

Effects of fire spread area and resource availability on tree survival **(A)**, mean biomass per surviving tree **(B)**, and biomass per planted seed **(C)** 16 years after planting. Each panel presents the coefficients (+- 95% confidence interval) from a separate multiple regression, showing the influence of standardized fire spread area and resource availability variables on trees. Full details of the regression structures are in Results.

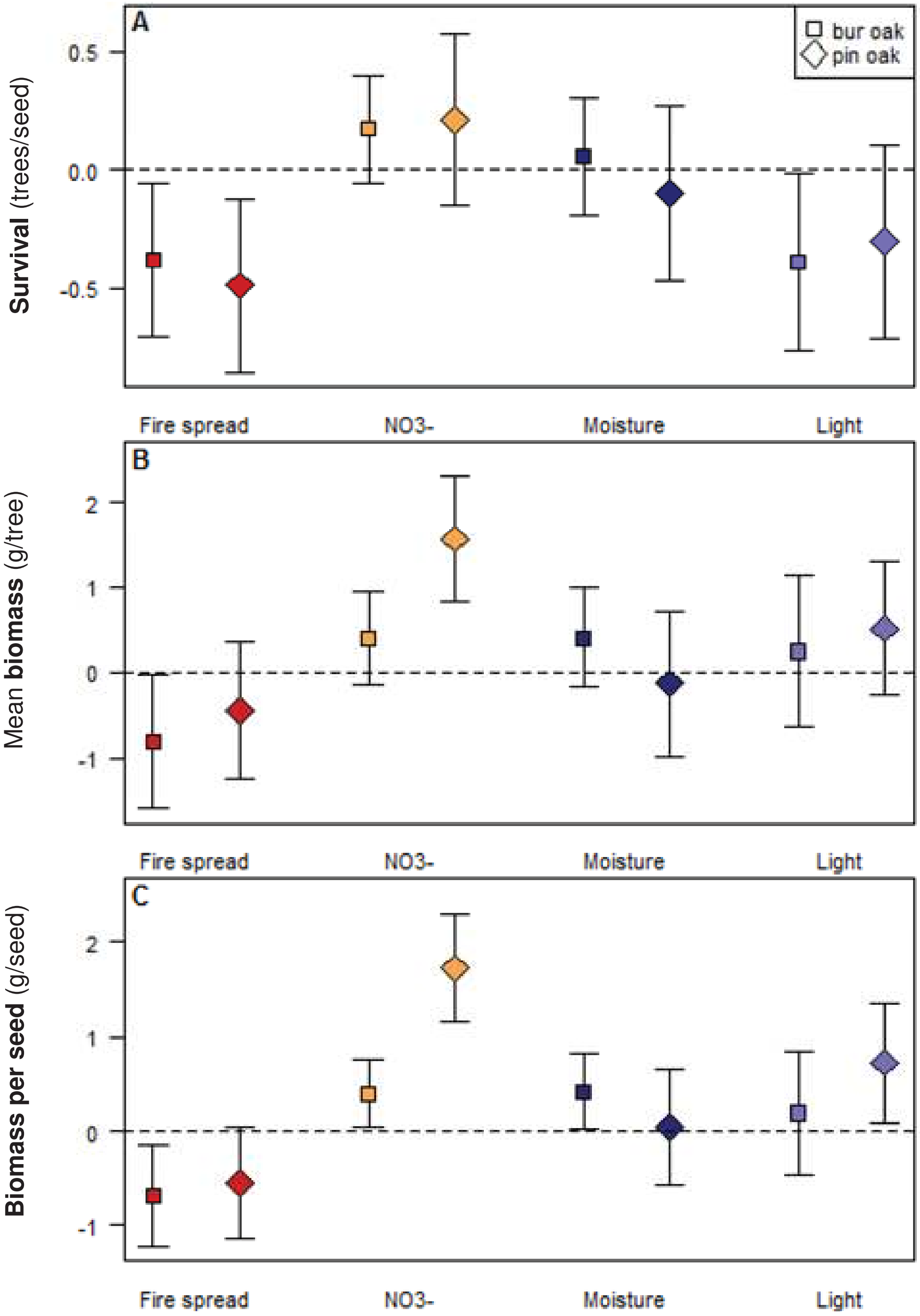


Figure 3-4: Associations between herbaceous functional groups, tree performance, and environmental factors.

Effects of the presence (left hand panels) or log biomass (right hand panels) of herbaceous functional groups on (A-B) savanna tree biomass per seed, (C-D) total biomass, and (E-J) environmental factors influencing savanna tree growth. Each panel presents the coefficients (+- 95% confidence interval) from a separate multiple regression, showing the influence of each functional group relative to a baseline with all herbaceous functional groups absent (left hand panels) or with zero biomass (right hand panels; 1 was added to functional group biomasses before log transforming them to make zero biomass zero on the log scale).

Figure 3-4 (caption on previous page).

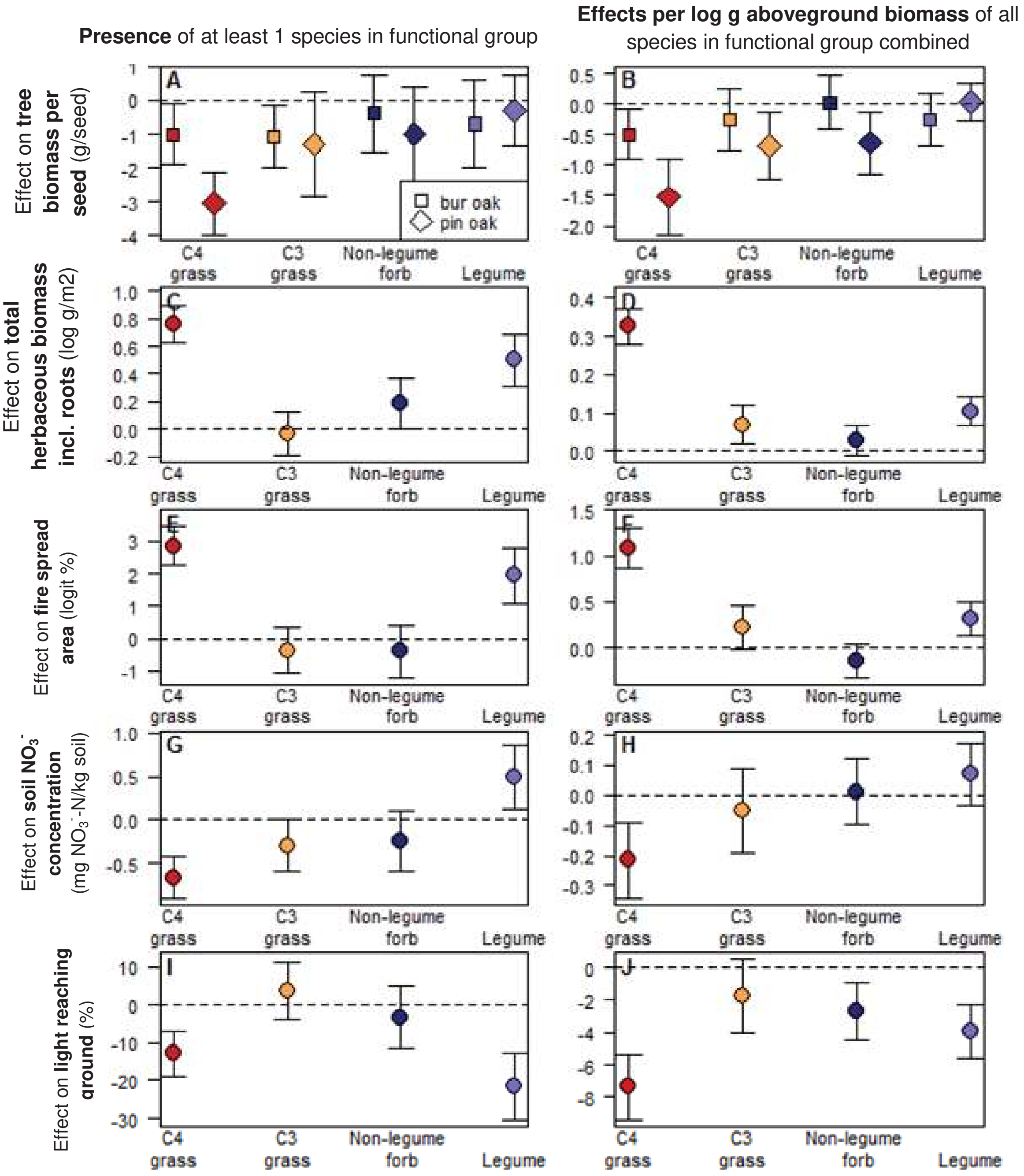


Figure 3-5: Associations between herbaceous biomass, tree top-kill, and tree growth rate. Savanna trees were more likely to be top-killed by fire in plots with more herbaceous biomass (**A, B**). Top-kill, scored as 1 if a tree was top-killed or 0 if it was not, is jittered for clarity; fit lines are logistic, with herbaceous biomass log-transformed. Top-killed trees had lower growth rates, i.e., increased less in biomass from before a fire through the subsequent growing season (**C-D**). With top-kill accounted for by fitting separate lines to top-killed (black) and non-top-killed (green) trees, growth was not significantly influenced by herbaceous biomass. Therefore, the green and black solid lines estimate intercepts but specify zero slopes. Together, these relationships can explain why trees tended to grow more slowly at higher herbaceous biomass. Averaging the expected growth rates for top-killed and non-top-killed trees, weighting the expectations at a given herbaceous biomass by the fitted probabilities of being top-killed and non-top-killed at that herbaceous biomass, yields expected growth rates as a function of herbaceous biomass (gray curves in C-D).

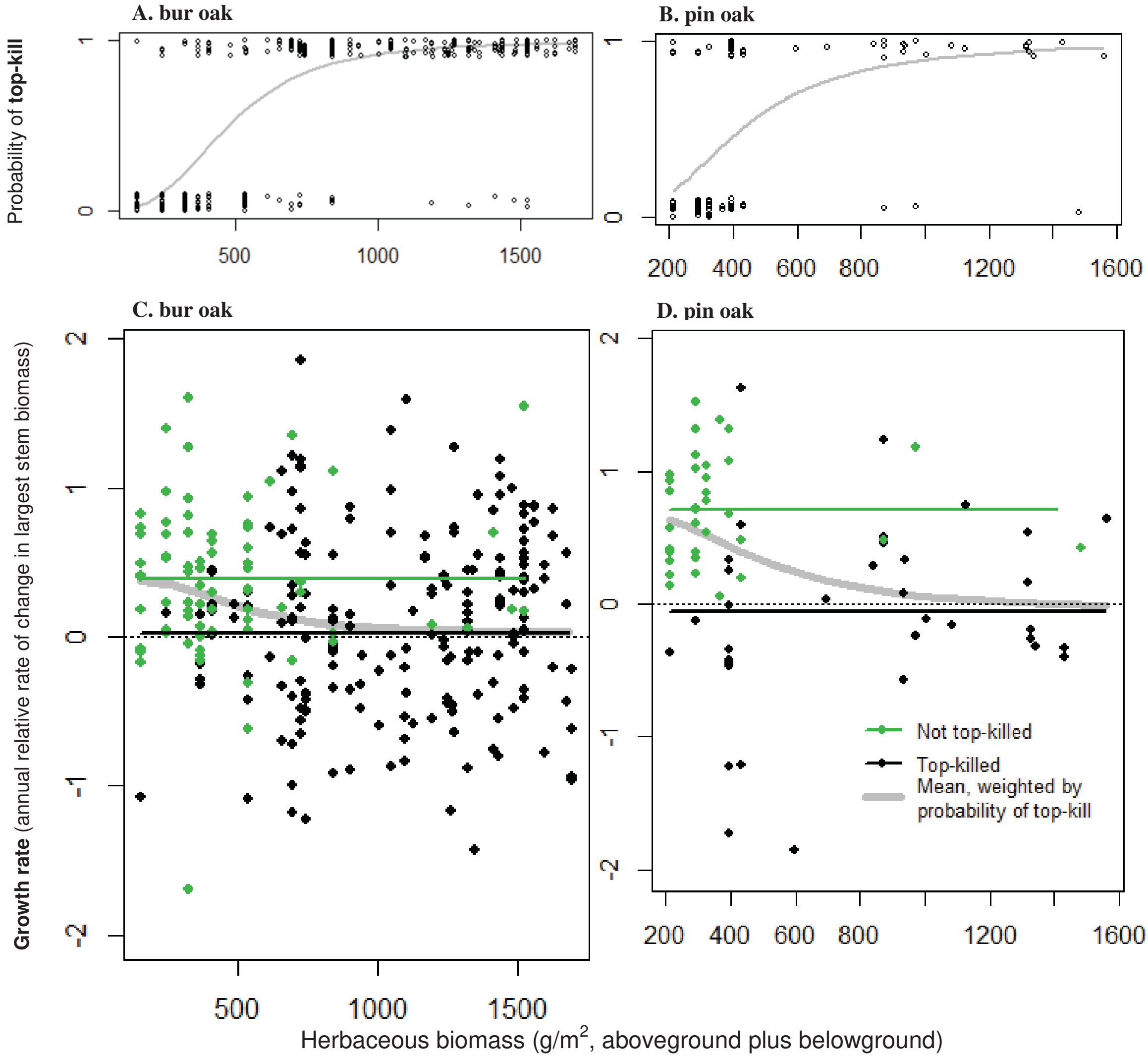


Figure 3-6: Associations between tree diameter, tree top-kill, and tree growth rate. The probability that a savanna tree was top-killed by fire decreased as its pre-fire diameter increased, most sharply at the inflection point of a logistic fit ("escape size") (**A-B**). For bur oaks (**A**), the data were sufficient to include the proportion of a 20 cm radius around each tree that burned and its interaction with pre-fire diameter in the regression. Individual tree growth rates (changes in biomass from before a fire through the subsequent growing season) depended on whether they were top-killed (**C-D**) and on their pre-fire stem diameters. Fit lines in C-D are linear. Averaging the expected rates of change of biomass for top-killed and non-top-killed trees, weighting the expectations at each pre-fire diameter by the fitted probabilities of being top-killed and non-top-killed trees at that diameter (A-B), yields expected growth rate as a function of pre-fire diameter and, for bur oaks, of burn percent ("Mean…" lines in C-D). For bur oaks (**E**) and less distinctly pin oaks (**F**), modal diameters of top-killed trees were similar to diameters at which top-killed trees tended to regrow to their pre-fire biomass (vertical gray lines, independent for the two savanna tree species).

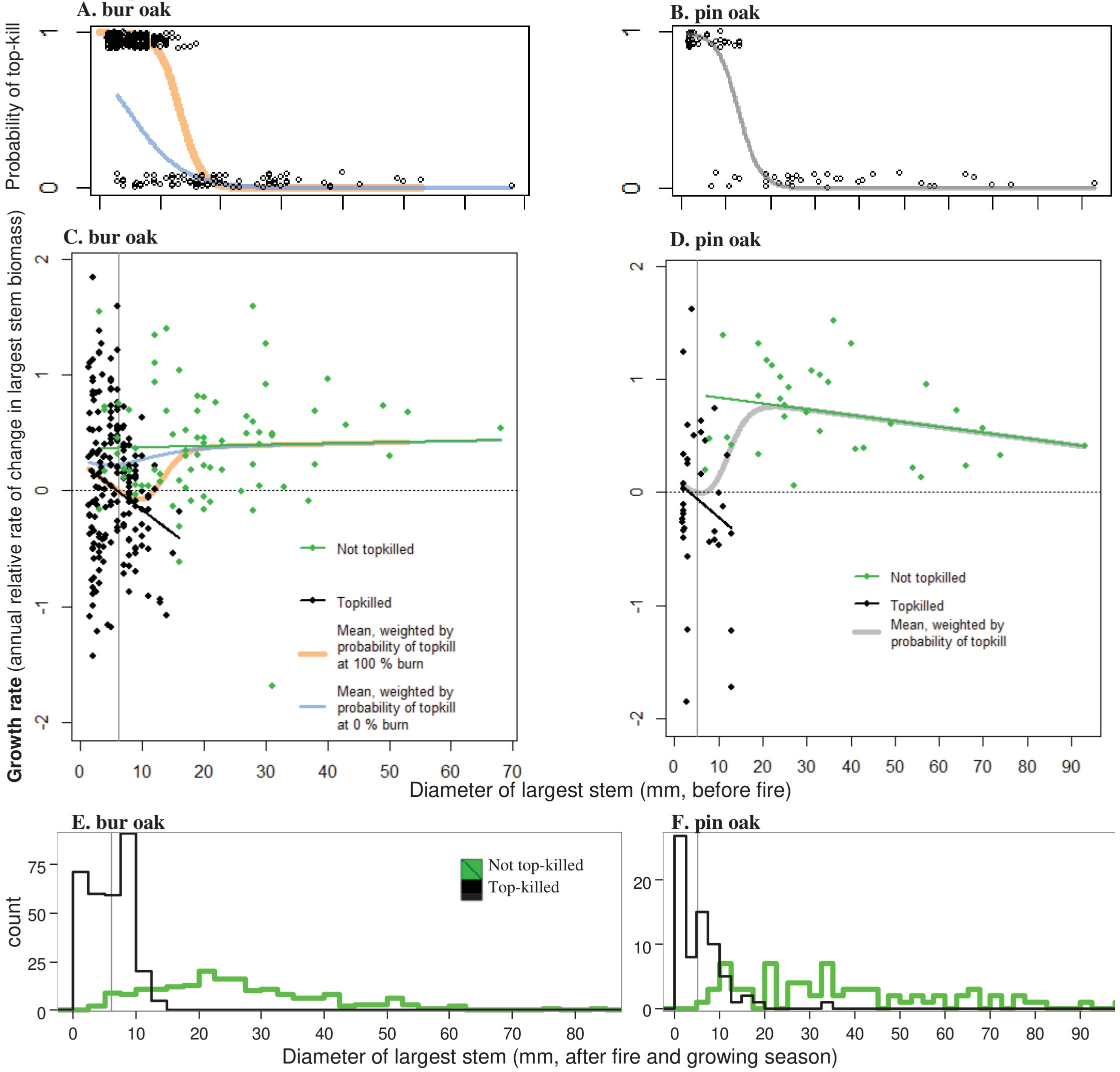


Figure 3-7: Summary diagram.

Our results, summarized in these diagrams, imply that herbaceous vegetation influenced savanna tree survival and growth by increasing fire spread and competitively reducing resource availability. A) Herbaceous biomass promoted fire spread that top-killed small trees; this slowed their growth, keeping them small and vulnerable to top-kill. Resource depletion by herbaceous biomass likely also reduced tree growth, increasing their vulnerability to top-kill. B) The presence of C4 grasses and legumes strongly increased the total amount of herbaceous biomass; black arrows show that over and above their effects via total herbaceous biomass, C4 grasses increased fire spread area and depleted NO_3^- and light, whereas legumes increased NO_3^-, more strongly per unit aboveground biomass than did other functional groups.

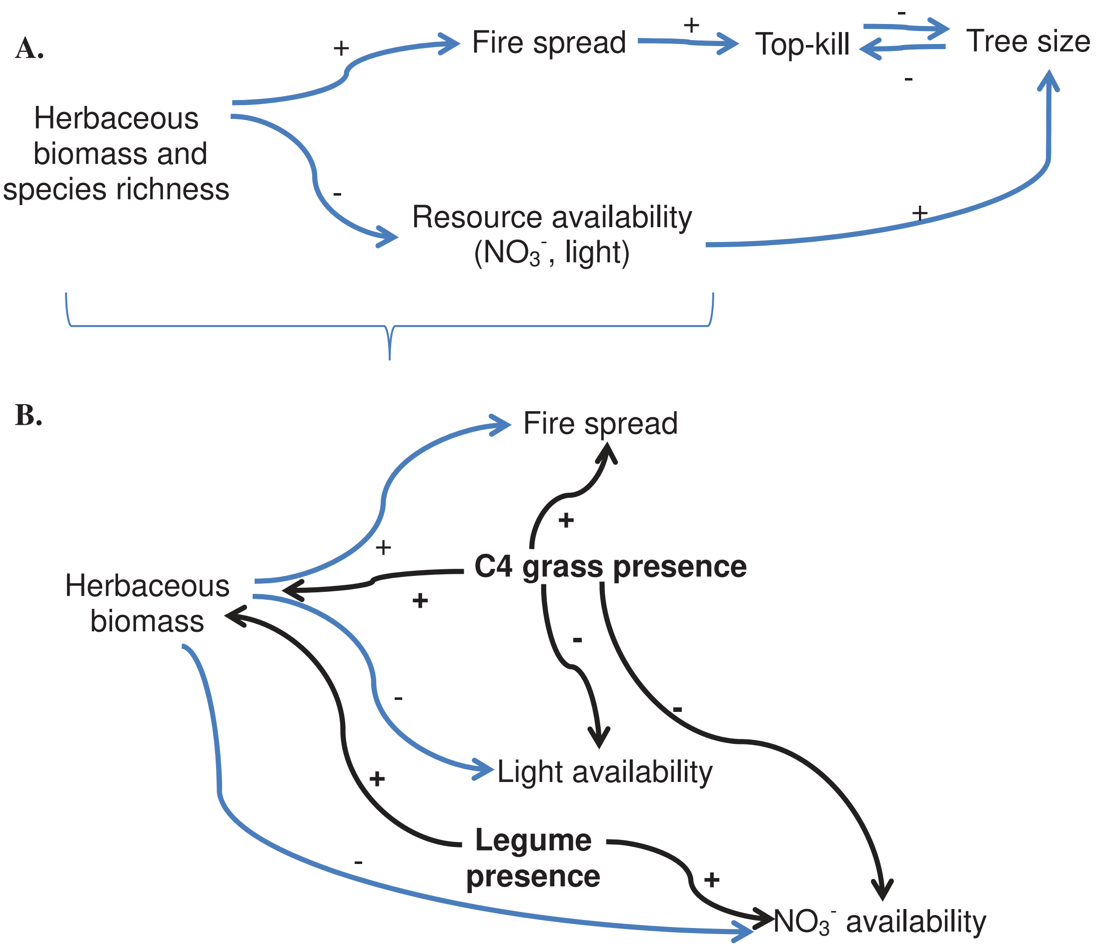


Figure 3-S1: Time course of tree size frequency distributions.

Frequency distributions are shown separately for plots with less than or more than 700 g/m^2 of herbaceous biomass (aboveground + belowground; 724 g/m^2 is the median herbaceous biomass across the 633 trees). Trees grew (i.e., the frequency distributions shifted to the right) very slowly in high herbaceous biomass plots, but rapidly in low herbaceous biomass plots. For 2010, both separate distributions for topkilled and non-topkilled trees and aggregate distributions are shown. <u>Figure methods:</u> At the end of each growing season from 2003-2006, the number of trees in each plot was counted and either all trees or a sample of trees were measured. In 2003 (176 trees measured) and 2004 (155 trees measured), the central 5 × 5 m of each plot was surveyed; all trees in that area were measured, except that a sample of 25-50% of trees were measured for plots with many trees. In autumn 2005 (449 trees measured) and autumn 2006 (341 trees measured), the entire 9 × 9 m plot was surveyed, except that for plots with many trees, one quarter of the plot -- bounded by the diagonals and an edge -- was measured. For each measured tree, we estimated each of its stems' biomass using its height and diameter and allometric equations developed from the 2010 harvest when all trees were measured, described in the main text. For 2009-2010 survey methods, see main text. We corrected for the incomplete sampling of plots with many (and typically larger) trees before 2010 to allow comparisons of size distributions through time: in plots in which not all trees were measured, we adjusted tree counts to sum to the total number actually counted (all trees were counted in all years, even though not all were measured) while retaining information on relative abundances of different tree sizes by creating *m* copies of the record for each tree, where *m* = number of trees counted ÷ number of trees surveyed; where *m* had a fractional as well as an integer part, we duplicated each tree an extra time with probability equal to its fractional part. This set of histograms is one realization of this stochastic process. The part of each bar that is black represents measured trees; the part that is gray represents inferred (duplicated) trees. Vertical lines are potential equilibrium sizes based on rates of change in biomass during 2010 (see Fig. 3-6C,D.)

Figure 3-S1 (caption on previous page).

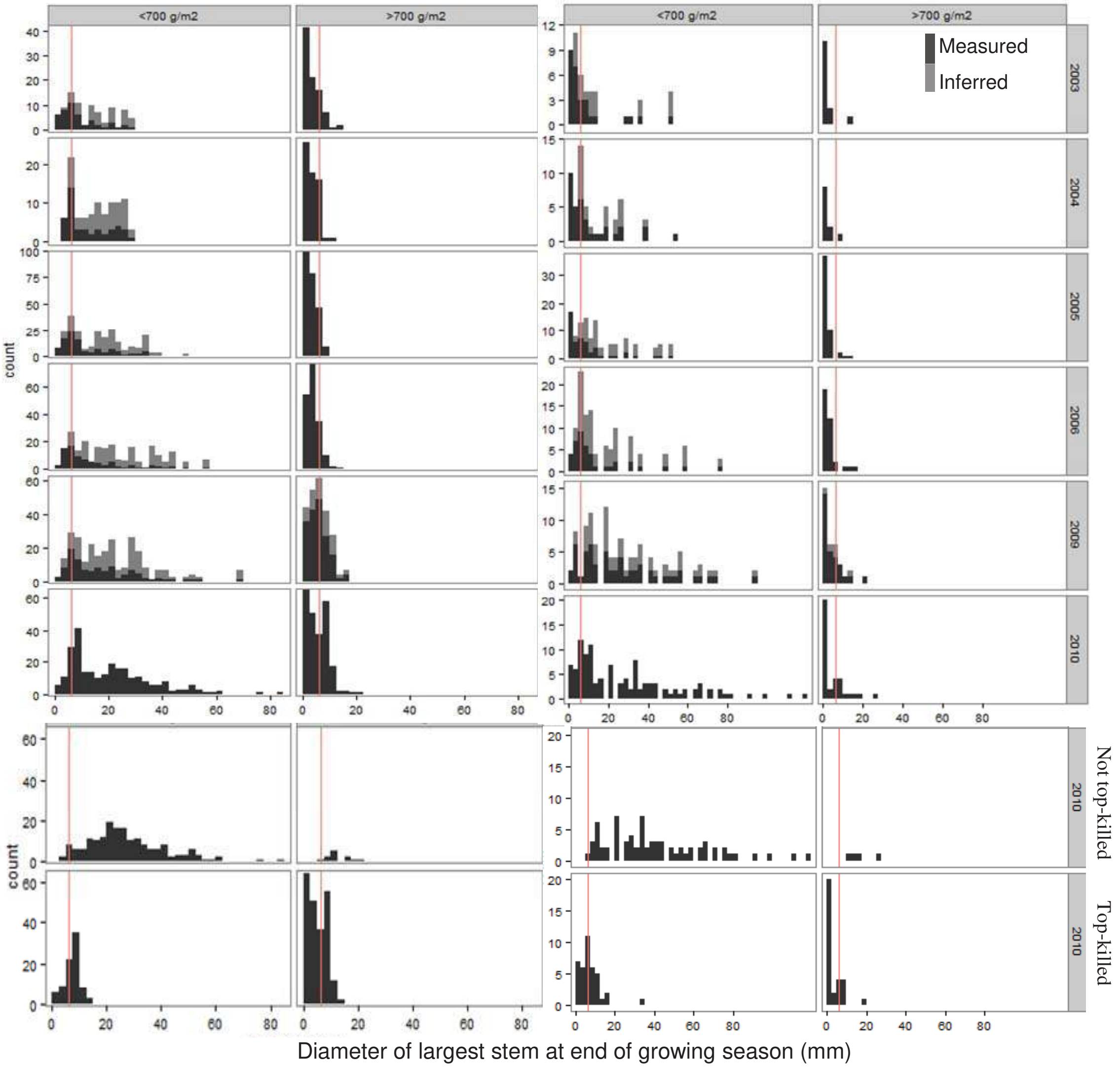


Figure 3-S2: Associations between species richness treatments and biomass of herbaceous functional groups.

Increasing planted species diversity increased herbaceous productivity, estimated as seasonal peak biomass excluding litter from previous years, **(a)** in total and **(b-e)** of each functional group. Values are averaged over all years**.** Lines are linear fits against log-transformed planted species number.

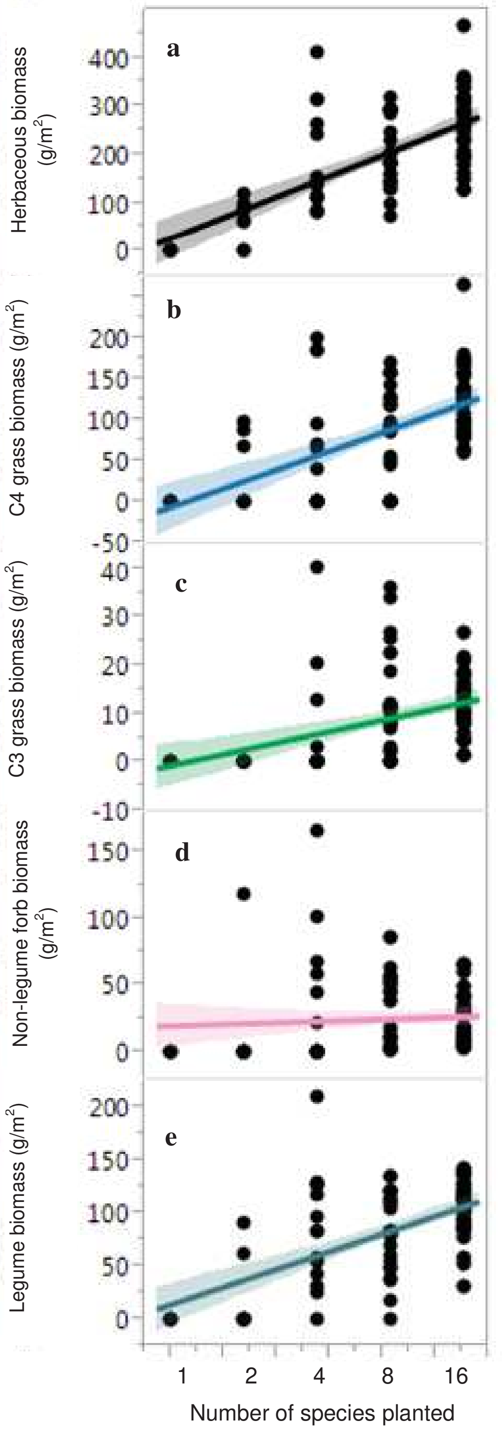


Figure 3-S3: Associations between tree performance and species richness treatments.

As for Fig. 3-1, but with planted species richness as the independent variable. Increasing planted species richness reduced tree survival **(A-B)**, mean biomass per surviving tree **(C-D)**, and biomass per planted seed **(E-F)** 16 years after planting. Planted species richness is jittered for display so that dots don't overlap; higher species richness values are jittered more because they are more numerous.

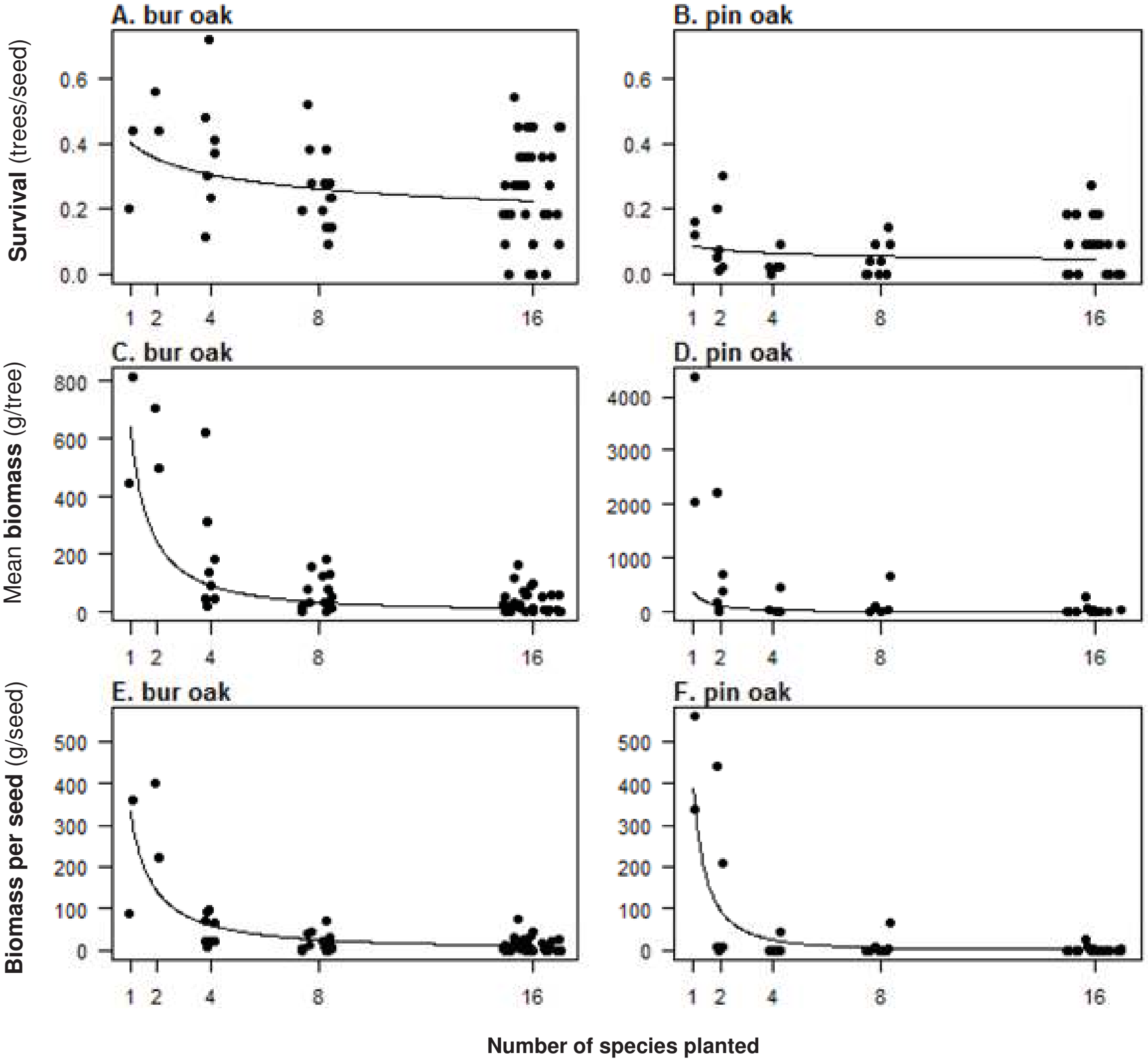


Figure 3-S4: Associations between environmental factors and species richness treatments. As for Fig. 3-2, but with planted species richness as the independent variable. Increasing planted species richness increased fire spread area (**A**), reduced NO_3 concentration (**B**), non-significantly increased soil moisture (**C**), and reduced light penetration (**D**). Planted species richness is jittered for display so that dots don't overlap; higher species richness values are jittered more because they are more numerous.

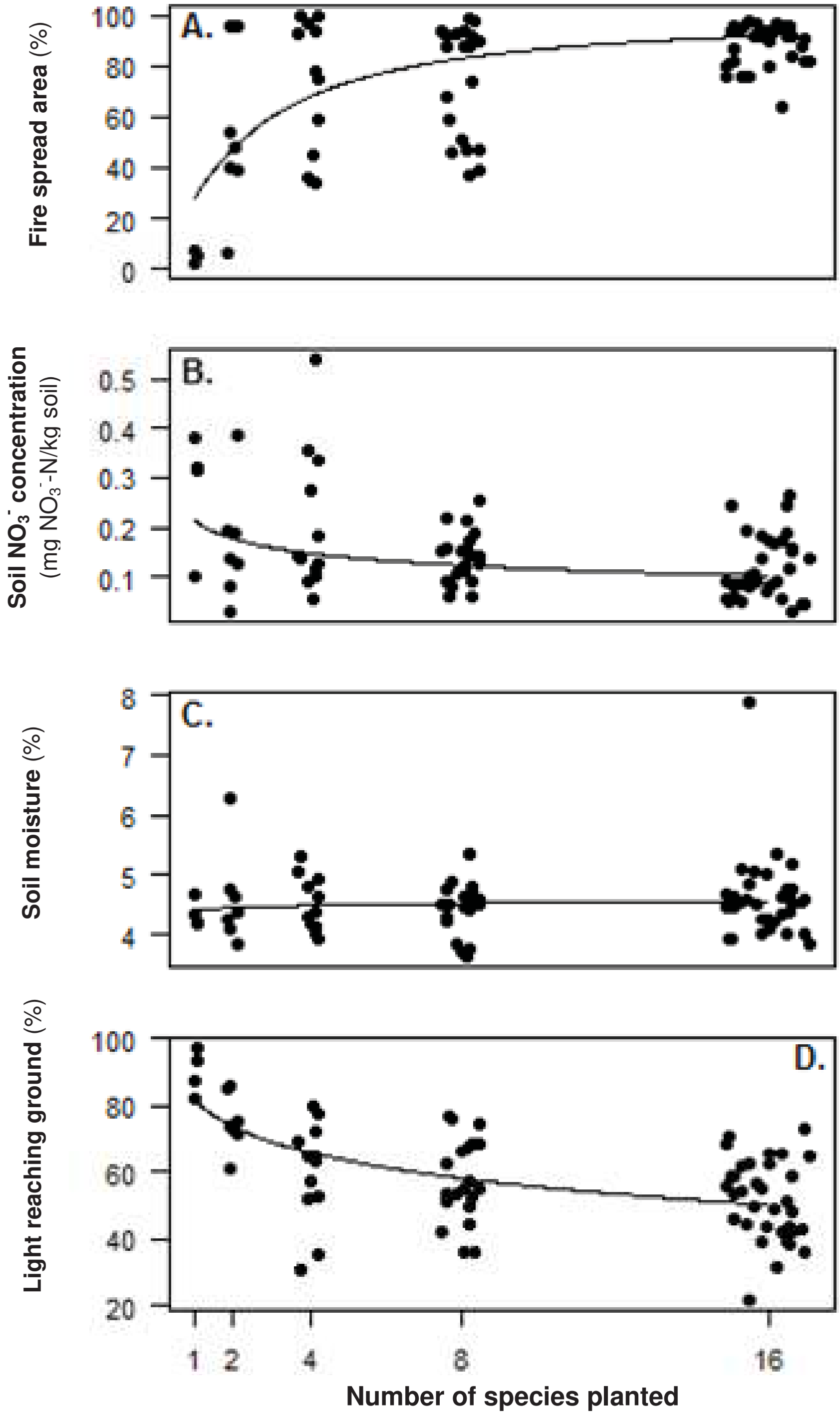


Figure 3-S5: Associations between tree height, tree top-kill, and tree growth rate.
As for Fig. 3-6, but using tree height instead of diameter as the metric of pre-fire size. For bur oaks, the upper mode in the height distribution of top-killed trees (E) was similar to the height at which top-killed trees tended to regrow to their pre-fire height (A).

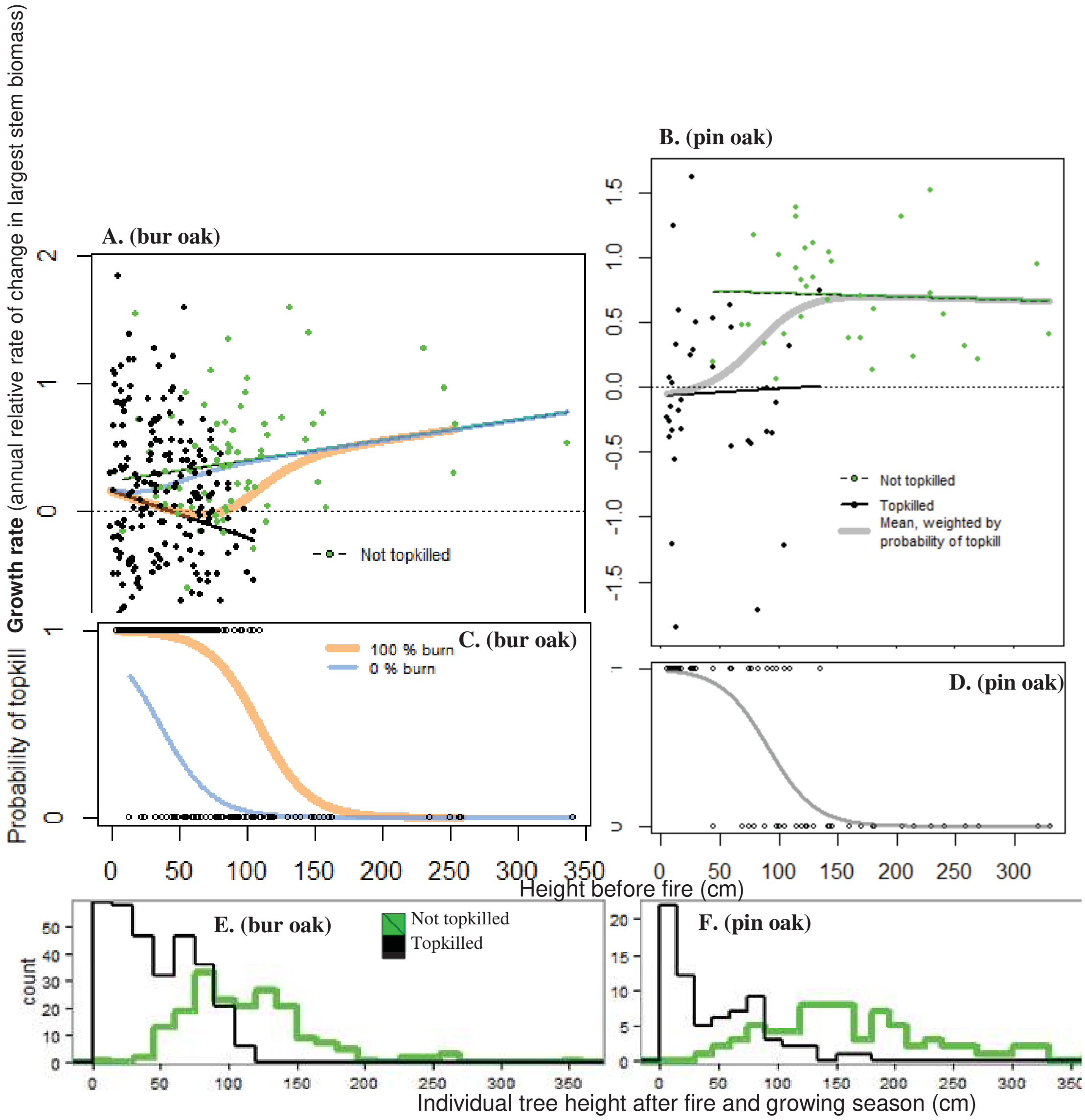


Figure 3-S6: Associations between tree biomass, tree top-kill, and tree growth rate.

As for Fig. 3-6, but using estimated biomass of the largest stem instead of diameter as the metric of pre-fire size. For bur oaks, the modal end-of-season biomass of top-killed trees (E) was similar to the biomass at which top-killed trees tended to regrow to their pre-fire biomass (A).

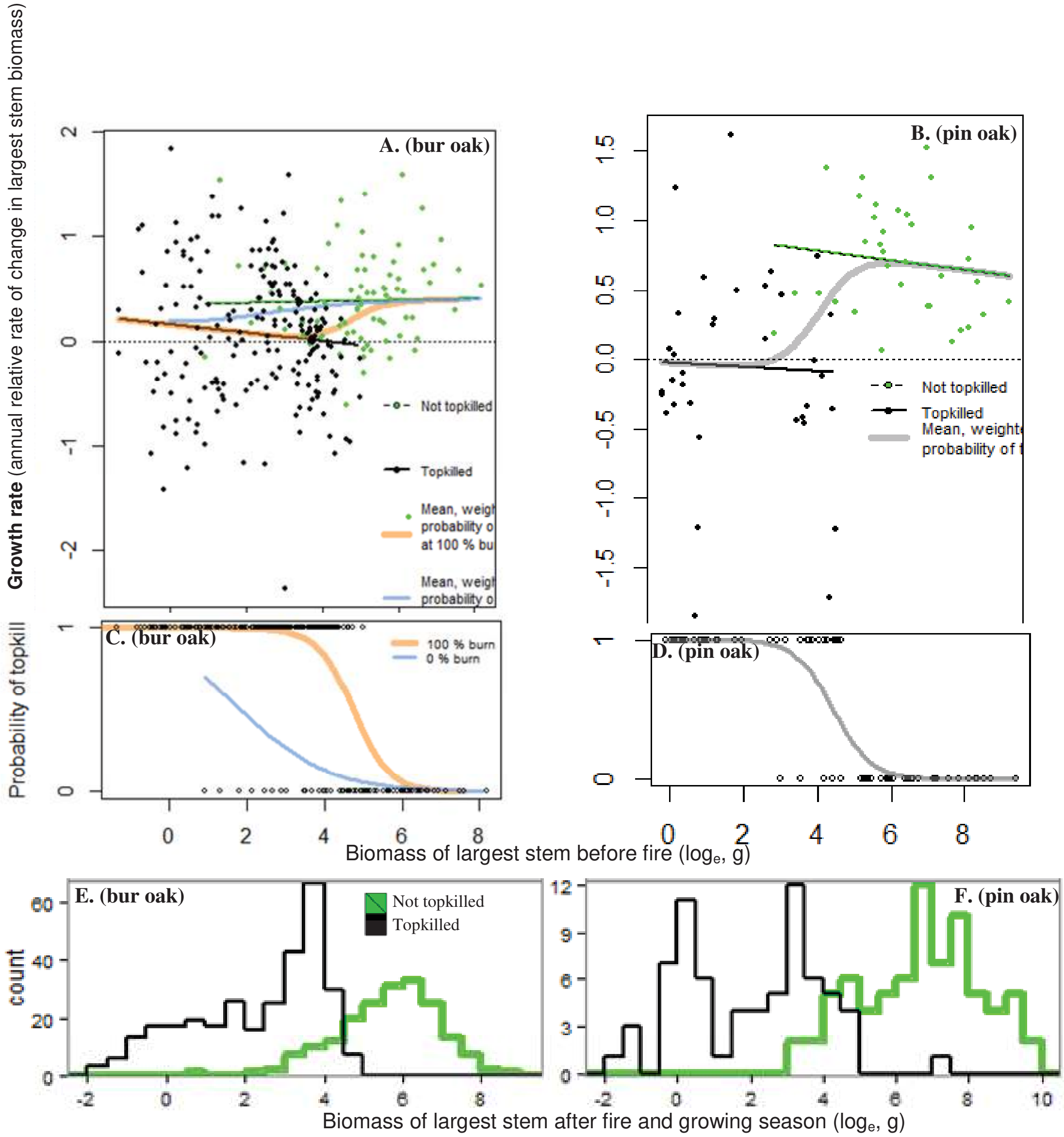
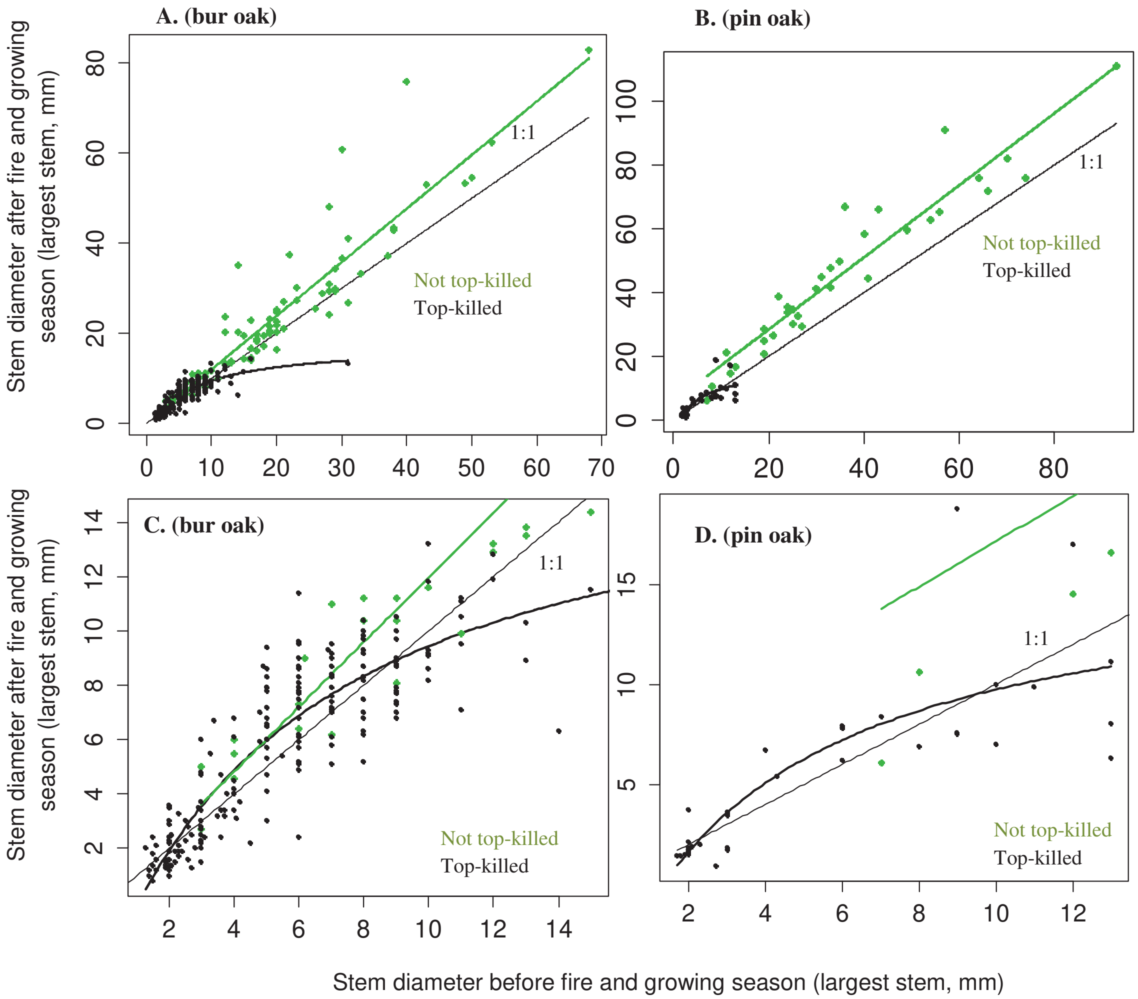


Figure 3-S7: Tree diameters pre- and post- fire.

An alternative representation of the data in Fig. 3-6, following and extending the graphical method of Grady & Hoffman (2012). The stem diameter of individual trees after a growing season (y axes) depended on their stem diameter before that growing season and a pre-growing-season fire (x axes) and whether they were top-killed. Fit lines for non-top-killed trees are linear regressions, and for top-killed trees are non-linear Michaelis-Menten functions. Top-killed trees of certain sizes (intersection of black lines) tended to regrow to their pre-fire size. c-d expand the data in the size range of topkilled trees, for clarity.



Chapter 4: Warming and diversity independently inhibit grassland plant recruitment

Summary

Rising temperatures are likely to alter the diversity and composition of plant communities as species' ranges track their climatic tolerances. However, it is unclear whether species' responses to climate change depend on how climate modifies their interactions with other species. Other species could moderate microclimate, or warming could alter the outcome of resource competition or disturbance-mediated interactions. Species respond to aspects of their environment (microclimate, resources, and disturbance) as influenced by the other species in the community in aggregate. Species richness and composition are aggregate properties of communities that influence plants' environment and are much altered by human actions. Therefore, we asked whether the effect of warming on seedling establishment depends on the diversity or composition of resident plants, and how these effects are mediated by microclimate, resource availability, and fire behavior.

We used a grassland field experiment to examine how factorial warming (+1.5 C or +3 C), resident diversity (1, 4, or 16 species) and resident functional composition treatments (presence of C3 and C4 grasses, legumes, and non-legume forbs) influenced the establishment of added seeds of 32 species, as well as seedling transplants and natural recruitment of resident species. Over five years, we measured the survival and growth of more than 20,000 seedlings.

Warming (+3 C) halved the number of seeded species that established and survived two growing seasons. Warming reduced the establishment success of most seeded species, mainly through reduced germination (emergence) at elevated temperature. The diversity and functional composition of the resident community also influenced plant establishment profoundly, though largely independently of the effects of warming. Diversity inhibited seedling survival and growth, mainly by reducing availability of light and nitrate resources. Responses to all treatments varied strongly by species and functional group.

The lower diversity of species establishing in warmed plots suggests that, as climate warms, low-diversity grasslands may gain diversity through seedling establishment less quickly than under current rates of seedling establishment. C3 grasses may become less abundant as climate warms, but species' responses to warming were explained only partially by their functional types. Nonetheless, our finding that the effects of increasing temperature on plant establishment are not contingent on the structure of the resident community suggests that developing a general understanding of plant species' and ecosystems' responses to global climate change is more tractable than it would otherwise be.

Introduction

Temperature is projected to rise 3 C or more this century in the US Midwest (Christensen *et al.* 2007, Galatowitsch *et al.* 2009), and this is likely to alter the diversity and composition of plant communities. One framework for such change is that species' ranges track shifts in the locations that meet their climate tolerances; this approach is exemplified by studies that have used climatic niche models to predict biotic responses to climate change (Thomas *et al.* 2004, Parmesan *et al.* 2011). However, it has long been recognized that species' responses to climate can depend not only on their intrinsic climatic tolerances but also on how climate modifies inter-specific interactions such as competition and facilitation. For example, MacArthur (1972) hypothesized that stronger competition at lower latitudes rather than climate *per se* limits polar species' equatorial range edges. A species' response to climate change may depend on the other species in a community for three reasons. First, other species may alter the influence of climate change on microclimate, for example by buffering against climate extremes (Wright *et al.* 2014). Second, warming may alter the outcome of competition with other species for limiting resources: the closer a species is to its optimum temperature, the lower may be its equilibrium resource requirements and the stronger a competitor it may be (Tilman and Pacala 1993, Chase and Leibold 2003, Tilman 2004). Third, warming may alter the outcome of disturbance- or herbivore-mediated interactions between plant species. In particular, warming may alter fire behavior and intensify fire regimes — by altering plant fuel and fire weather conditions — and thus advantage species that are more tolerant of fire. Here we investigate whether and how the effect of experimental warming on plant species' establishment — and ultimately, potentially, abundance — depends on the rest of the community in a North American tallgrass prairie grassland. Our results inform predictions of how grasslands varying in diversity and composition — from low diversity old fields to high diversity native prairie — will change in plant diversity and composition with climate change.

We hypothesize that two aspects of community structure may alter the effects of warming on a species' establishment: 1) species diversity, and 2) functional composition. Each influences species' establishment, and may interact with the effects of warming. First, species diversity reduces the ability of new species to invade (Elton 1958, Levine and D'Antonio 1999, Fargione *et al.* 2003, Fridley *et al.* 2007), through complementary use of limiting resources that results in higher productivity and leaves lower levels of resources unconsumed (Tilman *et al.* 1996). Warming increases aboveground productivity and standing biomass more strongly in more diverse communities (Cowles *et al.* in revision). Aboveground biomass in turn increases fire intensity (chapter 2) and light interception, so warming may reduce species' abundances more strongly through fire damage and light competition in more diverse communities; conversely, aboveground biomass can facilitate establishment by moderating microclimate, so the harsher summer microclimate created by warming may be ameliorated in more diverse communities (Wright *et al.* 2014). The net interactive effects of warming and diversity on soil moisture and nutrient availability are unclear.

For example, warming may directly reduce soil moisture and, through increased biomass, increase transpiration yet reduce evaporation. Similarly, warming may influence not only N mineralization rates (Rustad *et al.* 2001) (though not always, (Whittington *et al.* 2013)) but also N uptake by plants. Thus, we cannot predict the interactive effects of warming and diversity on species' establishment. This constrains our ability to predict and manage ecosystem responses to global warming, because diversity is widely reduced through agriculture and other land management. Here we address this gap in knowledge.

Second, functional composition — a combination of species composition and the functional types of those species — can influence species' establishment. This can be seen from two non-exclusive perspectives. First, according with a hypothesis that functionally similar species are more likely to compete (MacArthur and Levins 1967), species have been found to be less likely to invade communities dominated by resident species of the same functional type (Fox 1987, Leishman 1999, Fargione *et al.* 2003, Fargione and Tilman 2005a, Turnbull *et al.* 2005, Mwangi *et al.* 2007, Brown and Rice 2010, Hooper and Dukes 2010, Petermann *et al.* 2010) or similar functional traits (Funk *et al.* 2008) as the invader. Second, resident species can have strongly divergent impacts on environmental factors, for example through differences in their productivity (Tilman *et al.* 1997), which may result in resident species having different mean influences on invading species regardless of their similarity. C4 grasses and legumes promote productivity, which may promote fire and light interception and buffer climate extremes; C4 grasses also promote fire particularly strongly per unit abundance (chapter 2) and deplete available soil nitrate especially strongly, whereas legumes increase soil nitrate (Tilman *et al.* 1997). Warming may interact with functional composition by increasing the abundance of C4 grasses and legumes when they are present, as it did at our study site near the northern edge of the range of C4 grasses (Cowles *et al.* in revision). However, we cannot predict the net outcome of interactive effects of warming and functional composition on species' establishment. Functional composition is widely changed by land management and species invasions, so this gap, which we address also, is pertinent to managing climate change impacts.

Therefore, we examined how warming interacted with resident species richness and functional composition to influence the establishment of plant species, and thus the future composition and diversity of communities, using the Biodiversity And Climate (BAC) field experiment at Cedar Creek Ecosystem Science Reserve (Minnesota, USA). To generalize beyond the species pool of our experiment and understand why species respond as they do, we seek to understand species' responses to warming and functional composition in terms of their functional types and traits, i.e., do species' functional types and traits predict how their response to warming is influenced by resident diversity and functional composition? Other work in this experiment has shown that warming increased the abundance of C4 grasses and legumes and decreased the abundance of C3 grasses (Cowles *et al.* in revision). Here we used seed and seedling additions to examine the joint influences of warming, resident diversity and resident functional composition on establishment of a broad range of species, to gain further insight into how grasslands differing in diversity and functional composition respond to climate change.

Recruitment from seed is rare among most of the perennial species that dominate tallgrass prairie (see Results), yet is necessary for long-term viability of populations of many species, even long-lived ones, and is essential for species to migrate in response to climate change. Seedlings may be more affected by interactive effects of warming and resident diversity or composition than later life stages because their small size a) places them below the canopy of resident plants, making them susceptible to any influence of the residents on microclimate; b) limits their access to deep soil moisture, to nutrients that cannot be taken up from surface soil when it is dry, and to light above the plant canopy; and c) may make them more vulnerable to being killed by fire. Seed mass is a trait that determines initial seedling size and thus early access to, and requirements for, light and soil resources and thus may predict species' responses to warming and other treatments (Baker 1972, Grubb 1977, Buckley 1982, Jurado and Westoby 1992, Leishman and Westoby 1994, Caddick and Linder 2002, Moles and Westoby 2004, Kitajima and Myers 2008, Shevtsova *et al.* 2009). Experimental warming tended to reduce seedling establishment in previous grassland and shrubland studies, apparently by drying the soil (Sternberg *et al.* 1999, Lloret *et al.* 2004, Williams *et al.* 2007, Hovenden *et al.* 2008, Shevtsova *et al.* 2009). Seedling establishment was lower in communities with higher species numbers (Levine 2000, Hector *et al.* 2001, Kennedy *et al.* 2002, Levine *et al.* 2004), higher species evenness (Emery and Gross 2006), and higher functional group diversity (Symstad 2000) of residents. However, the interactive effects of warming and resident diversity or functional composition on seedling establishment are unknown.

Thus, we asked: (1) How do seedling species diversity, and the establishment success (germination, survival, and growth) of species and functional groups, respond to the interactive effects of warming and resident species diversity? (2) How do these same dependent variables respond to the interactive effects of warming and resident species functional composition? 3) How are treatment effects mediated by environmental factors, including microclimate, resource availability, and fire behavior?

We worked in a biodiversity experiment established in 1994-1995, within which nested heat treatments were established in 2009. We refer to species in plots where they were planted in 1994-1995 as part of the biodiversity experiment as resident species. We monitored natural recruitment from seed, but this was limited for many of these perennial resident species. To examine with far more power how treatments affect species' performance once they arrive at a site, we added to all plots a known and consistent quantity of seeds of the seven commonest resident species and 25 species native or naturalized at the site but not resident. All species were herbaceous biennials or perennials. We examined how establishment of these plants — measured as biomass per species per unit seeded area, or, for transplants, biomass per planted seedling, as well as particular aspects of establishment such as germination, survival and growth — responded to factorial warming, resident diversity, and resident functional composition treatments over two growing seasons, and how these responses were mediated by environmental factors.

Methods

Experimental design

The Biodiversity and Climate experiment (BAC, experiment number E249) is a split-plot experiment with 32 whole-plots differing in the number of grassland species planted (resident species: 1, 4, or 16) and the identities of those species (randomly selected from a pool of 18 species). The whole-plots are a subset of a larger 168-plot biodiversity experiment (experiment number E120) initiated in 1994 (Tilman *et al.* 1997), at Cedar Creek Ecosystem Science Reserve in Minnesota, USA. The pool of 18 species represented dominant and common species in the oak savanna that was the dominant pre-settlement vegetation of the area before fire suppression allowed forest encroachment. The species pool contained species of four herbaceous functional groups: C4 grasses, C3 grasses, legumes, and non-legume forbs; all were perennials, and most were native except the naturalized C3 grass *Poa pratensis* (Tilman *et al.* 1997). We characterized resident functional composition of each plot by whether each functional group was represented by at least one species. The species pool also included 2 savanna oak tree species, *Quercus macrocarpa* and *Q. ellipsoidalis,* but these were always rare in the BAC plots and were removed entirely in 2010 before many of the data reported here were collected (chapter 3), so presence of these woody resident species was not analyzed. Each plot was burned every spring, as soon as possible after snow-melt (chapter 2).

Since 2009, three subplots within each 9 * 9 m whole-plot, each 3 * 2.5 m, have received ambient, +1.5 C, or +3 C infrared heating treatments (Aronson and McNulty 2009, Whittington *et al.* 2013). The heat treatments were designed to warm bare ground by these nominal amounts, and temperature data reported in Results confirm that these targets were met approximately. However, realized soil temperatures below vegetation varied because the heating treatments were of fixed power output: the +1.5 C treatment used an 800 W infrared heater and the +3 C treatment used a 1600 W infrared heater, from March through November each year. The ambient subplot had a sham heater as a control.

Within each of the 96 subplots, we investigated seedling emergence and establishment -- both natural and arising from seed additions and seedling transplants -- within a 0.24 m^2 seedling microplot (experiment number E252).

Seed additions

In spring 2009, we planted seed of each of 25 perennial species that occur in prairie within 2 km of this experiment into each seedling microplot. We used species not established as resident species in the biodiversity experiment, both to prevent germination responses from being confounded by variation among plots in natural seed production and to examine the responses of a wider range of species. These species were selected to be potentially diverse in their temperature optima by including species near their eastern, northern, western or southern range edge as well as those near the center of their range in Minnesota, subject to the constraint that only species native to or naturalized at this reserve may be planted there. Seeds were supplied by Prairie Moon (Winona, MN, USA) and Prairie Restorations (Princeton, MN, USA) from seed sources in the upper Midwest, mostly Minnesota. Rhizobial innocula were mixed with legume seeds before planting, in a small amount of soil from the experimental field. To each microplot, we added a standard total mass of seed of every species (0.5 g of viable seed; if germination tests showed lower than 100 % viability, the mass was increased above 0.5 g to account for this). We used a standard total mass of seeds, instead of a standard total number of seeds, because species with smaller seeds tend to have lower emergence rates and so a standard total mass of seeds is more likely than a standard total number of seeds to yield approximately similar numbers of emerging seedlings across all species.

All of the resident grasses, and most of the resident forbs, had negligible natural seedling emergence. To examine the responses of these species that are dominant or common in regional remnant savannas, in 2012 we added 0.5 g of seed of each of 7 resident species to a 0.0675 m^2 portion of the seedling microplot. The resident oak trees were too small to produce seed, so in spring 2010 we planted 4 seeds per subplot of bur oak, *Quercus macrocarpa,* at a depth of 2 cm.

Seedling emergence, survival and growth

We monitored the emergence, survival and growth of all seedlings from natural and added seed monthly during the 2009-2013 growing seasons. More than 20,000 seedlings emerged. At each survey, newly emerged seedlings were uniquely marked with color-coded and mapped plastic toothpicks; we measured each seedling's height and number of leaves, from which we estimated its biomass using allometric equations in order to calculate relative growth rates. We derived equations relating each species' biomass to its height and number of leaves from seedlings of all relevant sizes harvested from monocultures planted in a disused, cleared 8-species plot, plus seedlings harvested from the BAC plots. Seedlings emerging from natural seed were monitored as long as they survived, up to five years. Only plants that were freshly emerged seedlings when first surveyed -- dicots with cotyledons, or equivalently-small monocots -- were measured. Species for which seeds were added were surveyed until the end of their second growing season. Most added species emerged almost exclusively in either the year of planting or the following year, but some species were more evenly split between two years. Each seedling arising from added seed was harvested at the end of its second growing season. For the 2009 addition of non-resident species, aboveground dry biomass was measured for a subset and the biomasses of the rest were estimated using the allometric equations; for the 2010 and 2012 additions of resident species, all seedlings were harvested and weighed. For analysis, we summarized these data as the total biomass per unit area of a given seeded species in a plot at the end of the second growing season.

Seedling transplants

To compare responses of newly germinated seedlings with those of much larger seedlings, consistently across all treatments, we planted two seedlings of each of four species (a C4 grass, a legume, and two non-legume forbs) at the edges of the seed addition microplots in spring 2009. The seedlings were six weeks old and had been grown in seedling trays by Minnesota Native Landscapes (Princeton, MN) and Prairie Restorations (Princeton, MN) from Minnesota seed sources. Most of the potting medium in which they had been grown was washed off before planting; they were watered during the first two weeks to reduce transplant shock, and the few plants that died during this time were replaced. At the end of the season, we harvested and weighed them.

Environmental factors

Soil temperatures were logged hourly during the growing season (May through September, except starting in July in 2009 and June in 2013) in two systematic locations per subplot, chosen to represent the thermal conditions (distance from heaters) in the seedling microplot. Temperatures were measured 1 cm below the soil surface every year, and 10 cm below ground in 2012-2013, using Thermochron iButtons (Thermodata Corp., Whitewater, WI). Soil temperature at 10 cm depth was tightly correlated with that at the surface (r = 0.98) and is not shown. Air temperature and relative humidity were also recorded at 10 cm above ground in 2012-2013, using Hygrochron iButtons (Thermodata Corp., Marblehead, MA) (Wright *et al.* 2014, Cowles *et al.* in revision). We calculated mean values across all years for each subplot at each depth; to avoid over-weighting the later part of the season for which data were collected in more years, we first averaged the values for each depth in each subplot on each day of year (averaging across years), and then averaged across these seasonal values.

Soil nitrate was measured in the top 20 cm of soil in each subplot, once each in July and August 2011 and every two weeks from 15 May through 15 August 2012. Each sampling event occurred a few days after rain. We composited 6 systematically located cores from each subplot, and extracted nitrate overnight using 0.01 M KCl. Nitrate was analyzed colorimetrically using a QuickChem 8500 series 2 Flow Injection Analysis System (Lachat, Loveland, CO).

Soil moisture was measured in the surface 6 cm of soil using a Thetaprobe (ML2x 2009-2011, SM300 2012-2013; Delta-T Devices, Cambridge, England), every two weeks in 2009 and 2013, and weekly in 2010-2012, from May (2010, 2012) or June (other years) through September. We measured 6-10 systematically located, permanently marked points around each seedling microplot, depending on the year. Measurements could not be taken on rainy days; to minimize bias, if rain was forecast for the systematically planned measurement day then measurements were instead taken either the previous or following day, alternating between the previous and following day. We verified that Thetaprobe soil moisture values were correctly calibrated against a dry-down sequence of gravimetric soil moisture measurements in the lab of soil samples from plots of each diversity level. These Thetaprobe measurements were also tightly correlated with gravimetric soil moisture in the 0-20 cm soil nitrogen cores in 2012 and 2013 (r = 0.92).

We measured light penetration to the soil surface relative to ambient light along two sides of each seedling microplot within two hours of solar noon every 2-3 weeks from May through September, from 2010-2013. We used an Accupar LP80 light meter (Decagon, Pullman, WA).

We estimated fire spread area as the percent of each seedling microplot that carried fire after each spring burn in 2010, 2011, and 2013. We also measured fire temperature at ground level using paints of varying melting points on copper tags (chapter 2). In each seedling microplot, we placed one tag in 2010 and two tags in 2011.

For each metric, we calculated growing season means per subplot over all years in order to average over variability arising from, for example, random variation in the depth of ibutton burial due to shifting surface soil and to allow us to use factors not measured every year. Using multi-year averages is unlikely to have masked signals because the treatment effects were consistent across years for these metrics (not shown) and for total herbaceous biomass (Cowles *et al.* in revision).

Analyses

Our units of analysis were subplots. Warming treatment and log resident species richness were treated as continuous variables; treating them as factors to allow for non-linear responses did not lead to qualitatively different conclusions.

We analyzed the response of seedling diversity to treatments using generalized linear mixed models, with plot as a random intercept to account for correlation between warming subplots within each diversity whole-plot. We analyzed species richness of seedlings using a binomial error distribution (with number of seeded species surviving as "successes" and number not surviving as "failures"), and effective species richness (e^H, where H is the Shannon diversity index, representing the equivalent number of equally abundant species and thus increasing with the evenness of species' abundances as well as the number of species present (Hill 1973)) using a Normal error distribution. The fixed effects were either warming, resident diversity, and their interaction; or warming, resident functional groups, and their interaction.

We analyzed the response of species' individual performances using total biomass per unit area in each subplot to integrate germination, survival and growth. The species' frequency distributions of total biomass per unit area had many zeros for plots without any establishment and a peak well above zero, making them unsuitable for standard error distributions. Therefore we used a compound Poisson error distribution (Zhang 2013), which is suitable if there is a Poisson-distributed number of individuals surviving and each of those survivors has a gamma-distributed biomass (both of which were empirically reasonable), and the biomasses of the individuals are summed (as they were). We analyzed seeded species' responses together in a single mixed model regression in order to test whether species differed significantly in their responses to treatments (using species x treatment interaction terms), as well as estimate each species' response and mean responses across species. We performed one such regression with seeded species' identity, warming, resident diversity, and all their interactions as fixed effects, and another with resident functional group presences substituted for resident diversity. We also performed similar regressions with seeded species' functional group, or seeded species' seed mass, substituted for seeded species' identity. In each case subplot nested within plot was a random intercept to account for correlations between species within a subplot arising from subplots varying in overall favorableness. These regressions included data from multiple years, for species that germinated in more than one year; to account for some years being more favorable to particular species, for example due to weather or seed inputs, each species-year combination was an additional random intercept. We analyzed seeded species, natural germination from resident species, and transplanted seedlings separately, using equivalent regressions.

To determine which life stages were influenced by the treatments and environmental factors, we performed similar regressions using, as responses, number germinating; survival and relative growth rate over the first growing season; and survival and relative growth rate from the end of the first growing season to the end of the second growing season. For seeded species, we report survival and relative growth rates for the second growing season because first season rates were noisier, perhaps because they were calculated over short time periods for plants that germinated late in the first season. However, first and second season rates were correlated. For number germinating we used a Poisson error distribution. A binomial distribution would have been more appropriate when the number of seeds added was known, but for seeded species emerging in more than one year the number of viable seeds remaining in a given year was unknown; a Poisson distribution was justified because it approximates a binomial distribution when the number of seeds is large and the proportion germinating is small, as was the case for most of our species. For survival, we used a binomial error distribution. For relative growth rate, we used a Normal error distribution. To account for overdispersion shown by regressions with Poisson or binomial error distributions, we included a random intercept with each observation as a different level.

We used R packages lme4 (Bates *et al.* 2014) and cplm (Zhang 2013) to fit generalized linear mixed models and compound Poisson mixed models, respectively. We report tests of individual coefficients from these regressions using Wald z scores, and joint tests of multiple coefficients from these regressions (i.e., tests involving categorical factors such as species x warming interactions) using Wald $\chi 2$ statistics from Type II ANOVAs that assess effects after accounting for all other non-interaction effects, using R package car (Fox and Weisberg 2011).

Results

Warming, resident diversity, and seedling performance: seed additions

The high warming treatment almost halved the number of species that established and survived in each plot (of 32 that were seeded) compared with controls (Fig. 4-1A, main effect of warming as a continuous variable: $P < 0.001$). The highest resident diversity treatment (16 species) had about a third as many species establish and survive compared with monocultures (Fig. 4-1A, main effect of log diversity as a continuous variable: $P < 0.001$). These effects did not interact. Diversity and warming had far weaker effects on the effective number of species surviving (Fig. 4-1B); this metric places less weight on rare species than the proportional number of species surviving, suggesting that the effects on species richness were largely due to failure of rare species to establish in diverse or warmed plots.

Across all 32 seeded species, biomass per seeded species per unit area at the end of the second growing season — which integrates germination, survival and growth rates — showed negative main effects of warming ($P < 0.001$) and resident species richness ($P < 0.001$) in an ANOVA with seeded species, warming treatment, and resident species richness, and their interactions, as independent variables (Fig. 4-2A). Seeded species varied significantly in their responses to warming and species richness (Fig. 4-S1 & Fig. 4-S2, species x warming and species x diversity interactions: $P < 0.001$). The C3 grass *Phleum pratense* and non-legume forbs *Anemone cylindrica* and *Liatris aspera* responded especially negatively to warming, whereas the non-legume forb *Penstemon grandiflorus* responded especially positively to warming. The C4 grass *Andropogon gerrardii* responded especially negatively to diversity whereas the forb *Anemone cylindrica* responded especially positively. The effects of warming and diversity did not interact significantly (diversity x warming: $P = 0.937$; species x diversity x warming: $P = 0.588$).

Warming inhibited seedlings mainly by reducing germination (Fig. 4-2B), not survival or growth (Fig. 4-2C,D), though the effects varied by species (Fig. 4-S2). In contrast, resident diversity inhibited seedlings mainly by reducing survival and relative growth rate (Fig. 4-2C,D), with a weaker (non-significant) negative effect on germination (Fig. 4-2B); again, these effects varied significantly by seeded species (Fig. 4-S2).

A similar analysis of biomass per unit area at the end of the second growing season with seeded species' identities replaced by their functional groups showed that seeded functional groups differed in their responses to warming (seeded functional group x warming interaction: $P = 0.031$) and resident diversity (seeded functional group x diversity interaction: $P < 0.001$) (Fig. 4-3). C3 grasses and C4 grasses were significantly more negatively affected by the warming treatment. All the herbaceous functional groups were significantly more negatively affected by diversity than was the woody oak species.

In a similar analysis with seeded species' identities replaced by their log seed mass, biomass per area after two growing seasons was reduced by diversity more strongly in smaller seeded species (log seed mass x resident diversity interaction: $P < 0.001$), but seed mass did not influence species' responses to warming.

Warming, resident diversity, and seedling performance: natural recruitment

Among the resident species, only legumes had seedling emergence from natural seed production that was high enough for informative analysis of treatment effects. During 2009-2011, the four legume species' mean emergence rates ranged from 4 to 28 seedlings/m^2/year in plots in which they were planted. Of the non-legume species, *Liatris aspera* and *Schizachyrium scoparium* each had 1.8 seedlings/m^2/year and the remaining species had fewer than 1 seedling/m^2/year.

For natural recruitment by the legumes we analyzed biomass per seeded species per germinant (rather than per area) at the end of the second growing season (Fig. 4-S3), because seed input was not controlled and warming may have already increased seed availability by increasing total legume biomass in warmed plots (Cowles *et al.* in revision). Only *Petalostemum purpureum (*synonym *Dalea purpurea)* responded significantly to the treatments, in a strongly interactive manner: it increased with warming in monoculture but decreased with warming in high diversity plots. None of the fitness components (survival in the first or second season, or growth rate the second season) responded significantly to the treatments when analyzed separately.

Warming, resident diversity, and seedling performance: seedling transplants

Across the four herbaceous species transplanted as seedlings, the warming treatments reduced biomass per transplanted seedling at the end of the growing season, which integrates the effects of survival and growth (Fig. 4-S4, main effect across species: $P = 0.008$, species x warming interaction not quite significant, $P = 0.055$). Diversity treatments even more strongly reduced biomass per planted seedling (Fig. 4-S4, main effect across species: $P < 0.001$, species x diversity interaction not significant). Again the trend was for the negative effect of warming to be weaker at higher diversity, but the warming x diversity interaction was not significant for any species. Averaging across species, warming reduced survival whereas resident diversity reduced growth rate. However, both survival and growth rate had species-specific responses to resident diversity.

The two transplanted species that also had enough germination from seed to compare their responses, the non-legume forb *Solidago nemoralis* and the legume *Baptisia alba*, had similar responses when seeded and transplanted.

Warming, resident functional composition, and seedling performance: seed additions

Seeded species responded differently to the presence of resident functional groups, but the resident functional groups generally did not modify (interact with) the effects of warming. In an ANOVA with seeded species, warming treatment, and the presence of each resident functional group, and their interactions as independent variables, the resident functional groups had distinct effects on seeded species' biomass per unit area after two growing seasons, and these differed between seeded species (resident functional group presence main effects and interactions with seeded species' identity: all $P < 0.001$). Resident presence of C3 grasses, C4 grasses and non-legume forbs all had negative main effects on seeded species' biomass per area (Fig. 4-4A, $P < 0.001$). There was one significant interaction of a resident functional group's presence with warming: the effect of warming on the non-legume forb *Penstemon* *grandiflorus* was slightly negative without resident legumes, but strongly positive with resident legumes; this drove a significant seeded species x warming x legume interaction. Inhibitory effects of resident non-legume functional groups were driven mainly by reduced seedling survival and growth rates (Fig. 4-4C,D); reduced germination rates also contributed to the suppressive effect of C3 grasses (Fig. 4-4B). The neutral net effect of legume presence on mass per area (Fig. 4-4A) resulted from negative effects on germination (Fig. 4-4B) and survival (Fig. 4-4C) opposed by non-significantly positive effects on growth rate (Fig. 4-4D).

A similar analysis with seeded species' identities replaced by their functional groups showed that seeded functional groups differed in their responses to the presences of resident functional groups (Fig. 4-5; seeded functional group x resident functional group interactions all $P < 0.001$). Resident C4 grasses, legumes, and non-legume forbs each most strongly inhibited seeded species of their own functional group. This can be seen in the highly negative effect of resident C4 grasses (y axis value of -4) on the point representing seeded C4 grass species in Fig. 4-5A, for example. The inhibitory effects of functional groups on themselves were driven mainly by effects on seedling growth rates. Resident C3 grasses inhibited C4 grasses most strongly. However, the effects of resident functional groups did not modify (interact with) the effects of warming.

Another similar analysis with seeded species' identities replaced by their log seed mass showed that the presence of each of C4 grasses, legumes, and non-legume forbs as residents was less inhibitory to seeded species with larger seed mass than with smaller seed mass (seed mass x resident functional group presence interactions significant). However, seed mass did not interact with the effects of warming.

We also performed all these analyses with the number of species planted in each resident functional group, instead of their planted presences, with similar results (not shown).

Warming, resident functional composition, and seedling performance: seedling transplants

The four herbaceous species transplanted as seedlings were influenced similarly to the seeded species by the planted presences of resident functional groups.

Warming, resident diversity, and environmental factors

The warming and resident diversity treatments altered environmental conditions, which could explain the treatment effects on seedling performance (Fig. 4-6, Fig. 4-S5).

Temperature was increased by warming and reduced, less strongly, by resident diversity. Warming and diversity interacted in their effects on soil temperature 1 cm below the surface: the high warming (nominally +3 C) treatment increased soil temperature by 3 C in monocultures, but by slightly less than 2 C in high diversity (16 species) plots (Fig. 4-S5, main and interaction effects $P < 0.001$). In contrast, warming and diversity effects were independent for air temperature, relative humidity, and vapor pressure deficit.

Warming increased air temperature 10 cm above ground by about 1 C in the low warming (nominally +1.5 C) treatment and about 2 C in the high warming (nominally +3 C) treatment (Fig. 4-6E and Fig. 4-S5, $P < 0.001$). Diversity independently reduced air temperature by up to 0.5 C in 16 species plots versus monocultures (Fig. 4-6E and Fig. 4-S5, marginally non-significant, $P = 0.065$). Vapor pressure deficit (VPD), too, increased with temperature and independently decreased with diversity; it was tightly correlated ($r = 0.98$) with relative humidity (Fig. 4-S5). Temperature 1 cm below the soil surface was closely correlated with both air temperature ($r = 0.80$) and VPD ($r = 0.93$).

Warming treatments increased available soil nitrate and decreased soil moisture, counter to but independent of the effects of resident diversity; warming and diversity synergistically reduced light availability. Soil nitrate at 0-20 cm depth increased with warming ($P < 0.001$) and decreased more strongly with diversity ($P = 0.019$, Fig. 4-6D and Fig. 4-S5). Surface soil moisture at 0-6 cm depth decreased with warming ($P < 0.001$) and increased more strongly with diversity ($P < 0.001$, Fig. 4-6C and Fig. 4-S5). Light availability at the ground decreased with warming ($P < 0.001$) and diversity ($P < 0.001$), and decreased more than additively when diverse plots were warmed (interaction $P = 0.003$, Fig. 4-6B and Fig. 4-S5).

Warming and diversity treatments independently increased fire spread and temperature. Fire spread increased marginally non-significantly with warming ($P = 0.077$) and far more strongly with diversity ($P < 0.001$, Fig. 4-6A and Fig. 4-S5). Fire temperature increased significantly with warming ($P = 0.049$) and more strongly with diversity ($P < 0.001$, Fig. 4-S5). Fire temperature and fire spread area were closely correlated ($r = 0.85$).

We selected a subset of relatively uncorrelated environmental variables to use as independent variables in multiple regressions in subsequent sections, to avoid multicollinearity. Correlations stronger than ±0.7 occurred between light and fire temperature ($r = -0.79$) and between surface soil temperature and surface soil moisture ($r = -0.74$), in addition to those above. Therefore, we used air temperature, soil nitrate, soil moisture, light, and fire spread area.

Warming, resident functional composition, and environmental factors

The resident functional groups had distinct effects on environmental conditions, which we examined using multiple regressions with the presence of each functional group, warming treatment, and the interaction of each functional group with warming as independent variables. Air temperature was not significantly affected by the presence of functional groups (Fig. 4-7E). Soil nitrate increased with the presence of legumes ($P < 0.001$), far more strongly than it increased with warming, but decreased with the presence of other functional groups, especially grasses ($P < 0.001$) (Fig. 4-7D). Soil moisture was not significantly affected by the presence of any functional groups (Fig. 4-7C). Light availability was reduced by warming more strongly when legumes were present (interaction: $P = 0.047$; legume main effect also significantly negative, $P < 0.001$), and independently by the presence of C_4 grasses ($P < 0.001$) and non-legume forbs (P

< 0.001) (Fig. 4-7B). Fire spread area increased with the presence of C4 and C3 grasses and legumes ($P < 0.001$), independently of the effects of warming (Fig. 4-7A).

Environment and seedling performance: seed additions

We used multiple regression to examine how seedling performance was influenced by environmental variables. The biomass per area of seeded species responded to air temperature and resource availability but not fire spread area (Fig. 4-8A). There were positive main effects (averaging across species) of soil nitrate ($P < 0.001$), soil moisture ($P = 0.010$), and light availability ($P < 0.001$), but seeded species varied significantly in how they responded to all of these factors, as well as air temperature which had a non-significantly negative main effect (interactions with seeded species: all $P < 0.005$; Fig. 4-S6). Increasing air temperature especially increased biomass per area of the non-legume forb *Asclepias tuberosa,* and decreased the C3 grasses *Dactylis glomerata* and *Phleum pratense* and the non-legume forb *Liatris aspera.* Whereas most species increased with nitrate, the non-legume forb *Liatris aspera* decreased with increasing nitrate. The non-legume forbs *Agastache foeniculum* and *Asclepias tuberosa* increased especially strongly with increasing moisture. With increasing light, *Asclepias tuberosa* increased especially strongly whereas the non-legume forb *Anemone cylindrica* decreased especially strongly. Fire spread area did not have significant effects.

Increased air temperature, the environmental factor that was influenced most strongly by the warming treatments, influenced seedlings mainly by reducing germination (Fig. 4-8B), though species varied in how strongly their germination was inhibited by temperature (Fig. 4-S6). Higher air temperature tended to increase survival (Fig. 4-8C) and growth (Fig. 4-8D) but these effects were not significant. In contrast, availability of light and soil nitrate, the environmental factors that were most strongly reduced by the resident diversity treatments, influenced seedlings mainly by increasing survival (Fig. 4-8C) and particularly growth rate (Fig. 4-8D); again, these effects varied significantly by seeded species (Fig. 4-S6). Soil moisture also tended to increase survival and growth rate (Fig. 4-8C,D); the main effect was not significant but species differed significantly in the effect of moisture on growth rate (Fig. 4-S6).

In a similar analysis with seeded species' identities replaced by their functional group, functional groups also differed significantly in their biomass per unit area responses to nitrate ($P < 0.001$), moisture ($P = 0.012$), light ($P = 0.003$), and air temperature ($P = 0.041$) (Fig. 4-9). In terms of biomass per unit area, C3 grasses decreased more strongly with increasing air temperature than did other functional groups; C4 grasses increased more strongly with increasing light and moisture than did other functional groups; and legumes and woody plants increased significantly less strongly with increasing nitrate than did other functional groups -- indeed, they were not significantly affected by nitrate.

In a similar analysis with seeded species' identities replaced by their log seed mass, the effects of environmental factors on biomass per area did not interact with seeded species' seed size.

Environment and seedling performance: seedling transplants

The transplanted species differed significantly in how their biomass per transplanted seedling responded to nitrate (species x nitrate interaction: P = 0.002). Nitrate inhibited the legume *Baptisia alba.* Conversely, nitrate had a slightly positive effect on the other species. The C4 grass *Bouteloua curtipendula* was unaffected by air temperature whereas the other species were significantly negatively affected by it (P = 0.004). None of the species were significantly influenced by light or moisture availability. (In assessing how the transplanted seedlings were affected by the environmental factors, we omitted fire spread area because they were harvested after one season, before burning.)

Discussion

Effects of warming were mostly independent of effects of diversity and functional composition

Warming strongly reduced the diversity of seeded species, as well as the establishment success (biomass per unit area) of most seeded species. The diversity and functional composition of the resident community also influenced establishment profoundly -- most species' establishment was generally inhibited by resident diversity and by the presence of grasses and non-legume forbs, or its own functional group (Fargione *et al.* 2003). However, effects of resident diversity and functional composition generally interacted weakly if at all with the effects of warming. This suggests that the effects of increasing temperature on seedling establishment are not highly contingent on the structure of the resident community in these grasslands.

Environmental factors mediate treatment effects

The lack of strong interactive effects of warming and diversity on seedling establishment contrasts with the strongly synergistic impact of warming and diversity on aboveground productivity, which increased more strongly with warming at higher diversity (Cowles *et al.* in revision). This may be because light availability was the only environmental factor we measured that showed the same interactive response as productivity. Air temperature, soil nitrate, and soil moisture all responded to warming independently of their responses to diversity, which accords with the independent effects of warming and resident diversity on seedling establishment. Similarly, the lack of strong interactions between the effects of warming and functional composition accords with the resident functional groups affecting nitrate, fire and light independently of how these variables were affected by warming. The exception was that warming reduced light more strongly in the presence of legumes, likely due to the increase in legume biomass with warming (Whittington *et al.* 2013, Cowles *et al.* in revision); N fixation by legumes also may have promoted increased productivity of other functional groups with warming.

Herbaceous productivity was strongly determined by diversity (Tilman *et al.* 1996, Reich *et al.* 2012) and composition (Tilman *et al.* 1997) in this experiment and so the effects of the treatments on productivity can explain many of their effects on the environmental factors that influenced seedlings, as discussed below. Thus, we hypothesize that resident biomass -- like resident diversity and functional composition -- will not alter the effect of warming on seedling establishment, even where biomass varies for other reasons.

Warming treatments increased nitrate availability, reduced soil moisture and ground level light availability, and increased fire spread, but most strongly influenced air temperature. The increase in available nitrate with warming is puzzling because the warming treatments did not significantly increase net N mineralization in 2009-2010 (Whittington *et al.* 2013) yet did increase aboveground productivity and — except at the highest diversity, root biomass (Cowles *et al.* in revision) — and therefore, presumably, increased nutrient uptake (Fornara and Tilman 2009). There are a few possible explanations. Reduced soil moisture with warming may have inhibited nitrate uptake by mass flow and diffusion, and perhaps reduced leaching losses of N from surface soil. Net N mineralization may have increased in warmed plots between the 2009-2010 measurements (the first two years of the warming treatments, (Whittington *et al.* 2013)) and the measurements of available nitrate in 2011-2012, as increased litter resulting from warming-increased productivity decomposed; in 2013, lab incubation measurements of net N mineralization were indeed higher in soil from warmed subplots than from control subplots, at high diversity (Cowles and Tilman in prep.). Finally, the available nitrate measurements reported here and the net N mineralization results of (Cowles and Tilman in prep.) may be more sensitive to subtle warming effects because they aggregated over spatial heterogeneity by using four or six cores per subplot compared with one per subplot for the 2009-2010 N mineralization assays (Whittington *et al.* 2013). The reduced soil moisture with warming may have been driven by increased evaporation from the soil and evapotranspiration by plants due to a higher vapor pressure deficit, as well as increased evapotranspiration by plants in response to increased heat loading. The decreased ground level light and increased fire spread area and temperature with warming are consistent with increased herbaceous biomass that intercepts light and is fuel for fire (chapter 2, \Cowles, in revision #9023}.

Resident species diversity had opposite effects to warming on air temperature, nitrate, and moisture, but concordant effects on light and fire spread. Resident diversity reduced air temperature, likely through shading by increased biomass (Cowles *et al.* in revision), as in other biodiversity experiments (Wright *et al.* 2014). However, the stronger effects of resident diversity (compared with the effects of warming) were on resource availability, particularly reduced soil nitrate and light at the ground as has been reported elsewhere (Tilman *et al.* 1996), as well as increased surface moisture and fire spread (chapter 2).

The presence of resident functional groups did not distinctly influence air temperature or moisture, but did influence other factors. Nitrate was increased by legumes but decreased by grasses, especially C4 grasses, consistent with N fixation by legumes and C4 grasses' low equilibrium soil nitrate concentrations (Tilman *et al.* 1997). Each of the functional groups except C3 grasses reduced light availability, likely through their contributions to biomass (Tilman *et al.* 1997), and grasses increased fire spread through their contributions to biomass and high biomass-specific flammability (chapter 2).

These effects of warming, resident diversity and resident functional composition treatments on environmental factors can partly explain the treatment effects on seedlings. The inhibitory effect of warming on seedling establishment appears to have resulted mainly from reduced germination (emergence) at elevated temperature. Elevated air temperatures may have caused thermal stress to seeds or newly germinated seedlings, which could have died before emerging or being surveyed. Elevated air temperatures may also have had indirect effects on germination through changes to soil moisture or nitrate availability, but such effects should have been accounted for by including these other factors in multiple regressions. In contrast, the inhibitory effect of diversity appears to have resulted mainly from reduced availability of resources, especially light and nitrate, which reduced seedling survival and growth. In drawing these inferences, we assume that the impacts of seedlings on resource levels were negligible relative to those of resident adults, due to vastly lower seedling biomass (Wedin and Tilman 1993). Most species responded positively to light, nitrate, and moisture resources. Nonetheless, species differed substantially in their responses to resources.

The C3 grass and C4 grass functional groups responded particularly negatively to warming treatments. This accords with C3 grasses being most strongly inhibited by warmer air temperatures, and with C4 grasses being particularly inhibited by low moisture and low light availability in this experiment, both of which were reduced by warming. The negative response of C3 grasses to warmer temperatures accords with their global distribution in relation to climate (Edwards and Smith 2010) and their physiology (Lambers *et al.* 1998) but the particularly negative response of C4 grasses to drying is surprising because their physiology tends to give them higher water use efficiency than C3 species and they tend to be most abundant in warmer and drier grasslands and savannas (Lambers *et al.* 1998). The non-legume forb *Liatris aspera* was particularly inhibited by warming treatments, and accordingly inhibited by high nitrate availability and air temperature (which were increased by warming). This accords with the decline in *Liatris aspera* in native grasslands at this site during the exceptionally dry and hot summer of 1988 (Tilman and El Haddi 1992). *Asclepias tuberosa* was not distinctly affected by warming, perhaps because its positive response to air temperature (increased by warming) offset its positive response to light availability (reduced by warming).

Implications for change in plant diversity

These results offer an indication of potential changes in plant diversity with increasing temperatures. Caution is needed in extrapolating from patterns of plant establishment over two years because some of the mechanisms by which warming appeared to influence the seedling community may have less influence on mature plants. Mature plants may have access to deeper water or light higher in the canopy, which may be less affected by warming than the surface moisture and ground level light accessed by seedlings. Therefore, other processes may influence the diversity response to warming over the longer term. Nonetheless, recruitment can be an important bottleneck, and these results suggest potential changes in community structure that are consistent with emerging responses in the mature biomasses of resident species (as assessed by clipping community biomass).

The lower diversity of species establishing under warming suggests that low-diversity communities, such as early-succession abandoned agricultural lands and extensive low-diversity grasslands established as part of the Conservation Reserve Program in the United States, may gain diversity through seedling establishment less quickly as climate warms. We suggest this hypothesis cautiously because the low-diversity treatments in this experiment, which were assembled by random draws, may not represent low-diversity grasslands in which species loss and gain have been non-random.

Restoring grassland by overseeding species (i.e., adding seed into an established plant community) into low-diversity grassland may be less successful in a warmer climate. The somewhat stronger effect of warming on species richness than on effective species richness (e^H) of seeded species suggests that rare species are most likely to fail to establish under warming. This may necessitate higher overseeding rates to successfully restore diversity in warmer climates. The lack of significant interactive effects of functional composition and warming on seedling diversity suggests that, though initial functional composition established during restoration may influence subsequent accumulation of diversity, the importance of this effect will not change with warming.

Species richness and effective species richness (e^H) of seeded species were also reduced by warming in experimental communities with high resident diversity. This is consistent with a near-significant reduction in species richness and significant reduction in effective species richness with warming among the resident species in 16-species plots, based on community biomass (J.M. Cowles, unpublished), associated with increasing dominance by C4 grasses and legumes with warming (Cowles *et al.* in revision). Together, these results hint at a loss of species richness from diverse prairie with warming. Given the longevity of adult plants, loss of species richness may be slow.

Implications for change in species and functional composition

The lack of strong interactions between warming treatments and either the diversity or functional composition of the resident community implies that shifts in composition with warming may be independent of community context.

Establishment of the mainly-exotic seeded C3 grasses tended to be strongly inhibited by warming, consistent with reduced productivity of resident C3 grass species (both native and exotic) with warming (Cowles *et al.* in revision). This suggests that both native C3 grasses, and the exotic C3 grasses that are naturalized and abundant through much of the North American tallgrass prairie, may decline with warming. The reduced establishment success of C4 grasses as a whole with warming is counter to increased productivity of resident C4 grasses with warming (Cowles *et al.* in revision), but consistent with the primary effect of warming being to reduce emergence, a process not directly connected to C4 photosynthesis and physiology. Neither functional group nor seed size provided an effective shortcut to predict species' responses to warming, so further work is needed to understand species' responses and predict changes in grassland composition and diversity with global change.

Species' responses and their ranges

Our data did not support the hypothesis that species' responses to warming (which also caused drying in our experiment) were related to which edge of their range is nearest. The species with their distributions centered to the hotter and drier south and west, particularly *Solidago nemoralis*, did not respond particularly positively to warming. Nor did the species with distributions centered to the cooler and wetter north and east show particularly negative responses to warming: *Agastache foeniculum, Baptisia alba, Lupinus perennis,* and *Lespedeza capitata.* However, our study does not provide a powerful test of this hypothesis because few of the seeded species were near an edge of their range.

The finding that seedlings established more readily in low- than high-diversity grasslands, here and in other studies, suggests that low-diversity grasslands such as those on abandoned agricultural lands could promote warming-driven poleward expansion of species' ranges (if dispersal limitation is overcome). (This inference is based on the main effect of diversity on seedling establishment, not on the effect of warming.)

Conclusion

The net effects of warming on plant establishment did not, in general, depend on the diversity or composition of the resident community, even though warming influenced the environmental conditions driving plant establishment in ways that both reinforced and opposed competitive and facilitative influences of resident plants. Thus, it may be tractable to develop a general understanding of plant species' responses to warming to better predict ecosystem responses to global climate change.

Figure 4-1: Effects of warming and resident species richness treatments on planted species richness. Interactive effects of warming (control, low warming of +1.5 C, high warming of +3 C) and resident species richness (1, 4, or 16 species) treatments on (**A**) species richness and (**B**) effective species richness e^H of 32 species planted as seeds. Diversity was assessed at the end of the second season after planting. Outlined symbols are treatment means, with 95% confidence intervals; other symbols are individual plots.

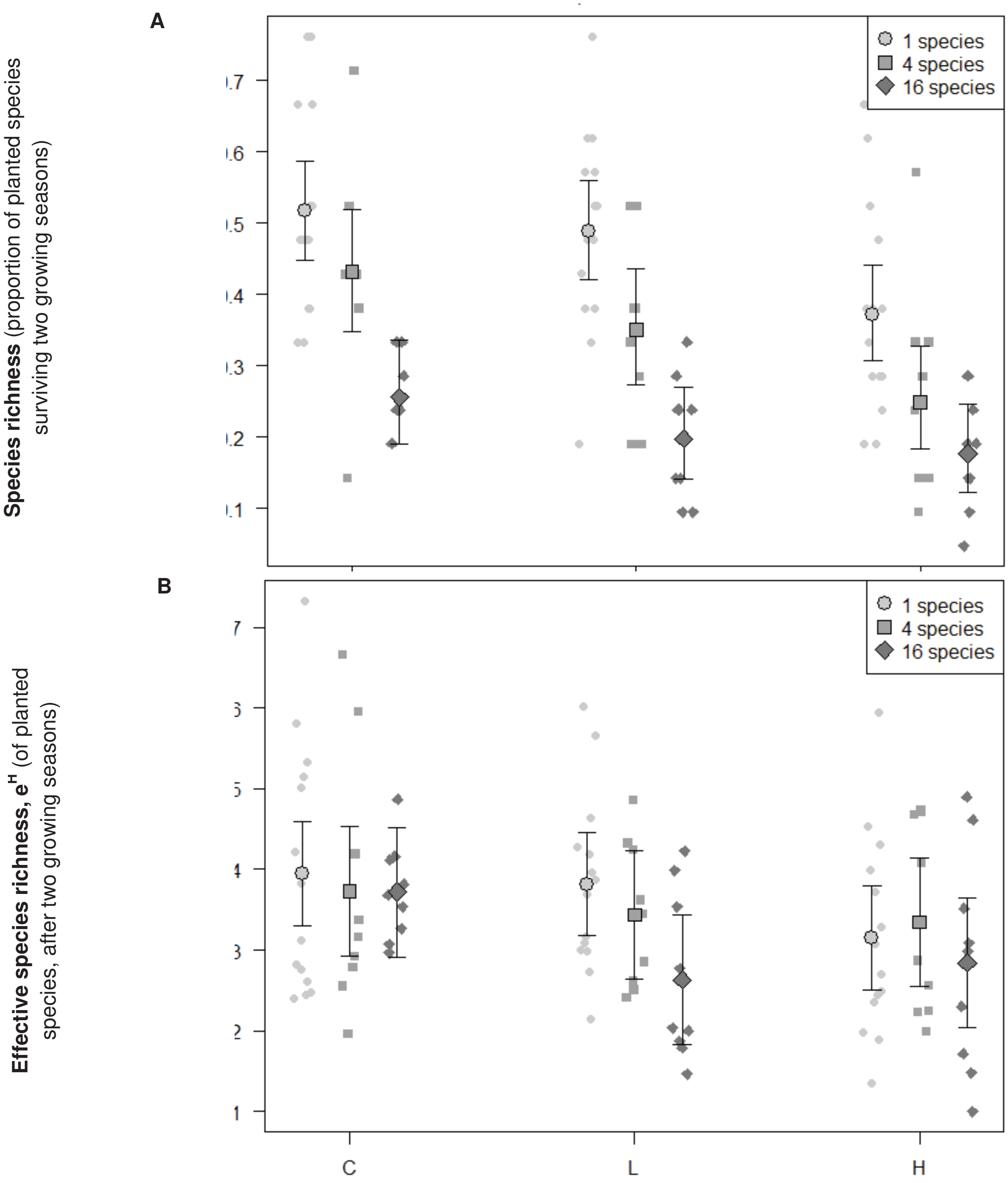


Figure 4-2: Interactive effects of warming and resident species richness (diversity) treatments on the average performance of seeded species.

A) aboveground biomass per unit area, a measure of species' performance that integrates performance components including B) number germinating, C) proportion of seedlings surviving from the end of the first growing season to the end of the second growing season, and D) aboveground relative growth rate (RGR) from the end of the first growing season to the end of the second growing season. Coefficients from the linear predictors of generalized linear mixed models are shown with 95 % confidence intervals; their magnitudes are not interpretable on the scale of the original data but they can be compared within each panel because warming and diversity (log species richness) were centered and scaled to unit variance. Note that a coefficient of zero for the warming:diversity interaction implies that the effects of warming and diversity are additive – not that they do not have effects.

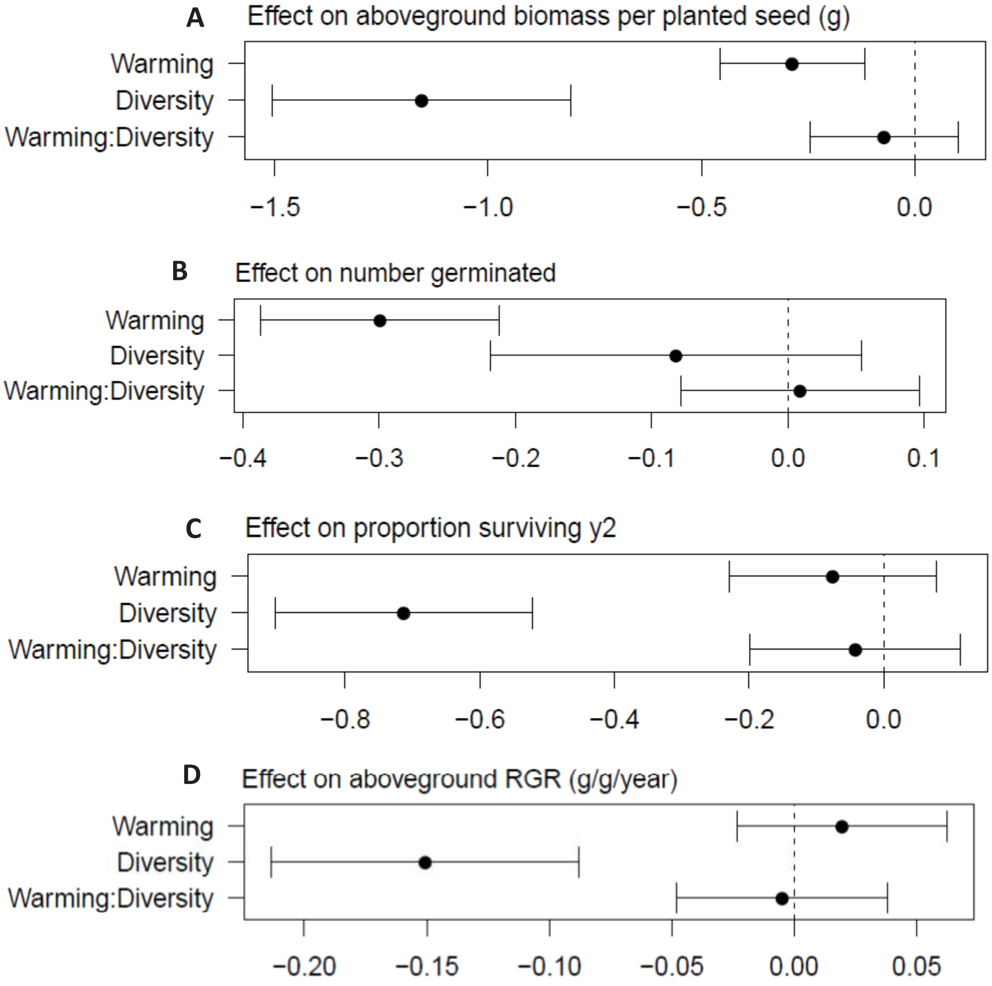


Figure 4-3: Effects of warming and resident species richness (diversity) treatments on biomass per area for each seeded functional group.

C3 = C3 grass; C4 = C4 grass; forb = non-legume forb. Effects are coefficients (with 95 % confidence intervals) from the linear predictor of a generalized linear mixed model with log diversity (1, 4, 16 species) and warming (0, +1.5 C, +3 C) and their interaction as continuous predictors, as well as interactions of these predictors with seeded functional group.

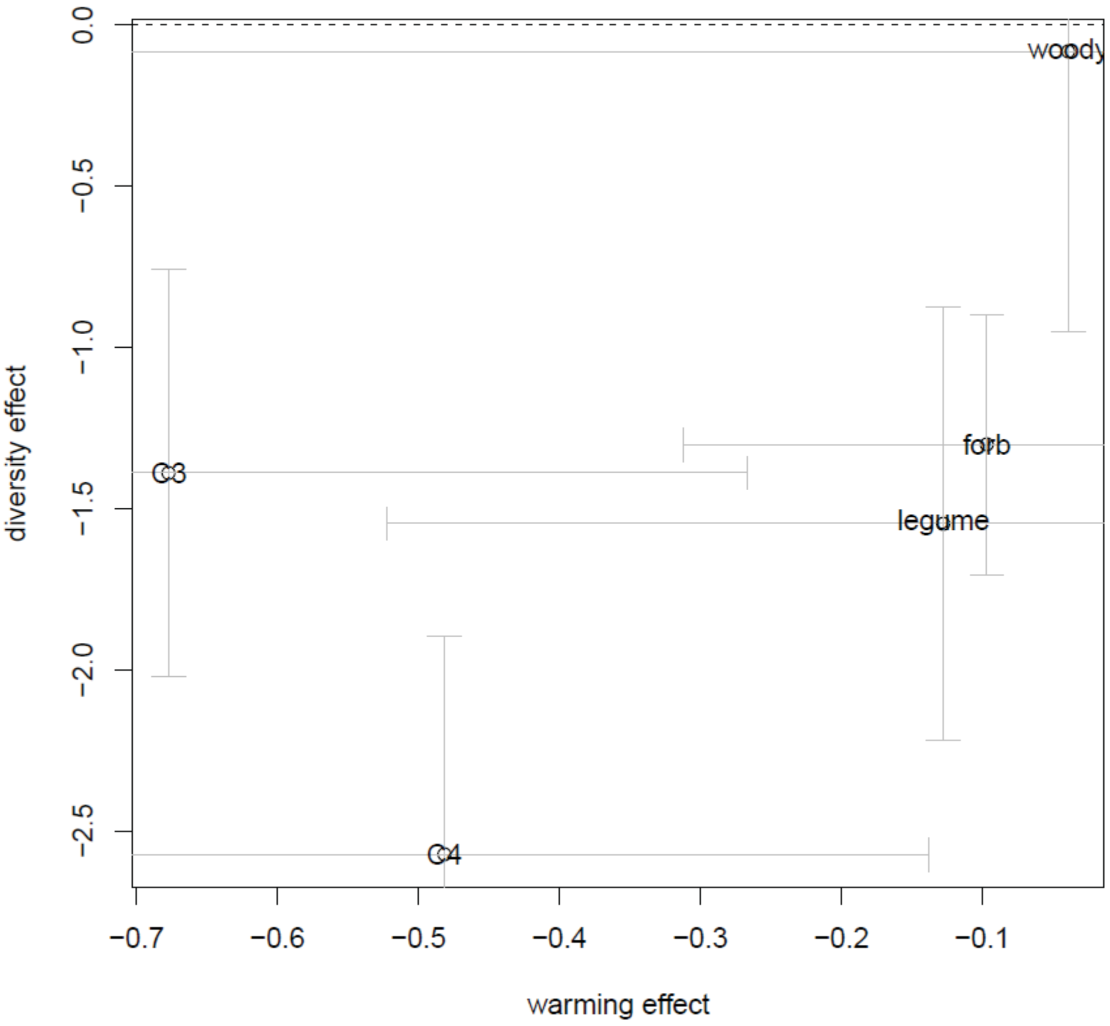


Figure 4-4: Interactive effects of warming and the presence of resident functional groups on the average performance of seeded species.

A) aboveground biomass per unit area, a measure of species' performance that integrates performance components including B) number germinating, C) proportion of seedlings surviving from the end of the first growing season to the end of the second growing season, and D) aboveground relative growth rate from the end of the first growing season to the end of the second growing season. Coefficients from the linear predictors of generalized linear mixed models are shown with 95 % confidence intervals. Note that a coefficient of zero for the interaction between two factors (e.g., Warming:C4) implies that the effects of the two factors (in this example, warming and presence of C4 grasses) are additive – not that these factors do not have effects. C3 = C3 grass; C4 = C4 grass; Forb = non-legume forb.

Figure 4-4 (caption on previous page).

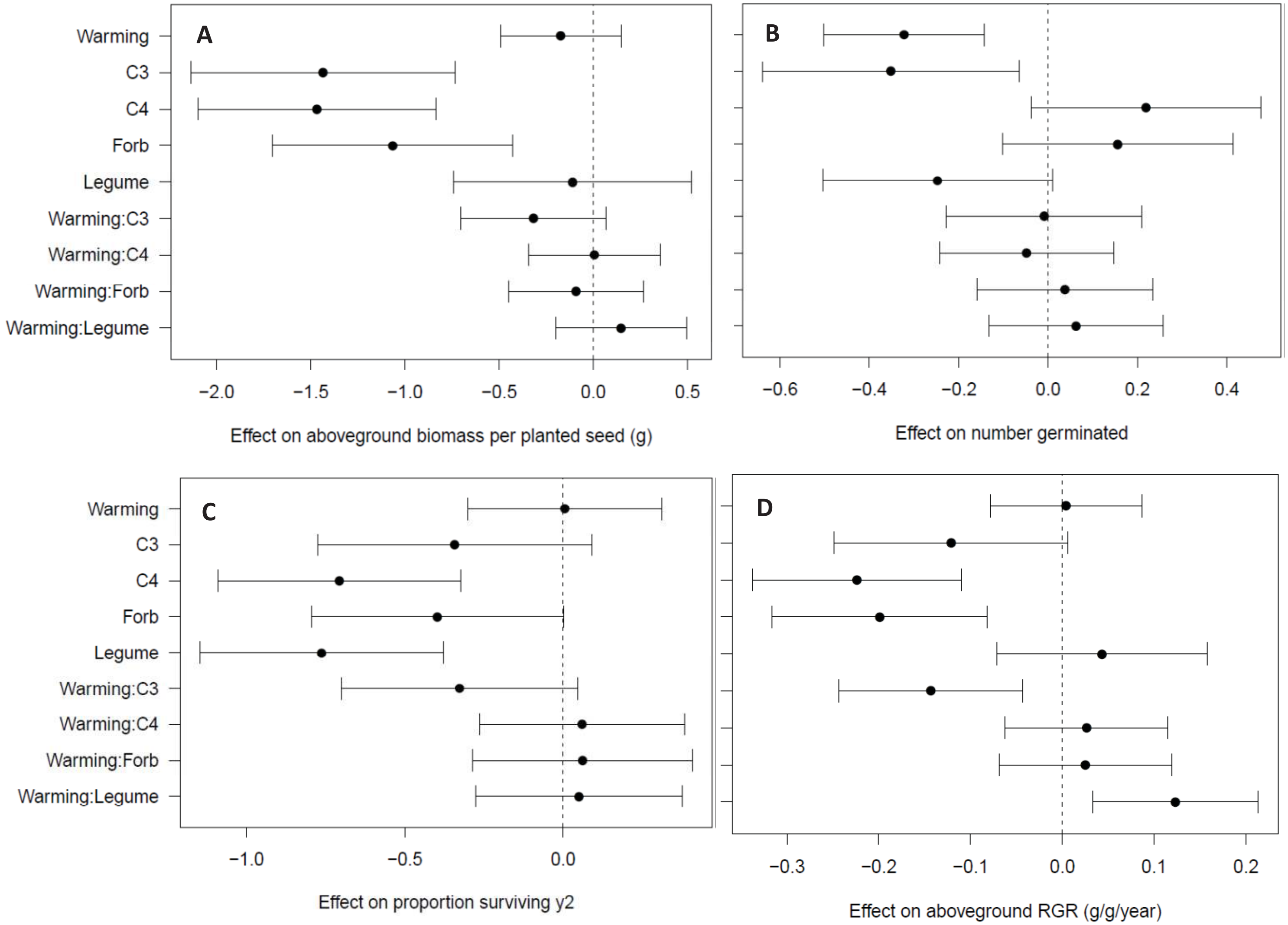


Figure 4-5: Effects of warming treatments and the presence of resident functional groups on biomass per area of each seeded functional group.

C3 = C3 grass; C4 = C4 grass; forb = non-legume forb. Effects are coefficients (with 95 % confidence intervals) from the linear predictor of a generalized linear mixed model with warming as a continuous predictor interacting with the resident functional groups as categorical predictors, as well as interactions of these predictors with seeded functional group.

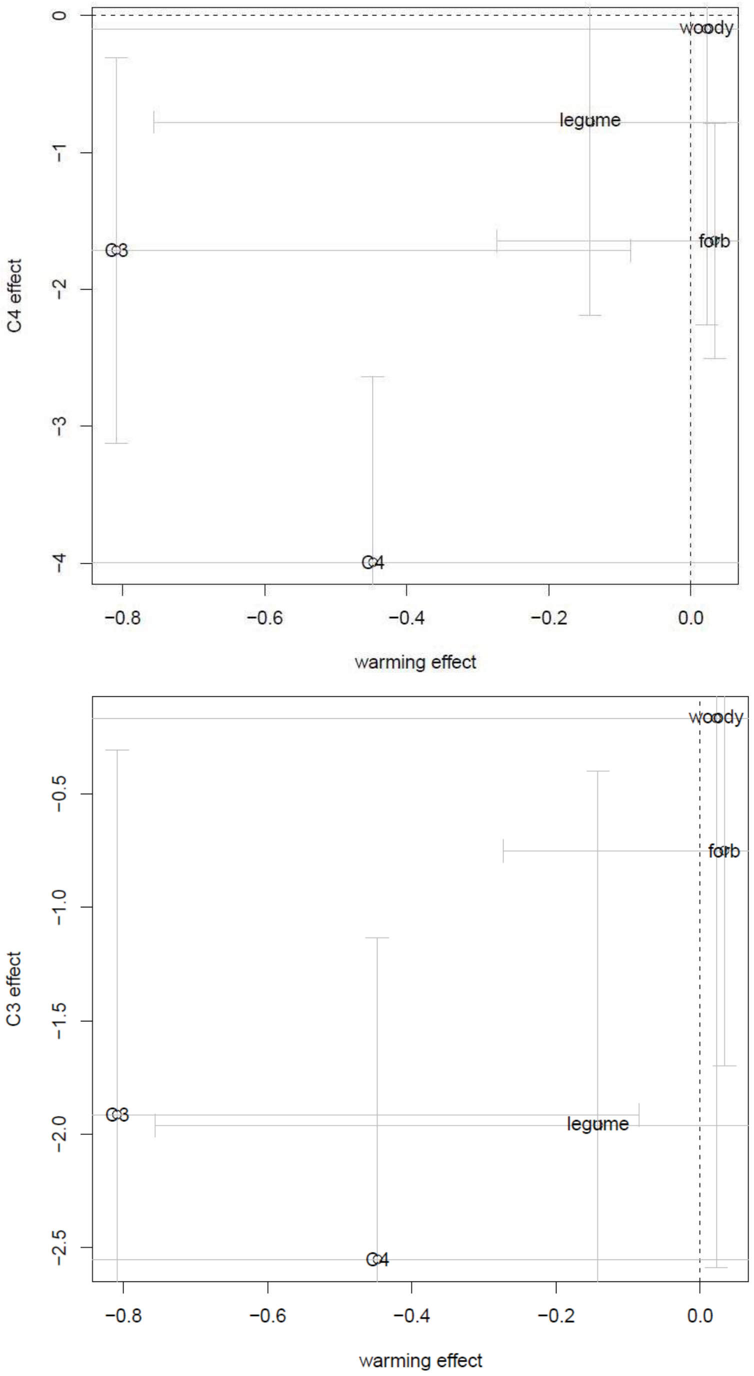
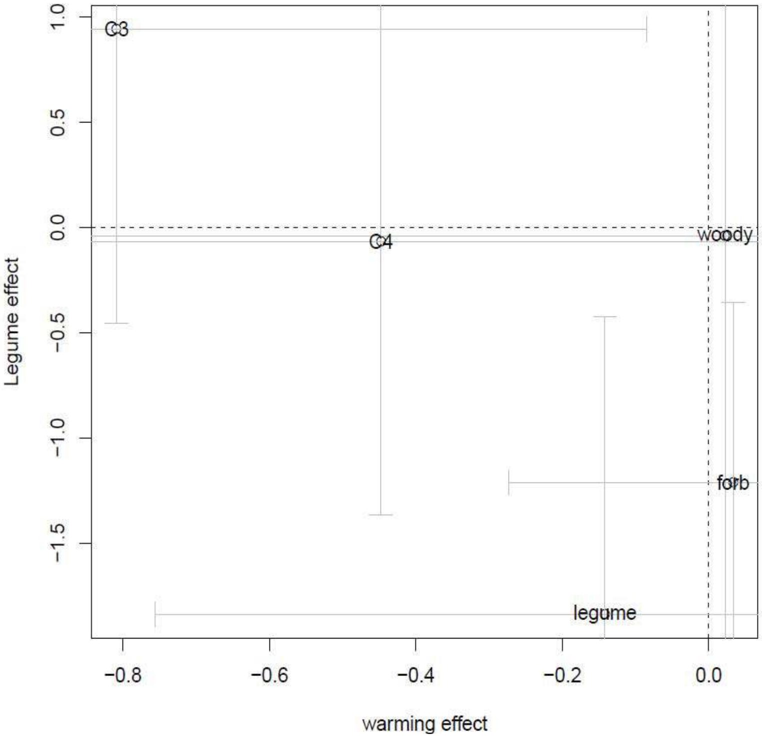


Figure 4-5 (continued).



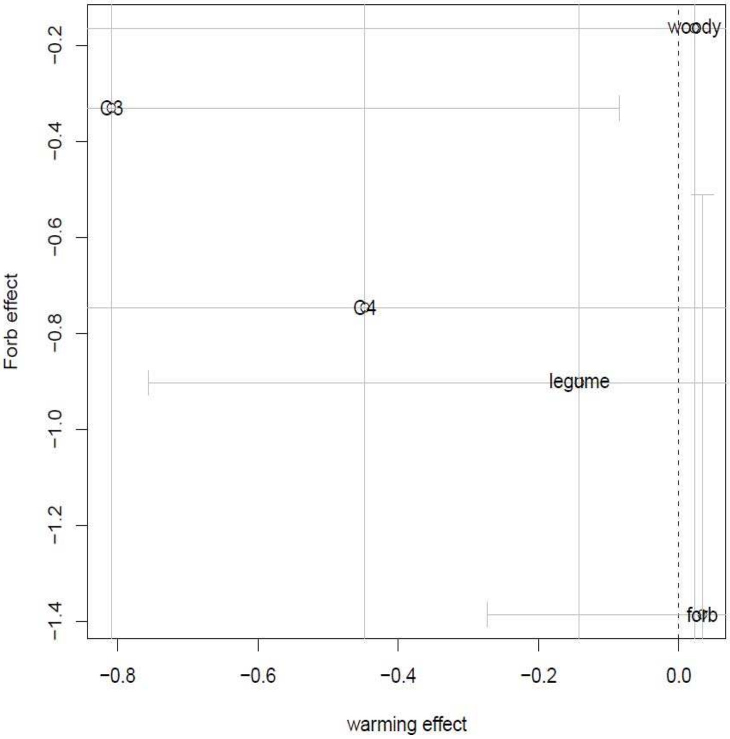


Figure 4-6: Interactive effects of warming and resident diversity treatments on environmental factors potentially affecting seedling performance.

Coefficients from linear mixed models are shown with 95 % confidence intervals; all variables were centered and scaled to unit variance to compare effect sizes. Soil nitrate was log-transformed. See Figure 4-2 caption for interpretation of Warming:Diversity interaction.

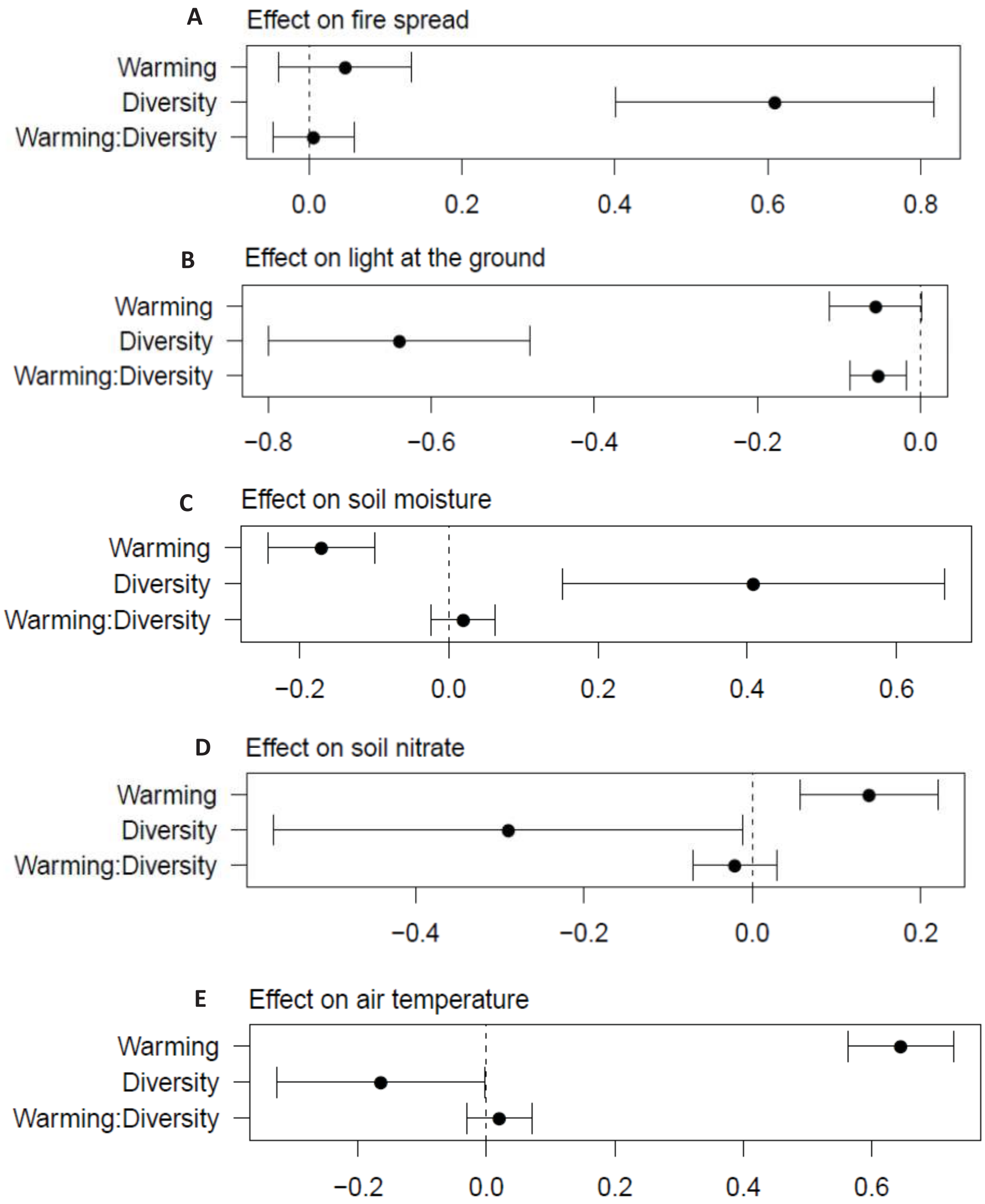


Figure 4-7: Interactive effects of warming and resident functional composition treatments on environmental factors potentially affecting seedling performance.

Coefficients from the linear predictors of generalized linear mixed models are shown with 95 % confidence intervals. Forb = Non-legume forb. See Figure 4-4 caption for interpretation of interaction effects.

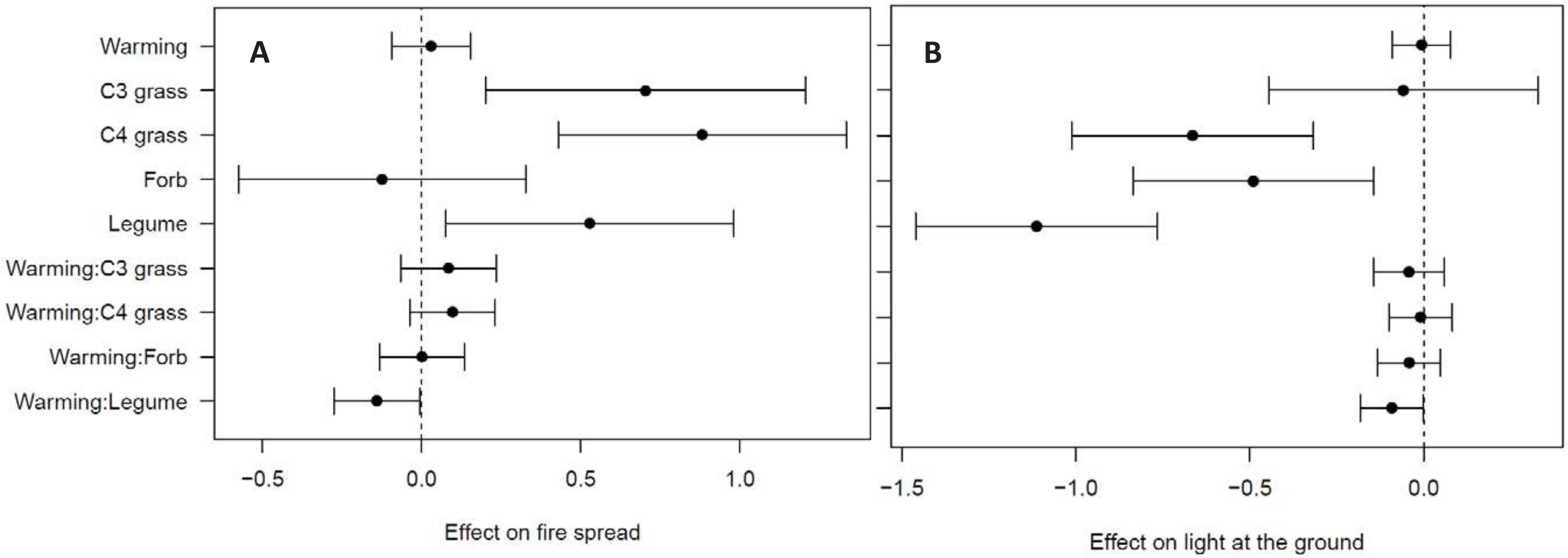


Figure 4-7 (continued).

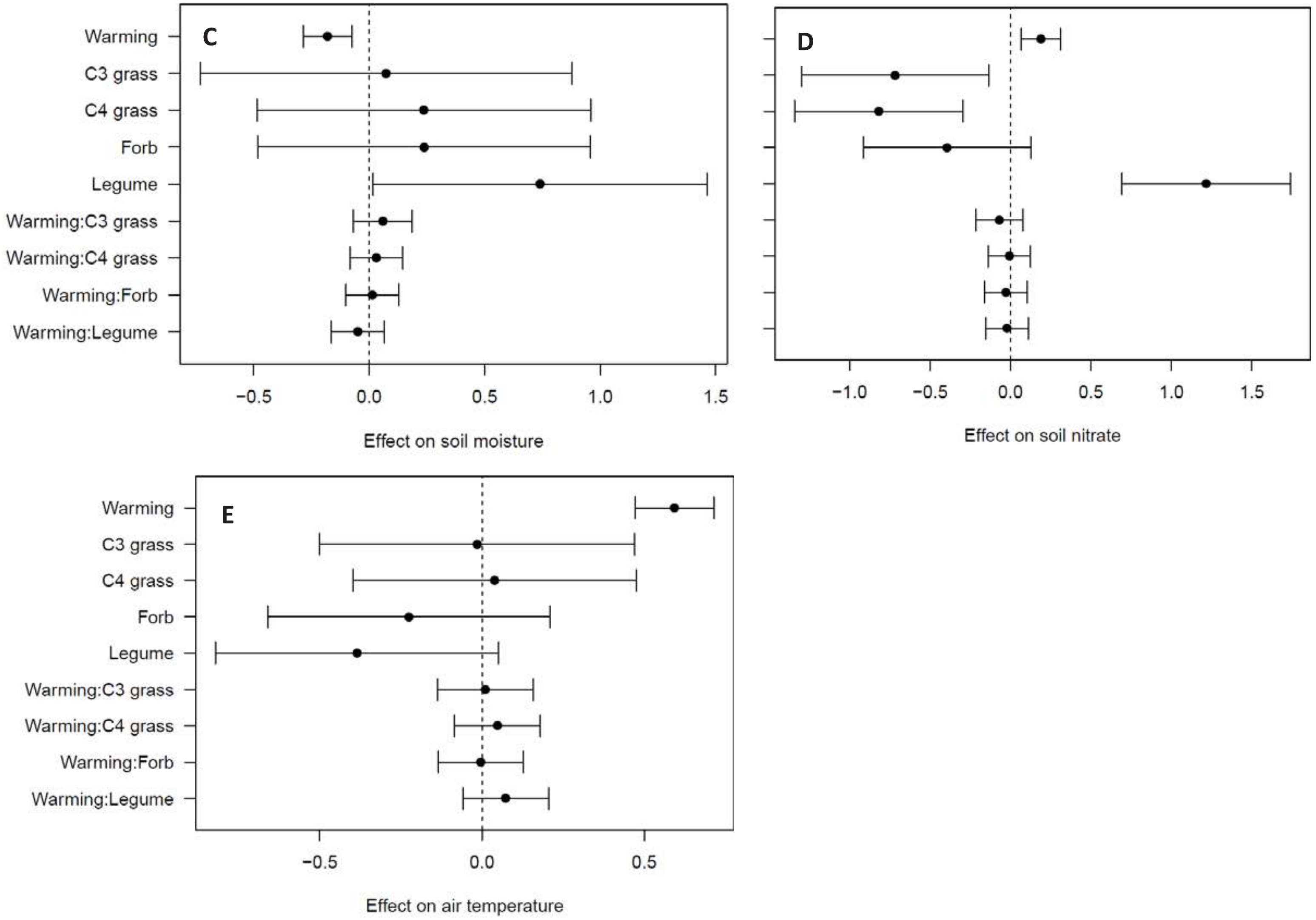


Figure 4-8: Joint effects of environmental factors on the average performance of seeded species. A) aboveground biomass per unit area, a measure of species' performance that integrates performance components including B) number germinating, C) proportion of seedlings surviving from the end of the first growing season to the end of the second growing season, and D) aboveground relative growth rate from the end of the first growing season to the end of the second growing season. Coefficients from the linear predictors of generalized linear mixed models are shown with 95 % confidence intervals. The environmental factors -- the proportion of the subplot through which fire spread, the proportion of light reaching the ground, soil moisture, soil nitrate, and air temperature -- were centered and scaled to unit variance so their effect sizes can be compared within panels.

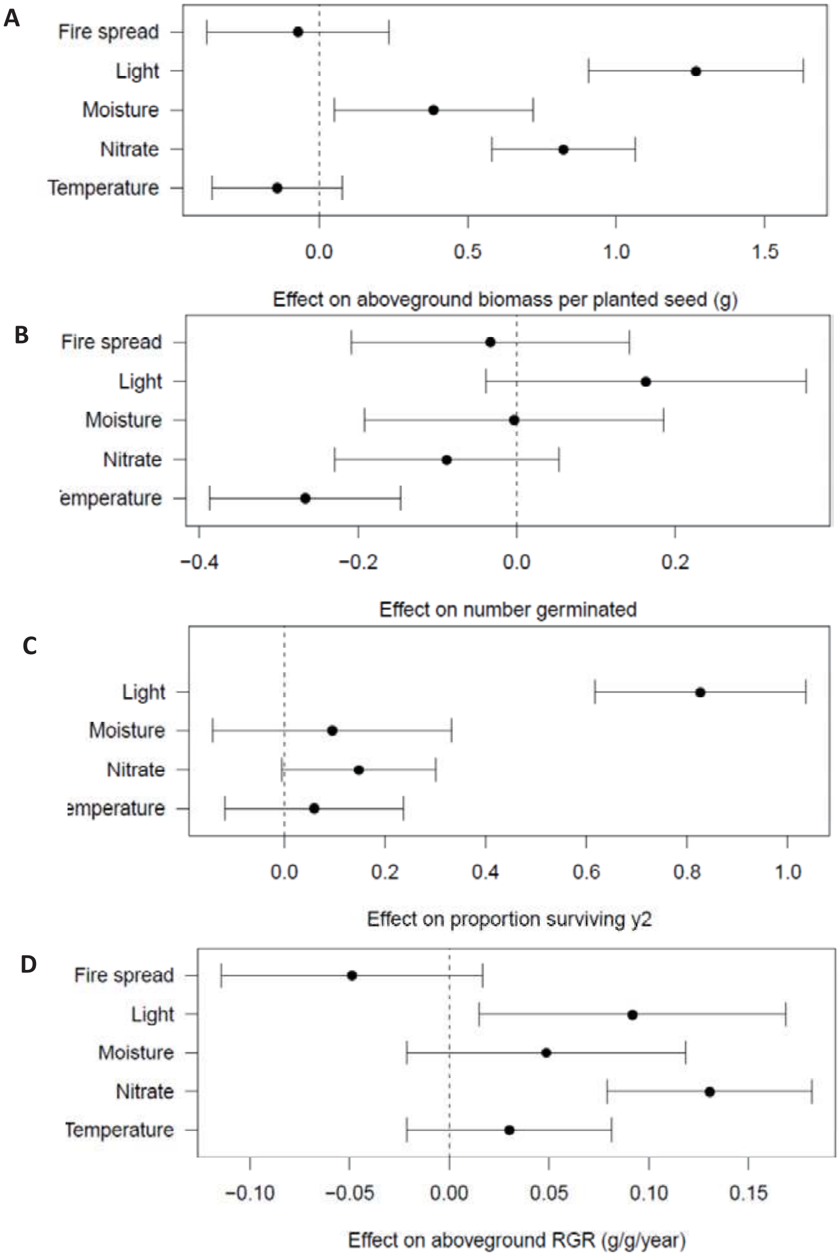


Figure 4-9: Effects of environmental factors on biomass per area of each seeded functional group. C3 = C3 grass; C4 = C4 grass; forb = non-legume forb. Effects are coefficients (with 95 % confidence intervals) from the linear predictor of a generalized linear mixed model with warming as a continuous predictor interacting with the resident functional groups as categorical predictors, as well as interactions of these predictors with seeded functional group.

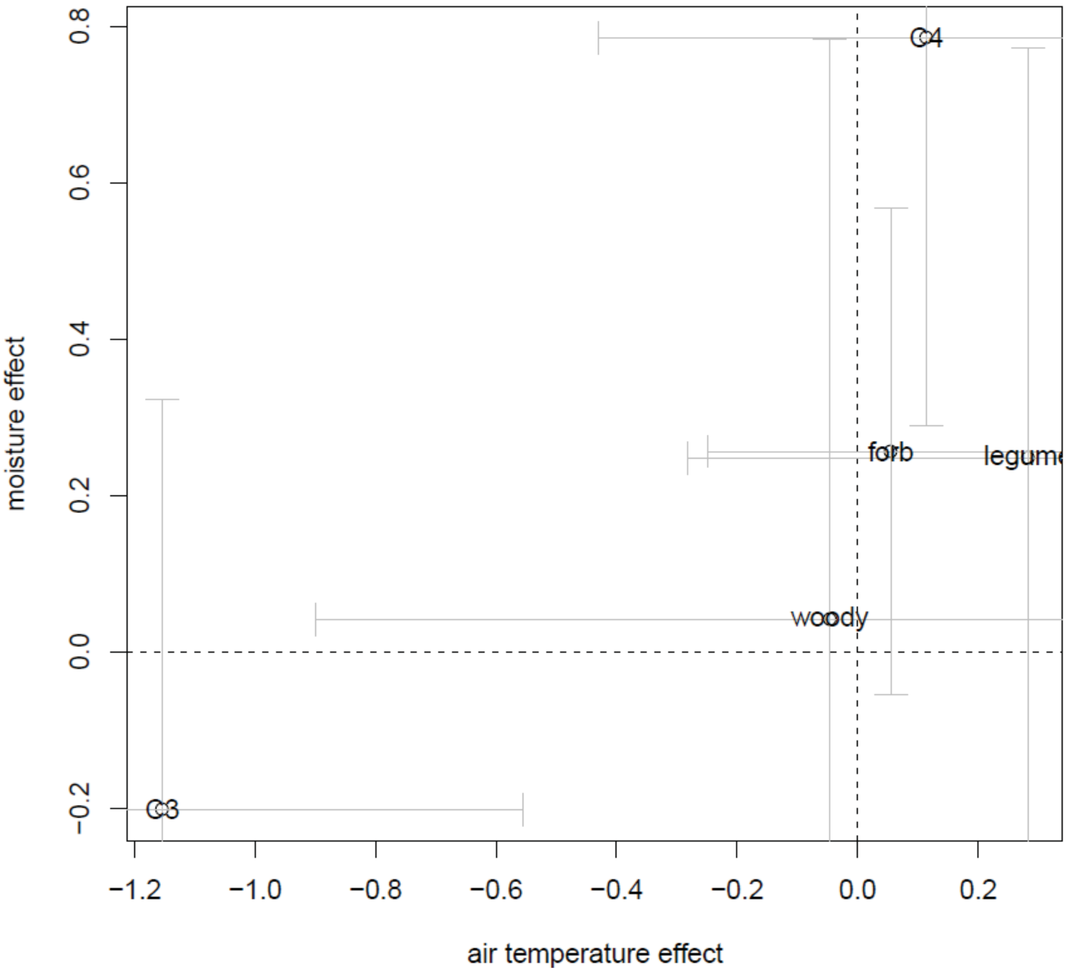


Figure 4-9 (continued).

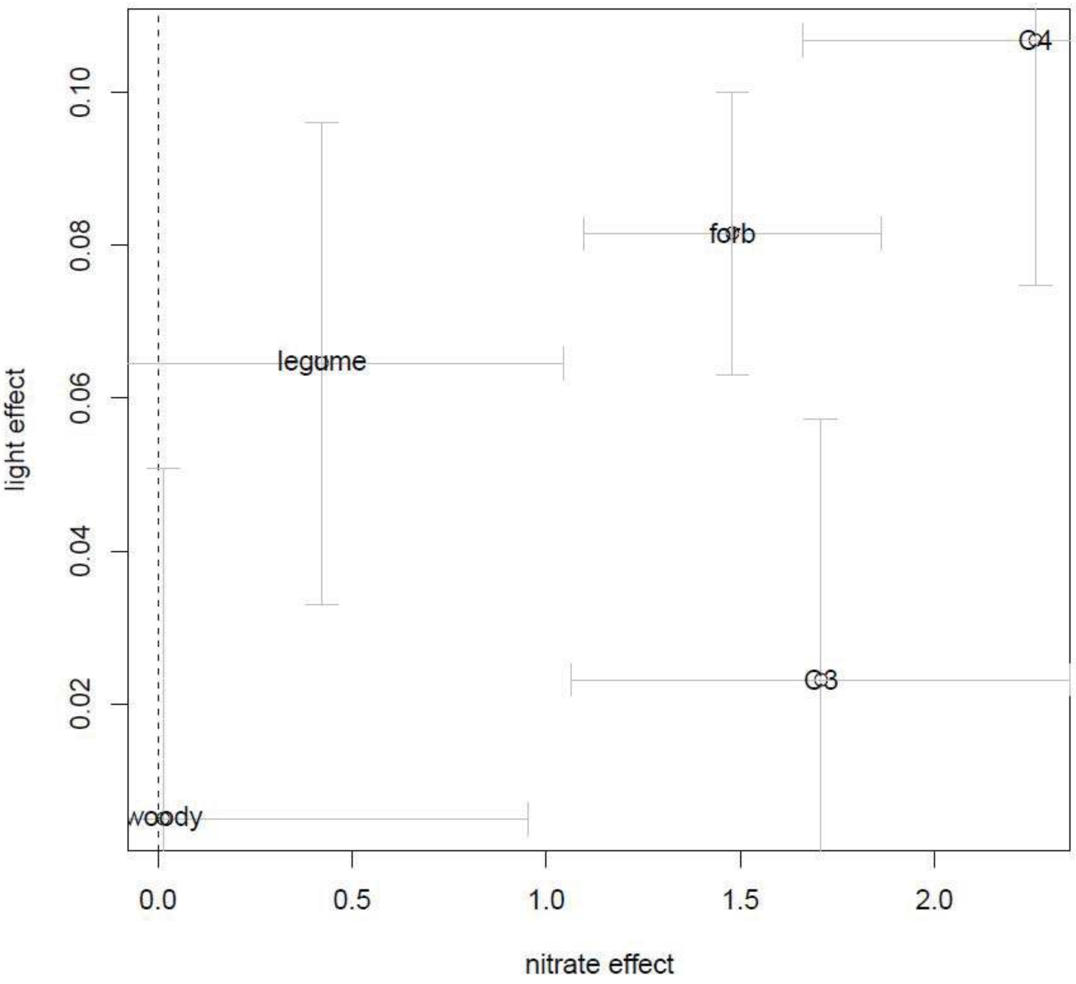


Figure 4-S1: Interactive effects of warming and resident diversity treatments on seeded species' aboveground biomass per unit area.

Reading the panels like a book, *Bromus* through *Phleum* are C3 grasses; *Andropogon* through *Sorghastrum* are C4 grasses; *Agastache* through *Solidago* are non-legume forbs; *Astragalus* through *Desmodium* are legume herbs; and *Quercus* is a tree. Outlined symbols are treatment means, with 95% confidence intervals.

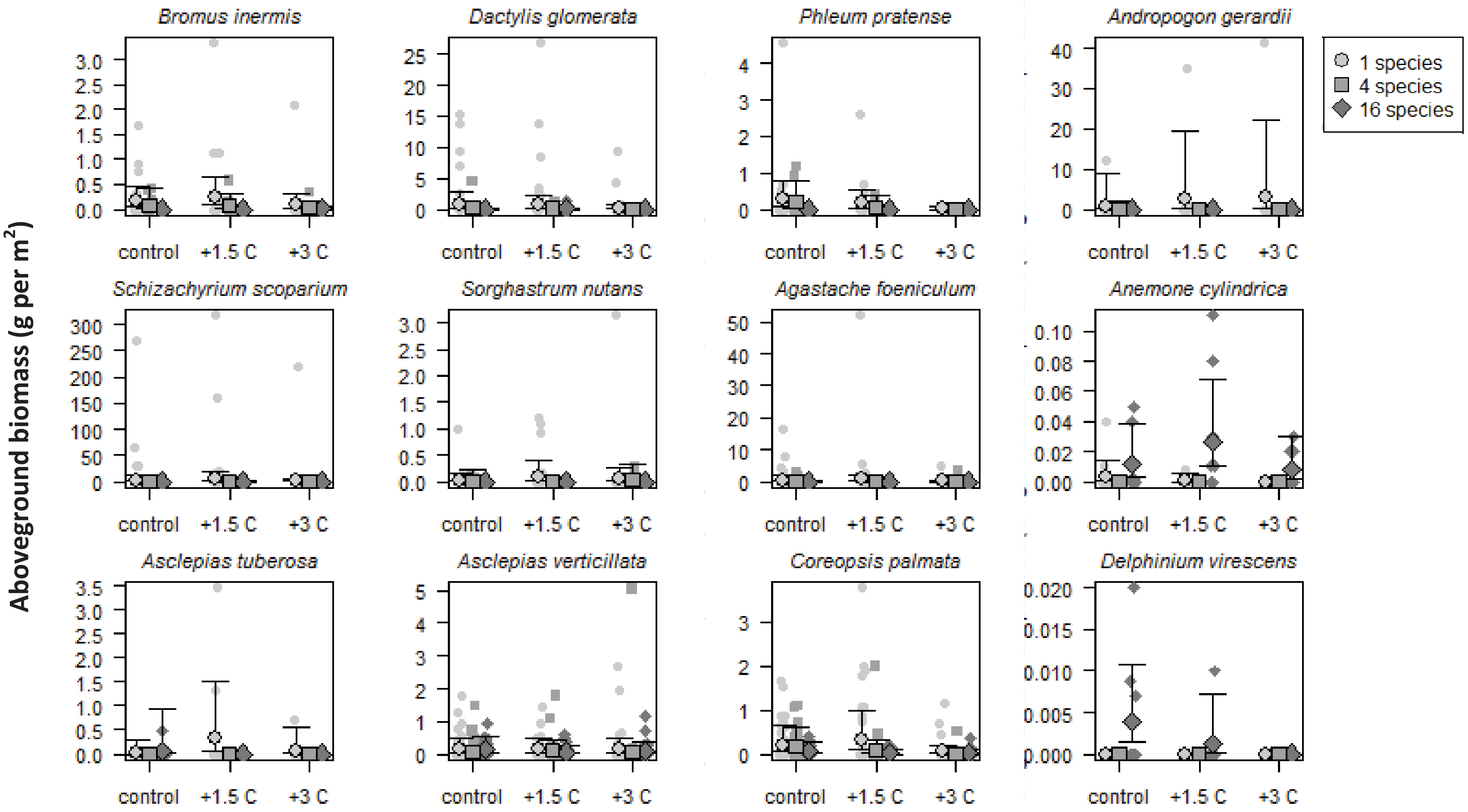


Figure 4-S1 (continued).

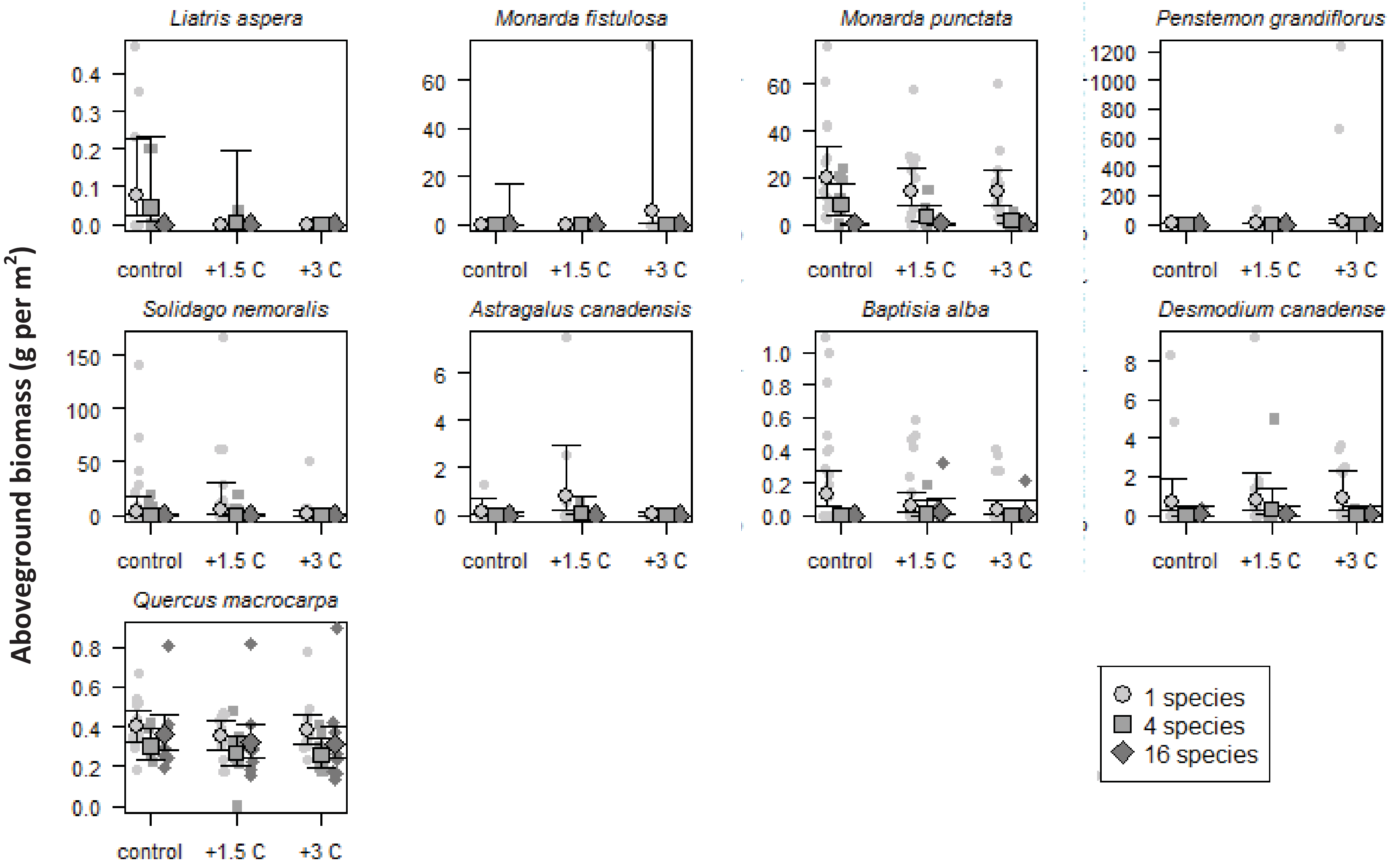
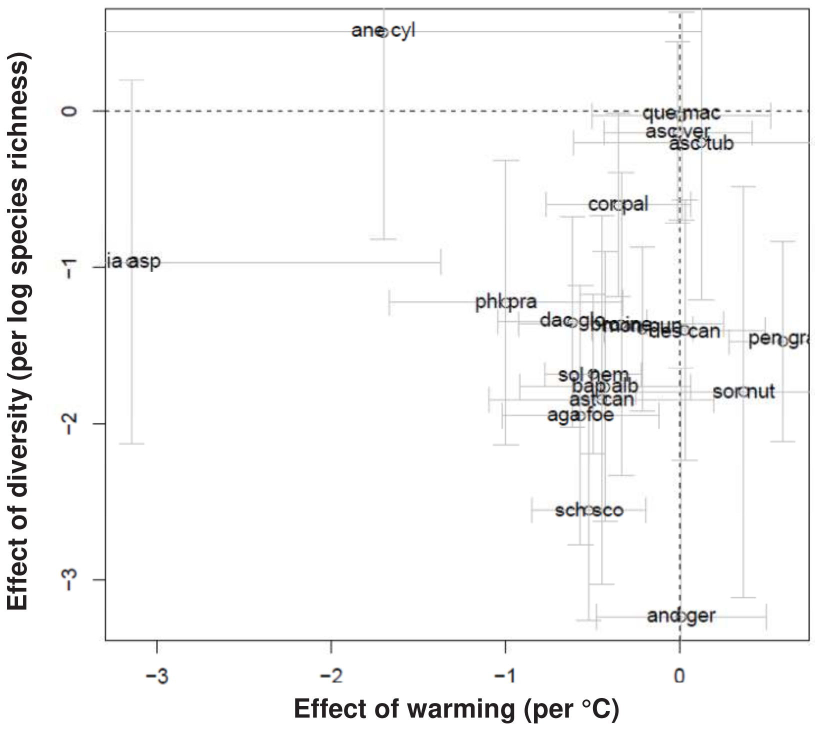


Figure 4-S2: Effects of warming and resident diversity treatments on various aspects of seeded species' performance.

Effects of warming and resident diversity on A) total biomass per area, an integrative measure of seeded species' performance, as well as performance components: B) number of seeds germinating, C) proportion of seedlings surviving from the end of the first year to the end of the second year, and D) aboveground relative growth rate of surviving seedlings from the end of the first year to the end of the second year. Effects are coefficients (with 95 % confidence intervals) from the linear predictors of generalized linear mixed models with log diversity (1, 4, 16 species) and warming (0, +1.5 C, +3 C) and their interaction as continuous predictors. Species abbreviations correspond to species names in Figure 4-S1. Rho is Spearman's rank correlation coefficient; r is Pearson's correlation coefficient, weighted by the mean standard error of each point. Spearman's correlation was significant only for the panel with a regression line, which is a major axis regression (not assigning dependent/independent roles to the variables) with 95 % confidence interval.

Figure 4-S2 (caption on previous page).


Biomass per area (g/m^2) rho = -0.08 r = 0.81

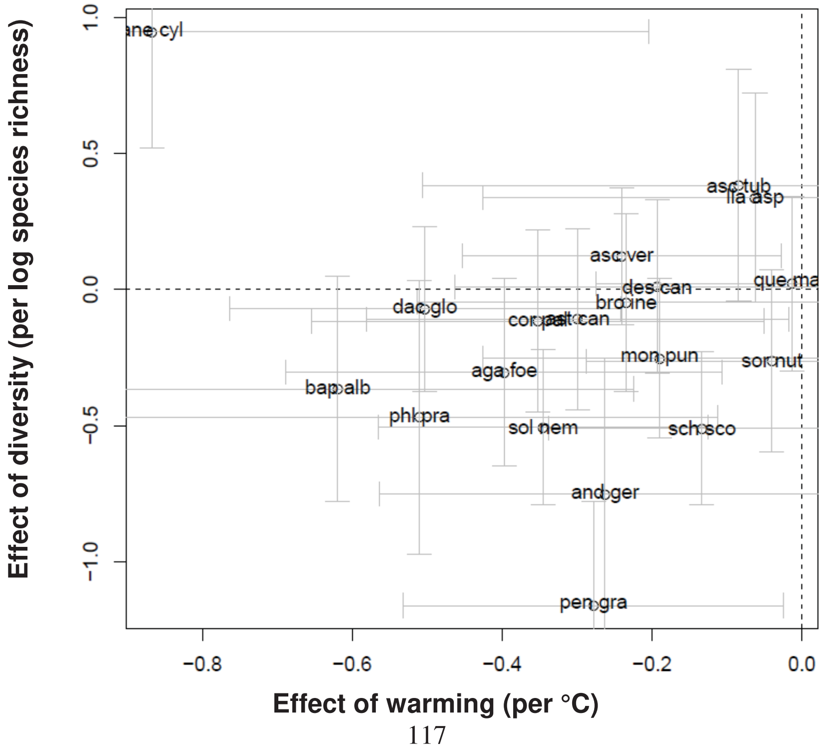
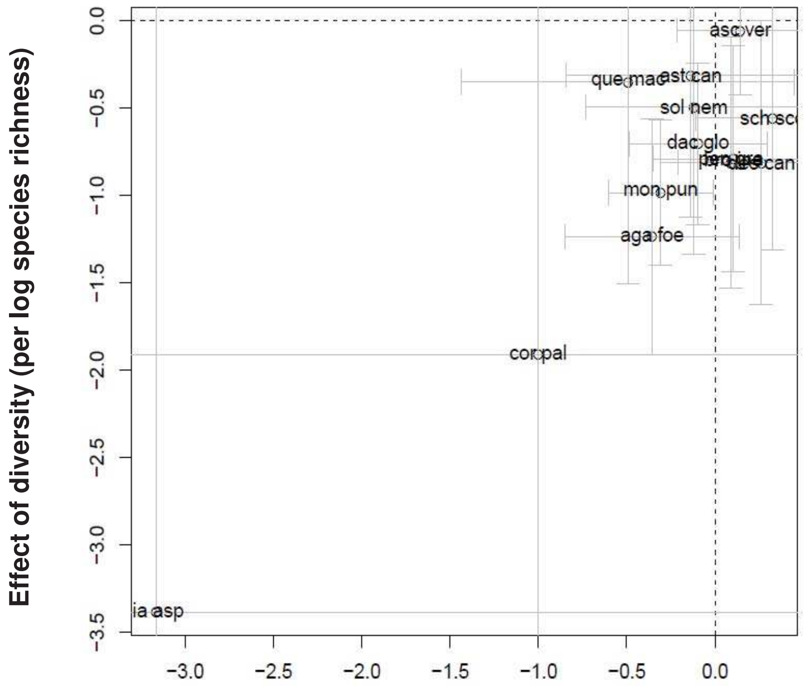

Number germinated rho= 0.22 r= −0.07

Figure 4-S2 (continued).


Proportion surviving y2 rho= 0.46 r= 0.74

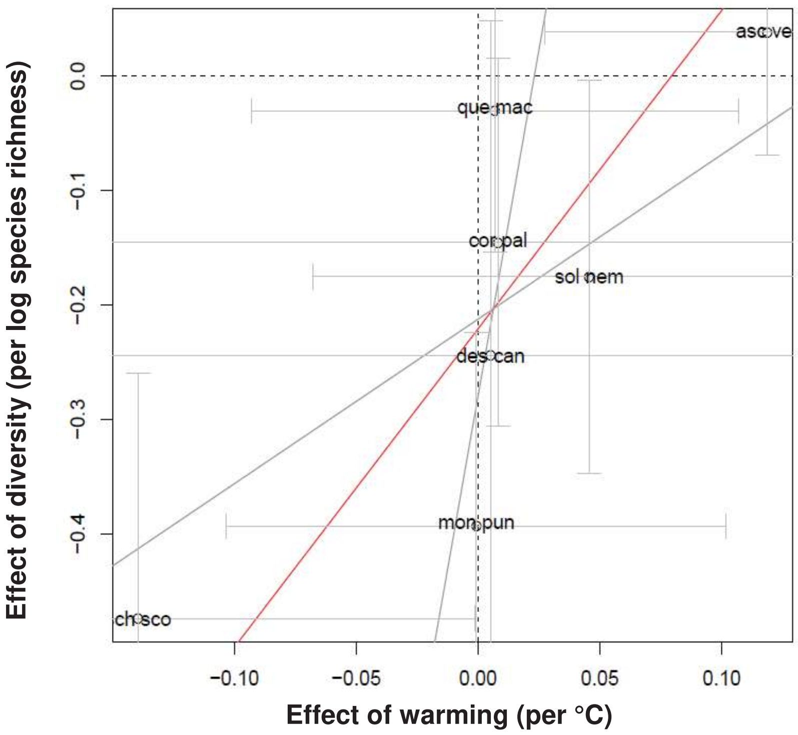

Aboveground RGR (g/g/year) rho= 0.86 r= 0.81

Figure 4-S3: Interactive effects of warming and resident diversity treatments on resident species' aboveground biomass per germinant.

All of these species are legumes; other resident species had too few seedlings for analysis. Outlined symbols are treatment means, with 95% confidence intervals.

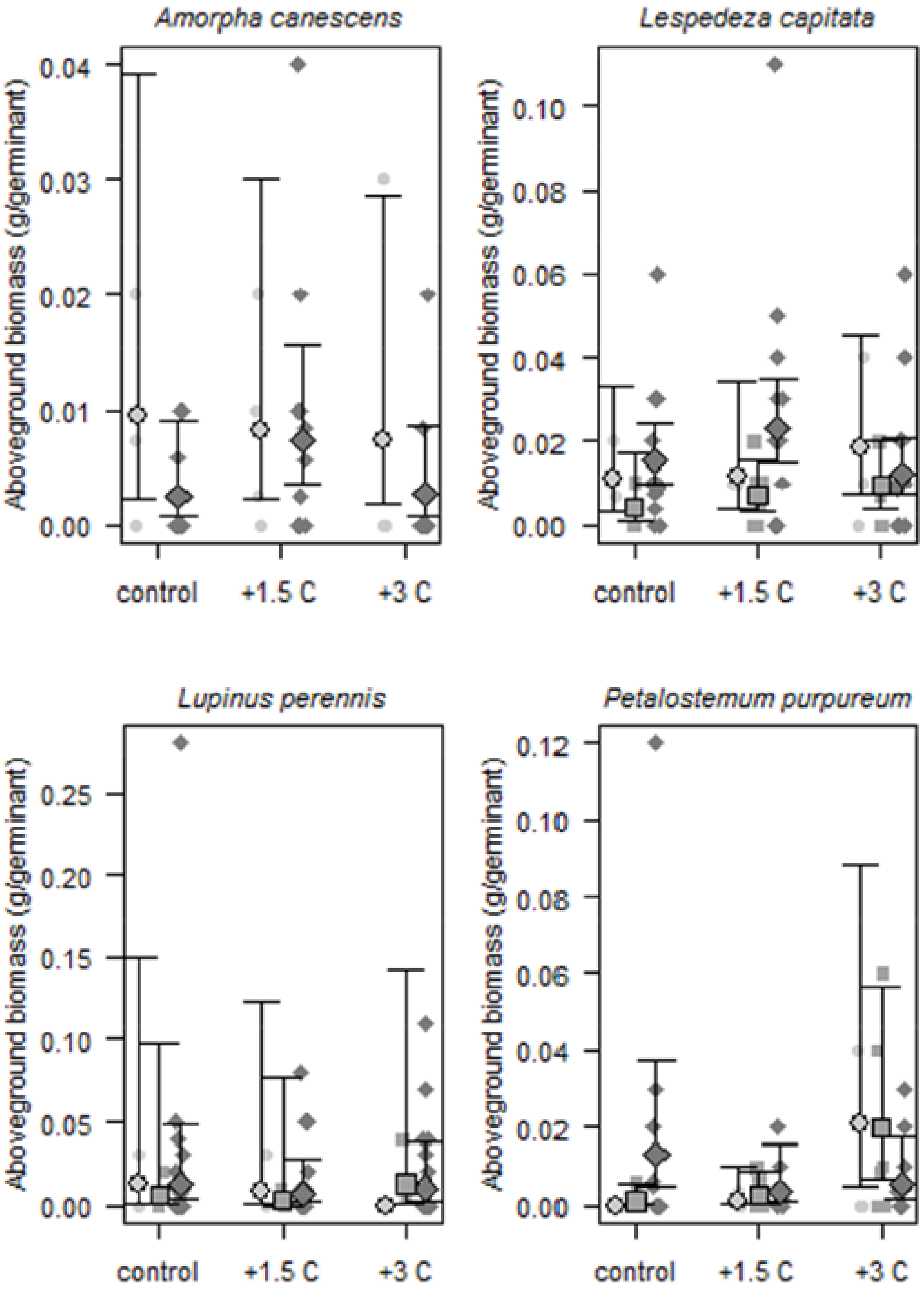


Figure 4-S4: Interactive effects of warming and resident diversity treatments on biomass per transplanted seedling.

Outlined symbols are treatment means, with 95% confidence intervals.

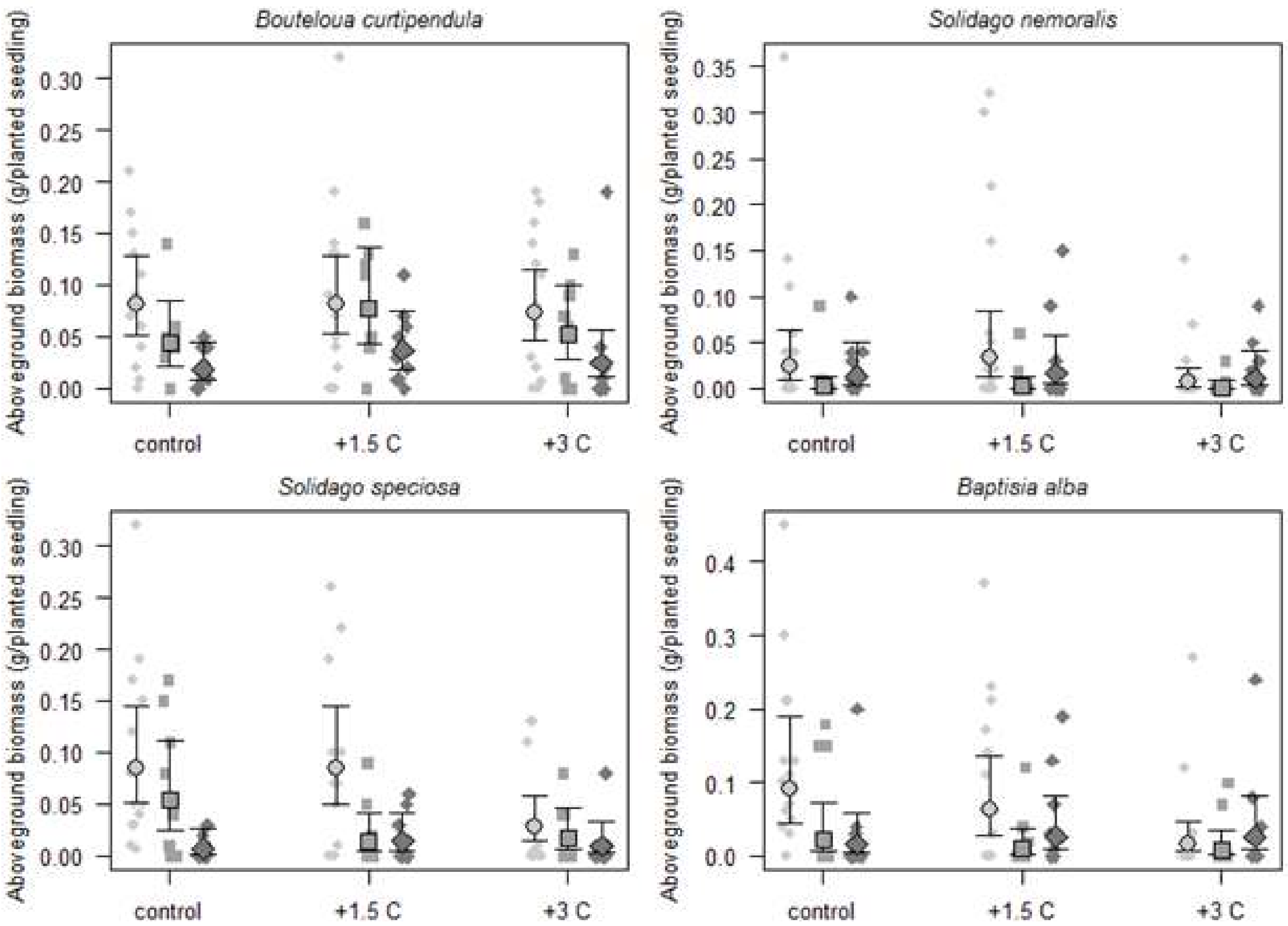


Figure 4-S5: Interactive effects of warming and resident diversity treatments on environmental factors.

Air temperature and vapor pressure deficit were measured 10 cm above ground. Outlined symbols are treatment means, with 95% confidence intervals.

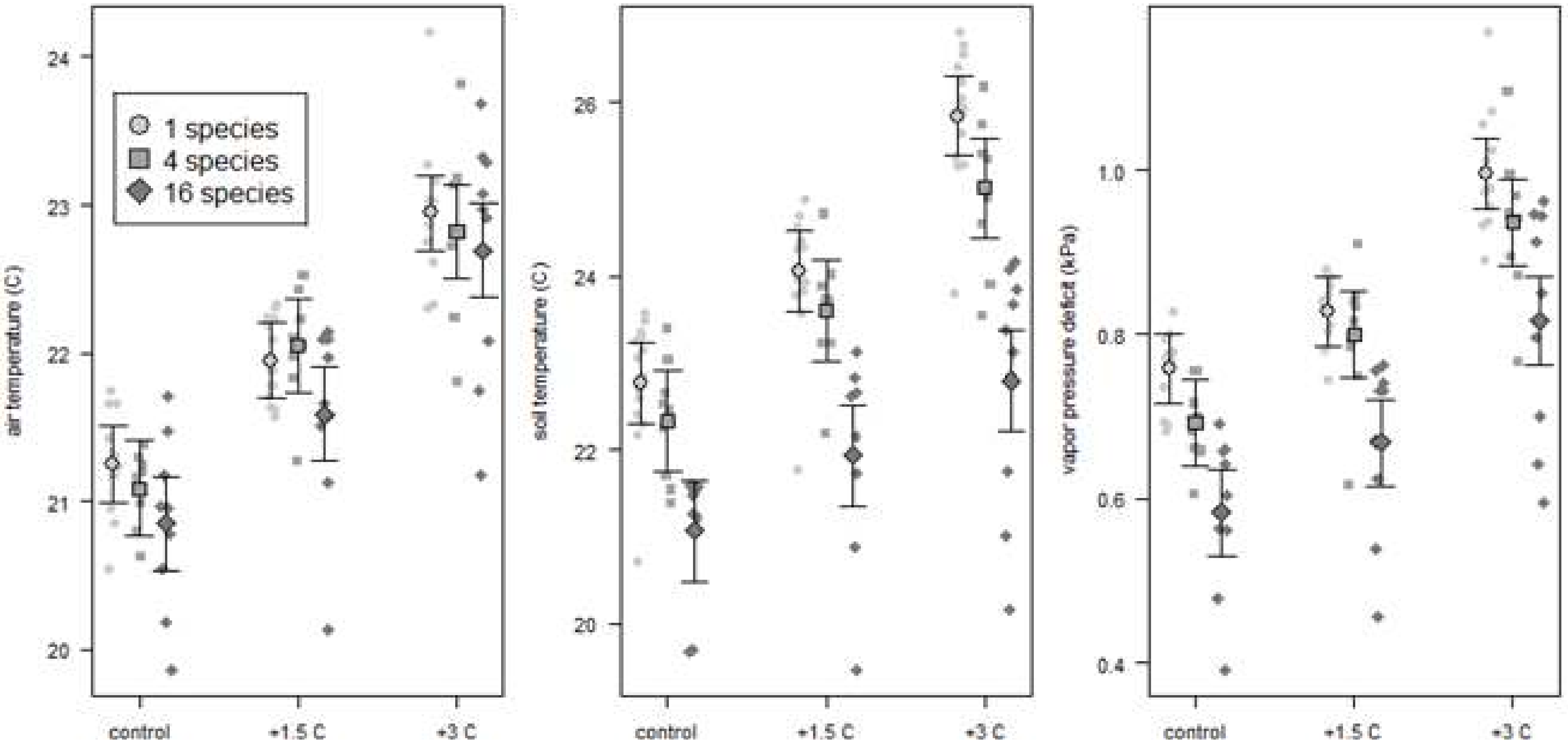


Figure 4-S5 (continued).

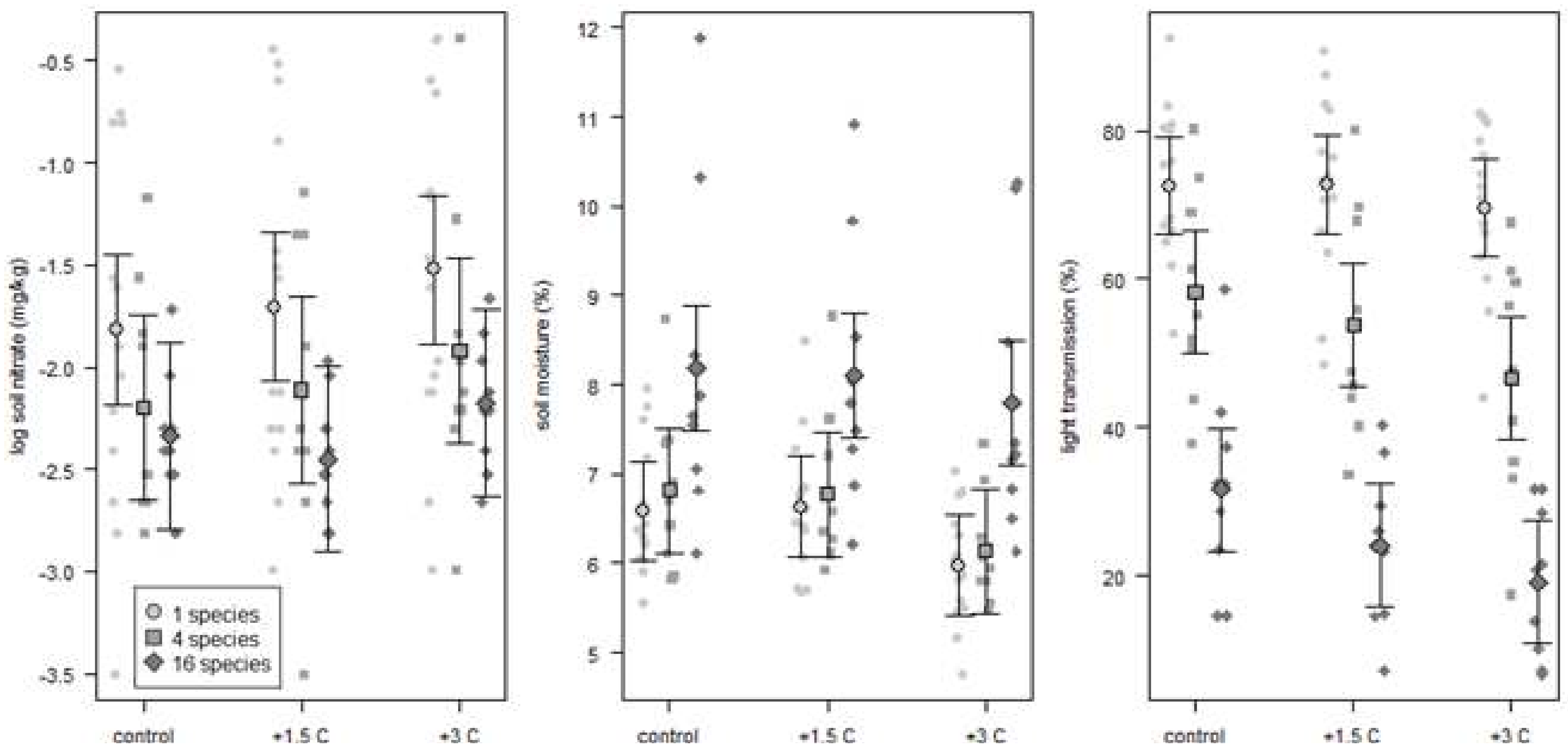


Figure 4-S5 (continued).

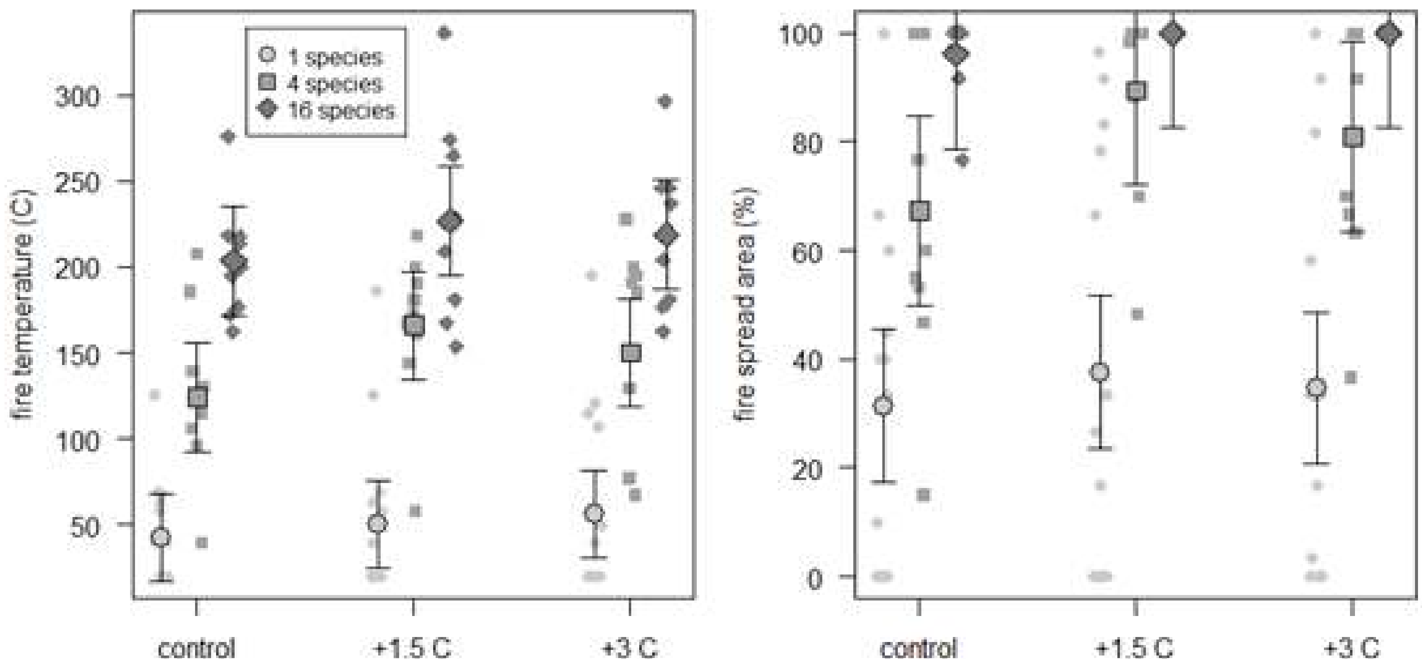
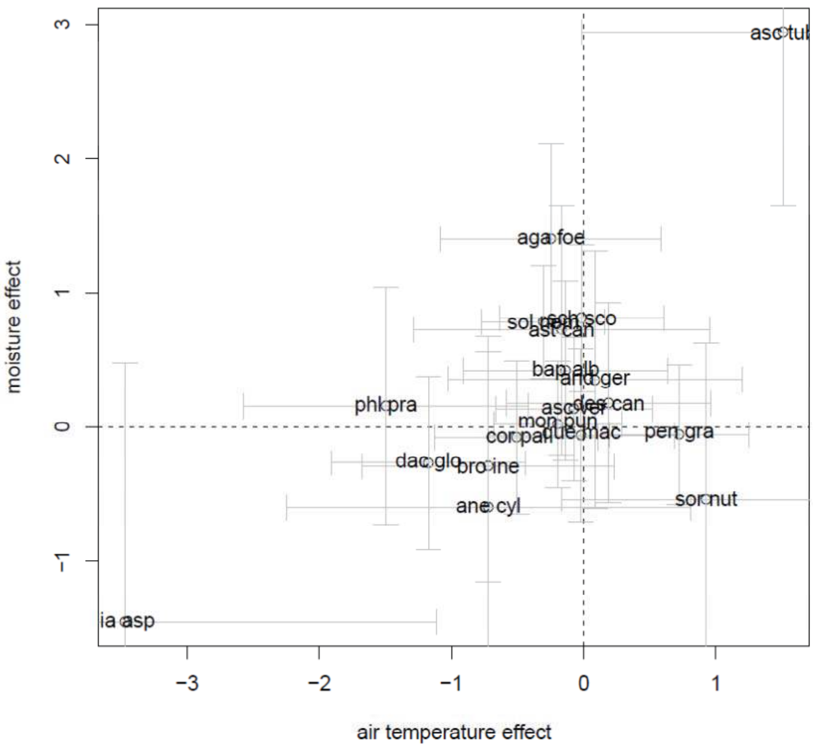


Figure 4-S6: Effects of environmental factors on total biomass per area, an integrative measure of seeded species' performance, as well as performance components.
Performance components: number of seeds germinating, proportion of seedlings surviving from the end of the first year to the end of the second year, and aboveground relative growth rate of surviving seedlings from the end of the first year to the end of the second year. Effects are coefficients (with 95 % confidence intervals) from the linear predictors of generalized linear mixed models with air temperature, light at the ground, soil moisture, soil nitrate, and fire spread as continuous predictors; fire spread did not have significant effects and is not shown. Species abbreviations correspond to species names in Figure 4-S1. Rho is Spearman's rank correlation coefficient; r is Pearson's correlation coefficient, weighted by the mean standard error of each point.

Figure 4-S6 (caption on previous page).

Biomass per area (g/m^2) rho = 0.40 r = 0.47



Aboveground biomass per planted seed (g) rho= 0.28 r= 0.23

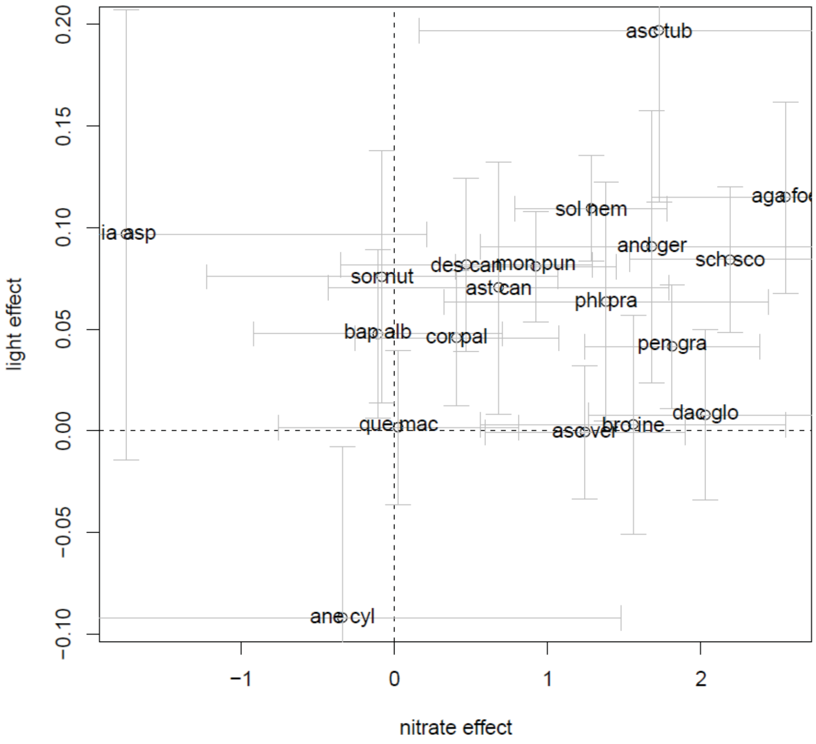
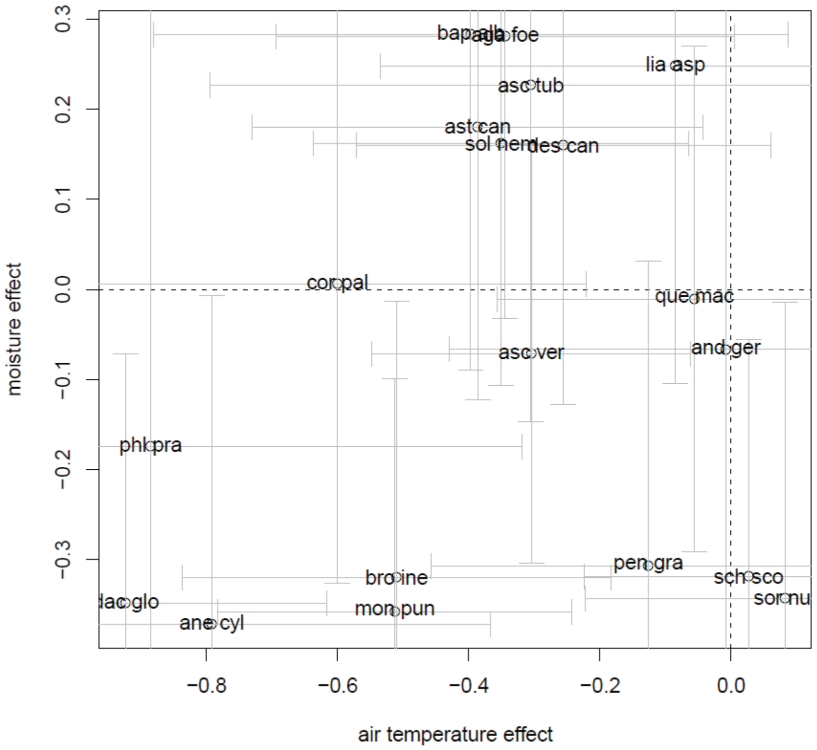
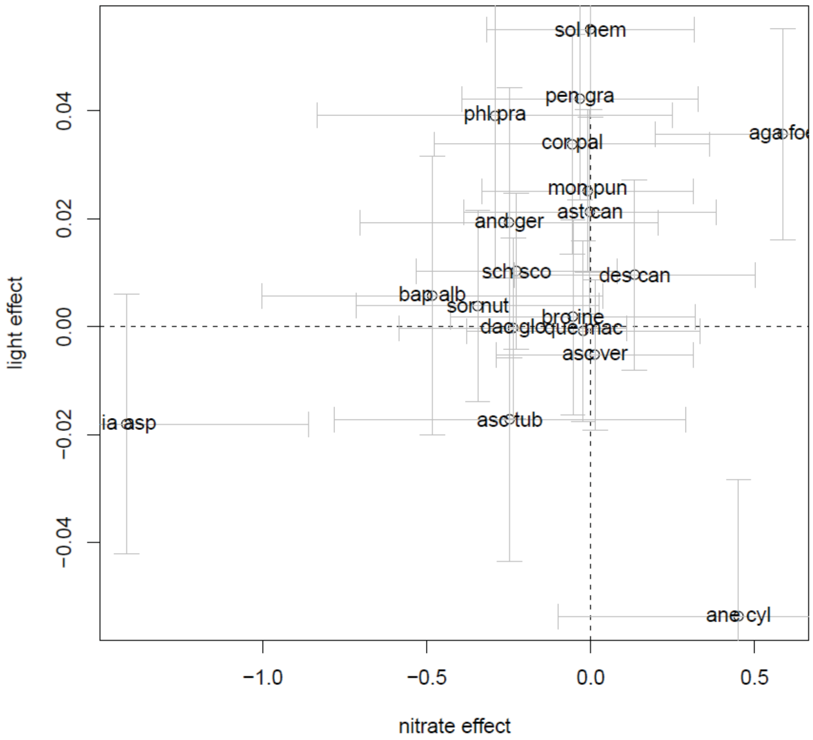


Figure 4-S6 (continued).



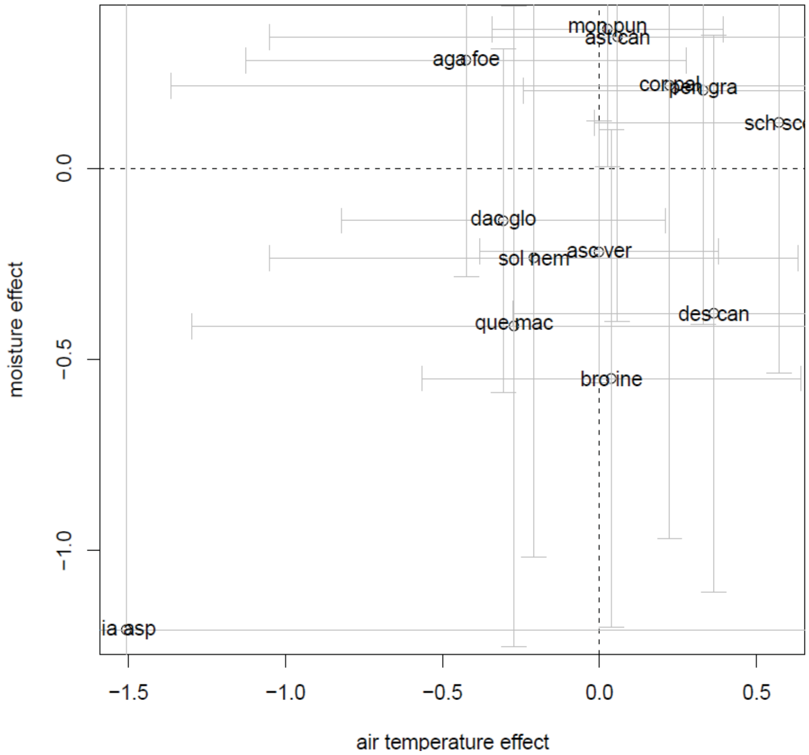
Number germinated rho= 0.18 r= 0.17



Number germinated rho= 0.15 r= 0.19

Figure 4-S6 (continued).

Proportion surviving y2 rho= 0.25 r= 0.33



Proportion surviving y2 rho= 0.09 r= −0.17

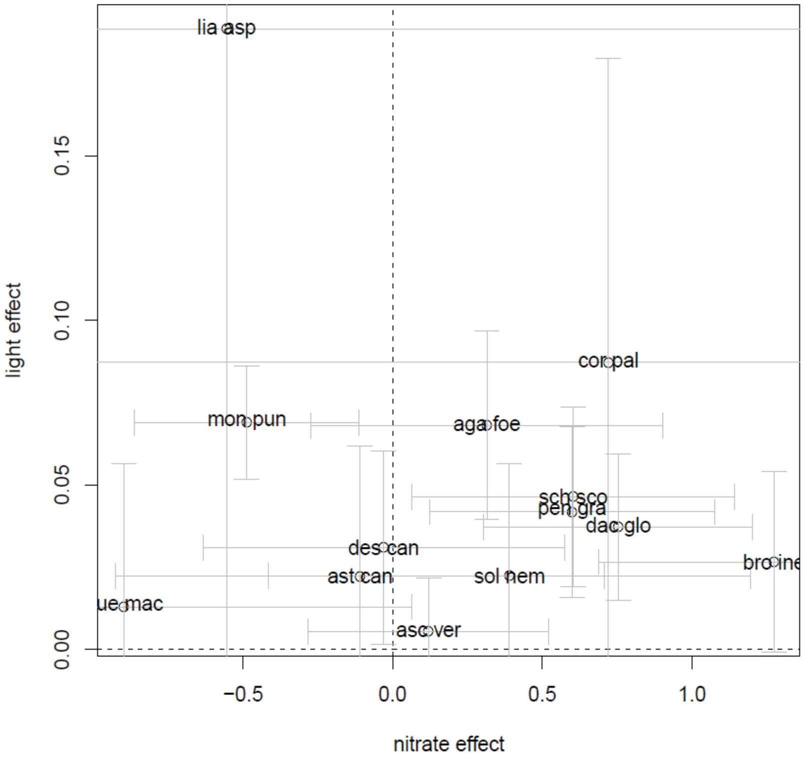
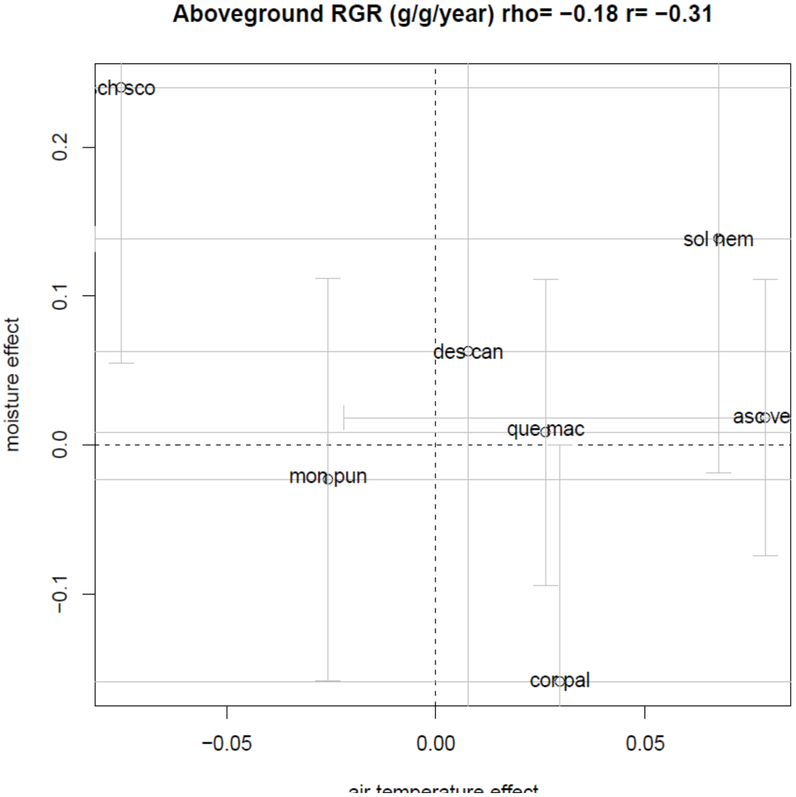
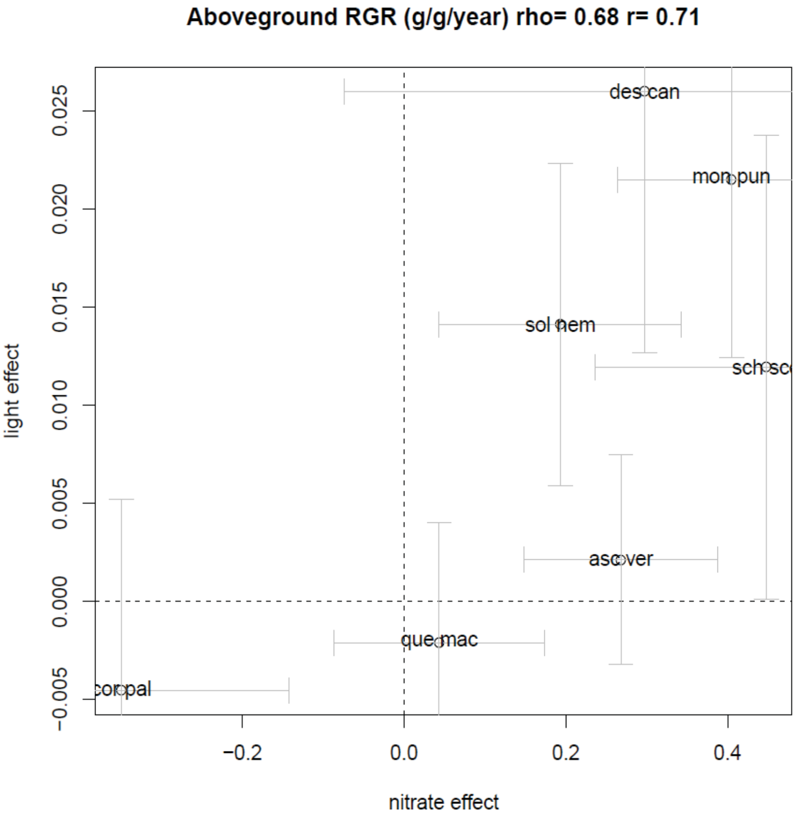


Figure 4-S6 (continued).





Chapter 5: Multiple nutrients control threatened grassland vegetation in eastern South Africa

Summary

Grassland types associated with highly weathered upland soils on dolerite and sandstone in eastern South Africa are rich in narrowly endemic plant species, many of which are of conservation concern because these grasslands have been much reduced in area. Anthropogenic changes in nutrient supply and herbivore abundance could threaten remaining fragments of these grasslands. Increasing N supply rates, including through widespread atmospheric deposition, commonly increase grassland productivity, reduce species diversity, and alter species composition. Supply rates of other nutrients also can influence grassland vegetation, especially where nutrients have been depleted by intense weathering, but this is less well understood. Herbivory is also commonly important in structuring grasslands, yet may be less important in these grasslands because large grazers have long been decimated and herbivory by large grazers may have been limited even pre-historically. Here we investigate the influence of multiple nutrients (N, P, K and micronutrients) and herbivory on grassland productivity, diversity, and composition on highly weathered dolerite- and sandstone-derived soils, as part of the globally distributed Nutrient Network experiment.

At two grassland sites, one on dolerite-derived soils (Gilboa) and one on sandstone-derived soils (Summerveld), we used factorial additions of N, P+Ca, and K+micronutrients to examine the role of nutrient limitation over four years. At the dolerite site, we also used factorial fencing and nutrient addition treatments to examine the interactive effects of herbivory and nutrient limitation.

Aboveground productivity increased with N and P+Ca addition, indicating that productivity was co-limited by these nutrients (independent co-limitation), at both sites. At the dolerite site, combined addition of N and P+Ca increased productivity more than expected from their separate effects, a case of synergistic co-limitation. Species richness and effective species richness tended to be lower with all three nutrient addition treatments at the dolerite site, and were significantly reduced by N addition at the sandstone site. At both sites, N addition increased total productivity mainly by increasing grass productivity, and thus increased the relative abundance of grasses at the expense of legumes; P+Ca addition increased the productivity of legumes more than it increased the productivity of grasses, and thus increased the relative abundance of legumes. Species composition, measured as cover, responded weakly to three years of nutrient addition. The most responsive species was a creeping legume that decreased with N addition at the sandstone site. Fencing at the dolerite site did not significantly influence aboveground productivity, diversity, or functional or species composition during the three year duration of this experiment.

Widespread increases in N supply rates, through atmospheric deposition and perhaps accelerated N mineralization with warming at high elevation, are likely to increase productivity, reduce diversity, and increase grass dominance at the expense of legumes in dolerite and sandstone grasslands. Diversity loss and reduced abundance of legumes, some of which are narrowly distributed and threatened with extinction, is

cause for conservation concern. Increased supply rates of other nutrients through fertilizer inputs may inhibit restoration of these grasslands. If our results from three years indicate long-term responses then loss of large grazers may not further impact these grasslands, at least where grazers are already at low abundance.

Introduction

The eastern summer rainfall region of South Africa holds mesic grasslands highly valued for their biodiversity, as well as cattle grazing and other ecosystem services such as streamflow and medicinal plants (Bond and Parr 2010). Agriculture and urbanization have caused extensive habitat loss and fragmentation (Driver *et al.* 2005, Mucina and Rutherford 2006). Accordingly, many range-restricted plant species endemic to vegetation types associated with distinctive soils and physiographic features are threatened with extinction (Raimondo *et al.* 2009). Further habitat loss poses a clear threat to biodiversity. It is less well known how biodiversity in remaining fragments of these grasslands may be threatened by anthropogenic environmental changes. In particular, nutrient supply rates and herbivory are changing through N deposition and changing grazer abundance. Here we investigate how changes in nutrient supply and grazer abundance influence grasslands of two types: Drakensberg Foothill Moist Grassland on dolerite outcrops, and KwaZulu-Natal Sandstone Sourveld on sandstone plateaus (Mucina and Rutherford 2006).

These grassland types are defined floristically and associated with particular soil types, which suggests that soil properties such as low nutrient supply rates might determine their species composition. If this is so then increasing nutrient supply may alter their species composition and diversity.

N commonly limits productivity and influences diversity and composition in grasslands (Tilman 1982, 1988, Vitousek and Howarth 1991, LeBauer and Treseder 2008). Nitrogen supply rate is increasing with widespread atmospheric deposition of inorganic N (Vitousek *et al.* 1997a). Nitrogen availability to plants also may increase with global warming in mountain grasslands that have high total soil N but low temperature-dependent N mineralization rates, including Drakensberg Foothill Moist Grasslands (Carbutt *et al.* 2013). Increased N supply commonly reduces diversity in N-limited grasslands (Clark and Tilman 2008, Bobbink *et al.* 2010), by mechanisms including increased productivity, reduced light availability (Hautier *et al.* 2009), and soil acidification (Stevens *et al.* 2010). Increased N supply rates can also alter functional and species composition by increasing the abundance of grasses at the expense of forbs (Tilman 1982, Stevens *et al.* 2006), reducing the competitive advantage of N fixation by legumes (Tilman 1982, Suding *et al.* 2005), and favoring species that compete better for light over those that compete better for N (Tilman 1988, Gough *et al.* 2012). If N is not limiting, increasing N supply may not threaten biodiversity through these competitive mechanisms but may still affect biodiversity by reducing soil pH and altering the availability of cations that act as limiting nutrients or toxins (Clark *et al.* 2007).

There is increasing recognition that nutrients other than N also commonly limit productivity and influence composition in grasslands (Tilman 1982, Tilman *et al.* 1994, Elser *et al.* 2007, Harpole *et al.* 2011, Fay *et al.* 2015). P, K and micronutrients are especially likely to be limiting on land surfaces that have been highly weathered through millions of years of exposure without major erosion or glaciation, such as parts of Australia, South America and the Cape of South Africa (Chadwick *et al.* 1999, Hopper 2009). Other such ancient surfaces are the quartzite plateaus underlying KwaZulu-Natal Sandstone Sourveld, which are geologically allied with the sandstones of the Cape (King 1982). The upland dolerite outcrops on which Drakensberg Foothill Moist Grassland occur are a more nutrient-rich parent material than sandstone but, like quartzite, are resistant to erosion and highly weathered. If non-N nutrients limit productivity then increases in their supply rates due to agricultural or other impacts could alter grassland productivity, diversity, and composition. Therefore, we ask whether and which nutrients limit productivity in these grasslands, and how they influence diversity and composition.

Another potential impact on these grasslands is changing abundances of large herbivores. Herbivores may influence plant diversity and composition by changing resource availability, as well as dispersing seeds, creating germination microsites, and selectively consuming more palatable species such as N-rich legumes (Olff and Ritchie 1998, Knops *et al.* 2000). Whereas on agricultural land cattle are typically stocked at rates high enough to strongly reduce standing biomass, in many grassland fragments not used for livestock native herbivores have become uncommon due to habitat fragmentation and hunting (O'Connor 2005). If herbivores maintain plant diversity then further loss of native herbivores may reduce plant diversity. On the other hand, further loss of large mammal herbivores (larger than mice) might, potentially, cause little further impact because grazer abundance in these grasslands has already been strongly reduced for over a century. Moreover, herbivory may not have been highly influential even pre-historically. Correlational studies found that in these grassland types moderate to heavy cattle grazing reduces diversity compared with little or no grazing (O’Connor *et al.* 2011, Scott-Shaw and Morris 2014); this is consistent with a trend for heavy grazing to reduce plant species richness rather than maintain it in nutrient-poor and unproductive ecosystems (Proulx and Mazumder 1998, Bakker *et al.* 2006). Fire has also been an important part of this landscape over evolutionary timescales, indicated by many species requiring smoke to germinate or flower (Bond and Parr 2010). Fire is widely used to manage grasslands — the sites we study are burned annually — and may serve some of the roles of lost grazers (Bond and Keeley 2005). Therefore, we hypothesize that excluding large herbivores from these fragmented, nutrient-poor, frequently burned grasslands is unlikely to strongly influence plant diversity and composition.

We ask how nutrients and herbivory influence the productivity, diversity, and composition of grassland vegetation by experimentally manipulating nutrient supply rates and herbivore abundance at two sites, as part of the globally distributed Nutrient Network experiment (Borer *et al.* 2014a). Conducting these experiments using standardized Nutrient Network methods placed these sites’ environmental characteristics (climate, vegetation and soil) and our results in a global context, and has contributed to global treatments of these questions reported elsewhere (Borer *et al.* 2014b, Fay *et al.* 2015). Here, we report results from these two experiments in more detail with a focus on their implications for biodiversity conservation in this region.

Specifically, we asked (1) how adding N, P, and other nutrients influences (a) aboveground net primary productivity, (b) plant diversity, and (c) functional group and species composition of these grasslands. We investigated functional and species composition responses both to understand which vegetation components drive overall productivity responses and to discover how the relative abundances of functional groups and species depend on nutrient supply. To understand some mechanisms by which nutrient additions might have influenced vegetation, we asked (2) how nutrient addition influenced plant resources, specifically light availability at the ground and soil nutrient levels. We also asked (3) how excluding large herbivores using fences influenced the same vegetation properties, and (4) influenced light and soil nutrients.

Methods

Study sites

We established similar experiments at two sites in KwaZulu-Natal province, South Africa. The Gilboa experiment is on the summit plateau of a dolerite mountain at an elevation of 1748 m, on a gently west-facing slope (~2 degree slope angle; 29.28424S, 30.29174E). The soil is a sandy loam. This site is similar in elevation and biota to the Drakensberg mountain range 100 km to the west. This site is classified as Drakensberg Foothill Moist Grassland (Mucina and Rutherford 2006), but also has species characteristic of Moist Midlands Mistbelt grasslands that surround it on lower slopes (Table 5-S1). It is in a private nature reserve managed by a timber company. The Summerveld experiment is on a sandstone (quartzite) plateau at an elevation of 679 m, on a gently south-facing slope (~4 degree slope angle; 29.81161S, 30.71573E). The soil ranges from sandy loam to loam. This site is in the KwaZulu-Natal Sandstone Sourveld vegetation type (Mucina and Rutherford 2006), on private land in a powerline right-of-way.

The two sites had similar precipitation regimes, with mean annual totals of 926 mm (Gilboa) and 939 mm (Summerveld), both strongly focused in summer (Table 5-1) (Hijmans *et al.* 2005). Gilboa was the cooler site: at Gilboa, temperatures were 0 C to 22 C (mean minimum in the coolest month to mean maximum in the warmest month), whereas at Summerveld temperatures were 7 C to 26 C. Both sites were burned annually in winter (June - August). Burning every 1-3 years is common in these grasslands, especially where grazing is light; less frequent burning results in encroachment by woody plants (Titshall *et al.* 2000). Both sites were lightly grazed by antelope, hares and other small mammals. Domestic livestock were absent from both sites but had been present in the past, at low abundance at Gilboa and moderate abundance at Summerveld. Neither site had been transformed by ploughing. Both sites were strongly dominated by native/indigenous perennial plants (Seabloom *et al.* 2013). Gilboa had two annual species, both rare (maximum cover 1 %, in no more than a handful of plots each year); Summerveld had no annuals.

Experimental design

Experimental design and sampling followed the Nutrient Network protocol (Borer *et al.* 2014a), which we summarize with some site-specific details. At each site 30 plots, each 2 x 2 m, were established in September 2009 in a 6 x 5 grid with 1 m buffers between plots. Each grid was divided into three blocks of 2 x 5 plots. Within each block, eight randomly selected plots were randomly allocated the factorial combinations of N, P+Ca, and K+micronutrients. At Gilboa, there was also a partially overlapping fence experiment. To generate the four factorial combinations of fencing and all nutrients combined, the remaining two plots per block were randomly allocated to fenced but unfertilized, and fenced and fertilized treatments; the two unfenced treatments were shared with the nutrient experiment. At Summerveld, fences were not erected; one of the remaining two plots in each block was used as an extra unfenced no-nutrient control, and the other was unused.

Following a pre-treatment sampling year, nutrients were applied each spring (October-December) from 2010 onwards. Each year, N was applied at 10 g m^{-2} in the form of Multicote 42-0-0 slow-release urea N_2H_4CO pellets, P at 10 g m^{-2} in the form of triple superphosphate $Ca(H_2PO_4)_2$, and K at 10 g m^{-2} in the form of potassium sulphate K_2SO_4. The triple superphosphate included Ca 8.1 g m^{-2}, Mg 0.3 m^{-2}, and S 0.5 g m^{-2}. Micronutrients were applied in the first year in the form of Scott's Micromax: Ca 6.0 g m^{-2}, Mg 3.0 g m^{-2}, S 15.9 g m^{-2}, B 0.1 g m^{-2}, Cu 1.0 g m^{-2}, Fe 17.0 g m^{-2}, Mn 2.5 g m^{-2}, Mo 0.1 g m^{-2}, Zn 1.0 g m^{-2}. Treatments were applied in spring after plants had re-established substantial cover following winter burning. Nutrients apparently did not spread between treatments: four months after treatments were applied, nutrient granules were observed only in plots where they had been added, and the nutrient treatments had clear effects on levels of most nutrient after two years.

Also in spring following the pre-treatment sampling year, fences were erected around designated plots. These excluded jumping antelope using wire strands up to 2 m, and smaller animals using 1 cm mesh forming a flange 50 cm high around the base. There was no evidence that burrowing animals entered fenced plots. Indeed, the only evidence of soil disturbance even in unfenced areas was modest porcupine digging at Gilboa.

Sampling

The 2 x 2 m plots were divided into four 1 x 1 m subplots, systematically assigned to (subplot A) non-destructive percent cover and light interception estimates, (subplots B and C) annual biomass sampling and soil/root sampling, and (subplot D) extra biomass sampling in the final year. All sampling occurred when biomass peaked in late summer (late February to late March).

We clipped aboveground biomass in two 1 m x 0.1 m strips per plot, in a new location each year. In the final year, 2013, we clipped an additional two strips per plot to increase statistical power. The biomass from each pair of strips was separately sorted to graminoids (grasses and sedges), legumes, and non-legume forbs, dried, and weighed. These biomass measures are estimates of aboveground net primary productivity because all plots were completely burned every year. We excluded the small amount of litter remaining from earlier years, which was mostly charred stems of *Protea* subshrubs.

We sampled species diversity and composition by estimating the absolute percent cover of each plant species, as well as bare ground and rock, in a permanent 1 m^2 quadrat in each plot. Estimates were to the nearest 1%, allowing total cover to exceed 100 %. We calculated both species richness (the number of species recorded in each plot) and effective species richness (e^H, where H is the Shannon diversity index, representing the equivalent number of equally abundant species (Hill 1973)). Species richness weights the presence of all species equally regardless of there abundance, whereas effective species richness increases with the evenness of species' abundances as well as the number of species present.

We measured light interception by taking a light measurement across both diagonals of the cover subplot using an AccuPAR light meter, as well as an ambient measurement above each plot.

We sampled soil to 10 cm depth before applying the treatments (October 2010) and again after two treatment seasons (February 2012). We systematically located 5 cores per plot, each 3 cm in diameter. Soils were analyzed alongside samples from other Nutrient Network sites for total C and N; extractable forms of other nutrients; and, in 2012, root biomass (Borer *et al.* 2014a). We measured soil depth in 2010 by hammering a reinforcing bar into the ground until it hit rock, at 6 systematically distributed points per plot.

Analyses

We analyzed the effects of the factorial nutrient addition treatments using a separate Analysis of Variance (ANOVA) for each response variable at each site. In each ANOVA, we included the three nutrient addition treatments (N, P+Ca, and K+micronutrients) and their two-way interactions. We omitted blocks from our analyses to maximize statistical power, because the blocks were not discrete in space and did not differ significantly in major environmental variables such as soil depth, rock cover, or pre-treatment productivity (not shown).

To assess shifts in functional composition, we performed ANOVAs as described above separately for the abundances of each functional group (graminoids, legumes, and non-legume forbs) versus the nutrient treatments. To hold the total Type I error rate at 0.05 for each site-specific set of three functional group analyses, we used a Dunn-Sidak adjusted significance threshold of $p = 0.017$ instead of $p = 0.050$ (Quinn and Keough 2002).

Because the treatment effects strengthened through time, we focused on results in the third (final) treatment year. Identical analyses of pre-treatment data showed that treatment effects were not significant before treatments were applied, except in a few cases indicated in Results. We also assessed treatment effects across all years in which treatments were applied using mixed-effects ANOVAs that additionally included year and year*treatment interactions as fixed effects, and plot as a random intercept to account for correlation between multiple measures of the same plot. These analyses showed treatment effects averaging across all treatment years that matched the final-year results, except where noted in Results. We fit the single-year fixed-effect linear models using base R (R Development Core Team 2013), and the multi-year mixed-effects linear models using lme4 (Bates *et al.* 2014) in R.

To assess the effects of the nutrient treatments on species composition in the final year, we used a multivariate extension of generalized linear models (Warton *et al.* 2012), implemented in the R package mvabund (Wang *et al.* 2012). This method fitted separate negative binomial regressions to the cover of each species versus the main effects and two-way interaction effects of the nutrient treatments, and used randomization to test the effects of the treatments on multivariate species composition accounting for correlations between species' responses. It also yielded univariate tests of treatment effects for each species, adjusting p-values for multiple testing to ensure a total Type I error rate of 0.05 across all species.

We analyzed the joint effects of excluding herbivores and adding all nutrients similarly.

Results

Vegetation, climate, and soil in a global context

We placed these sites in a global context by comparing pre-treatment measurements with those from 55-77 other Nutrient Network grassland sites (depending on the measure), averaging across plots at each site (Table 5-1). Nutrient Network sites are clustered in North America; Australia and Europe are also well represented, and there are fewer sites on other continents. In aboveground net primary productivity, Gilboa (280 g/m^2) and Summerveld (313 g/m^2) ranked in the middle of Nutrient Network sites. Root biomass to 10 cm depth was higher at Gilboa (mean +- SD 3,228 +- 961 g/m^2) and Summerveld (2,122 +- 794 g/m^2) than at the 27 other sites sampled; this metric is derived from control plots in the second treatment year whereas other metrics are from all plots, pre-treatment. Summerveld likely had more additional roots below 10 cm because soils were deeper at Summerveld (mean depth 16.7 cm) than at Gilboa (mean depth 4.3 cm). Diversity was in the top 10 percent for both Gilboa (richness: 24 species/m^2; effective richness, e^H: 11 species/m^2) and Summerveld (richness: 31 species/m^2; effective richness, e^H: 16 species/m^2). Similarly diverse Nutrient Network sites were in Estonia, Chinese and Swiss mountains, and North American tallgrass prairie.

Gilboa had a relatively low mean maximum temperature in the warmest month (22.3 C, 21st percentile, Table 5-1), consistent with its relatively high elevation (88th percentile). Conversely, Summerveld had a particularly high mean minimum temperature in the coolest month (7.3 C, 92nd percentile), consistent with its coastal and relatively low latitude (29.8 S, 16th percentile) position in this largely temperate dataset.

Modeled N deposition in this region was intermediate, at 0.5 g/m^2/year.

Gilboa had among the highest soil C (20.6% C) and N (1.16% N) of any Nutrient Network site, alongside some sites in Europe and northern North America; Summerveld was at the 71st percentile for C (6.5% C) and 60th percentile for N (0.33% N) (Table 5-1). S was also relatively high at Gilboa (89th percentile) and Summerveld (67th percentile). Conversely, most other soil nutrients — P, K, Mg, Ca, Zn, and Mn — were relatively low at both sites. Given vegetation responses to the P+Ca treatment, we note that P was 25th percentile at Gilboa and 11th percentile at Summerveld, alongside sites in Australia and parts of the US Great Plains, and Ca was 2nd percentile at Gilboa and 18th percentile at Summerveld. Soil acidity was relatively high: pH was 5.13 at Gilboa and 5.14 at Summerveld (16-18th percentiles). Rock cover averaged 6.3% at Gilboa and 0.1% at Summerveld.

Effects of nutrients on productivity

Aboveground productivity increased with N and P+Ca addition at both sites, in separate ANOVAs for each site of productivity in the final year versus the nutrient addition treatments and their two-way interactions (main effects of N and P+Ca: $p < 0.001$, except $p = 0.005$ for P+Ca at Summerveld) (Fig. 5-1A). The positive effects of N and P+Ca were super-additive (positive interaction: $p = 0.003$) at Gilboa. Productivity also increased marginally non-significantly with K+micronutrients at Summerveld ($p = 0.099$). By chance, the Gilboa P+Ca effect was significant even in the pre-treatment year ($p = 0.003$). However, it is unlikely that the effect of adding P+Ca was due entirely to chance because productivity increased significantly more from the pre-treatment year to the final year where P+Ca was added.

The Gilboa grasslands were short but extremely dense, and with N and P+Ca addition became so dense aboveground that plants such as the prostrate-leaved forb *Ledebouria ovatifolia* were pushed upright by grass growth. The Summerveld grasslands were taller and sparser.

Root biomass to 10 cm depth had not responded significantly to the nutrient treatments at either site by the end of the second treatment season.

Effects of nutrients on species diversity

Species richness and effective species richness (e^H, where H is Shannon diversity) decreased with nutrient additions. At Gilboa, species richness and effective species richness tended to be lower with all three nutrient addition treatments (Fig. 5-2A,B, all main effects negative but not significant, $p > 0.12$, in factorial ANOVAs). At Summerveld, adding N significantly reduced species richness ($p = 0.039$) and effective species richness ($p = 0.019$) in the final year, also shown by factorial ANOVAs.

Effects of nutrients on functional composition

We examined the effects of the factorial nutrient addition treatments on functional composition in the final year using separate ANOVAs for each functional group's biomass (Fig. 5-3). At both sites, N addition significantly increased graminoid biomass ($p < 0.001$) and decreased legume biomass ($p = 0.012$-0.013). N addition increased non-legume forb biomass at Summerveld ($p < 0.001$) but not at Gilboa (not significant). At both sites, P+Ca addition significantly increased graminoid biomass ($p < 0.021$), but also increased legume biomass ($p < 0.036$). P+Ca addition increased non-legume forbs at Summerveld ($p < 0.001$), but not at Gilboa. The interactive effect of N and P+Ca significantly increased graminoid biomass at Gilboa ($p = 0.002$), consistent with the positive N * P+Ca interaction for total productivity. At both sites, adding K+micronutrients tended to increase legume biomass, though this was not significant. Adding P+Ca and K+micronutrients together resulted in the highest mean biomass of legumes at both sites, substantially higher than the P+Ca only treatment, but this effect was not significant, either as a P+Ca * K+micronutrients interaction in a factorial ANOVA or as a pairwise difference between the P+Ca plus K+micronutrient and P+Ca treatments assessed using a Tukey test following a one-way (non-factorial) ANOVA.

By chance, at Gilboa the plots receiving P+Ca addition had higher legume biomass even before treatments were applied ($p = 0.011$). However, the positive effect of the P+Ca treatments on legume abundance may be real because legume cover also increased with P+Ca addition at this site (marginally non-significantly in the final year, and significantly across all treatment years in a mixed-effects ANOVA, $p = 0.006$) yet was *not* significantly higher in P+Ca addition plots pre-treatment. A negative P+Ca * K+micronutrients interaction effect on graminoid biomass at Summerveld was also near-significant even before the treatments were applied, though it is consistent with a shift in competitive advantage to legumes which responded near-significantly positively to this interaction.

These shifts in absolute biomass resulted in shifts in the relative biomass of functional groups, which we analyzed using similar ANOVAs (Fig. 5-S1). N addition tended to increase graminoids' relative biomass (Gilboa: $p = 0.033$; Summerveld: $p = 0.185$; effects on relative cover were concordant and significant), at the cost of reduced relative biomass of legumes (Gilboa: $p = 0.017$; Summerveld: $p = 0.006$). Conversely, P+Ca addition tended to increase legumes' relative biomass (Gilboa: $p = 0.240$; Summerveld: $p = 0.040$), at the cost of reduced relative biomass of graminoids (not significant). Non-legume forbs' relative abundance did not respond to the treatments consistently across years. Functional groups' covers (both absolute and relative) responded to nutrients in ways that were similar to, but mostly less distinct than, their biomasses.

Effects of nutrients on species composition

Multivariate analyses of species' abundances showed no significant effects of the factorial nutrient treatments on species composition at either site, when both main and two-way interactive effects were included. Omitting interactions revealed some significant main effects. At Gilboa, P+Ca addition significantly affected species composition ($p = 0.042$). Two species responded significantly to P+Ca: the sedge *Cyperus* sp. decreased from a mean of 11% in plots without P+Ca to 6% in plots with P+Ca added ($p = 0.001$), and the non-legume forb *Helichrysum aureum* increased from 0% in plots without P+Ca to a mean of 1% in plots with P+Ca added ($p = 0.036$). At Summerveld, N addition significantly affected species composition ($p = 0.014$). Only the prostrate, creeping legume *Tephrosia macropoda* responded significantly: it decreased in cover from a mean abundance of 9% in plots without N to 2% in plots with N added ($p = 0.010$).

All species were native except for one exotic, *Conyza canadensis*, which entered a plot receiving all nutrient additions at Gilboa in the final year (outside the cover subplot, personal observation).

Effects of nutrients on light availability and soil

To assess potential mechanisms by which the nutrient addition treatments may have influenced vegetation, as well as their efficacy, we analyzed their effects on light and soil properties using factorial ANOVAs like those used for vegetation responses.

Light availability at ground level decreased with N and P+Ca addition at Gilboa and Summerveld in the final year (Fig. 5-S2A, $p < 0.003$, except $p = 0.089$ for P+Ca addition at Summerveld), consistent with increased light-intercepting biomass in these treatments.

Adding nutrients increased soil nutrient levels as intended in most cases, measured after two years of treatments. Neither soil C nor N had responded significantly to treatments at either site. Plots with P+Ca added had extractable P that was 17-fold higher at Gilboa ($p < 0.001$) and 10-fold higher at Summerveld ($p < 0.001$) than plots not receiving P+Ca. Plots with P+Ca added, in the form of $Ca(H_2PO_4)_2$ including 8.1 g/m^2/year Ca, also had extractable Ca that was 2-fold higher at Gilboa ($p = 0.002$) and 1.5-fold higher at Summerveld ($p = 0.003$) than plots not receiving P+Ca . In contrast, the K+micronutrient addition including a one-time application of 6.0 g/m^2 Ca in the form of dolomite did not significantly increase extractable Ca at either site. The K+micronutrient treatment increased K, S, Zn, Mn and Cu at both sites and increased Fe at Gilboa ($p < 0.05$, except Mn not significant at Gilboa), but failed to increase Mg or B. Instead, Fe increased marginally non-significantly with N addition at Summerveld ($p = 0.072$), and Mg increased with P+Ca addition at Gilboa ($p = 0.012$), perhaps because the P+Ca treatment also included 0.3 g/m^2/year of Mg (though this was considerably less than the 3 g/m^2 one-time application of Mg in the form of dolomite in the K+micronutrient treatment).

pH was reduced significantly by N addition at both sites ($p < 0.001$), but less so when P+Ca was also added (positive interaction, $p < 0.017$). Compared with untreated controls, plots with only N added had pH 0.91 units lower at Gilboa and 0.38 units lower at Summerveld, whereas plots with N and P+Ca added were only only 0.33 units lower at Gilboa and 0.18 units lower at Summerveld.

Effects of fencing on productivity, composition, and diversity

Aboveground productivity did not respond significantly to fencing independently of nutrient addition at Gilboa (Fig. 5-1, fence main effect: $p = 0.204$ in an ANOVA of productivity the final year versus nutrient addition and fencing and their interaction). There was a negative effect of fencing when all the nutrients were added (negative interaction: p = 0.033), but this may be an artefact of biased allocation of plots to treatments by chance because there was an opposite positive interactive effect on light availability (negatively correlated with biomass) even before treatments were applied (p = 0.034). Root biomass had not responded significantly to fencing when sampled in the second treatment year. Neither species richness, nor effective species richness, nor functional group relative abundance (biomass or cover), nor species composition, nor soil properties responded significantly to fencing or the interaction of fencing with nutrient addition (Fig. 5-2C,D).

Discussion

At both sites, aboveground productivity was co-limited by N, by P and/or Ca, and perhaps by other nutrients. Species richness tended to be reduced by all three nutrient addition treatments at the dolerite site, and was significantly reduced by N addition at the sandstone site. Nutrient addition may have reduced species richness partly by reducing light availability. At both sites, N addition favored grasses whereas P+Ca addition favored legumes. Fencing had no significant effects during our three years of treatments.

Efficacy of nutrient treatments

The clear-cut effects of nutrient treatments on extractable levels of most soil nutrients suggests that the treatments effectively increased nutrient supply rates. N addition did not significantly increase total soil N, but this is unsurprising given the already-high N content. N content and organic matter were especially high at Gilboa (1.16% N, 20.6% C), perhaps because the relatively cool summer temperatures at this mountain site inhibit decomposition of organic matter (Carbutt *et al.* 2013). Even at Summerveld where total soil N is lower (0.33%), if soil bulk density was 1 g/cm^3 (towards the lower end of common values; not measured) then total N would be 330 g/m^2 in the top 10 cm of soil; adding N at 10 g/m^2/year would increase this stock very slowly. Nonetheless, much of this N is in forms unavailable to plants. Adding N presumably increased the supply of plant-available inorganic N considerably to increase productivity as we observed. The marginally non-significant increase in extractable Fe with N addition at Summerveld may have been due to acidification, which can make Fe more available (Haynes and Swift 1985).

We cannot separate the effects of P and Ca because adding P+Ca as triple superphosphate strongly increased soil P and Ca, whereas the K+micronutrients treatment did not significantly raise soil Ca (or P). Both P and Ca had relatively low levels in our experiments, and indeed Ca was extraordinarily scarce at Gilboa where adding P+Ca increased productivity more strongly, so P or Ca or both could have limited productivity. The fact that Ca limitation is less well known than P limitation in grasslands (McLaughlin and Wimmer 1999, Elser *et al.* 2007) may suggest that P is more likely to have driven the P+Ca treatment effects. However, syntheses of the effects of P limitation in previous experiments may also be partly confounded with limitation by Ca or other cations, because most previous studies have also added P as a salt. Therefore, it is unclear whether P or Ca or both are influential in these grasslands.

Nutrients controlled productivity

Aboveground productivity was apparently limited by N as well as P or Ca, at both sites. N limitation of productivity is widespread in grasslands (Tilman *et al.* 1994, LeBauer and Treseder 2008, Fay *et al.* 2015). Though Gilboa had a total soil N content among the highest in the Nutrient Network, N likely accumulated in the soil due to low rates of mineralization so it is not surprising that plant growth was limited by N nonetheless. N mineralization may have been slowed by cool temperatures. Across the global Nutrient Network, N tends to be most limiting at cooler temperatures (Fay *et al.* 2015), and relative to most other Nutrient Network sites Gilboa had low maximum temperatures in the warmest month (which coincides with rainfall and the growing season). Locally, N mineralization in the Drakensberg mountains, near and at similar elevation to Gilboa, has been shown to be limited by cool growing season temperatures (Carbutt *et al.* 2013). If increasing global temperatures increase N mineralization rates in these mountain grasslands (Carbutt *et al.* 2013), this could increase productivity and reduce diversity. N availability to plants at Gilboa may also have been reduced by high microbial uptake, enabled by the high soil C content (Kuzyakov and Xu 2013).

Limitation by P or Ca is consistent with these nutrients being relatively low at these highly weathered sites. Positive effects on productivity of P+Ca -- and the positive interactive effect of P+Ca and N at Gilboa -- are consistent with findings at many other Nutrient Network sites (Fay *et al.* 2015) and at a 60 year grass fertilization experiment at Ukulinga, located between Gilboa and Summerveld, which found positive effects of N and P and their interaction (Fynn and O'Connor 2005, Tsvuura and Kirkman 2013). The biomass responses to N and P indicate independent co-limitation (Harpole *et al.* 2011), additive at Summerveld and super-additive (synergistic) at Gilboa. The marginally non-significant K+micronutrients effect at Summerveld is consistent with a positive effect of K and micronutrients at some other sites (Tilman 1982, Fay *et al.* 2015). Thus, the apparent co-limitation by N, P or Ca, and potentially other nutrients at these sites bolsters an emerging understanding that co-limitation of productivity by multiple nutrients is widespread in grasslands (Tilman 1982, Tilman *et al.* 1994, Harpole *et al.* 2011, Fay *et al.* 2015).

That we did not detect significant belowground biomass responses to treatments during three years is unsurprising because belowground biomass consists partly of long-lived storage organs with slow turnover, and because the small sampling area of soil cores yielded high sampling variation.

Nutrients controlled diversity

The loss of plant diversity with nutrient addition — significant at Summerveld and a distinct though not significant trend at Gilboa — is consistent with findings from other nutrient addition and correlation studies and with theoretical predictions. In particular, N, which significantly reduced diversity at Summerveld, has strongly reduced diversity elsewhere when added as fertilizer (Gough *et al.* 2000, Clark and Tilman 2008) and deposited atmospherically (Stevens *et al.* 2006). The trend for other nutrients to also reduce diversity, especially at Gilboa, may become significant in time: these communities are comprised almost entirely of long-lived perennial plants with large storage organs so compositional change is slow.

Eutrophication can reduce plant diversity by reducing light penetration to the ground because of greater living aboveground biomass or litter (Hautier *et al.* 2009, Borer *et al.* 2014b). Consistent with this potential explanation, diversity responses did largely mirror productivity and light responses to nutrient addition in our experiments. However, there are several other potential mechanisms by which eutrophication could have affected diversity. Increased productivity can competitively reduce availability of water and nutrients to plants even when total nutrient supply increases (Tilman *et al.* 1996, Davis *et al.* 1999), which could reduce diversity. N addition reduced pH, which can be associated with reduced species richness, especially of forbs (Tilman 1982, Fynn and O'Connor 2005, Stevens *et al.* 2010). Diversity could have been reduced by N addition but not by P+Ca addition at Summerveld because pH was reduced by N addition but not by P+Ca addition, or simply because productivity was increased more by adding N than by adding P+Ca. More generally, adding limiting resources can disrupt tradeoffs in species' responses to different limiting factors that allow species to coexist (Harpole and Tilman 2007).

Nutrients controlled functional composition

The effects of nutrient addition on productivity were driven mainly by positive responses of graminoids (mostly grasses) to N and P+Ca. Legumes also increased in abundance with P+Ca addition, and non-significantly with K+micronutrient addition, at both sites, consistent with their ability to fix N giving them a competitive advantage when limitation by other nutrients is removed (Tilman 1982). Increased legume productivity may have increased total productivity both directly and indirectly by increasing N supply to C4 grasses that have high N use efficiency (Tilman *et al.* 1997).

N addition reduced absolute and relative legume biomass at Summerveld and reduced relative legume biomass at Gilboa, perhaps by reducing the advantage conferred by N fixation as other resources became more limiting. In particular, the abundant prostrate legume *Tephrosia macropoda* decreased significantly in cover at Summerveld with N addition, perhaps due to reduced light availability. This is consistent with reduced abundance abundance of legumes (Suding *et al.* 2005) and short species with runners (Gough *et al.* 2012) in other N addition experiments. Conversely, P+Ca addition increased legumes' relative abundance, both by increasing the abundance of legumes more than it increased the abundance of grasses and, at Gilboa, by reducing the abundance of a common sedge (*Cyperus* sp.), perhaps because some sedges have root structures that allow them to extract soil P particularly efficiently in low-P environments (Lambers and Shane 2007). K+micronutrients also tended to increase legumes' relative abundance at both sites, especially in combination with P; though not statistically significant, this is consistent with N-fixation conferring the strongest advantage on legumes when limitation by all nutrients other than N is removed (Tilman 1982).

Species composition, measured using percent cover, may have been slow to respond to treatments because the plants were long-lived. Another contributing factor may have been that cover is not sensitive to changes in the third dimension, plant height: functional groups responded to treatments more clearly when their abundance was measured as biomass, which does capture height, than as cover.

Exotic (non-native) species were initially absent from both experiments. This is consistent with exotic species being very rare in regularly burned, lightly grazed, un-ploughed, infertile grasslands in this region (personal observation). These two experiments — with among the highest grass species richness in the Nutrient Network (18 species at Gilboa and 26 species at Summerveld, totaled across all plots) — contributed to a global trend for pre-treatment exotic species richness and cover to be lower at sites with higher native grass species richness, perhaps because native grasses offer competitive biotic resistance to invasion (Seabloom *et al.* 2013). In the final treatment year, two high-nutrient-associated species appeared in these experiments for the first time, both in fertilized plots: the exotic forb *Conyza canadensis* at Gilboa and the native grass *Eragrostis curvula* at Summerveld. Both occurred previously in disturbed habitats about 20 m away from the experiments. This suggests that species adapted to high nutrient conditions, including many exotics, may progressively enter these experiments as seeds reach fertilized plots and as nutrient effects on vegetation and soil accumulate. This would be consistent with a tendency for increased exotic cover with N and P+Ca addition across all nutrient network sites (Seabloom *et al.* 2015).

Large herbivores did not affect productivity, diversity, or composition

Excluding large herbivores at Gilboa did not significantly affect plant productivity, diversity, or composition over three years. The lack of a significant effect of fencing on aboveground net primary productivity or light availability is consistent with low abundance of large herbivores. A low intensity of herbivory, with little impact on availability of light — or, presumably, other resources — also explains why fencing did not significantly affect diversity (Borer *et al.* 2014b) or composition. The fences did not exclude small mammals such as mice, or insects; we did not observe any evidence that their impact was substantial (personal observations).

Herbivory may influence composition in the longer term, especially because the grazers at these sites are small- to medium-sized antelope that tend to feed selectively, which could favor less palatable species. It is also plausible that herbivores have little effect at Gilboa and Summerveld and other infertile grasslands with light grazing. Our study sites, and many of the other remaining sandstone and dolerite grasslands in this region, are fragments in a matrix of agricultural and urban land uses and thus have been without abundant large herbivores for over a century. Larger, less selective herbivores such as rhino occurred historically, migrating between seasonally palatable upland grasslands in the summer growing season and perennially palatable lowland grasslands in winter, but these migrations were disrupted by fragmentation.

Moreover, grazing may never have been important in maintaining diversity in such infertile grasslands (Proulx and Mazumder 1998, Bakker *et al.* 2006). However, the joint effects of nutrient enrichment and herbivory in sandstone and dolerite grasslands that are more heavily grazed remains an open question.

N deposition may increase grass abundance and reduce plant diversity, even with annual burning

The level of N added in the N addition treatment (10 g/m^2/year) was far larger than typical values of N deposition, which were modeled to range up to 3.5 g/m^2 at Nutrient Network sites (0.5 g/m^2/year in the study region). Nonetheless, these results may indicate effects of N deposition because in other experiments adding even a little N (1 g/m^2/year, comparable with deposition rates in regions with intense agriculture and industry) strongly reduced diversity, comparable with adding N at much higher rates (Clark and Tilman 2008). Our results suggest that, as elsewhere, N deposition may increase grass abundance and reduce plant diversity. Depending on which grass species are favored, increased grass abundance may enhance or compromise livestock production, because grasses include both the most and the least nutritionally valuable species in these grasslands (Tainton 1999). Most of the threatened species in these grasslands are non-grasses, so reduced relative abundance of non-grasses and loss of diversity may be cause for conservation concern (Raimondo *et al.* 2009). The reduction in legume abundance with added nitrogen suggests that the many threatened legume species may be particularly vulnerable.

It has been proposed that frequent burning may prevent N deposition from reducing plant diversity in North American tallgrass prairie (McLauchlan *et al.* 2014), by volatilizing N (Raison *et al.* 1985) and reducing litter accumulation. Our finding that N addition reduced diversity even with annual burning suggests that frequent burning may not always counteract the effects of nutrient addition. This accords with findings in other South African and North American grasslands that adding N at 10 g/m^2/year reduced diversity independently of whether they were unburned or annually burned (Kirkman *et al.* 2014). However, it remains a critical question whether — and in what conditions — the effects of actual N deposition rates on diversity can be mitigated by frequent burning.

Multiple nutrient limitation may inhibit restoration

An influence of nutrient limitation on plant diversity and functional composition may make it more difficult to restore grasslands on abandoned agricultural lands after their nutrient supply rates have been increased using fertilizers. Multiple nutrients are added as fertilizer when sandstone grasslands are cultivated for sugar cane and when montane grasslands are cultivated for timber or potatoes. Depending how long these added nutrients remain available, this may be another reason why it is so difficult in this region to re-establish diverse grassland similar in composition to untransformed grassland, in addition to low availability of plant propagules and perhaps symbionts such as mycorrhizae or rhizobia.

Conclusion

Nutrient limitation is an important factor structuring the productivity, diversity and functional composition of threatened dolerite and sandstone grasslands of eastern South Africa. Widespread increases in inputs and perhaps mineralization of nutrients likely pose a challenge to conserving biodiversity in these grasslands; even in conserved grasslands that are frequently burned, diversity might be reduced by N deposition and, perhaps, a warming-driven increase in N mineralization in mountain grasslands. At least during its three year duration, the experiment in dolerite grasslands provided no evidence that herbivores are currently playing a major role in the ecology of these fragmented, nutrient-poor grasslands, at least when they are frequently burned.

Table 5-1: Environmental properties of Nutrient Network sites.

Vegetation, climate and soil properties in dolerite grassland at Gilboa and sandstone grassland at Summerveld before treatments were applied, averaged across all plots. Percentiles place these sites' values in the context of 55-77 other Nutrient Network grassland sites (depending on the measure). N deposition is from the modeled output of (Dentener 2006), and climate variables are from WORLDCLIM (Hijmans *et al.* 2005).

	Gilboa		Summerveld	
	Value	*Percentile*	Value	*Percentile*
Vegetation				
Aboveground productivity (g/m^2/year)	280.5	*52%*	313.1	*61%*
Effective species richness (species per m^2)	11.2	*91%*	15.6	*96%*
Species richness (species per m^2)	23.8	*91%*	30.4	*96%*
Total species at site	74	*79%*	105	*94%*
Physical factors				
Elevation (m)	1748	*88%*	679	*66%*
Absolute latitude (degrees)	29.3	*13%*	29.8	*16%*
Mean maximum temperature, warmest month (C)	22.3	*21%*	26.0	*40%*
Mean minimum temperature, coolest month (C)	0.3	*55%*	7.3	*92%*
Mean annual precipitation (mm)	926	*68%*	939	*70%*
Mean precipitation in the wettest month (mm)	146	74%	150	75%
Mean precipitation in the dryest month (mm)	13	35%	19	44%
N deposition (g/m^2/yr)	0.50	*55%*	0.50	*55%*
Soil				
C (%)	20.6	*98%*	6.5	*71%*
N (%)	1.16	*96%*	0.33	*60%*
P (ppm)	17.7	*25%*	12.1	*11%*
K (ppm)	129.3	*40%*	84.0	*11%*
Mg (ppm)	44.4	*5%*	124.0	*33%*
Ca (ppm)	269.2	*2%*	536.6	*18%*
S (ppm)	47.1	*89%*	32.5	*67%*
Na (ppm)	36.5	*53%*	53.2	*75%*
Zn (ppm)	2.0	*36%*	1.3	*15%*
Mn (ppm)	16.4	*13%*	11.1	*4%*
Fe (ppm)	113.5	*33%*	141.9	*42%*
Cu (ppm)	2.3	*60%*	1.5	*20%*
B (ppm)	0.4	*38%*	0.6	*55%*
pH	5.1	*16%*	5.1	*18%*

Table 5-S1: Lists of plant species recorded at each Nutrient Network site during four years of cover sampling.

Voucher specimens of most species are lodged at NU Herbarium, Pietermaritzburg, KwaZulu-Natal, South Africa.

Gilboa

Family	**Species**
Amaryllidaceae	*Tulbaghia acutiloba*
Apocynaceae	*Aspidonepsis reenensis*
Asteraceae	*Euryops laxus*
Asteraceae	*Gazania* sp.
Asteraceae	*Helichrysum acutatum*
Asteraceae	*Helichrysum adenocarpum*
Asteraceae	*Helichrysum aureum*
Asteraceae	*Helichrysum cephaliodeum*
Asteraceae	*Helichrysum krebsianum*
Asteraceae	*Helichrysum pallidum*
Asteraceae	*Senecio adnatus*
Asteraceae	*Senecio coronatus*
Asteraceae	*Senecio erubescens*
Asteraceae	*Senecio speciosus*
Asteraceae	*Senecio subcoriaceus*
Campanulaceae	*Craterocapsa tarsodes*
Commelinaceae	*Cyanotis speciosa*
Crassulaceae	*Crassula vaginata*
Cyperaceae	*Abildgaardia* sp.
Cyperaceae	*Bulbostylis* sp.
Cyperaceae	*Cyperus* sp.
Ericaceae	*Erica woodii*
Euphorbiaceae	*Acalypha peduncularis*
Fabaceae	*Eriosema distinctum*
Fabaceae	*Eriosema kraussianum*
Fabaceae	*Eriosema salignum*
Fabaceae	*Indigofera sp.*
Fabaceae	*Pearsonia grandifolia*
Fabaceae	*Rhynchosia totta*
Gentianaceae	*Sebaea filiformis*

Hyacinthaceae	*Ledebouria ovatifolia*
Hypoxidaceae	*Hypoxis gerrardii*
Hypoxidaceae	*Hypoxis* sp.
Iridaceae	*Dierama* sp.
Iridaceae	*Dierama insigne*
Iridaceae	*Hesperantha baurii*
Iridaceae	*Moraea stricta*
Iridaceae	*Watsonia lepida*
Molluginaceae	*Psammotropha* sp.
Orchidaceae	*Disa pulchra*
Orchidaceae	*Disa stachyoides*
Orchidaceae	*Disa versicolor*
Poaceae	*Andropogon schirensis*
Poaceae	*Digitaria diagonalis*
Poaceae	*Diheteropogon amplectens*
Poaceae	*Diheteropogon filifolius*
Poaceae	*Elionurus muticus*
Poaceae	*Eragrostis racemosa*
Poaceae	*Festuca scabra*
Poaceae	*Heteropogon contortus*
Poaceae	*Loudetia simplex*
Poaceae	*Melinis nerviglumis*
Poaceae	*Microchloa caffra*
Poaceae	*Monocymbium ceresiiforme*
Poaceae	*Panicum ecklonii*
Poaceae	*Rendlia altera*
Poaceae	*Sporobolus centrifugus*
Poaceae	*Trachypogon spicatus*
Poaceae	*Tristachya leucothrix*
Polygalaceae	*Muraltia saxicola*
Polygalaceae	*Polygala hottentotta*
Proteaceae	*Protea simplex*
Rubiaceae	*Anthospermum herbaceum*
Rubiaceae	*Kohautia amatymbica*
Santalaceae	*Thesium goetzeanum*
Scrophulariaceae	*Alectra sessiliflora*

Scrophulariaceae	*Diclis reptans*
Scrophulariaceae	*Graderia scabra*
Scrophulariaceae	*Hebenstretia dura*
Scrophulariaceae	*Zaluzianskya natalensis*

<u>Summerveld</u>

Family	**Species**
Acanthaceae	*Chaetacanthus burchellii*
Acanthaceae	*Crabbea nana*
Acanthaceae	*Thunbergia atriplicifolia*
Agavaceae	*Chlorophytum angulicaule*
Amaryllidaceae	*Tulbaghia acutiloba*
Apiaceae	*Alepidea longifolia*
Apocynaceae	*Asclepias albens*
Asteraceae	*Aster bakerianus*
Asteraceae	*Gazania krebsiana*
Asteraceae	*Gerbera ambigua*
Asteraceae	*Gerbera piloselloides*
Asteraceae	*Gerbera viridifolia*
Asteraceae	*Helichrysum adenocarpum*
Asteraceae	*Helichrysum aureonitens*
Asteraceae	*Helichrysum aureum*
Asteraceae	*Helichrysum longifolium*
Asteraceae	*Helichrysum miconiifolium*
Asteraceae	*Helichrysum mixtum*
Asteraceae	*Helichrysum pannosum*
Asteraceae	*Helichrysum pilosellum*
Asteraceae	*Lactuca* sp.
Asteraceae	*Senecio erubescens*
Asteraceae	*Senecio glaberrimus*
Asteraceae	*Senecio umgeniensis*
Asteraceae	*Senecio variabilis*
Asteraceae	*Vernonia gerrardii*
Asteraceae	*Vernonia natalensis*
Asteraceae	*Vernonia oligocephala*
Caryophyllaceae	*Dianthus zeyheri*

Commelinaceae	*Commelina africana*
Commelinaceae	*Cyanotis speciosa*
Convolvulaceae	*Convolvulus natalensis*
Cyperaceae	*Abildgaardia ovata*
Cyperaceae	*Bulbostylis contexta*
Cyperaceae	*Cyperus obtusiflorus*
Cyperaceae	*Cyperus tenax*
Euphorbiaceae	*Euphorbia gueinzii*
Euphorbiaceae	*Euphorbia striata*
Euphorbiaceae	*Phyllanthus glaucophyllus*
Fabaceae	*Aeschynomene micrantha*
Fabaceae	*Argyrolobium rotundifolium*
Fabaceae	*Argyrolobium tuberosum*
Fabaceae	*Chamaecrista capensis*
Fabaceae	*Desmodium dregeanum*
Fabaceae	*Eriosema kraussianum*
Fabaceae	*Indigofera hilaris*
Fabaceae	*Indigofera rubroglandulosa*
Fabaceae	*Lotononis corymbosa*
Fabaceae	*Rhynchosia totta*
Fabaceae	*Tephrosia macropoda*
Fabaceae	*Zornia capensis*
Gentianaceae	*Sebaea grandis*
Gentianaceae	*Sebaea* sp.
Hyacinthaceae	*Albuca setosa*
Hypoxidaceae	*Hypoxis gerrardii*
Hypoxidaceae	*Hypoxis rigidula*
Iridaceae	*Aristea cognata*
Iridaceae	*Aristea woodii*
Iridaceae	*Dierama argyreum*
Iridaceae	*Moraea elliotii*
Iridaceae	*Watsonia densiflora*
Lamiaceae	*Becium obovatum*
Lamiaceae	*Rotheca hirsuta*
Lamiaceae	*Syncolostemon parviflorus*
Molluginaceae	*Psammotropha myriantha*

Orchidaceae	*Brachycorythis ovata*
Orchidaceae	*Eulophia* sp.
Orchidaceae	*Eulophia tenella*
Orchidaceae	*Habenaria dives*
Orchidaceae	*Satyrium longicauda*
Poaceae	*Alloteropsis semialata*
Poaceae	*Andropogon schirensis*
Poaceae	*Aristida junciformis*
Poaceae	*Brachiaria serrata*
Poaceae	*Cymbopogon excavatus*
Poaceae	*Digitaria eriantha*
Poaceae	*Diheteropogon amplectens*
Poaceae	*Elionurus muticus*
Poaceae	*Eragrostis capensis*
Poaceae	*Eragrostis curvula*
Poaceae	*Eragrostis racemosa*
Poaceae	*Eulalia villosa*
Poaceae	*Heteropogon contortus*
Poaceae	*Hyparrhenia hirta*
Poaceae	*Loudetia simplex*
Poaceae	*Melinis nerviglumis*
Poaceae	*Microchloa caffra*
Poaceae	*Monocymbium ceresiiforme*
Poaceae	*Panicum ecklonii*
Poaceae	*Panicum natalense*
Poaceae	*Rendlia altera*
Poaceae	*Sporobolus africanus*
Poaceae	*Sporobolus stapfianus*
Poaceae	*Themeda triandra*
Poaceae	*Trachypogon spicatus*
Poaceae	*Tristachya leucothrix*
Polygalaceae	*Polygala hottentotta*
Rubiaceae	*Agathisanthemum chlorophyllum*
Rubiaceae	*Anthospermum herbaceum*
Rubiaceae	*Kohautia amatymbica*
Rubiaceae	*Pentanisia angustifolia*

Rubiaceae	*Pentanisia prunelloides*
Scrophulariaceae	*Selago peduncularis*
Scrophulariaceae	*Tetraselago natalensis*
Scrophulariaceae	*Zaluzianskya natalensis*

Figure 5-1: Effects of nutrient and fencing treatments on aboveground biomass.

Effects of A) factorial nutrient additions and B) addition of all nutrients and fencing on aboveground productivity after applying treatments for three years, approximated by peak standing biomass in these annually burned grasslands. Symbols are treatment means (+- standard errors). P represents the P+Ca treatment and K represents the K+micronutrients treatment.

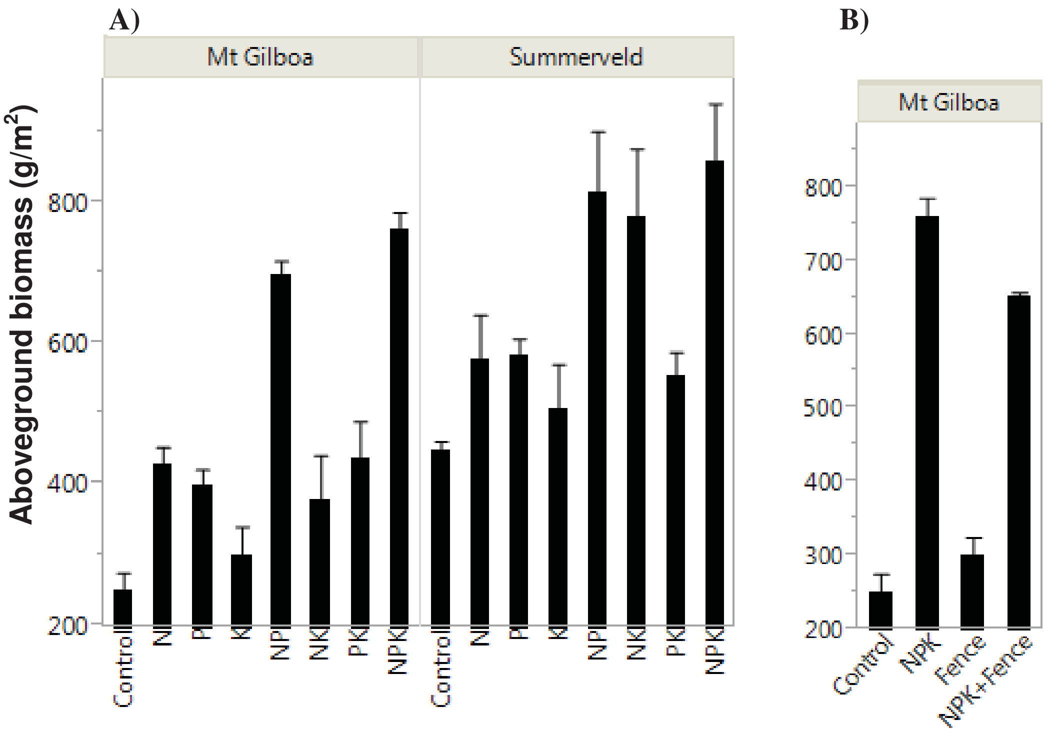


Figure 5-2: Effects of nutrient and fencing treatments on species richness.

Effects of (A,B) factorial nutrient additions and (C,D) addition of all nutrients and fencing on species richness and effective species richness, e^H, after applying treatments for three years. Symbols are treatment means (+- standard errors). P represents the P+Ca treatment and K represents the K+micronutrients treatment.

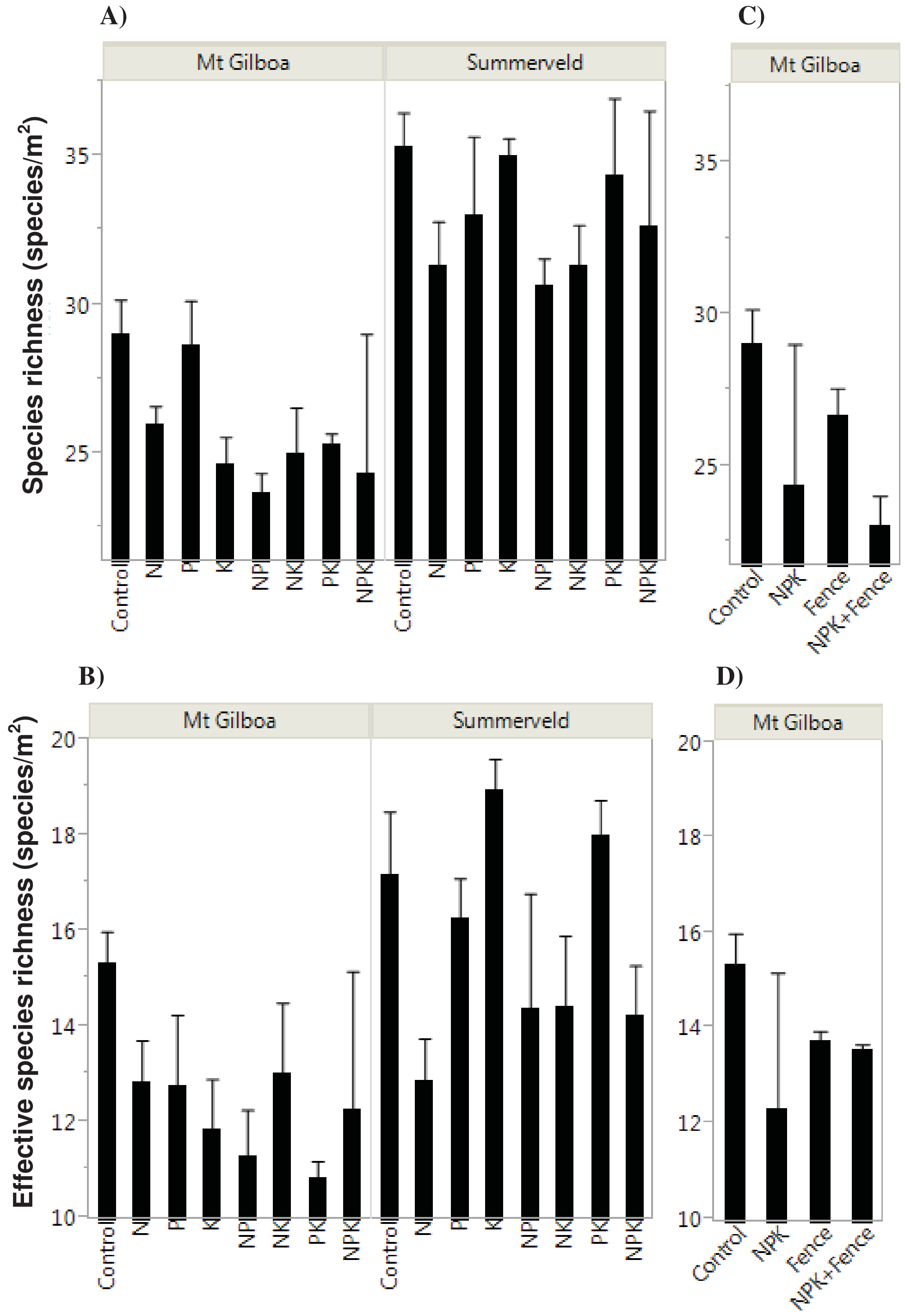


Figure 5-3: Effects of factorial nutrient additions on biomass of functional groups, after applying treatments for three years.

Symbols are treatment means (+- standard errors). P represents the P+Ca treatment and K represents the K+micronutrients treatment. Note different y axis scales.

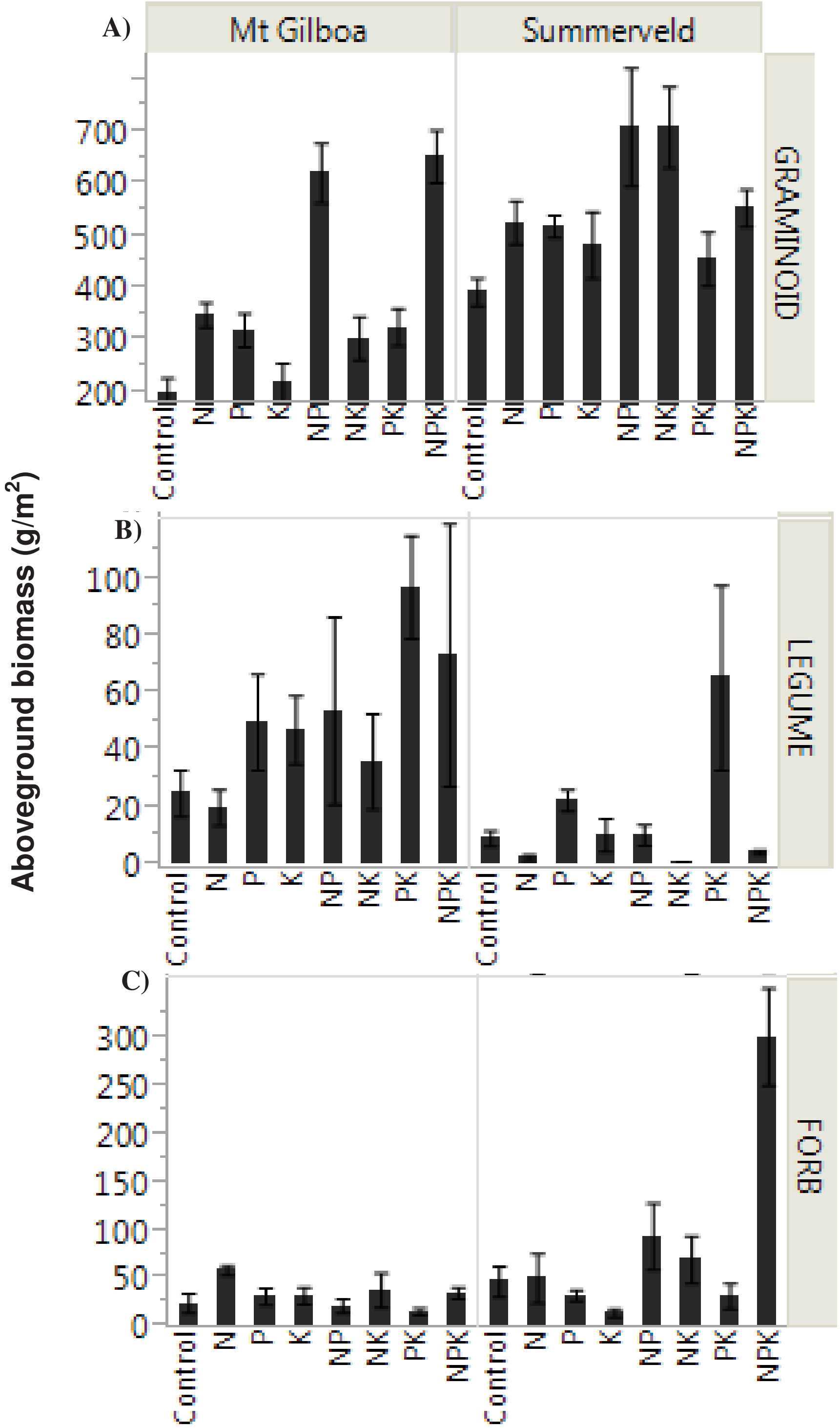


Figure 5-S1: Effects of factorial nutrient additions on relative biomass of functional groups, after applying treatments for three years.

Symbols are treatment means (+- standard errors). P represents the P+Ca treatment and K represents the K+micronutrients treatment. Note different y axis scales.

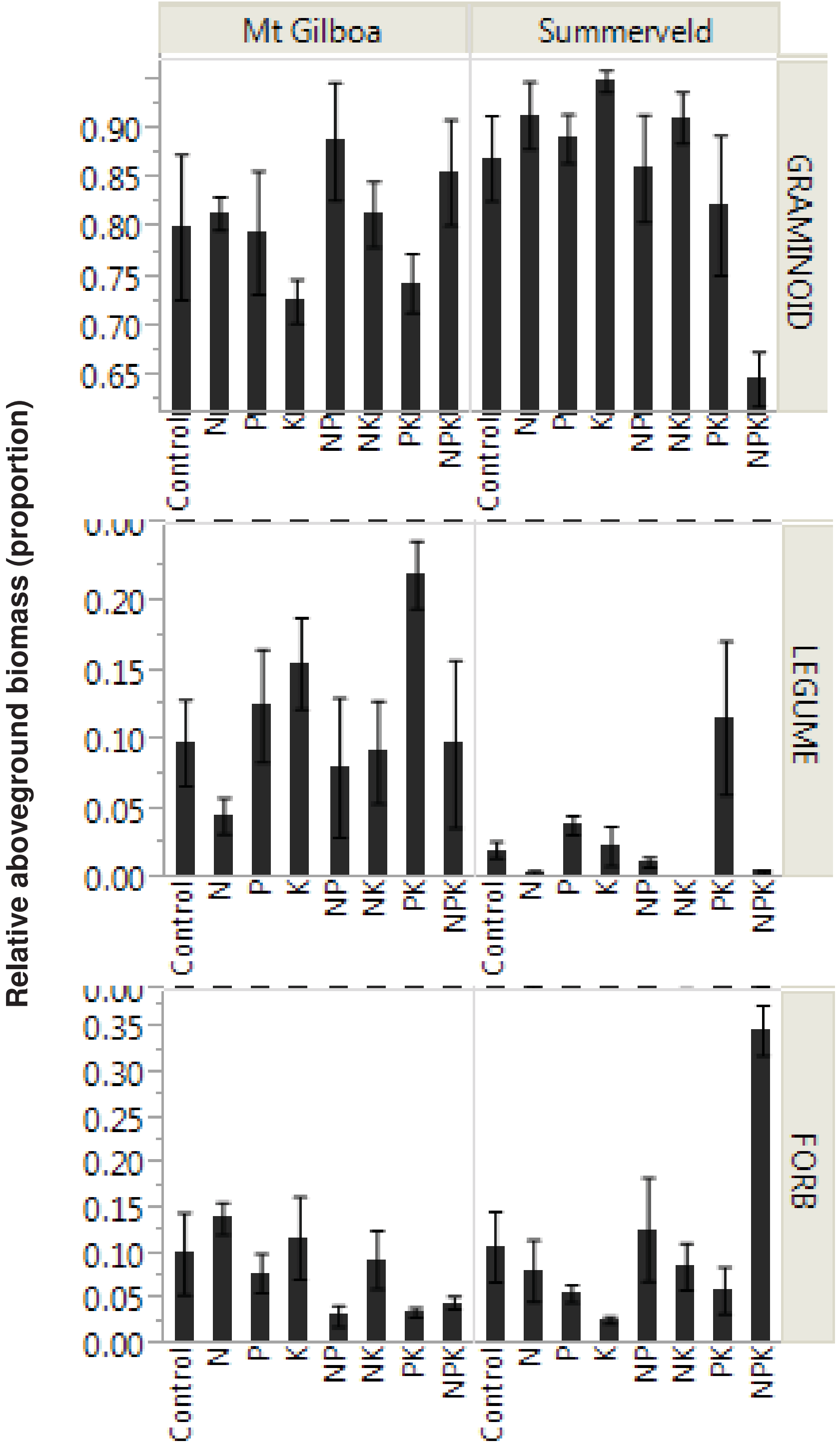
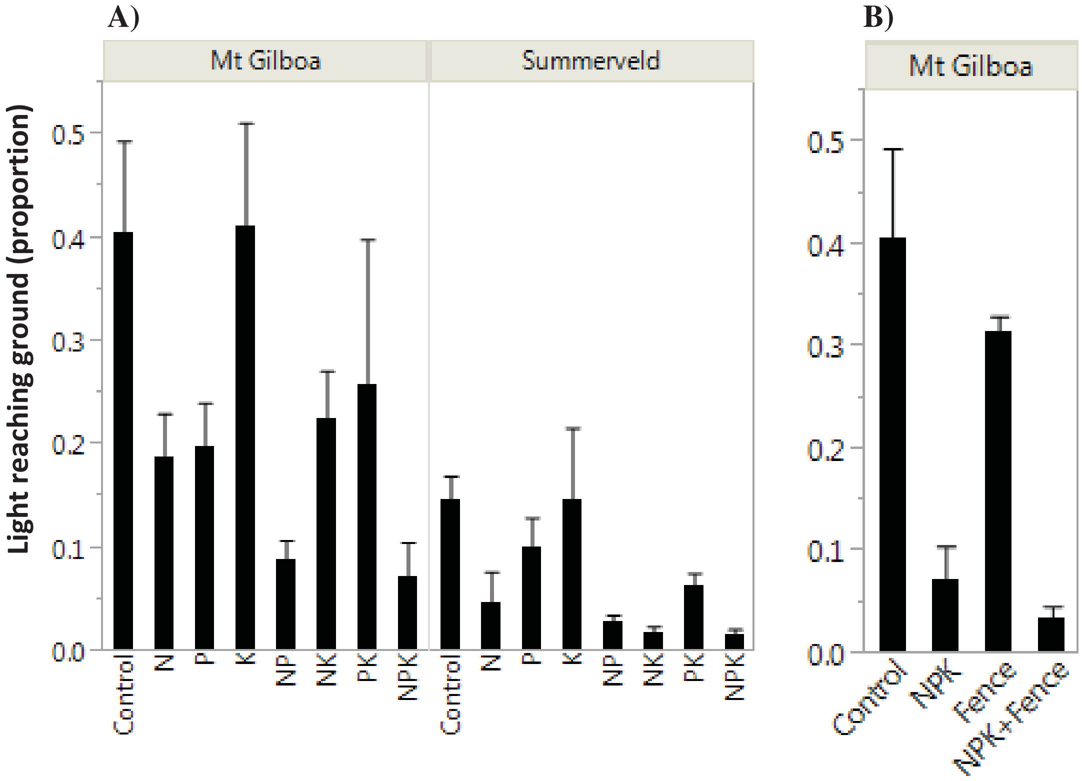


Figure 5-S2: Effects of nutrient and fencing treatments on light availability.

Effects of A) factorial nutrient additions and B) adding all nutrients and fencing on light availability, after applying treatments for three years. Symbols are treatment means (+- standard errors). P represents the P+Ca treatment and K represents the K+micronutrients treatment.



Bibliography

Abades, S. R., A. Gaxiola, and P. A. Marquet. 2014. Fire, percolation thresholds and the savanna forest transition: a neutral model approach. Journal of Ecology 102:1386-1393.

Adler, P. B., E. W. Seabloom, E. T. Borer, H. Hillebrand, Y. Hautier, A. Hector, W. S. Harpole, L. R. O'Halloran, J. B. Grace, T. M. Anderson, J. D. Bakker, L. A. Biederman, C. S. Brown, Y. M. Buckley, L. B. Calabrese, C.-J. Chu, E. E. Cleland, S. L. Collins, K. L. Cottingham, M. J. Crawley, E. I. Damschen, K. F. Davies, N. M. DeCrappeo, P. A. Fay, J. Firn, P. Frater, E. I. Gasarch, D. S. Gruner, N. Hagenah, J. H. R. Lambers, H. Humphries, V. L. Jin, A. D. Kay, K. P. Kirkman, J. A. Klein, J. M. H. Knops, K. J. La Pierre, J. G. Lambrinos, W. Li, A. S. MacDougall, R. L. McCulley, B. A. Melbourne, C. E. Mitchell, J. L. Moore, J. W. Morgan, B. Mortensen, J. L. Orrock, S. M. Prober, D. A. Pyke, A. C. Risch, M. Schuetz, M. D. Smith, C. J. Stevens, L. L. Sullivan, G. Wang, P. D. Wragg, J. P. Wright, and L. H. Yang. 2011. Productivity Is a Poor Predictor of Plant Species Richness. Science 333:1750-1753.

Alexander, M. E. and M. G. Cruz. 2012. Interdependencies between flame length and fireline intensity in predicting crown fire initiation and crown scorch height. International Journal of Wildland Fire 21:95-113.

Archibald, S., C. E. R. Lehmann, J. L. Gómez-Dans, and R. A. Bradstock. 2013. Defining pyromes and global syndromes of fire regimes. Proceedings of the National Academy of Sciences 110:6442-6447.

Archibald, S., D. P. Roy, B. W. Van Wilgen, and R. J. Scholes. 2009. What limits fire? An examination of drivers of burnt area in Southern Africa. Global Change Biology 15:613-630.

Aronson, E. L. and S. G. McNulty. 2009. Appropriate experimental ecosystem warming methods by ecosystem, objective, and practicality. Agricultural and Forest Meteorology 149:1791-1799.

Baker, H. G. 1972. Seed weight in relation to environmental conditions in California. Ecology 53:997-1010.

Bakker, E. S., M. E. Ritchie, H. Olff, D. G. Milchunas, and J. M. H. Knops. 2006. Herbivore impact on grassland plant diversity depends on habitat productivity and herbivore size. Ecology Letters 9:780-789.

Balch, J. K., B. A. Bradley, C. M. D'Antonio, and J. Gómez-Dans. 2013. Introduced annual grass increases regional fire activity across the arid western USA (1980–2009). Global Change Biology 19:173-183.

BassiriRad, H., J. Lussenhop, H. Sehtiya, and K. Borden. 2015. Nitrogen deposition potentially contributes to oak regeneration failure in the Midwestern temperate forests of the USA. Oecologia 177:53-63.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. Fitting Linear Mixed-Effects Models using lme4. ArXiv e-prints 1406.5823.

Baudena, M., S. C. Dekker, P. M. van Bodegom, B. Cuesta, S. I. Higgins, V. Lehsten, C. H. Reick, M. Rietkerk, S. Scheiter, Z. Yin, M. A. Zavala, and V. Brovkin. 2015. Forests, savannas, and grasslands: bridging the knowledge gap between ecology and Dynamic Global Vegetation Models. Biogeosciences 12:1833-1848.

Bellingham, P. J. and A. D. Sparrow. 2000. Resprouting as a life history strategy in woody plant communities. Oikos 89:409-416.

Berkowitz, A. R., C. D. Canham, and V. R. Kelly. 1995. Competition vs facilitation of tree seedling growth and survival in early successional communities. Ecology 76:1156-1168.

Blair, J. M. 1997. Fire, N availability, and plant response in grasslands: A test of the transient maxima hypothesis. Ecology 78:2359-2368.

Bobbink, R., K. Hicks, J. Galloway, T. Spranger, R. Alkemade, M. Ashmore, M. Bustamante, S. Cinderby, E. Davidson, F. Dentener, B. Emmett, J. W. Erisman, M. Fenn, F. Gilliam, A. Nordin, L. Pardo, and W. De Vries. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications 20:30-59.

Bond, W. J. 2008. What limits trees in C_4 grasslands and savannas? Annual Review of Ecology, Evolution, and Systematics 39:641-659.

Bond, W. J. and J. E. Keeley. 2005. Fire as a global ‘herbivore’: the ecology and evolution of flammable ecosystems. Trends in Ecology & Evolution 20:387-394.

Bond, W. J., G. F. Midgley, and F. I. Woodward. 2003. What controls South African vegetation – climate or fire? South African Journal of Botany 69:79-91.

Bond, W. J. and J. J. Midgley. 1995. Kill thy neighbor - an individualistic argument for the evolution of flammability. Oikos 73:79-85.

Bond, W. J. and J. J. Midgley. 2003. The evolutionary ecology of sprouting in woody plants. International Journal of Plant Sciences 164:S103-S114.

Bond, W. J. and C. L. Parr. 2010. Beyond the forest edge: Ecology, diversity and conservation of the grassy biomes. Biological Conservation 143:2395-2404.

Bond, W. J. and B. W. van Wilgen. 1996. Fire and Plants. Chapman & Hall, London.

Bond, W. J., F. I. Woodward, and G. F. Midgley. 2005. The global distribution of ecosystems in a world without fire. New Phytologist 165:525-538.

Borer, E. T., W. S. Harpole, P. B. Adler, E. M. Lind, J. L. Orrock, E. W. Seabloom, and M. D. Smith. 2014a. Finding Generality In Ecology: A Model For Globally Distributed Experiments. Methods in Ecology and Evolution 5:65-73.

Borer, E. T., E. W. Seabloom, D. S. Gruner, W. S. Harpole, H. Hillebrand, E. M. Lind, P. B. Adler, J. Alberti, T. M. Anderson, J. D. Bakker, L. Biederman, D. Blumenthal, C. S. Brown, L. A. Brudvig, Y. M. Buckley, M. Cadotte, C. Chu, E. E. Cleland, M. J. Crawley, P. Daleo, E. I. Damschen, K. F. Davies, N. M. DeCrappeo, G. Du, J. Firn, Y. Hautier, R. W. Heckman, A. Hector, J. HilleRisLambers, O. Iribarne, J. A. Klein, J. M. H. Knops, K. J. La Pierre, A. D. B. Leakey, W. Li, A. S. MacDougall, R. L. McCulley, B. A. Melbourne, C. E. Mitchell, J. L. Moore, B. Mortensen, L. R. O'Halloran, J. L. Orrock, J. Pascual, S. M. Prober, D. A. Pyke, A. C. Risch, M. Schuetz, M. D. Smith, C. J. Stevens, L. L. Sullivan, R. J. Williams, P. D. Wragg, J. P. Wright, and L. H. Yang. 2014b. Herbivores and nutrients control grassland plant diversity via light limitation. Nature 508:517-520.

Bowles, M. L. and M. D. Jones. 2012. Repeated burning of eastern tallgrass prairie increases richness and diversity, stabilizing late successional vegetation. Ecological Applications 23:464-478.

Bradstock, R. A. and T. D. Auld. 1995. Soil Temperatures During Experimental Bushfires in Relation to Fire Intensity: Consequences for Legume Germination and Fire Management in South-Eastern Australia. Journal of Applied Ecology 32:76-84.

Bragg, T. B. 1982. Seasonal Variations in Fuel and Fuel Consumption by Fires in a Bluestem Prairie. Ecology 63:7-11.

Briggs, J. M., G. A. Hoch, and L. C. Johnson. 2002. Assessing the Rate, Mechanisms, and Consequences of the Conversion of Tallgrass Prairie to Juniperus virginiana Forest. Ecosystems 5:578-586.

Britton, C. M., J. D. Dodd, and A. T. Weichert. 1976. Energy Values of Plant Species and Litter of an Andropogon-Paspalum Grassland. Journal of Biogeography 3:389-395.

Brooks, M. L., C. M. D'Antonio, D. M. Richardson, J. B. Grace, J. E. Keeley, J. M. Ditomaso, R. J. Hobbs, M. Pellant, and D. Pyke. 2004. Effects of invasive alien plants on fire regimes. BioScience 54:677-688.

Brown, C. S. and K. J. Rice. 2010. Effects of belowground resource use comlementarity on invasion of constructed grassland plant communities. Biological Invasions 12:1319-1334.

Bruno, J. F., J. J. Stachowicz, and M. D. Bertness. 2003. Inclusion of facilitation into ecological theory. Trends in Ecology & Evolution 18:119-125.

Buckley, R. C. 1982. Seed size and seedling establishment in tropical arid dunecrest plants. Biotropica 14:314-315.

Burt-Smith, G. S., J. P. Grime, and D. Tilman. 2003. Seedling resistance to herbivory as a predictor of relative abundance in a synthesised prairie community. Oikos 101:345-353.

Byram, G. M. 1959. Combustion of forest fuels. Pages 61-89 *in* K. P. Davis, editor. Forest fire: control and use. McGraw-Hill, New York.

Caddick, L. R. and P. L. Linder. 2002. Evolutionary strategies for reproduction and dispersal in African Restionaceae. Australian Journal of Botany 50:339-355.

Cadotte, M. W., J. Cavender-Bares, D. Tilman, and T. H. Oakley. 2009. Using phylogenetic, functional and trait diversity to understand patterns of plant community productivity. PLoS ONE 4:e5695.

Canty, A. and B. Ripley. 2015. boot: Bootstrap R (S-Plus) Functions. R package version 1.3-15.

Carbutt, C., T. J. Edwards, R. W. S. Fynn, and R. P. Beckett. 2013. Evidence for temperature limitation of nitrogen mineralisation in the Drakensberg Alpine Centre. South African Journal of Botany 88:447-454.

Cardinale, B. J., K. L. Matulich, D. U. Hooper, J. E. Byrnes, E. Duffy, L. Gamfeldt, P. Balvanera, M. I. O'Connor, and A. Gonzalez. 2011. The functional role of producer diversity in ecosystems. American Journal of Botany 98:572-592.

Cavender-Bares, J., K. Kitajima, and F. A. Bazzaz. 2004. Multiple trait associations in relation to habitat differentiation among 17 Floridian oak species. Ecological Monographs 74:635-662.

Cavender-Bares, J. and P. B. Reich. 2012. Shocks to the system: community assembly of the oak savanna in a 40-year fire frequency experiment. Ecology 93:52-69.

Chadwick, O. A., L. A. Derry, P. M. Vitousek, B. J. Huebert, and L. O. Hedin. 1999. Changing sources of nutrients during four million years of ecosystem development. Nature 397:491.

Chase, J. M. and M. A. Leibold. 2003. Ecological Niches: Linking Classical and Contemporary Approaches. University of Chicago Press, Chicago.

Cheney, N., J. Gould, and W. Catchpole. 1993. The Influence of Fuel, Weather and Fire Shape Variables on Fire-Spread in Grasslands. International Journal of Wildland Fire 3:31-44.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology & Systematics 31:343-366.

Christensen, J. H., B. Hewitson, A. Busuioc, A. Chen, X. Gao, I. Held, R. Jones, R. K. Kolli, W.-T. Kwon, R. Laprise, V. M. Rueda, L. Mearns, C. G. Menéndez, J. Räisänen, A. Rinke, A. Sarr, and P. Whetton. 2007. Regional Climate Projections.*in* S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Clark, C. M., E. E. Cleland, S. L. Collins, J. E. Fargione, L. Gough, K. L. Gross, S. C. Pennings, K. N. Suding, and J. B. Grace. 2007. Environmental and plant community determinants of species loss following nitrogen enrichment. Ecology Letters 10:596-607.

Clark, C. M. and D. Tilman. 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature 451:712-715.

Clarke, P. J., M. J. Lawes, J. J. Midgley, B. B. Lamont, F. Ojeda, G. E. Burrows, N. J. Enright, and K. J. E. Knox. 2013. Resprouting as a key functional trait: how buds, protection and resources drive persistence after fire. New Phytologist 197:19-35.

Collins, S. L. 1987. Interaction of Disturbances in Tallgrass Prairie: A Field Experiment. Ecology 68:1243-1250.

Collins, S. L. and M. D. Smith. 2006. Scale-Dependent Interaction Of Fire And Grazing On Community Heterogeneity In Tallgrass Prairie. Ecology 87:2058-2067.

Cornwell, W. K., J. H. C. Cornelissen, K. Amatangelo, E. Dorrepaal, V. T. Eviner, O. Godoy, S. E. Hobbie, B. Hoorens, H. Kurokawa, N. Perez-Harguindeguy, H. M. Quested, L. S. Santiago, D. A. Wardle, I. J. Wright, R. Aerts, S. D. Allison, P. van Bodegom, V. Brovkin, A. Chatain, T. V. Callaghan, S. Diaz, E. Garnier, D. E. Gurvich, E. Kazakou, J. A. Klein, J. Read, P. B. Reich, N. A. Soudzilovskaia, M. V. Vaieretti, and M. Westoby. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters 11:1065-1071.

Cottam, G. 1949. The Phytosociology of an Oak Woods in Southwestern Wisconsin. Ecology 30:271-287.

Cowles, J. M. and D. Tilman. in prep. Deep soil net nitrogen mineralization, rooting depth, and aboveground productivity in a grassland biodiversity and warming experiment.

Cowles, J. M., P. D. Wragg, A. J. Wright, J. S. Powers, and D. Tilman. in revision. Shifting plant community structure drives positive interactive effects of warming and diversity on aboveground net primary productivity.

Cox, J. T. and R. Durrett. 1988. Limit theorems for the spread of epidemics and forest fires. Stochastic Processes and their Applications 30:171-191.

Craine, J. M., D. M. Berin, P. B. Reich, D. G. Tilman, and J. M. H. Knops. 1999. Measurement of leaf longevity of 14 species of grasses and forbs using a novel approach. New Phytologist 142:475-481.

Craine, J. M., D. Tilman, D. Wedin, P. Reich, M. Tjoelker, and J. Knops. 2002. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. Functional Ecology 16:563-574.

D'Antonio, C. M. 2000. Fire, plant invasions, and global changes. Pages 65-93 *in* H. A. Mooney and R. J. Hobbs, editors. Invasive species in a changing world. Island Press.

D'Antonio, C. M. and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics:63-87.

Davis, M. A., L. Bier, E. Bushelle, C. Diegel, A. Johnson, and B. Kujala. 2005. Non-indigenous grasses impede woody succession. Plant Ecology 178:249-264.

Davis, M. A., P. B. Reich, M. J. B. Knoll, L. E. E. Dooley, M. Hundtoft, and I. Attleson. 2007. Elevated atmospheric CO_2: a nurse plant substitute for oak seedlings establishing in old fields. Global Change Biology 13:2308-2316.

Davis, M. A., K. J. Wrage, and P. B. Reich. 1998. Competition between tree seedlings and herbaceous vegetation: support for a theory of resource supply and demand. Journal of Ecology 86:652-661.

Davis, M. A., K. J. Wrage, P. B. Reich, M. G. Tjoelker, T. Schaeffer, and C. Muermann. 1999. Survival, growth, and photosynthesis of tree seedlings competing with herbaceous vegetation along a water-light-nitrogen gradient. Plant Ecology 145:341-350.

de Magalhães, R. M. Q. and D. W. Schwilk. 2012. Leaf traits and litter flammability: evidence for non-additive mixture effects in a temperate forest. Journal of Ecology 100:1153-1163.

Dentener, F. 2006. Global Maps of Atmospheric Nitrogen Deposition, 1860, 1993, and 2050. Data set. Available on-line [http://daac.ornl.gov/] from Oak Ridge National Laboratory Distributed Active Archive Center, Oak Ridge, Tennessee, USA.

Driver, A., K. Maze, M. Rouget, A. T. Lombard, J. Nel, J. K. Turpie, R. M. Cowling, P. Desmet, P. Goodman, J. Harris, Z. Jonas, B. Reyers, K. Sink, and T. Strauss. 2005. National Spatial Biodiversity Assessment 2004: Priorities for Biodiversity Conservation in South Africa. South African National Biodiversity Institute, Pretoria.

Dybzinski, R., J. E. Fargione, D. R. Zak, D. Fornara, and D. Tilman. 2008. Soil fertility increases with plant species diversity in a long-term biodiversity experiment. Oecologia 158:85-93.

Edwards, E. J. and S. A. Smith. 2010. Phylogenetic analyses reveal the shady history of C_4 grasses. Proceedings of the National Academy of Sciences 107:2532-2538.

Ehleringer, J. R. and R. K. Monson. 1993. Evolutionary and Ecological Aspects of Photosynthetic Pathway Variation. Annual Review of Ecology and Systematics 24:411-439.

Elser, J. J., M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters 10:1135-1142.

Elton, C. S. 1927. Animal Ecology. London.

Elton, C. S. 1958. The Ecology of Invasions by Animals and Plants. Methuen, London.

Emery, S. M. and K. L. Gross. 2006. Dominant species identity regulates invasibility of old-field plant communities. Oikos 115:549-558.

Engber, E. A. and J. M. Varner. 2012. Patterns of flammability of the California oaks: the role of leaf traits. Canadian Journal of Forest Research 42:1965-1975.

Engle, D. M. and T. G. Bidwell. 2001. Viewpoint: The Response of Central North American Prairies to Seasonal Fire. Journal of Range Management 54:2-10.

Fargione, J., C. S. Brown, and D. Tilman. 2003. Community assembly and invasion: An experimental test of neutral versus niche processes. Proceedings of the National Academy of Sciences of the United States of America 100:8916-8920.

Fargione, J. and D. Tilman. 2005a. Niche differences in phenology and rooting depth promote coexistence with a dominant C_4 bunchgrass. Oecologia 143:598-606.

Fargione, J. and D. Tilman. 2006. Plant species traits and capacity for resource reduction predict yield and abundance under competition in nitrogen-limited grassland. Functional Ecology 20:533-541.

Fargione, J., D. Tilman, R. Dybzinski, J. Lambers, C. Clark, W. Harpole, J. Knops, P. Reich, and M. Loreau. 2007. From selection to complementarity: shifts in the causes of biodiversity-productivity relationships in a long-term biodiversity experiment. Proceedings of the Royal Society B: Biological Sciences:FirstCite-FirstCite.

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

Fay, P. A., S. M. Prober, H. W. Stanley, J. M. H. Knops, J. D. Bakker, E. T. Borer, E. M. Lind, A. S. MacDougall, E. W. Seabloom, P. D. Wragg, P. B. Adler, D. M. Blumenthal, Y. M. Buckley, C. Chu, E. E. Cleland, S. L. Collins, K. F. Davies, G. Du, X. Feng, J. Firn, D. S. Gruner, N. Hagenah, Y. Hautier, R. W. Heckman, V. L. Jin, K. P. Kirkman, J. Klein, L. M. Ladwig, Q. Li, R. L. McCulley, B. A. Melbourne, C. E. Mitchell, J. L. Moore, J. W. Morgan, A. C. Risch, M. Schütz, C. J. Stevens, D. A. Wedin, and Y. L. H. 2015. Grassland productivity limited by multiple nutrients. Nature Plants 1.

Fornara, D. and D. Tilman. 2009. Ecological mechanisms associated with the positive diversity-productivity relationship in an N-limited grassland. Ecology 90:408-418.

Fornara, D. A. and D. Tilman. 2008. Plant functional composition influences rates of soil carbon and nitrogen accumulation. Journal of Ecology 96:314-322.

Fornara, D. A., D. Tilman, and S. E. Hobbie. 2009. Linkages between plant functional composition, fine root processes and potential soil N mineralization rates. Journal of Ecology 97:48-56.

Forrestel, E. J., M. J. Donoghue, and M. D. Smith. 2014. Convergent phylogenetic and functional responses to altered fire regimes in mesic savanna grasslands of North America and South Africa. New Phytologist 203:1000-1011.

Fox, B. J. 1987. Species assembly and the evolution of community structure. Evolutionary Ecology 1:201-213.

Fox, J. and S. Weisberg. 2011. An {R} Companion to Applied Regression, Second Edition, Thousand Oaks CA.

Fridley, J. D., J. J. Stachowicz, S. Naeem, D. F. Sax, E. W. Seabloom, M. D. Smith, T. J. Stohlgren, D. Tilman, and B. Von Holle. 2007. The Invasion Paradox: Reconciling pattern and process in species invasions. Ecology 88:3-17.

Fuhlendorf, S. D. and D. M. Engle. 2004. Application of the fire-grazing interaction to restore a shifting mosaic on tallgrass prairie. Journal of Applied Ecology 41:604-614.

Funk, J. L., E. E. Cleland, K. N. Suding, and E. S. Zavaleta. 2008. Restoration through reassembly: plant traits and invasion resistance. Trends in Ecology & Evolution 23:695-703.

Fynn, R. W. S., R. J. Haynes, and T. G. O'Connor. 2003. Burning causes long-term changes in soil organic matter content of a South African grassland. Soil Biology & Biochemistry 35:677-687.

Fynn, R. W. S., C. D. Morris, and T. J. Edwards. 2005. Long-term compositional responses of a South African mesic grassland to burning and mowing. Applied Vegetation Science 8:5-12.

Fynn, R. W. S. and T. G. O'Connor. 2005. Determinants of community organization of a South African mesic grassland. Journal of Vegetation Science 16:93-102.

Galatowitsch, S., L. Frelich, and L. Phillips-Mao. 2009. Regional climate change adaptation strategies for biodiversity conservation in a midcontinental region of North America. Biological Conservation 142:2012-2022.

Ganguli, A. C., D. M. Engle, P. M. Mayer, and E. C. Hellgren. 2008. Plant community diversity and composition provide little resistance to Juniperus encroachment. Botany 86:1416-1426.

Gelman, A. and J. Hill. 2007. Data Analysis Using Regression and Multilevel/Hierarchical Models. Cambridge University Press, Cambridge.

Gibson, D. J., D. C. Hartnett, and G. L. S. Merrill. 1990. Fire Temperature Heterogeneity in Contrasting Fire Prone Habitats: Kansas Tallgrass Prairie and Florida Sandhill. Bulletin of the Torrey Botanical Club 117:349-356.

Gibson, D. J. and L. C. Hulbert. 1987. Effects of fire, topography and year-to-year climatic variation on species composition in tallgrass prairie. Plant Ecology 72:175-185.

Gill, D. S. and P. L. Marks. 1991. Tree and Shrub Seedling Colonization of Old Fields in Central New York. Ecological Monographs 61:183-205.

Gough, L., K. Gross, E. Cleland, C. Clark, S. Collins, J. Fargione, S. Pennings, and K. Suding. 2012. Incorporating clonal growth form clarifies the role of plant height in response to nitrogen addition. Oecologia 169:1053-1062.

Gough, L., C. W. Osenberg, K. L. Gross, and S. L. Collins. 2000. Fertilization effects on species density and primary productivity in herbaceous plant communities. Oikos 89:428-439.

Govender, N., W. S. W. Trollope, and B. W. Van Wilgen. 2006. The effect of fire season, fire frequency, rainfall and management on fire intensity in savanna vegetation in South Africa. Journal of Applied Ecology 43:748-758.

Grady, J. M. and W. A. Hoffmann. 2012. Caught in a fire trap: Recurring fire creates stable size equilibria in woody resprouters. Ecology 93:2052-2060.

Grigulis, K., S. Lavorel, I. D. Davies, A. Dossantos, F. Lloret, Vil, and Montserrat. 2005. Landscape-scale positive feedbacks between fire and expansion of the large tussock grass, *Ampelodesmos mauritanica* in Catalan shrublands. Global Change Biology 11:1042-1053.

Grimm, E. C. 1983. Chronology and dynamics of vegetation change in the prairie-woodland region of southern Minnesota, U.S.A. New Phytologist 93:311-350.

Grimm, E. C. 1984. Fire and other factors controlling the Big Woods vegetation of Minnesota in the mid-nineteenth century. Ecological Monographs 54:291-311.

Grubb, P. J. 1977. The maintenance of species-richness in plant communities: the importance of the regeneration niche. Biological Reviews 52:107-145.

Haddad, N. M., G. M. Crutsinger, K. Gross, J. Haarstad, J. M. H. Knops, and D. Tilman. 2009. Plant species loss decreases arthropod diversity and shifts trophic structure. Ecology Letters 12:1029-1039.

Harpole, W. S., J. T. Ngai, E. E. Cleland, E. W. Seabloom, E. T. Borer, M. E. S. Bracken, J. J. Elser, D. S. Gruner, H. Hillebrand, J. B. Shurin, and J. E. Smith. 2011. Nutrient co-limitation of primary producer communities. Ecology Letters 14:852-862.

Harpole, W. S. and D. Tilman. 2007. Grassland species loss resulting from reduced niche dimension. Nature 446:791-793.

Harte, J. and R. Shaw. 1995. Shifting dominance within a montane vegetation community - results of a climate-warming experiment. Science 267:876-880.

Hautier, Y., P. A. Niklaus, and A. Hector. 2009. Competition for Light Causes Plant Biodiversity Loss After Eutrophication. Science 324:636-638.

Haynes, R. J. and R. S. Swift. 1985. Effects of soil acidification on the chemical extractability of Fe, Mn, Zn and Cu and the growth and micronutrient uptake of highbush blueberry plants. Plant and Soil 84:201-212.

Haynes, W. M., T. J. Bruno, and D. R. Lide, editors. 2014. CRC Handbook of Chemistry and Physics, 95th Edition, Internet Version 2015. CRC Press, Boca Raton.

Hector, A., K. Dobson, A. Minns, E. Bazeley-White, and J. H. Lawton. 2001. Community diversity and invasion resistance: An experimental test in a grassland ecosystem and a review of comparable studies. Ecological Research 16:819-831.

Higgins, S. I., W. J. Bond, E. C. February, A. Bronn, D. I. W. Euston-Brown, B. Enslin, N. Govender, L. Rademan, S. O'Regan, A. L. F. Potgieter, S. Scheiter, R. Sowry, L. Trollope, and W. S. W. Trollope. 2007. Effects of four decades of fire manipulation on woody vegetation structure in savanna. Ecology 88:1119-1125.

Higgins, S. I., W. J. Bond, and W. S. W. Trollope. 2000. Fire, resprouting and variability: a recipe for grass-tree coexistence in savanna. Journal of Ecology 88:213-229.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25:1965-1978.

Hill, M. O. 1973. Diversity and evenness: A unifying notation and its consequences. Ecology 54:427-432.

Hirota, M., M. Holmgren, E. H. Van Nes, and M. Scheffer. 2011. Global Resilience of Tropical Forest and Savanna to Critical Transitions. Science 334:232-235.

Hobbie, S. E. 1992. Effects of plant species on nutrient cycling. Trends in Ecology & Evolution 7:336-339.

Hobbs, N. T., D. S. Schimel, C. E. Owensby, and D. S. Ojima. 1991. Fire and Grazing in the Tallgrass Prairie: Contingent Effects on Nitrogen Budgets. Ecology 72:1374-1382.

Hoffmann, W. A. and O. T. Solbrig. 2003. The role of topkill in the differential response of savanna woody species to fire. Forest Ecology and Management 180:273-286.

Holt, R. D., J. Grover, and D. Tilman. 1994. Simple Rules for Interspecific Dominance in Systems with Exploitative and Apparent Competition. American Naturalist 144:741-771.

Hooper, D. U. and J. S. Dukes. 2010. Functional composition controls invasion success in a California serpentine grassland. Journal of Ecology 98:764-777.

Hopper, S. 2009. OCBIL theory: towards an integrated understanding of the evolution, ecology and conservation of biodiversity on old, climatically buffered, infertile landscapes. Plant and Soil 322:49-86.

Hovenden, M. J., P. C. D. Newton, K. E. Wills, J. K. Janes, A. L. Williams, J. K. Vander Schoor, and M. J. Nolan. 2008. Influence of warming on soil water potential controls seedling mortality in perennial but not annual species in a temperate grassland. New Phytologist 180:143-152.

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

Inouye, R. S., T. D. Allison, and N. C. Johnson. 1994. Old Field Succession on a Minnesota Sand Plain: Effects of Deer and Other Factors on Invasion by Trees. Bulletin of the Torrey Botanical Club 121:266-276.

Iverson, L. R., D. A. Yaussy, J. Rebbeck, T. F. Hutchinson, R. P. Long, and A. M. Prasad. 2004. A comparison of thermocouples and temperature paints to monitor spatial and temporal characteristics of landscape-scale prescribed fires*. International Journal of Wildland Fire 13:311-322.

Jurado, E. and M. Westoby. 1992. Seedling growth in relation to seed size among species of arid Australia. Journal of Ecology 80:407-416.

Kauffman, J. B., D. L. Cummings, and D. E. Ward. 1994. Relationships of Fire, Biomass and Nutrient Dynamics along a Vegetation Gradient in the Brazilian Cerrado. Journal of Ecology 82:519-531.

Kennard, D., K. Outcalt, D. Jones, and J. O'Brien. 2005. Comparing techniques for estimating flame temperature of prescribed fires. Fire Ecology.

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

Kgope, B. S., W. J. Bond, and G. F. Midgley. 2010. Growth responses of African savanna trees implicate atmospheric [CO2] as a driver of past and current changes in savanna tree cover. Austral Ecology 35:451-463.

Kidnie, S. M. 2009. Fuel load and fire behaviour in the southern Ontario tallgrass prairie. Thesis. University of Toronto.

King, L. 1982. The Natal Monocline: Explaining the Origin and Scenery of Natal, South Africa. 2nd edition. University of Natal Press, Pietermaritzburg.

Kirkman, K. P., S. L. Collins, M. D. Smith, A. K. Knapp, D. E. Burkepile, C. E. Burns, R. W. S. Fynn, N. Hagenah, S. E. Koerner, K. J. Matchett, D. I. Thompson, K. R. Wilcox, and P. D. Wragg. 2014. Responses to fire differ between South African and North American grassland communities. Journal of Vegetation Science 25:793-804.

Kitajima, K. and J. A. Myers. 2008. Seedling ecophysiology: strategies toward achievement of positive net carbon balance. Pages 172-188 *in* M. A. Leck, V. T. Parker, and R. L. Simpson, editors. Seedling Ecology and Evolution. Cambridge University Press, Cambridge.

Knapp, A. K. and T. R. Seastedt. 1986. Detritus Accumulation Limits Productivity of Tallgrass Prairie. BioScience 36:662-668.

Knops, J. M. H., K. L. Bradley, and D. A. Wedin. 2002. Mechanisms of plant species impacts on ecosystem nitrogen cycling. Ecology Letters 5:454-466.

Knops, J. M. H., M. E. Ritchie, and D. Tilman. 2000. Selective herbivory on a nitrogen fixing legume (*Lathyrus venosus*) influences productivity and ecosystem nitrogen pools in an oak savanna. Ecoscience 7:166-174.

Knops, J. M. H., D. Wedin, and D. Tilman. 2001. Biodiversity and decomposition in experimental grassland ecosystems. Oecologia 126:429-433.

Kuster, T. M., P. Schleppi, B. Hu, R. Schulin, and M. S. Günthardt-Goerg. 2013. Nitrogen dynamics in oak model ecosystems subjected to air warming and drought on two different soils. Plant Biology 15:220-229.

Kuzyakov, Y. and X. Xu. 2013. Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. New Phytologist 198:656-669.

Lambers, H., S. F. Chapin, and T. Pons. 1998. Plant Physiological Ecology. Springer-Verlag, New York.

Lambers, H. and M. W. Shane. 2007. Phosphorus nutrition of Australian Proteaceae and Cyperaceae: A strategy on old landscapes with prolonged oceanically buffered climates. South African Journal of Botany 73:274-275.

Lavorel, S. and E. Garnier. 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Functional Ecology 16:545-556.

LeBauer, D. S. and K. K. Treseder. 2008. NITROGEN LIMITATION OF NET PRIMARY PRODUCTIVITY IN TERRESTRIAL ECOSYSTEMS IS GLOBALLY DISTRIBUTED. Ecology 89:371-379.

Lehmann, C. E. R., T. M. Anderson, M. Sankaran, S. I. Higgins, S. Archibald, W. A. Hoffmann, N. P. Hanan, R. J. Williams, R. J. Fensham, J. Felfili, L. B. Hutley, J. Ratnam, J. San Jose, R. Montes, D. Franklin, J. Russell-Smith, C. M. Ryan, G. Durigan, P. Hiernaux, R. Haidar, D. M. J. S. Bowman, and W. J. Bond. 2014. Savanna Vegetation-Fire-Climate Relationships Differ Among Continents. Science 343:548-552.

Leishman, M. R. 1999. How well do plant traits correlate with establishment ability? Evidence from a study of 16 calcareous grassland species. New Phytologist 141:487-496.

Leishman, M. R. and M. Westoby. 1994. The role of seed size in seedling establishment in dry soil conditions: experimental evidence from semi-arid species. Journal of Ecology 82.

Levine, J. M. 2000. Species diversity and biological invasions: Relating local process to community pattern. Science 288:852.

Levine, J. M., P. B. Adler, and S. G. Yelenik. 2004. A meta-analysis of biotic resistance to exotic plant invasions. Ecology Letters 7:975-989.

Levine, J. M. and C. M. D'Antonio. 1999. Elton revisited: a review of evidence linking diversity and invasibility. Oikos 87:15-26.

Li, W., X. Zuo, and J. M. H. Knops. 2013. Different Fire Frequency Impacts Over 27 Years on Vegetation Succession in an Infertile Old-Field Grassland. Rangeland Ecology & Management 66:267-273.

Lind, E. M., E. Borer, E. Seabloom, P. Adler, J. D. Bakker, D. M. Blumenthal, M. Crawley, K. Davies, J. Firn, D. S. Gruner, W. S. Harpole, Y. Hautier, H. Hillebrand, J. Knops, B. Melbourne, B. Mortensen, A. C. Risch, M. Schuetz, C. Stevens, and P. D. Wragg. 2013. Life-history constraints in grassland plant species: a growth-defence trade-off is the norm. Ecology Letters 16:513-521.

Lloret, F., J. Peñuelas, and M. Estiarte. 2004. Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. Global Change Biology 10:248-258.

Loreau, M. and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412:72-76.

MacArthur, R. and R. Levins. 1967. The limiting similarity, convergence, and divergence of coexisting species. American Naturalist 101:377-385.

MacArthur, R. H. 1972. Geographical Ecology: Patterns in the Distribution of Species. Princeton University Press, Princeton.

Mack, R. N. and J. N. Thompson. 1982. Evolution in Steppe with Few Large, Hooved Mammals. The American Naturalist 119:757-773.

Martin, R. E., C. T. Cushwa, and R. L. Miller. 1969. Fire as a physical factor in wildland management. Proceedings of the Ninth Annual Tall Timbers Fire Ecology Conference:271-288.

Masubelele, M. L., W. J. Bond, and W. D. Stock. 2007. How savanna grasses decompose? South African Journal of Botany 73:301-301.

McLauchlan, K. K., J. M. Craine, J. B. Nippert, and T. W. Ocheltree. 2014. Lack of eutrophication in a tallgrass prairie ecosystem over 27 years. Ecology 95:1225-1235.

McLaughlin, S. B. and R. Wimmer. 1999. Calcium physiology and terrestrial ecosystem processes. New Phytologist 142:373-417.

Mitchell, C. E., D. Tilman, and J. V. Groth. 2002. Effects of grassland plant species diversity, abundance, and composition on foliar fungal disease. Ecology 83:1713-1726.

Moles, A. T. and M. Westoby. 2004. Seedling survival and seed size: a synthesis of the literature. Journal of Ecology 92:372-383.

Moncrieff, G. R., S. Scheiter, W. J. Bond, and S. I. Higgins. 2013. Increasing atmospheric CO_2 overrides the historical legacy of multiple stable biome states in Africa. New Phytologist:Early View.

Mucina, L. and M. C. Rutherford, editors. 2006. The Vegetation of South Africa, Lesotho and Swaziland. South African National Biodiversity Institute, Pretoria.

Mueller, K. E., S. E. Hobbie, D. Tilman, and P. B. Reich. 2013. Effects of plant diversity, N fertilization, and elevated carbon dioxide on grassland soil N cycling in a long-term experiment. Global Change Biology 19:1249-1261.

Murphy, B. P. and D. M. J. S. Bowman. 2012. What controls the distribution of tropical forest and savanna? Ecology Letters 15:748-758.

Murray, B. R., L. K. Hardstaff, and M. L. Phillips. 2013. Differences in Leaf Flammability, Leaf Traits and Flammability-Trait Relationships between Native and Exotic Plant Species of Dry Sclerophyll Forest. PLoS ONE 8:e79205.

Mwangi, P., M. Schmitz, C. Scherber, C. Roscher, J. Schumacher, M. Scherer-Lorenzen, W. Weisser, and B. Schmid. 2007. Niche pre-emption increases with species richness in experimental plant communities. Journal of Ecology 95:65-79.

Myster, R. 1993. Tree invasion and establishment in old fields at Hutcheson Memorial Forest. The Botanical Review 59:251-272.

Neary, D. G., C. C. Klopatek, L. F. DeBano, and P. F. Ffolliott. 1999. Fire effects on belowground sustainability: a review and synthesis. Forest Ecology and Management 122:51-71.

O'Connor, T. G. 2005. Influence of land use on plant community composition and diversity in Highland Sourveld grassland in the southern Drakensberg, South Africa. Journal of Applied Ecology 42:975-988.

O'Neill, R. V., B. T. Milne, M. G. Turner, and R. H. Gardner. 1988. Resource utilization scales and landscape pattern. Landscape Ecology 2:63-69.

O’Connor, T. G., G. Martindale, C. D. Morris, A. Short, T. F. Witkowski, and R. Scott-Shaw. 2011. Influence of Grazing Management on Plant Diversity of Highland Sourveld Grassland, KwaZulu-Natal, South Africa. Rangeland Ecology & Management 64:196-207.

O’Neill, R. V., R. H. Gardner, M. G. Turner, and W. H. Romme. 1992. Epidemiology theory and disturbance spread on landscapes. Landscape Ecology 7:19-26.

Ohtsuki, T. and T. Keyes. 1986. Biased percolation: forest fires with wind. Journal of Physics A: Mathematical and General 19:L281.

Ojima, D., D. S. Schimel, W. J. Parton, and C. E. Owensby. 1994. Long- and short-term effects of fire on nitrogen cycling in tallgrass prairie. Biogeochemistry 24:67-84.

Olff, H. and M. E. Ritchie. 1998. Effects of herbivores on grassland plant diversity. Trends in Ecology & Evolution 13:261-265.

Onodi, G., M. Kertesz, Z. Botta-Dukat, and V. Altbacker. 2008. Grazing effects on vegetation composition and on the spread of fire on open sand grasslands. Arid Land Research and Management 22:273-285.

Parmesan, C., C. Duarte, E. Poloczanska, A. J. Richardson, and M. C. Singer. 2011. Overstretching attribution. Nature Climate Change 1:2-4.

Pausas, J. G. 2014. Bark thickness and fire regime. Functional Ecology 29: 315-327.

Pellegrini, A. F. A., L. O. Hedin, A. C. Staver, and N. Govender. 2014. Fire alters ecosystem carbon and nutrients but not plant nutrient stoichiometry or composition in tropical savanna. Ecology.

Petermann, J. S., A. J. F. Fergus, C. Roscher, L. A. Turnbull, A. Weigelt, and B. Schmid. 2010. Biology, chance, or history? The predictable reassembly of temperate grassland communities. Ecology 91:408-421.

Peterson, D. and P. Reich. 2008. Fire frequency and tree canopy structure influence plant species diversity in a forest-grassland ecotone. Plant Ecology 194:5-16.

Peterson, D. W. and P. B. Reich. 2001. Prescribed fire in oak savanna: fire frequency effects on stand structure and dynamics. Ecological Applications 11:914-927.

Peterson, D. W., P. B. Reich, and K. J. Wrage. 2007. Plant functional group responses to fire frequency and tree canopy cover gradients in oak savannas and woodlands. Journal of Vegetation Science 18:3-12.

Pierce, R. L. 1954. Vegetation cover types and land use history of the Cedar Creek Natural History Reservation, Anoka and Isanti Counties, Minnesota. Thesis. University of Minnesota, St. Paul, Minnesota, USA.

Porensky, L. M. and K. E. Veblen. 2012. Grasses and browsers reinforce landscape heterogeneity by excluding trees from ecosystem hotspots. Oecologia 168:749-759.

Poulsen, J. R., C. W. Osenberg, C. J. Clark, D. J. Levey, and B. M. Bolker. 2007. Plants as Reef Fish: Fitting the Functional Form of Seedling Recruitment. The American Naturalist 170:167-183.

Price, J. N. and J. W. Morgan. 2008. Woody plant encroachment reduces species richness of herb-rich woodlands in southern Australia. Austral Ecology 33:278-289.

Prober, S. M., J. W. Leff, S. T. Bates, E. T. Borer, J. Firn, W. S. Harpole, E. M. Lind, E. W. Seabloom, P. B. Adler, J. D. Bakker, E. E. Cleland, N. M. DeCrappeo, E. DeLorenze, N. Hagenah, Y. Hautier, K. S. Hofmockel, K. P. Kirkman, J. M. H. Knops, K. J. La Pierre, A. S. MacDougall, R. L. McCulley, C. E. Mitchell, A. C. Risch, M. Schuetz, C. J. Stevens, R. J. Williams, and N. Fierer. 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. Ecology Letters 18:85-95.

Proulx, M. and A. Mazumder. 1998. Reversal of grazing impact on plant species richness in nutrient-poor vs. nutrient-rich ecosystems. Ecology 79:2581-2592.

Quinn, G. P. and M. J. Keough. 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press, Cambridge, UK.

R Development Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Raimondo, D., L. von Staden, W. Foden, J. Victor, N. Helme, R. Turner, D. Kamundi, and P. Manyama, editors. 2009. Red List of South African plants. South African National Biodiversity Institute, Pretoria.

Raison, R. J., P. K. Khanna, and P. V. Woods. 1985. Mechanisms of element transfer to the atmosphere during vegetation fires. Canadian Journal of Forest Research 15:132-140.

Ratajczak, Z. and J. B. Nippert. 2012. Comment on “Global Resilience of Tropical Forest and Savanna to Critical Transitions”. Science 336:541.

Ratajczak, Z., J. B. Nippert, and S. L. Collins. 2011. Woody encroachment decreases diversity across North American grasslands and savannas. Ecology 93:697-703.

Reich, P. B., C. Buschena, M. G. Tjoelker, K. Wrage, J. Knops, D. Tilman, and J. L. Machado. 2003. Variation in growth rate and ecophysiology among 34 grassland and savanna species under contrasting N supply: a test of functional group differences. New Phytologist 157:617-631.

Reich, P. B., D. W. Peterson, D. A. Wedin, and K. Wrage. 2001. Fire and vegetation effects on productivity and nitrogen cycling across a forest–grassland continuum. Ecology 82:1703-1719.

Reich, P. B., D. Tilman, F. Isbell, K. Mueller, S. E. Hobbie, D. F. B. Flynn, and N. Eisenhauer. 2012. Impacts of Biodiversity Loss Escalate Through Time as Redundancy Fades. Science 336:589-592.

Reich, P. B., I. J. Wright, and C. H. Lusk. 2007. Predicting leaf physiology from simple plant and climate attributes: a global GLOPNET analysis. Ecological Applications 17:1982-1988.

Ripley, B., G. Donald, C. P. Osborne, T. Abraham, and T. Martin. 2010. Experimental investigation of fire ecology in the C_3 and C_4 subspecies of *Alloteropsis semialata*. Journal of Ecology 98:1196-1203.

Roques, K. G., T. G. O'Connor, and A. R. Watkinson. 2001. Dynamics of shrub encroachment in an African savanna: relative influences of fire, herbivory, rainfall and density dependence. Journal of Applied Ecology 38:268-280.

Ruggiero, P. G. C., M. A. Batalha, V. R. Pivello, and S. T. Meirelles. 2002. Soil-vegetation relationships in cerrado (Brazilian savanna) and semideciduous forest, Southeastern Brazil. Plant Ecology 160:1-16.

Rustad, L. E., J. L. Campbell, G. M. Marion, R. J. Norby, M. J. Mitchell, A. E. Hartley, J. H. C. Cornelissen, J. Gurevitch, and N. Gcte. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126:543-562.

Sankaran, M., N. P. Hanan, R. J. Scholes, J. Ratnam, D. J. Augustine, B. S. Cade, J. Gignoux, S. I. Higgins, X. Le Roux, F. Ludwig, J. Ardo, F. Banyikwa, A. Bronn, G. Bucini, K. K. Caylor, M. B. Coughenour, A. Diouf, W. Ekaya, C. J. Feral, E. C. February, P. G. H. Frost, P. Hiernaux, H.

Hrabar, K. L. Metzger, H. H. T. Prins, S. Ringrose, W. Sea, J. Tews, J. Worden, and N. Zambatis. 2005. Determinants of woody cover in African savannas. Nature 438:846-849.

Scheiter, S., S. I. Higgins, C. P. Osborne, C. Bradshaw, D. Lunt, B. S. Ripley, L. L. Taylor, and D. J. Beerling. 2012. Fire and fire-adapted vegetation promoted C4 expansion in the late Miocene. New Phytologist 195:653-666.

Schertzer, E., A. C. Staver, and S. A. Levin. 2014. Implications of the spatial dynamics of fire spread for the bistability of savanna and forest. Journal of Mathematical Biology:1-13.

Scholes, R. J. and S. R. Archer. 1997. Tree-grass interactions in savannas. Annual Review of Ecology and Systematics 28:517-544.

Schwilk, D. W. 2003. Flammability is a niche construction trait: canopy architecture affects fire intensity. American Naturalist 162:725-733.

Scott-Shaw, R. and C. D. Morris. 2014. Grazing depletes forb species diversity in the mesic grasslands of KwaZulu-Natal, South Africa. African Journal of Range & Forage Science 32:21-31.

Seabloom, E., E. T. Borer, Y. Buckley, W. E. Cleland, K. Davies, J. Firn, W. S. Harpole, Y. Hautier, E. Lind, A. MacDougall, J. L. Orrock, S. M. Prober, P. Adler, J. Alberti, T. M. Anderson, J. D. Bakker, L. A. Biederman, D. Blumenthal, C. S. Brown, L. A. Brudvig, M. Caldeira, C. Chu, M. J. Crawley, P. Daleo, E. I. Damschen, C. M. D'Antonio, N. M. DeCrappeo, C. R. Dickman, G. Du, P. A. Fay, P. Frater, D. S. Gruner, N. Hagenah, A. Hector, A. Helm, H. Hillebrand, K. S. Hofmockel, H. C. Humphries, O. Iribarne, V. L. Jin, A. Kay, K. P. Kirkman, J. A. Klein, J. M. H. Knops, K. J. La Pierre, L. M. Ladwig, J. G. Lambrinos, A. D. B. Leakey, Q. Li, W. Li, R. McCulley, B. Melbourne, C. E. Mitchell, J. L. Moore, J. Morgan, B. Mortensen, L. R. O'Halloran, M. Pärtel, J. Pascual, D. A. Pyke, A. C. Risch, R. Salguero-Gómez, M. Sankaran, M. Schuetz, A. Simonsen, M. Smith, C. Stevens, L. Sullivan, G. M. Wardle, E. M. Wolkovich, P. D. Wragg, J. Wright, and L. Yang. 2013. Predicting invasion in grassland ecosystems: is exotic dominance the real embarrassment of richness? Global Change Biology 19:3677-3687.

Seabloom, E. W., E. T. Borer, Y. M. Buckley, E. E. Cleland, K. F. Davies, J. Firn, W. S. Harpole, Y. Hautier, E. M. Lind, A. S. MacDougall, J. L. Orrock, S. M. Prober, P. B. Adler, T. M. Anderson, J. D. Bakker, L. A. Biederman, D. M. Blumenthal, C. S. Brown, L. A. Brudvig, M. Cadotte, C. Chu, K. L. Cottingham, M. J. Crawley, E. I. Damschen, C. M. Dantonio, N. M. DeCrappeo, G. Du, P. A. Fay, P. Frater, D. S. Gruner, N. Hagenah, A. Hector, H. Hillebrand, K. S. Hofmockel, H. C. Humphries, V. L. Jin, A. Kay, K. P. Kirkman, J. A. Klein, J. M. H. Knops, K. J. La Pierre, L. Ladwig, J. G. Lambrinos, Q. Li, W. Li, R. Marushia, R. L. McCulley, B. A. Melbourne, C. E. Mitchell, J. L. Moore, J. Morgan, B. Mortensen, L. R. O'Halloran, D. A. Pyke, A. C. Risch, M. Sankaran, M. Schuetz, A. Simonsen, M. D. Smith, C. J. Stevens, L. Sullivan, E. Wolkovich, P. D. Wragg, J. Wright, and L. Yang. 2015. Plant species' origin predicts dominance and response to nutrient enrichment and herbivores in global grasslands. Nature Communications 6.

Shevtsova, A., B. J. Graae, T. Jochum, A. Milbau, F. Kockelbergh, L. Beyens, and I. Nijs. 2009. Critical periods for impact of climate warming on early seedling establishment in subarctic tundra. Global Change Biology 15:2662-2680.

Snyman, H. A. 2002. Fire and the dynamics of a semi-arid grassland: influence on soil characteristics. African Journal of Range & Forage Science 19:137-145.

Staver, A. C., S. Archibald, and S. A. Levin. 2011. The Global Extent and Determinants of Savanna and Forest as Alternative Biome States. Science 334:230-232.

Staver, A. C., W. J. Bond, W. D. Stock, S. J. van Rensburg, and M. S. Waldram. 2009. Browsing and fire interact to suppress tree density in an African savanna. Ecological Applications 19:1909-1919.

Staver, A. C. and S. A. Levin. 2012. Integrating theoretical climate and fire effects on savanna and forest systems. The American Naturalist 180:211-224.

Sternberg, M., V. K. Brown, G. J. Masters, and I. P. Clarke. 1999. Plant community dynamics in a calcareous grassland under climate change manipulations. Plant Ecology 143:29-37.

Stevens, C. J., N. B. Dise, D. J. G. Gowing, and J. O. Mountford. 2006. Loss of forb diversity in relation to nitrogen deposition in the UK: regional trends and potential controls. Global Change Biology 12:1823-1833.

Stevens, C. J., E. M. Lind, Y. Hautier, W. S. Harpole, E. T. Borer, S. Hobbie, E. W. Seabloom, L. Ladwig, J. D. Bakker, C. Chu, S. Collins, K. F. Davies, J. Firn, H. Hillebrand, K. J. L. Pierre, A.

MacDougall, B. Melbourne, R. L. McCulley, J. Morgan, J. L. Orrock, S. M. Prober, A. C. Risch, M. Schuetz, and P. D. Wragg. 2015. Anthropogenic nitrogen deposition predicts local grassland primary production worldwide. Ecology 96:1459-1465.

Stevens, C. J., L. C. Maskell, S. M. Smart, S. J. M. Caporn, N. B. Dise, and D. J. G. Gowing. 2009. Identifying indicators of atmospheric nitrogen deposition impacts in acid grasslands. Biological Conservation 142:2069-2075.

Stevens, C. J., K. Thompson, J. P. Grime, C. J. Long, and D. J. G. Gowing. 2010. Contribution of acidification and eutrophication to declines in species richness of calcifuge grasslands along a gradient of atmospheric nitrogen deposition. Functional Ecology 24:478-484.

Stronach, N. R. H. and S. J. McNaughton. 1989. Grassland Fire Dynamics in the Serengeti Ecosystem, and a Potential Method of Retrospectively Estimating Fire Energy. Journal of Applied Ecology 26:1025-1033.

Suding, K. N., S. L. Collins, L. Gough, C. Clark, E. E. Cleland, K. L. Gross, D. G. Milchunas, and S. Pennings. 2005. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. Proceedings of the National Academy of Sciences of the United States of America 102:4387-4392.

Symstad, A. J. 2000. A test of the effects of functional group richness and composition on grassland invasibility. Ecology 81:99-109.

Tainton, N., editor. 1999. Veld Management in South Africa. University of Natal Press, Pietermaritzburg.

Taylor, B. R., D. Parkinson, and W. F. J. Parsons. 1989. Nitrogen and Lignin Content as Predictors of Litter Decay Rates: A Microcosm Test. Ecology 70:97-104.

Tester, J. R. 1989. Effects of fire frequency on oak savanna in east-central Minnesota. Bulletin of the Torrey Botanical Club 116:134-144.

Thomas, C. D., A. Cameron, R. E. Green, M. Bakkenes, L. J. Beaumont, Y. C. Collingham, B. F. N. Erasmus, M. F. de Siqueira, A. Grainger, L. Hannah, L. Hughes, B. Huntley, A. S. van Jaarsveld, G. F. Midgley, L. Miles, M. A. Ortega-Huerta, A. Townsend Peterson, O. L. Phillips, and S. E. Williams. 2004. Extinction risk from climate change. Nature 427:145-148.

Tilman, D. 1982. Resource Competition and Community Structure. Princeton University Press, Princeton, New Jersey.

Tilman, D. 1987. Secondary Succession and the Pattern of Plant Dominance Along Experimental Nitrogen Gradients. Ecological Monographs 57:190-214.

Tilman, D. 1988. Plant Strategies and the Dynamics and Structure of Plant Communities. Princeton University Press, Princeton.

Tilman, D. 2004. Niche tradeoffs, neutrality, and community structure: A stochastic theory of resource competition, invasion, and community assembly. Proceedings of the National Academy of Sciences of the United States of America 101:10854-10861.

Tilman, D., M. Dodd, J. Silvertown, P. Poulton, A. Johnston, and M. Crawley. 1994. The park grass experiment-insights from the most long-term ecological study. Pages 287-303 *in* R. A. Leigh and A. E. Johnston, editors. Long-term Experiments in Agricultural and Ecological Sciences, Wallingford, UK.

Tilman, D. and A. El Haddi. 1992. Drought and biodiversity in grasslands. Oecologia 89:257-264.

Tilman, D., F. Isbell, and J. M. Cowles. 2014. Biodiversity and Ecosystem Functioning. Annual Review of Ecology, Evolution, and Systematics 45:471-493.

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

Tilman, D. and S. Pacala. 1993. The maintenance of species richness in plant communities. Pages 13-25 *in* R. E. Ricklefs and D. Schluter, editors. Species Diversity in Ecological Communities. University of Chicago Press, Chicago.

Tilman, D., P. Reich, and J. Knops. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature 441:629 - 632.

Tilman, D., P. Reich, H. Phillips, M. Menton, A. Patel, E. Vos, D. Peterson, and J. Knops. 2000. Fire suppression and ecosystem carbon storage. Ecology 81:2680-2685.

Tilman, D., P. B. Reich, J. Knops, D. Wedin, T. Mielke, and C. Lehman. 2001. Diversity and productivity in a long-term grassland experiment. Science 294:843-845.

Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379:718-720.

Tilman, G. D. 1984. Plant Dominance Along an Experimental Nutrient Gradient. Ecology 65:1445-1453.

Titshall, L. W., T. G. O'Connor, and C. D. Morris. 2000. Effect of long-term exclusion of fire and herbivory on the soils and vegetation of sour grassland. African Journal of Range & Forage Science 17:70-80.

Trollope, W. S. W., L. A. Trollope, and D. C. Hartnett. 2002. Fire behaviour a key factor in the fire ecology of African grasslands and savannas.*in* D. X. Viegas, editor. Forest Fire Research & Wildland Fire Safety. Proceedings of the IV International Conference on Forest Fire Research. Millpress, Rotterdam.

Trollope, W. S. W., L. A. Trollope, A. F. L. Potgieter, and N. Zambatis. 1996. SAFARI-92 characterization of biomass and fire behavior in the small experimental burns in the Kruger National Park. Journal of Geophysical Research-Atmospheres 101:23531-23539.

Tsvuura, Z. and K. P. Kirkman. 2013. Yield and species composition of a mesic grassland savanna in South Africa are influenced by long-term nutrient addition. Austral Ecology:n/a-n/a.

Turnbull, L. A., S. Rahm, O. Baudois, S. Eichenberger-Glinz, L. Wacker, and B. Schmid. 2005. Experimental invasion by legumes reveals non-random assembly rules in grassland communities. Journal of Ecology 93:1062-1070.

Uys, R. G., W. J. Bond, and T. M. Everson. 2004. The effect of different fire regimes on plant diversity in southern African grasslands. Biological Conservation 118:489-499.

van Langevelde, F., C. A. D. M. van de Vijver, L. Kumar, J. van de Koppel, N. de Ridder, J. van Andel, A. K. Skidmore, J. W. Hearne, L. Stroosnijder, W. J. Bond, H. H. T. Prins, and M. Rietkerk. 2003. Effects of fire and herbivory on the stability of savanna ecosystems. Ecology 84:337-350.

van Wagtendonk, J. W. 2006. Fire as a physical process. Pages 38-57 *in* N. G. Sugihara, J. van Wagtendonk, S. K. E, J. Fites-Kaufman, and A. E. Thode, editors. Fire in California's ecosystems. University of California Press, Berkeley.

Vellend, M., L. Baeten, I. H. Myers-Smith, S. C. Elmendorf, R. Beauséjour, C. D. Brown, P. De Frenne, K. Verheyen, and S. Wipf. 2013. Global meta-analysis reveals no net change in local-scale plant biodiversity over time. Proceedings of the National Academy of Sciences 110:19456-19459.

Vile, D., É. Garnier, B. Shipley, G. Laurent, M.-L. Navas, C. Roumet, S. Lavorel, S. Díaz, J. G. Hodgson, F. Lloret, G. F. Midgley, H. Poorter, M. C. Rutherford, P. J. Wilson, and I. J. Wright. 2005. Specific Leaf Area and Dry Matter Content Estimate Thickness in Laminar Leaves. Annals of Botany 96:1129-1136.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997a. Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications 7:737-750.

Vitousek, P. M. and R. W. Howarth. 1991. Nitrogen Limitation on Land and in the Sea: How Can It Occur? Biogeochemistry 13:87-115.

Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997b. Human domination of Earth's ecosystems. Science 277:494-499.

Wakeling, J. L., A. C. Staver, and W. J. Bond. 2011. Simply the best: the transition of savanna saplings to trees. Oikos 120:1448-1451.

Wally, A. L., E. S. Menges, and C. W. Weekley. 2006. Comparison of three devices for estimating fire temperatures in ecological studies. Applied Vegetation Science 9:97-108.

Wan, S., D. Hui, and Y. Luo. 2001. Fire effects on nitrogen pools and dynamics in terrestrial ecosystems: a meta-analysis. Ecological Applications 11:1349-1365.

Wang, Y., U. Naumann, S. T. Wright, and D. I. Warton. 2012. mvabund– an R package for model-based analysis of multivariate abundance data. Methods in Ecology and Evolution 3:471-474.

Ward, D. 2005. Do we understand the causes of bush encroachment in African savannas? African Journal of Range & Forage Science 22:101-105.

Warner, R. R. and P. L. Chesson. 1985. Coexistence mediated by recruitment fluctuations: a field guide to the storage effect. American Naturalist 125:769-787.

Warton, D. I. and F. K. C. Hui. 2010. The arcsine is asinine: the analysis of proportions in ecology. Ecology 92:3-10.

Warton, D. I., S. T. Wright, and Y. Wang. 2012. Distance-based multivariate analyses confound location and dispersion effects. Methods in Ecology and Evolution 3:89-101.

Wedin, D. and D. Tilman. 1993. Competition among grasses along a nitrogen gradient - initial conditions and mechanisms of competition. Ecological Monographs 63:199-229.

Whittaker, R. H. 1975. Communities and Ecosystems. 2nd edition. Macmillan, New York.

Whittington, H. R., D. Tilman, and J. S. Powers. 2013. Consequences of elevated temperatures on legume biomass and nitrogen cycling in a field warming and biodiversity experiment in a North American prairie. Functional Plant Biology 40:1147-1158.

Whittington, H. R., D. Tilman, P. D. Wragg, and J. S. Powers. in press. Phenological responses of prairie plants vary among species and year in a three-year experimental warming study. Ecosphere.

Willems, J. H. and M. G. L. van Nieuwstadt. 1996. Long-term after effects of fertilization on above-ground phytomass and species diversity in calcareous grassland. Journal of Vegetation Science 7:177-184.

Williams, A. L., K. E. Wills, J. K. Janes, J. K. V. Schoor, P. C. D. Newton, and M. J. Hovenden. 2007. Warming and free-air CO_2 enrichment alter demographics in four co-occurring grassland species. New Phytologist 176:365-374.

Williams, R., A. Gill, and P. Moore. 1998. Seasonal Changes in Fire Behaviour in a Tropical Savanna in Northern Australia. International Journal of Wildland Fire 8:227-239.

Williams, R. J., G. A. Duff, D. M. J. S. Bowman, and G. D. Cook. 1996. Variation in the composition and structure of tropical savannas as a function of rainfall and soil texture along a large-scale climatic gradient in the Northern Territory, Australia. Journal of Biogeography 23:747-756.

Willis, C. G., M. Halina, C. Lehman, P. B. Reich, A. Keen, S. McCarthy, and J. Cavender-Bares. 2010. Phylogenetic community structure in Minnesota oak savanna is influenced by spatial extent and environmental variation. Ecography 33:565-577.

Wolf, J. O. Y. 2004. A 200-year Fire History in a Remnant Oak Savanna in Southeastern Wisconsin. The American Midland Naturalist 152:201-213.

Wright, A., S. A. Schnitzer, I. A. Dickie, A. R. Gunderson, G. A. Pinter, S. A. Mangan, and P. B. Reich. 2013. Complex facilitation and competition in a temperate grassland: loss of plant diversity and elevated CO2 have divergent and opposite effects on oak establishment. Oecologia 171:449-458.

Wright, A., S. A. Schnitzer, and P. B. Reich. 2014. Living close to your neighbors - the importance of both competition and facilitation in plant communities. Ecology.

Xia, J., S. Niu, and S. Wan. 2009. Response of ecosystem carbon exchange to warming and nitrogen addition during two hydrologically contrasting growing seasons in a temperate steppe. Global Change Biology 15:1544-1556.

Xia, J. and S. Wan. 2008. Global response patterns of terrestrial plant species to nitrogen addition. New Phytologist 179:428-439.

Zak, D. R., W. E. Holmes, D. C. White, A. D. Peacock, and D. Tilman. 2003. Plant diversity, soil microbial communities, and ecosystem function: Are there any links? Ecology 84:2042-2050.

Zhang, Y. 2013. Likelihood-based and Bayesian methods for Tweedie compound Poisson linear mixed models. Statistics and Computing 23:743-757.

Ziegler, S. S., E. R. Larson, J. Rauchfuss, and G. P. Elliott. 2008. Tree Establishment During Dry Spells at an Oak Savanna in Minnesota. Tree-Ring Research 64:47-54.