MECHANISMS DRIVING WOODY ENCROACHMENT IN THE TALLGRASS PRAIRIE: AN ANALYSIS OF FIRE BEHAVIOR AND PHYSIOLOGICAL INTEGRATION

by

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Abstract

Woody encroachment has altered the vegetative structure of grasslands worldwide and represents a potentially irreversible shift in grassland dynamics and biodiversity. Clonal woody species appear to be one of the greatest contributors to the shift from graminoid to woody dominance in the tallgrass prairie. Part of the high success rate of clonal species may be attributed to an ability to circumvent recruitment filters through the integration of environmental heterogeneity and acropetal translocation of resources from mother to daughter ramets. The clonal shrub *Cornus drummondii* persists in a tension zone of the graminoid-dominated tallgrass prairie, where the dominance structure is primarily maintained through the direct and indirect effects of fire. The competitive displacement of native herbaceous vegetation associated with the establishment and expansion of *C. drummondii* causes a major alteration in the fuel dynamics responsible for the propagation and sustainment of fire, potentially contributing to biofeedback mechanisms that facilitate shrub expansion. The goal of this research was to quantify fire behavior parameters (temperature, intensity, rate of spread, and heat flux) in relation to *C. drummondii* invasions and to test physiological integration as a mechanism driving encroachment, using manipulation experiments at the Konza Prairie Biological Station.

We observed a significant decrease in fireline intensity associated with the encroachment of *C. drummondii*, which was amplified by the effects of stem density and shrub island area. This alteration in fire behavior also led to reduced heat flux at stems within shrub islands, reducing the likelihood of tissue necrosis and top-kill. With additional fuel, temperatures and fire intensities were higher, similar to open grasslands. In severing rhizomes, and effectively severing the integration of clonal ramets, we observed a higher risk of mortality of daughter ramets. These rhizome severed ramets were more water stressed, had lower photosynthetic rates, and lower woody and foliar biomass production. These results indicate that *C. drummondii* significantly alters fire behavior, releasing ramets from the fire trap of successive top-killing, while the integration of intraclonal ramets allows daughter ramets to survive mid-summer drought and increases the likelihood of successful establishment and further clonal reproduction.

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Acknowledgements

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Chapter 1 - Woody encroachment in the tallgrass prairie

Introduction:

The transition of graminoid dominated grasslands to shrublands and even closed forests is a phenomenon that has been observed worldwide, across diverse habitats and evolutionary histories (Ratajczak *et al.* 2012). This conversion to woody vegetation has far-reaching consequences for the global carbon cycle (Knapp *et al.* 2008), biodiversity (Ratajczak *et al.* 2012), and socioeconomics (Briggs *et al.* 2005), as grasslands comprise over 40% of the global terrestrial landscape. The tallgrass prairie occupies a tension zone in the east-west precipitation gradient of the Great Plains, where the vegetative dominance structure can shift rapidly from C_4 graminoids to C_3 trees and shrubs within a few decades in response to management and climate variability, as there is sufficient precipitation to sustain both functional types (Axelrod 1985; Briggs *et al.* 2005). Historically, most of the tallgrass prairie was lost to row-crop agriculture, but the increasing abundance of woody vegetation from the expansion of bottomland hardwood forests and increases in upland shrub cover have presented a unique contemporary threat to the last remaining tracts of tallgrass prairie (Briggs *et al.* 2002). Over the last 25 years, native woody trees and shrubs have increased in abundance and cover in a nearly linear fashion within the tallgrass prairie, becoming a major component of the grassland vegetation (Briggs *et al.* 2005). Understanding the mechanisms driving this conversion will be necessary to mitigate problems associated with woody encroachment and possibly conserve the functionality of the tallgrass prairie.

The C_4-dominated tallgrass prairie exists in an alternate stable state that is maintained primarily through the interaction of disturbance by fire and differential plant growth responses (Briggs *et al.* 2002; Lett and Knapp 2003; McCarron and Knapp 2003; Heisler *et al.* 2004).

Alterations in disturbance regimes and land-use practices have greatly reduced the extent and viability of grassland habitats worldwide, making them one of the most threatened biomes (Samson *et al.* 2004; Hoekstra *et al.* 2005). Woody encroachment not only reduces viable grassland habitat by changing the vegetation structure and composition, it is also associated with declines in plant species diversity (Knapp *et al.* 2008; Ratajczak *et al.* 2012). This may also contribute to a nearly irreversible change in the disturbance regimes responsible for maintaining the plant species diversity and the graminoid dominance structure of these grassland systems (Gibson *et al.* 1990; Riginos and Grace 2008; Staver *et al.* 2011; Ratajczak *et al.* 2011).

Fire is an important process for grasslands, as it inhibits the establishment of woody vegetation and provides the conditions necessary to foster the development of native grasses and forbs (Reich *et al.* 1990; Howe 1995; Arabas 2000). Increases in aboveground net primary productivity are associated with fire events, and the post-fire environment provides a transient period of resource availability for the growth and development of herbaceous vegetation (Knapp *et al.* 1993; Gilliam 1998; Lett and Knapp 2003; McCarron & Knapp 2003; Rollins *et al.* 2004). The differential survival of seedlings following a fire disturbance event determines the floral development trajectory and is the primary causative factor in maintaining the open characteristic of the tallgrass prairie (Harttnet 1987; Reich *et al.* 1990). Without fire, the native trees and shrubs are able to establish and competitively displace the graminoid and forb vegetation (Oborny *et al.* 2000; Lett and Knapp 2003), changing not only the vegetation composition and structure of the grassland, but the nutrient, water, and disturbance dynamics as well (Schlesinger *et al.* 1990; Reich *et al.* 1990; Blair 1997; D’Odorico *et al.* 2010).

High fire frequency is one of the strongest recruitment filters for shrub seedlings in the tallgrass prairie, as shown by Heisler *et al.* (2003), with the evident absence of shrub species where fire return intervals are less than two years. Given a sufficient refuge from these recruitment filters associated with fire effects, shrubs are able to escape the fire-trap of successive top killing, further reinforcing their position within the plant community with rapid resprouting responses from perennating buds, leading to the eventual exclusion of herbaceous vegetation and high intensity fire events (Heisler *et al.* 2003; Buitenwerf *et al.* 2012). Once established, woody plants like *Cornus drummondii* C.A. Mey (rough-leaf dogwood) will competitively displace the native grass and forb community (Oborny *et al.* 2000; Lett and Knapp 2003; Ratajczak *et al.* 2012). The exclusion of herbaceous vegetation that comes with the closure of shrub canopies consequently reduces the fine fuels necessary to carry fire (Lett and Knapp 2003; Gibson *et al.* 1990; Finney *et al.* 2010; Pearce *et al.* 2010; Ratajczak *et al.* 2011; Staver *et al.* 2011). The herbaceous productivity of the tallgrass prairie generally leads to high intensity fires (Gibson *et al.* 1990, Knapp *et al.* 1993), but the development of monspecific islands of *C. drummondii* leads to intensified biofeedback mechanisms that reduce fire intensity within and around the shrub island, mitigating potential population recruitment filters associated with excessive top-killing in high intensity fires (Briggs *et al.* 2005; Finney *et al.* 2010; Ratajczak *et al.* 2011).

Under elevated atmospheric CO_2 concentrations, woody species exhibit a higher water use efficiency that may also help drive further encroachment by decreasing the limitation of water availability (Schlesinger *et al.* 1990; Scholes and Archer 1997; Bond 2008; Ratajczak *et al.* 2011; Bond and Midgley 2012). The clonal structure of *C.drummondii* may facilitate the success of daughter ramets through greater capacity for resource acquisition, storage, and allocation by means of intraclonal sharing of resources (Hartnett and Bazzaz 1985; Hartnett 1993; Ratajczak et al. 2011). The intraclonal translocation of resources such as water can greatly reduce the effects of interspecific competition in a heterogeneous environment, and as a result, increase daughter ramet performance (Hartnett and Bazzaz 1985; Hartnett 1993).

Through the acropetal translocation of nutrients and water from parent ramets to daughter ramets, *C. drummondii* is capable of circumventing recruitment filters and competitive interactions. The ability to plastically alter water acquisition and move it throughout the clonal architecture may prove advantageous during the mid-summer droughts that limit water availability to the deeper soil horizons, which daughter ramets would otherwise have no access to without the physiological connection provided by the connective rhizome (Nippert and Knapp 2007; Ratajczak *et al.* 2011). Ratajczak *et al.* (2011) demonstrated that up to 50 per-cent of xylem water in daughter ramets persisting in the grassland/shrub matrix is traceable to these deeper, more reliant water tables, even though excavations of offspring ramet roots revealed that they are not in direct physical contact with these deeper soil horizons and water tables. The source water profiles of offspring ramets therefore offers compelling evidence for the acropetal translocation of water resources between intraclonal ramets, acting as a mechanism facilitating shrub expansion.

Integration may reduce the strength of recruitment filters and potential demographic bottle-necks related to competition and fire induced top-kill by increasing the performance of daughter ramets and increasing the likelihood of establishment and long-term survival. With greater survival rates and reduced effects of competition, clonal integration may facilitate further clonal expansion, conferring persistence to woody species under disturbance (Hartnett and Bazzaz 1985; Hartnett 1993; Bond and Midgley 2012; Ratajczak 2011). Even though clonal reproduction may cause a decrease in genet performance when individual ramets exert a strong resource sink (Schmid and Bazzaz 1987; Abrahamson *et al.* 1991), the continual iteration of clonal ramets may allow for the eventual escape from fire traps of successive top-killing as they fundamentally alter the effects of fire behavior (Barney *et al.* 2010) by competitively excluding herbaceous vegetation (Oborny *et al.* 2000; Lett and Knapp 2003).

There also appears to be a life-history trade-off between seed reproduction and resprouting that accounts for the tolerance of high frequency and high intensity fires (Benson and Hartnett 2006). Seedling establishment may be important for maintaining genetic diversity and allow for rapid establishment in newly disturbed habitats followed by sufficient refuges from fire effects (Benson and Hartnett 2006), but the integration of environmental heterogeneity through clonal reproduction may facilitate daughter ramet success by circumventing physiological and biotic recruitment filters (Hartnett and Bazzaz 1983). Clonal reproduction may also aid in the growth of daughter ramets and further contribute to the escape from excessive top-killing (Buitenwerf *et al.* 2012). The perennating organs of *C. drummondii* exhibit a quick response to disturbance with early spring emergence, drawing from the high nitrogen and water reserves of the clonal genet (Lett *et al.* 2004; McCarron and Knapp 2003), where leaf-level carbon assimilation is enhanced to meet the demands of resprouting and tissue regeneration (Bond and Midgley 2012; Paula and Pausas 2006). The higher nitrogen and water availability in post burned shrub islands contributes to higher photosynthetic rates, and subsequently greater tissue growth, and may even be able to compensate for competitive interactions as well (McCarron and Knapp 2003). This recovery mechanism may also be directly proportional to carbon reserves and allocation provided by the clonal rooting strategy (Polley *et al.* 1997; Bond and Midgley 2012), limiting the need to down-regulate photosynthesis for tissue repair and root growth (Buitenwerf 2012).

The establishment and expansion of *C. drummondii* in the tallgrass prairie is driven by a complex interaction of fire effects and its clonal rooting strategy. By reducing fire intensity through the exclusion of herbaceous vegetation, *C. drummondii* is able to escape the fire-trap of successive top-killing of daughter ramets. Extensive post-fire resprouting insures persistence in the fire-prone tallgrass prairie. Through the intraclonal sharing of resources, these daughter ramets have greater access to resources which imparts a competitive advantage that further facilitates the dominance shift to woody vegetation. Furthermore, enriched atmospheric CO_2 concentrations may favor clonal woody species like *C. drummondii*, exacerbating issues with grassland biodiversity, carbon cycling, and resource availability associated with woody encroachment.

The objectives of this research were to quantify various aspects of fire behavior in relation to *Cornus drummondii* invasions and examine how altered fire behavior relates to ramet survival and biomass production, and to test the reliance of daughter ramets on the physiological integration with parent ramets. Chapter 2 of this thesis illustrates the dynamics of fire behavior in response to *C. drummondii* invasions and the biotic responses of intraclonal ramets in the post-fire environment. I examine the effects of various fuel loads on fire behavior and how this may affect the intensity of fires and how biomass production is affected by the reallocation of resources to tissue repair and regrowth following fire. In Chapter 3, I examine the degree to which intraclonal ramets of *C. drummondii* rely on physiological integration through a rhizome severing experiment. I examined the effects of blocking the acropetal translocation of resources on physiological parameters and ramet growth. Chapter 4 concludes with a conceptual model of fire behavior and physiological integration and how the two interact to facilitate the expansion of shrub cover. This model also conveys the importance of clonal reproduction for persistence in the fire prone tallgrass prairie and how population dynamics are affected by this strategy.

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Chapter 2 - Fire behavior and intraclonal ramet responses of the C_3 shrub *Cornus drummondii* in the tallgrass prairie.

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Abstract.

Woody encroachment has altered grasslands worldwide and represents a potentially irreversible shift in grassland structure, dynamics, and biodiversity. The clonal shrub *Cornus drummondii* persists in a tension zone of the graminoid-dominated tallgrass prairie, where the dominance structure is primarily maintained through the direct and indirect effects of fire. Once established *C. drummondii* competitively displaces herbaceous vegetation, which has been suggested to lead to significantly altered fire behavior, where the shrub can eventually escape top-kill and population filters associated with fire disturbance. We measured fire behavior (temperature, intensity, duration, and rate of spread) in monospecific islands of *C. drummondii* and the adjacent open grasslands in order to gain an understanding of the direct effects of this shrub invasion on fire processes in the tallgrass prairie. We also experimentally manipulated fine fuel loading within shrub islands to try to restore the high fire intensity observed in open grasslands. Shrubs were monitored throughout the 2011 growing season for long-term effects of fire on ramet mortality, density, and biomass production.

The reduction in fine fuels associated with the establishment of *C. drummondii* significantly decreased the mean maximum fire temperatures within the shrub islands (353.97 ± 35.14 °C) when compared to the grasslands (539.74 ± 21.65 °C). The heterogeneity of fine fuels within shrub islands also contributed to ignition instability, leading to decreased fireline intensity and patchy fires. We also found that the fireline intensity was negatively correlated with the density of stems within a shrub island, decreasing by 8.5% as density increased. These effects on fire behavior were slightly mitigated by the addition of fine fuels to the shrub islands, making a more homogeneous fuel bed that allowed for the sustained propagation of fire through the shrub island. The biotic responses of this species were complex. Ramets in islands that received additional fuel generally had greater biomass production, however, there was also an overall decrease in total biomass production associated with greater fire severity. The encroachment of *C. drummondii* has unequivocally reduced the intensity of grassland fires through the exclusion of fine fuels, allowing for the eventual escape from ramet mortality by reducing the net heat flux at stems.

Key words.

Cornus drummondii, fire intensity, fire severity, tallgrass prairie, woody encroachment

Introduction.

Over the last century, the cover and abundance of woody vegetation in grassland habitats has increased worldwide (Briggs *et al.* 2002, Ratajczak *et al.* 2011). Historically, the greatest threat to the North American tallgrass prairie was land use change and conversion to row-crop agriculture (Briggs *et al.* 2002), but now woody encroachment presents a greater and possibly irreversible threat (Howe 1995, Briggs *et al.* 2005). Changes in historic disturbance regimes along with climatic and environmental variability have led to shifts in the vegetation structure of tallgrass prairie towards shrub-dominance (McCarron and Knapp 2003, Lett *et al.* 2004, Briggs *et al.* 2005). Driving factors include changes in atmospheric carbon dioxide concentration, nitrogen deposition, grazing pressure, and the effects of fire intensity and return interval (Hobbs *et al.* 1984, Schlesinger *et al.* 1990, Briggs 2002, Briggs *et al.* 2005; Bond and Midgley 2012). The encroachment of woody vegetation in grasslands is of particular concern, as it is associated with declines in grassland biodiversity and changes in fundamental ecosystem dynamics (Rollins *et al.* 2004, Dellasala *et al.* 2004, Ratajczak *et al.* 2012), and further represents a potentially irreversible shift in vegetation structure (Ratajczak *et al.* 2011, Bond and Midgley 2012).

Fire is an important process for C_4-dominated grasslands like the tallgrass prairie, as it inhibits the establishment of woody vegetation, as well as provides conditions necessary to foster the development of native grasses and forbs (Reich *et al.* 1990, Howe 1995, Arabas 2000). Fire provides a transient period of resource availability for the growth and development of herbaceous vegetation and increases aboveground net primary productivity in the post-fire environment (Briggs and Knapp 1995, Gilliam 1998, Lett and Knapp 2003, McCarron & Knapp 2003, Rollins *et al.* 2004). The differential survival of seedlings following a fire event determines the floral development trajectory and is the primary factor in maintaining the graminoid dominance structure and the open characteristics of the tallgrass prairie (Hartnet 1987, Reich *et al.* 1990). Without fire, the native trees and shrubs are able to outcompete and displace the herbaceous species (Oborny *et al.* 2000, Lett and Knapp 2003), changing not only the vegetation structure of the grassland, but the nutrient, water and disturbance dynamics as well (Reich *et al.* 1990; Blair 1997; Peterson & Reich 2001).

Fire return interval is one of the strongest recruitment filters for shrub seedlings in the tallgrass prairie, as shown by Briggs *et al.* (2002) and Heisler *et al.* (2003), with the evident absence of shrub species on sites with fire return interval of less than two years. Given a sufficient refuge from population recruitment filters associated with fire effects, shrubs are able to escape the fire-trap of successive top killing. Once woody plants have established, fire acts as a promoter of growth, with rapid resprouting responses from perennating buds and greater growth rates following a fire event (Heisler *et al.* 2003; Buitenwerf *et al.* 2012). This growth response confers persistence to woody species under high fire frequency and intensity fire regimes (Buitenwerf *et al.* 2012).

Woody plants like *Cornus drummondii* C.A. Mey, an abundant and aggressive shrub species within the tallgrass prairie (Briggs *et al.* 2002; Heisler *et al.* 2003), will competitively displace the herbaceous species as it reaches larger size classes (Oborny *et al.* 2000, Lett and

Knapp 2003, Ratajczak *et al.* 2012). The exclusion of herbaceous vegetation that comes with the closure of shrub canopies consequently reduces the fine fuels necessary to carry fire (Lett and Knapp 2003, Riginos and Grace 2008, Finney *et al.* 2010, Pearce *et al.* 2010, Ratajczak *et al.* 2011, Staver *et al.* 2011). This further alters soil water relations and nutrient dynamics (Gilliam 1998, McCarron and Knapp 2003, Bond and Midgley 2012) facilitating further expansion and growth of this shrub species (Briggs *et al.* 2005). The high level of herbaceous productivity of the tallgrass prairie generally leads to high intensity fires (Gibson *et al.* 1990), but the development of monspecific islands of *C. drummondii* may lead to intensified biofeedback mechanisms that may reduce fire intensity within and around the shrub island, mitigating potential population bottle-necks associated with excessive top-killing in high intensity fires (Briggs *et al.* 2005, Finney *et al.* 2010, Ratajczak *et al.* 2011).

Due to the impact of shrub encroachment on carbon and nitrogen cycling (Knapp *et al.* 2008), grassland biodiversity (Ratajczak *et al.* 2012), and resource availability (McCarron and Knapp 2003), there is a developing need for empirical studies examining the mechanisms of shrub expansion and the direct effects of woody encroachment on fire characteristics in the tallgrass prairie (Gibson *et al.* 1990, Heisler *et al.* 2004).

The objective of this study was to quantify various aspects of fire behavior (fireline intensity and burn severity, Keeley 2009) in relation to *C. drummondii* invasions, as well as effects of fire on shrub growth and mortality. We hypothesize that: 1.) due to reduced fuel loads within *C. drummondii* islands, the net heat output, or fireline intensity, will be greatly reduced. 2.) The reduction in fine fuels should also reduce burn severity and the effects on ramet populations, decreasing the incidence of top-kill and increasing ramet recruitment. 3.) In addition, increasing fuel load, will lead to greater fire intensities and burn severities, possibly damaging perennating organs and leading to reduced genet performance through reduced biomass production and ramet recruitment.

Materials and Methods.

Study site:

All field work was conducted at the Konza Prairie Biological Station (KPBS). KPBS is a 3487-ha native tallgrass prairie preserve, located in the Flint Hills of northeast Kansas, USA (39°05' N. 96°35'). It has been part of the Long-Term Ecological Research program (LTER) since 1981, managing experimental watersheds (~55) of varying fire frequency and grazing treatments. Most of the burning at KPBS occurs in the spring (March-April), the most common fire management practice in the area. The site is characterized by rolling topography with elevation ranging from 320 to 444 m above sea level. Mean annual precipitation is 835 mm, with 75% falling between April and September (Hayden 1998). Plant communities are dominated by warm season C_4 grasses *Andropogon geradii* Vitman, *A. scoparius* Michx., and *Sorghastrum nutans* (L.) Nash (Freeman 1998). Forbs (primarily C_3 dicots) account for the majority of plant species diversity (~600 species of higher plants) despite being much less abundant (Freeman 1998). The C_3 shrubs *Cornus drummondii* C.A. Mey, *Rhus glabra* L., and *Prunus americana* Marsh. are the most predominant woody species, occurring historically along seeps and intermittent lowland streams, becoming less abundant towards the upland slopes (Briggs *et al.* 2002, McCarron and Knapp 2003). The density and cover of the woody plants are primarily influenced by fire history and intensity, with long-term unburned sites having a higher abundance of woody plants (Briggs et al 2005).

This study focused on *C. drummondii,* as it is the dominant species involved in the woody encroachment transition of KPBS' grasslands (Briggs *et al.* 2002, 2005, Ratajczak *et al.*

2011). This species is a medium sized (1-4 m high) shrub, found commonly in grasslands and old fields from southern Ontario westward to Illinois, Iowa and South Dakota and southward to Alabama and Texas (Stephens 1969). The fruits of *C. drummondii* are bird dispersed and therefore this species is not affected by dispersal limitation (Wilson and Whelan 1993). The shrub is long-lived, clonal, and produces numerous stems in high-density patches termed “shrub islands”. This is an ideal plant for our manipulations since it is one of the most common shrubs on KPBS and responds rapidly to fire suppression in the tallgrass prairie, while also persisting in sites with a high fire frequency (Briggs *et al.* 2002).

Manipulation:

Sixty-four *C. drummondii* islands, ranging in size from 5 m^2 to 100 m^2 were randomly selected from two annually burned and ungrazed watersheds on similar soil types and topography. Each island was randomly assigned a number generated in program R V2.14.0 (R Development Core Team, Vienna, Austria). This permutation led to the random assignment of fuel manipulation (supplement fuel and no fuel addition – control) to each *C. drummondii* island. We increased the fine fuel load within half of the islands (n = 32) with supplemental fuel, approximately 2527.68 ± 432.21 g m^{-2} (mean additional fuel load ± se) of prairie hay, collected within the watershed and applied evenly throughout the island. The purpose of the fuel addition was to increase the fine fuels within islands, and as a result increase the intensity and severity of the prescribed fires.

Fire temperature measurements:

Prescribed burns were conducted with a mean RH of 65.49% and wind speed of 4.08 m s^{-1} (LTER CLIMDB http://www.fsl.orst.edu/climhy/), falling within the boundaries of prescription parameters. Three Campbell Scientific dataloggers coupled with AM25T solid-state multiplexers

(Campbell Scientific Inc., Logan, UT, U.S.) were programmed to take continuous temperature measurements at 27 points every second. These measurements were taken within three of the 32 *C. drummondii* islands in each watershed for the entire duration of the prescribed fire. The type-K thermocouples (Omega Engineering Inc., Stamford, CT, U.S.) attached to the dataloggers were systematically located around shrub islands at the ground level to give both the rate of spread and a two-dimensional temperature profile of the fire front as it moved through the shrub island. Fireline intensity, the rate of heat released per unit of fire line, was calculated from Byran's equations (1959) as $I = HWS$. Where H is the average low heat of combustion of surface fuels ($H = 16{,}357$ kJ kg^{-1}; Trollope *et al.* 2002), W is fuel consumption (kg m^{-2}), and S is the rate of spread of the flame (m s^{-1}), to get the net energy release expressed in kW m^{-1}. We also calculated the time integrated net heat flux, expressed as Q'' kJ m^{-2}, as fireline intensity alone cannot explain the net energy output at each point along the fireline (Bova and Dickenson 2005). These calculated indices of fire behavior were used primarily because they are directly related to tissue necrosis and burn severity (Bova and Dickenson 2005). An additional 54 measurements of fire temperature were taken in open grasslands during the summer burns and included in the models for information on grass fires. Before inclusion, a studentized t-test was used to insure summer grassland fire behavior was similar to the grassland fires in the spring ($t = 1.30$, $p = 0.25$).

Twenty "thermocolor" pyrometers (see Hobbs *et al.* 1984 for more details) per island were also deployed as a means to record maximum fire temperatures along two intersecting, perpendicular transects running the length of each *C. drummondii* island. An additional two transects comprised of 20 pyormeters were placed in the open grassland at a spacing of one meter. These pyrometers were made by painting 21 spots of OMEGALAQ liquid temperature indicating paint (Omega Engineering Inc. Stamford, CT) on the unglazed side of 10 × 10 cm ceramic tiles. Each of these 21 spots of dry paint melt at a specific temperature between 75°C and 649°C in 21 irreversible steps. These thermocolor pyrometers were placed at ground level, facing the anticipated direction of the advancing fire front, allowing us a more efficient way to gain greater spatial coverage of the fire temperatures within and surrounding the shrub islands.

Biotic measurements:

Prior to burning, fuel load and fuel moisture were sampled in order to assess potential factors influencing fire behavior. Vegetation was clipped at ground level in five 0.1-m^2 quadrats adjacent to and within each of the shrub islands. Leaf litter was then placed over the harvested areas to restore the fuel bed to original conditions. The clippings were dried at 60°C for 48 hours and weighed for estimates of fine fuel loading (g m^{-2}), succulence, and to calibrate disk pasture meter measurements. A disk pasture meter was used as a non-destructive way to estimate fuel load in the grassland matrix around the shrub islands (Karl & Nicholson 1987).

Before the fires and at the end of the growing season, two intersecting belt transects running the length of each *C. drummondii* island were used to estimate island area, ramet density, and the ratio of living to dead ramets. Total biomass was estimated from 20 random stems within each island using allometric relationships developed by Lett *et al*. (2004). Ramets were also recorded as to their relative position within the island to account for possible insulation from thermal flux from the grassland fires. Immediately after burning, total fuel consumed (% burn), char heights on ramets (an indirect measure of fireline intensity), and % top kill were measured in each island and compiled as an index of burn severity (Table 2.1).

Statistical analysis:

All data were analyzed with program SAS V. 9.2 (SAS Institute Inc., Cary, NC, USA) using either the GLIMMIX or PROC REG procedure. For mixed models, islands within watersheds were defined as random factors. Satterthwaite's approximation for degrees freedom was used for all ANOVA results and Tukey's LSD was used for all pairwise comparisons. Regression models were run as a stepwise function at an alpha level of 0.05. Akaike's Information Criteria (AIC) was used to systematically select models based on the evaluated fit of each model. The response variables fireline intensity and biomass production were both log transformed in order to meet assumptions of model validity.

Results

Fire behavior:

Maximum fire temperatures achieved in the fuel loading experiment, as recorded by the dataloggers, approached the maximum temperatures observed within open grasslands (880 °C and 990 °C, respectively), while the mean residence time was greater with the additional fuel (257.41 ± 36.00, mean seconds ± 1 SE) than in the open grasslands (104.00 ± 26.00 seconds). These temperatures and residence times were generally greater than those observed within shrub islands that did not receive additional fuel (max temperature 654.7 °C and mean residence time 112.00 ± 14.37; Fig. 2.1). These fire temperatures were primarily influenced by fuel load (g m^{-2}), island area, stem density, and stem basal diameter (r^2 adjusted = 0.1874, $p = 0.0059$). Fire temperatures increased by 0.31°C with increasing fuel load, and as stem density increased, fire temperature also increased 26.98°C. As island area and stem basal diameter increased, temperatures decreased by 3.15°C and 73.40°C respectively (Table 2.2).

The pyrometer data indicated a similar trend with fuel additions showing a greater maximum temperature (621°C) than the shrub islands with no additional fuel (593 °C). Our results also indicate that the fuel loading heterogeneity within shrub islands affects fire temperatures, resulting in significantly altered fire behavior from the grasslands to the center of the island (*df* = 2, 253.7, *F* = 5.95, $p = 0.003$). The mean maximum fire temperatures for fires in the shrub/grass matrix (ecotone) was 258.32 °C (± 17.76) for no fuel addition and 300.58 °C (± 17.46) with additional fuel (Fig. 2.1). At the island center, the mean maximum recorded fire temperatures was 134.14 °C (± 15.63) for no fuel addition and 230.60 °C (± 22.23) with additional fuel. Pairwise comparisons between temperatures observed in the island center and the area adjacent to the grassland matrix within islands without additional fuel indicated that reduced fuel loads and patchy fuel sources are significantly altering fire temperature within each island ($p < 0.01$), while islands with supplemental fuel additions showed no significant difference in temperatures with pyrometer placement ($p > 0.05$). Mean temperatures in the grassland fires was 141.67 °C (± 7.94).

Mean fireline intensity in shrub islands without additional fuel was 1273.64 kW m^{-1} (± 244.90) and fireline intensity in shrub islands with additional fuel was 3003.32 kW m^{-1} (± 706.69), while fireline intensity within the grass fires was more variable, with a mean of 594.31 kW m^{-1} (± 4000.75). Fireline intensity was primarily affected by the density of stems within the island (r^2 adjusted = 0.51, $p < 0.0001$; Fig. 2.2). With each incremental increase in stem density, fire intensity decreased by 8.5 percent. The net heat output at each stem, max net heat flux, also exhibited a strong relationship with fuel loading (r^2 adjusted = 0.531, $p < 0.001$). Heat flux at stems with no additional fuel was 9225.93 kJ m^{-2} (± 519.96), and with additional fuel the max net heat flux was 15857.88 kJ/m^{-2} (± 1577.30).

Fuel loading also played a significant role in affecting burn severity, interacting with stem density, and the area of each island (r^2 adjusted = 0.7581, $p < 0.0001$). Burn severity was positively affected by fuel loading (parameter estimate = 0.04) and stem density (parameter estimate = 1.73), and negatively affected by island area (parameter estimate = -1.36). In the fuel additions, we observed 56.84 % (± 21.40) combustion of fuels due to higher fuel moisture resulting from precipitation events in the 24 hours preceding the fires. Islands that did not have additional fuel had 80.22 % (±14.14) of fine fuel combustion. This generally led to lower indices of burn severity in the fuel loaded islands (3.35 ± 0.12), than the islands with no additional fuel (4.75 ± 0.17), as % top-kill and char heights showed no significant differences in the fuel loading experiments ($p > 0.05$). This did not appear to affect fireline intensity or maximum temperatures observed in islands with fuel additions. Furthermore, fireline intensity did not appear to influence burn severity either ($p > 0.05$).

Biotic responses:

Mean total biomass within shrub islands with fuel was 1479.12 g m^{-2} (95% CI: 1230.27-1778.28) and with no additional fuel was 1230.27 g m^{-2} (95% CI: 977.24-1513.56; Fig. 2.3). The mean total woody biomass of *C. drummondii* stems within each island at the end of the growing season was influenced by burn severity and island area (r^2 adjusted = 0.4492, $p < 0.0001$). Greater burn severity was negatively correlated with total biomass, decreasing woody biomass by 9% as burn severity increased. Island size, however, was positively correlated with woody biomass, increasing by 0.4% with each incremental increase in island area (m^2). These results were reflected in the stem heights, where shrub islands without fuel additions had a mean stem height of 0.99 m (± 0.26), and mean stem heights with additional fuel was 1.16 m (± 0.27). With the addition of fireline intensity, in a reduced dataset, woody biomass showed a similar negative correlation with severity and fireline intensity (r^2 adjusted = 0.8310, $p < 0.0001$).

Discussion.

Fire provides a buffer against the recruitment and establishment of *C. drummondii* seedlings (Heisler *et al.* 2003); however, clonal integration between ramets allows for this species to respond favorably to disturbance, drought, and competition once established (Hartnett and Bazzaz 1983, McCarron & Knapp 2003, Ratajczak *et al.* 2011). In clonal plant species, translocation of nutrients and water from parent to daughter ramets allows for rapid recovery and resource acquisition immediately following fire events (Hartnett and Bazzaz 1983; Hartnett 1993, Ratajczak *et al.* 2011, Bond and Midgley 2012). Integration of environmental heterogeneity also increases the competitive advantage of clonal species like *C. drummondii* (Hartnett and Bazzaz 1983, Oborny *et al.* 2000), leading to the eventual displacement of herbaceous vegetation in the sub canopy (Heisler *et al.* 2003).

The change in fuel dynamics that results from the closure of the shrub canopy greatly alters the behavior and the effects of fire. Our results indicate this trend in decreasing fire behavior characteristics, where maximum temperature drops from 990.0 °C in the open grasslands to 654.7 °C within the shrub islands. Mean temperatures also showed a similar decline from grasslands (539.74 ± 21.65 °C; mean ± se) to shrub islands (353.97 ± 35.14 °C). As suggested by the dataloggers and pyrometer data, it becomes clear that this is primarily influenced by the heterogeneity of fuel availability responsible for the propagation of fire through the fuel bed. Without sufficient fuel loading, continuity is disrupted and convective heat transfer and turbulent flow of the flaming front are unable to ignite adjacent fuels and carry fire through the shrub islands. The differences in fire temperatures between the two methods of measurement may be attributed to the lag in temperature recording for pyrometers, whereas the dataloggers are recording instantaneously. Therefore the pyrometers may be more indicative of mean fire temperatures (Alaa *et al.* 2006).

Fuel moisture within the islands is generally much higher than the open grasslands due to reduced incidence of incoming light (Heisler *et al.* 2004), further compounding the effects of reduced fuel loading, resulting in reduced temperatures, fireline intensity, and net heat flux within shrub islands (Zhou 2007, Plucinski *et al.* 2010). Fireline intensity was also negatively affected by stem density, as stem density affects factors such as wind speed, fine fuel heterogeneity, and fuel moisture resulting in ignition instability. These results are congruent with studies in other biomes and lab settings where live-fuel moisture, wind, shrub density, and fuel continuity were significant drivers of fire sustainability and intensity (Bradstock and Gill 1993, Thaxton *et al.* 2006, Weise *et al.* 2005, Zhou *et al.* 2007).

With additional fuel supplementation, mean fireline intensity was increased by 1733.46 kW m^{-1}. Even with additional fine fuels, stem density played a significant role in fireline intensity. The fine fuel structure, in spatial heterogeneity and fuel loading, was also responsible for the net heat flux at each point within the islands, where the exclusion of fine fuels within islands significantly decreased the net heat flux at the stem, reducing the probability of mortality for above- and belowground meristems (Beadle 1940, Bova and Dickinson 2005). As tissue necrosis is a rate process (Bova and Dickinson 2005), it is unlikely that surface fires within the shrub islands are able to reach and maintain critical thresholds for belowground tissue mortality (Bova & Dickinson 2005, Dickinson and Johnson 2004, Dickinson *et al.* 2004, Jones et al 2006, Michaletz & Johnson 2007). The interaction of pyrometer placement and fuel loading shows that fuel continuity is a major factor in determining fire spread and maximum fire temperature within shrub islands and may contribute to the survival of aboveground tissues and meristems.

There is a complex biotic response to fire in this clonal species due to different levels and dynamics of population structure and the multiple modes of recruitment in clonal species (Hartnett 1987). Based on the review by Michaletz and Johnson (2007), the vitality of a tree or shrub exposed to fire is dependent on direct processes such as tissue necrosis, related to heat transfer into the stem from thermal flux, and indirect processes such as altered physiology and pathogenic infection. Though there were no noticeable changes in mortality or stem density throughout the growing season in response to the factors tested, there were significant changes in total biomass production. These changes in total biomass and stem heights were likely due to burn severity and fire intensity as well as alterations in the post-fire microclimate (Gibson *et al.* 1990) related to resource availability immediately following the fire disturbance (Heisler *et al.* 2004, McCarron and Knapp 2003). Since total biomass and height were negatively influenced by the fireline intensity and the severity of the burn, we have good evidence that the costs of fire disturbances are high for *C. drummondii*, especially as most of the aboveground biomass is allocated to supporting structures (Heisler et al. 2004). The higher incidence of top-killing following greater fire intensity results in a large resource sink for resprouting post-fire, as this species must reconfigure its growth trajectory for tissue repair and regrowth following fire disturbance (Heisler et al. 2004).

Conclusion.

The invasion of the C_3 shrub *Cornus drummondii* greatly alters the fuel structure of grasslands, resulting in significant alterations in fire behavior (temperature, intensity, duration, and rate of spread). The interactions of the shrub with fire is complex, as fire both acts as a limiting filter for seedling recruitment, but also promotes a high level of resprouting following fire disturbance. The high level of resprouting indicates that heat flux at the stem is insufficient to cause mortality in the perennating belowground buds responsible for population persistence. It is possible that we did not observe any pronounced effects for mortality under higher fire intensity due to the limited temporal extent of this experimental design. Future studies would need to address this, extending fire manipulations over multiple years to ensure that either meristem viability is diminished or that carbon reserves are depleted. A greater temporal extent to this study may provide some insight into the viability of clonal shrubs, such as *C. drummondii*, under greater fire disturbance effects.

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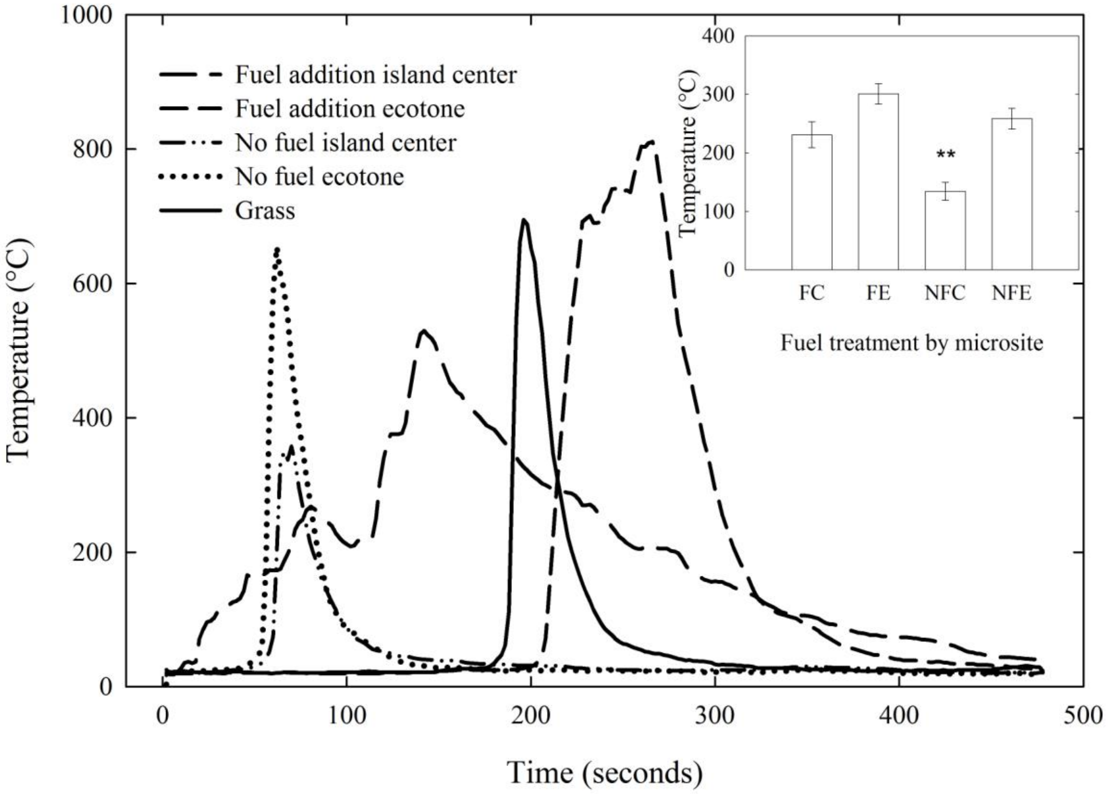

Fig. 2.1. Temperature profiles for fires in each of the shrub and grassland microsites and fuel manipulations. Insert: Pyrometer means for fuel addition island center (FC), fuel addition ecotone (FE), no fuel island center (NFC), and no fuel ecotone (NFE). ANOVA results indicate a significant difference in maximum fire temperatures between open grasslands, shrub islands with the fuel addition, and shrub islands without the fuel addition (*df* = 3, 3.11, *F* = 23.27, *p* = 0.0125). Asterisks represent significant differences in pairwise comparisons for maximum temperature recorded for fuel type by island placement ($p < 0.05$).

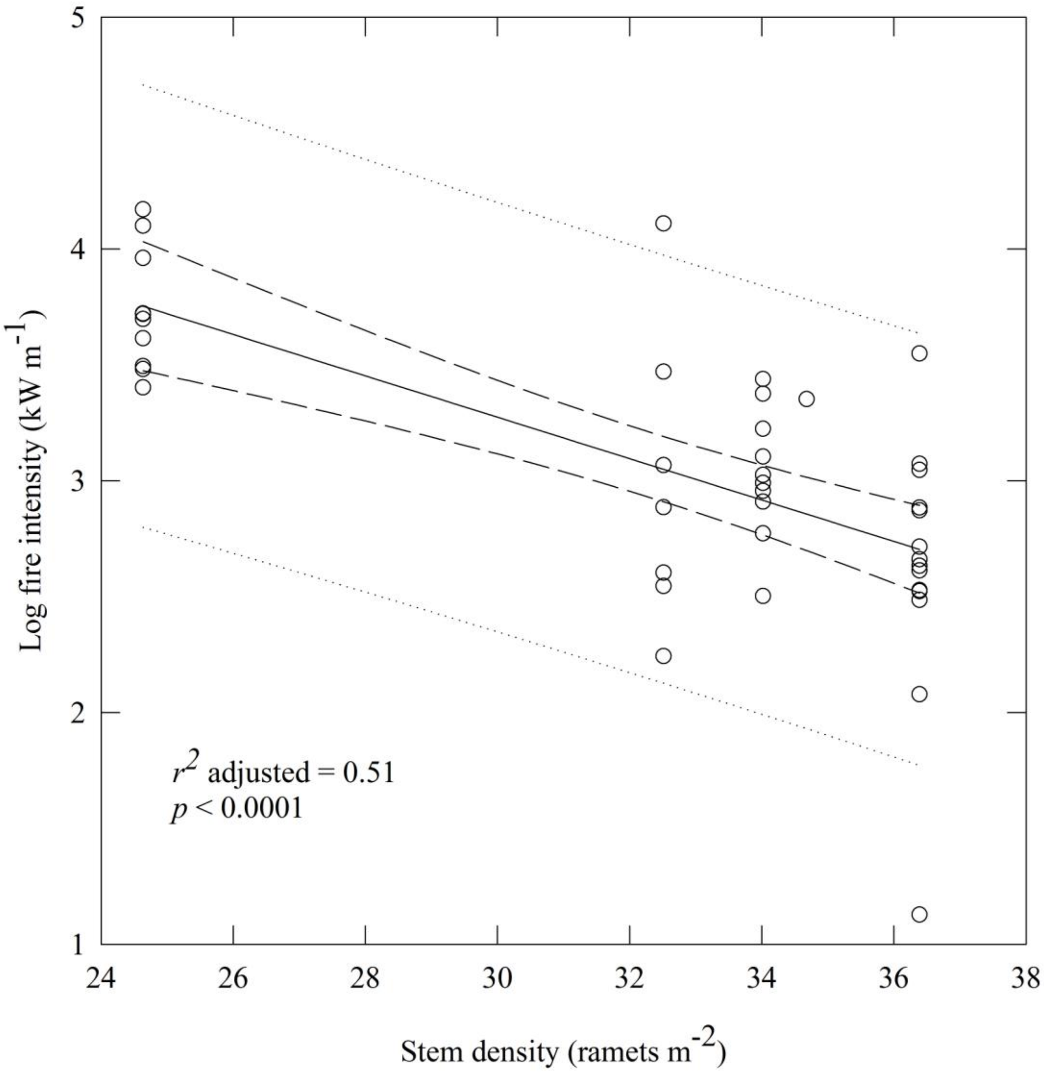

Fig. 2.2. Regression analysis for fire intensity by stem density within islands of C. drummondii.

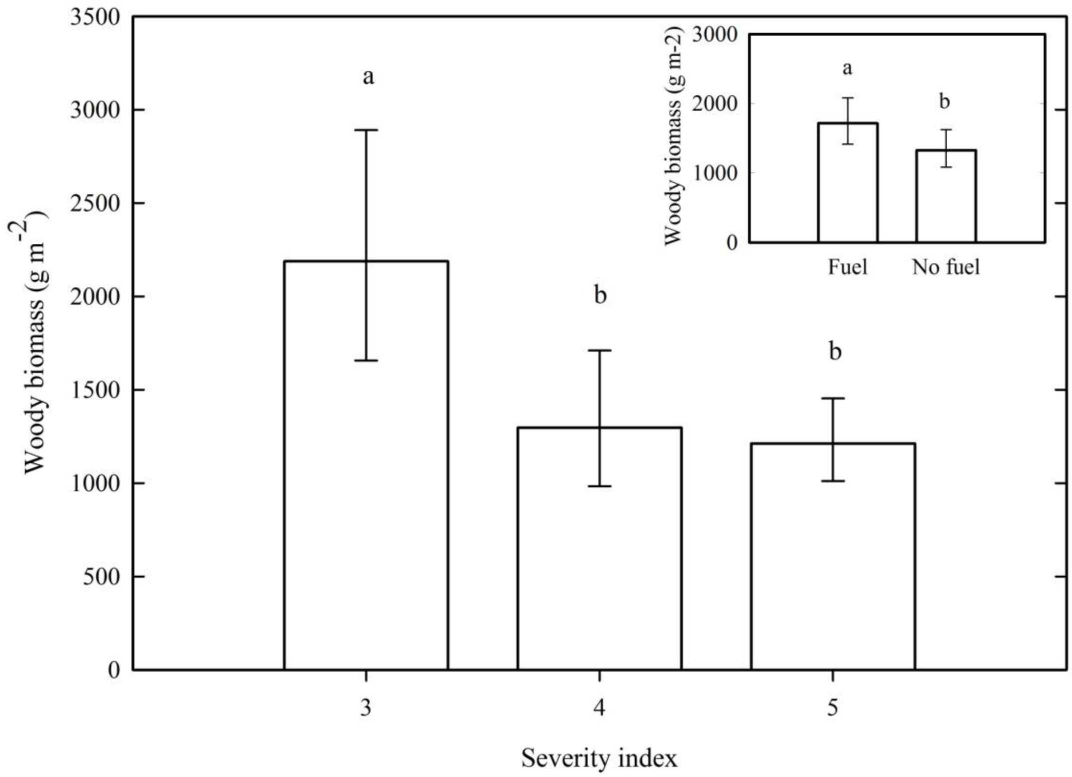

Fig. 2.3. Mean woody biomass (error bars are ± 95% CI) for burn severity. Letters indicate significant differences in biomass ($p < 0.05$). Insert: Mean woody biomass production (error bars are ± 95% CI) from the shrub island fuel manipulations. Letters indicate a marginal significant difference in biomass ($p = 0.068$).

Table 2.1. Description of burn severity index derived from ramet top-kill, char heights, and total fuel consumption.

Severity index	Percent top-kill	Char height (m)	Fuel consumed (%)	Description
1	< 20	< 0.25	< 50	Majority of stems retain green leaves, no direct effects from radiant heat
2	20 – 49	0.25 – 0.5	< 50	Mostly unburned, with some leaf retention, and scorched stems
3	50 – 89	0.25 – 0.5	50 – 75	Significant top-kill, surface litter mostly consumed, scorched stems and leaves
4	90 – 100	0.5 – 0.75	> 75	Complete to nearly complete top-kill, surface litter consumed, and scorch evidence indicating moderately high intensity fire
5	90 – 100	> 0.75	> 75	Complete to nearly complete top-kill, surface litter consumed, and scorch evidence indicating high intensity fire.

Table 2.2. Fire temperature (°C) at different sites and fuel manipulations.

Microsite	n	Mean	SE	Min	Max	CV
			Dataloggers			
Grass	45	539.74	21.65	32.71	990.00	26.91
Shrub no fuel	22	353.97	35.14	14.80	654.70	46.56
Shrub fuel	78	327.83	28.11	16.07	880.0	75.72
			Pyrometers			
Grass	21	141.67	7.94	107.00	191.00	25.70
Shrub no fuel	131	210.92	13.53	0.00	593.00	73.44
Shrub fuel	130	276.35	14.03	0.00	621.00	57.88

Table 2.3. Fireline intensity (kW m^{-1}) within shrub islands by fuel manipulation.

Microsite	n	Mean	SE	Min	Max	CV
Shrub no fuel	16	1273.64	244.90	318.06	2745.92	60.81
Shrub fuel	40	3007.10	706.18	119.98	14830.23	132.84

Chapter 3 - Physiological integration of *Cornus drummondii* and the mechanisms driving woody encroachment in the tallgrass prairie

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Abstract

Clonal woody species appear to be one of the greatest contributors to woody encroachment in graminoid-dominated mesic grasslands. Part of the high success rate may be due to the ability of clonal species to circumvent intensive recruitment filters through the acropetal translocation of nutrients and water from mother to daughter ramets. The integration of environmental heterogeneity through the clonal architecture can also provide a buffer against competitive effects of neighboring grasses, making species like *Cornus drummondii* well adapted to respond to resource limitations and disturbances. This may further impart a competitive advantage to intraclonal daughter ramets, facilitating the competitive displacement of native graminoids and forbs. To assess the physiological integration hypothesis as an adaptive trait for clonal expansion of *C. drummondii* in fire prone habitats, we implemented a rhizome severing experiment on 384 clonal ramets across six watersheds at the Konza Prairie Biological Research Station.

We found that rhizome severed ramets had a higher incidence of mortality (over 58%), had lower photosynthetic rates, were more water stressed, and had less total biomass production throughout the growing season. In addition to the influence of severing on photosynthetic rates ($p < 0.0001$), burning and CO_2 availability also played a significant role in affecting photosynthesis ($p = 0.0129$). The effects of burning and severing were also major factors influencing water potential throughout the growing season, with the differences in severed versus intact ramets becoming more pronounced in the higher temperatures and greater water deficits of mid-summer. This also translated to a general decline in nodal leaf populations ($p < 0.0001$) and lower biomass production ($p = 0.0244$) over the growing season.

Synthesis:

Our results indicate that daughter ramets of *C. drummondii* are physiologically reliant on the connective vasculature in the rhizomes to support growth and respond favorably to fire. Physiological connection to parent ramets that have deeper roots and access to more reliant water sources allows daughter ramets to survive mid-summer drought and increases the likelihood of successful establishment and further clonal reproduction. This may explain how clonal woody plant species are able to establish new ramets and persist in suboptimal conditions.

Key words:
Clonal integration, *Cornus drummondii*, tallgrass prairie, woody encroachment

Introduction

Alterations in disturbance regimes and land-use practices have greatly reduced the extent of viable and intact grasslands, making them one of the most threatened biomes worldwide (Samson *et al.* 2004; Hoekstra *et al.* 2005). Coupled with habitat fragmentation and destruction, the dominance structure has shifted towards native trees and shrubs over the last century, presenting a unique contemporary threat to grassland systems (Briggs *et al.* 2005; Buitenwerf 2012). Many of the C_4-dominated mesic grasslands of the North American Great Plains prairie exist in an alternate stable state that is maintained primarily through the interaction of fire and plant growth responses (Briggs *et al.* 2002; Lett and Knapp 2003; McCarron and Knapp 2003; Heisler *et al.* 2004). Woody encroachment not only reduces viable grassland habitat by changing the vegetation structure, it is also associated with declines in plant species diversity (Knapp *et al.* 2008; Ratajczak *et al.* 2012), as well as alterations in light, water, and nutrient dynamics (Schlesinger *et al.* 1990; D’Odorico *et al.* 2010). This contributes to a nearly irreversible change in the disturbance regimes responsible for maintaining the herbaceous diversity and the graminoid dominance of these systems (Gibson *et al.* 1990; Riginos and Grace 2008; Staver *et al.* 2011; Ratajczak *et al.* 2011).

Woody encroachment has traditionally been linked to anthropogenically altered disturbance regimes (Briggs *et al.* 2002; McCarron and Knapp 2003; Buitenwerf *et al.* 2012), but atmospheric CO_2 concentration appears to be strongly correlated with increases in woody cover (Bond and Midgley 2000; Buitenwerf *et al.* 2012) and may favor the development and expansion of clonal shrubs (Bond *et al.* 2003). Under elevated atmospheric CO_2 concentrations, woody species exhibit a higher water use efficiency that may also help drive further encroachment by decreasing the limitation of water availability (Schlesinger *et al.* 1990; Scholes and Archer 1997; Bond 2008; Ratajczak *et al.* 2011; Bond and Midgley 2012). The clonal structure of many of the C_3 shrub species that are driving the change in the vegetative structure in these grasslands (Ratajczak *et al.* 2011) may further the success of ramets through greater capacity for resource acquisition, storage, and allocation by means of intraclonal sharing of resources (Hartnett and Bazzaz 1985; Hartnett 1993; Nippert and Knapp 2007). The intraclonal sharing of resources such as water can greatly reduce the effects of interspecific competition in a heterogeneous environment, and as a result, increase daughter ramet performance (Hartnett and Bazzaz 1985; Hartnett 1993).

The clonal shrub *Cornus drummondii*, an abundant shrub species in the tallgrass prairie, is hypothesized to be buffered against resource limitation and disturbance by means of its clonal structure. Through the acropetal translocation of nutrients and water from parent ramets to daughter ramets, *C. drummondii* is capable of circumventing recruitment filters and neighborhood competitive effects (McCarron and Knapp 2003; Nippert and Knapp 2007; Ratajczak *et al.* 2011). The ability to plastically alter water acquisition based on availability and leaf water stress and translocate these water resources throughout the clonal architecture may prove advantageous during the mid-summer droughts that limit water availability to the deeper soil horizons, which daughter ramets would otherwise have no access to without the physiological connection provided by the connective rhizome (Nippert and Knapp 2007; Ratajczak *et al.* 2011). Ratajczak *et al.* (2011) demonstrated that up to 50 per-cent of xylem water in daughter ramets persisting in the grassland/shrub matrix is traceable to these deeper, more reliant water tables, offering compelling evidence for the sharing of resources between intraclonal ramets as a mechanism facilitating shrub expansion.

There also appears to be a life-history trade-off between seed reproduction and resprouting that accounts for the tolerance of high frequency and high intensity fires, where leaf-level carbon assimilation is enhanced to meet the demands of resprouting and tissue regeneration (Bond and Midgley 2012; Paula and Pausas 2006). The perennating organs of *C. drummondii* exhibit a quick response to disturbance with early spring emergence, drawing from the high nitrogen and water reserves of the clonal genet (Lett *et al.* 2004; McCarron and Knapp 2003). The higher nitrogen and water availability in post-burned shrub islands contributes to higher photosynthetic rates, and subsequently greater tissue growth, and may even be able to compensate for competitive interactions as well (McCarron and Knapp 2003). This recovery mechanism may also be directly proportional to carbon reserves and allocation provided by the clonal rooting strategy (Polley *et al.* 1997; Bond and Midgley 2012), limiting the need to down-regulate photosynthesis for tissue repair and root growth (Buitenwerf et al. 2012).

The goal of this study was to experimentally test the role of clonal integration in the expansion of *C. drummondii* in the fire-prone tallgrass prairie. To assess the physiological integration hypothesis (Hartnett and Bazzaz 1983) as an adaptive trait for clonal expansion of *C.*

drummondii, we implemented a rhizome severing experiment meant to determine the degree of physiological interdependency among intraclonal ramets. In severing the connective rhizome, we hypothesize that severed daughter ramets will have: 1.) reduced survivorship rates, 2.) greater water stress, but higher water use efficiency, 3.) lower photosynthetic capacity under increasing CO_2 concentrations, and 4.) reduced growth and biomass production.

Materials and Methods:

Study site

All research was conducted at the Konza Prairie Biological Station (KPBS), a 3487-ha native tallgrass prairie preserve located in the northern reaches of the Flint Hills of northeast Kansas, USA (39°05’ N. 96°35’ W). KPBS is a part of the Long-Term Ecological Research program (LTER), with ~55 managed watersheds of various fire and grazing treatments. Elevation at KPBS ranges from 320 to 444 m above sea level. Mean annual rainfall is 835 mm with 75% falling between April and September (Hayden 1998). Plant communities are dominated by the warm-season C_4 grasses *Andropogon geradii* Vitman, *A. scoparius* Michx., and *Sorghastrum nutans* (L.) Nash. C_3 shrubs *Cornus drummondii* C.A. Mey, *Rhus glabra* L., and *Prunus americana* Marsh (Freeman and Hulbert 1985; Abrams and Hulbert 1987; Gibson and Hulbert 1987; Knapp *et al.* 1993). The C_3 clonal shrub species *C. drummondii* was chosen for this study because this species is abundant and widespread on KPBS (Briggs *et al.* 2002; Heisler *et al.* 2003). *C. drummondii* is a medium sized (1-4 m high) shrub, found commonly in grasslands and old fields from southern Ontario westward to Illinois, Iowa and South Dakota and southward to Alabama and Texas (Stephens 1969). The shrub is long-lived, clonal, and produces numerous stems in high-density patches termed “shrub islands”. These islands can be quite large and it is not uncommon for a single shrub island to be over 5m in diameter. This is an ideal plant for our experiments since it is one of the most common shrubs on KPBS and responds rapidly to fire suppression in the tallgrass prairie, while also persisting in sites with a high fire frequency (Briggs *et al.* 2002).

Experimental design

Six watersheds at KPBS were chosen for this experiment. Three of these watersheds were burned annually in the spring (mid to late April) for at least three years prior to this study and three watersheds were not burned within the three years prior to this study. In each of the six watersheds, we randomly selected 32 distinctly isolated, monospecific islands of *C. drummondii*. Shrub islands were selected based on size (mean 50.7 ± 4.2 m^2), slope position, and soil type within each watershed. Soils were primarily silty-clay loams overlaying shale bedrock. Two ramets per island, one to two nodes towards the center of the island from the grassland matrix, were chosen as replicate pairs to either have their rhizomes severed or left intact. One ramet was used for all physiological responses and the other was used for measuring leaf demography to avoid confounding leaf population values. Rhizomes were severed close to the nearest neighbor with a sharpened steel blade inserted into the soil in a full circle to minimize damage to the root system while insuring that all connections to parent ramets were severed. This procedure left each ramet with an intact nodal section of the rhizome and an intact root system. Severing occurred in early June on all watersheds and each ramet was permanently tagged and monitored throughout the growing season.

Leaf level photosynthesis

Leaf level photosynthesis measurements were taken mid-July between 1000 and 1500 hours (CST). This mid-July time period is when the maximum growth rates occur prior to late summer senescence and is usually marked by high air temperatures and greater mid-season water stress (Turner and Knapp 1996; Nippert *et al.* 2007). This mid-July time period was specifically chosen to account for low resource availability and higher use efficiencies, as water availability and soil nitrogen are generally lower during this time (Nippert *et al.* 2007).

Upper canopy leaves on six randomly selected ramets per watershed, three from each experimental manipulation on the rhizome, were used to measure gas exchange. Rates of instantaneous net CO_2 assimilation (A), stomatal conductance to water vapor diffusion (g), and transpiration (E) were measured using a LI-COR 6400 IRGA with an artificial red/blue LED light source (6400-02B, LI-COR Inc., Lincoln, NE, USA). $A_{\max}$ was measured at varying levels of reference CO_2 concentrations (C_a). Leaves were placed inside the leaf chamber and allowed to reach steady-state photosynthesis at sub-ambient (210 µmol CO_2 mol^{-1} air), ambient (370 µmol CO_2 mol^{-1} air), and super-ambient (650 µmol CO_2 mol^{-1} air) CO_2 concentrations, at saturating light intensity (2000 µmol $m^{-2}s^{-1}$). Between each CO_2 concentration change, A, g, and flow rate were allowed to stabilize within the IRGA cuvette. Leaf temperature was also allowed to increase with ambient daily air temperature. Instantaneous water-use efficiency (WUE) at each C_a level was calculated using $A_{\max}$ at C_a divided by the corresponding transpiration rate (E).

Water potential and water use efficiency

Water stress was assessed by measuring pre-dawn and mid-day leaf water potentials (ψ) with a pressure chamber (PMS-1000, PMS Instruments, Corvallis, OR, USA). Water potential was measured three times over the growing season at evenly spaced intervals (approximately 30 days). Leaf tissue was also collected at these times to determine intrinsic seasonal water use efficiency (iWUE). Samples were dried at 60°C for 48 hours, ground to powder, and a 2 mg subsample was combusted in a mass spectrometer at the KSU Stable Isotope Mass Spectrometry Lab (SIMSL) using standard methods on a Thermo Finnigan Delta Plus mass spectrometer

(Thermo Fisher Scientific Inc., Waltham, MA, USA). Using $\delta^{13} C_a$ values (-8.525 ‰) from flask samples of atmospheric air around the time of sampling (NOAA Earth System Research Laboratory 2012), we were able to calculate foliar δ^{13}C isotope discrimination (Δ) and intrinsic water use efficiency using equations 1 through 3 from McLauchlan *et al.* (2010).

Leaf demography

Immediately following the severing treatment, a metal ring was affixed towards the apical meristem on one of the sister ramets of the set of two that received the severing treatment for each island. This design allowed for easy tracking of leaf births and deaths by considering each node on the plant as a population of leaves, providing a proxy estimate of foliar biomass in a non-destructive measurement. From the rates of births and deaths, we were able to calculate the finite rate of increase (λ) for each nodal leaf population. Nodal leaf populations were monitored every 18 days from the day of the severing to leaf senescence and abscission in early October. We also measured stem heights and stem basal diameters at each sample date and estimated end of season aboveground biomass (g m^{-2}) using the allometric relationships developed by Lett et al. (2004).

Statistical analysis

All data were analyzed using mixed effects models with repeated measures in a fully factorial design with a split-plot. Models were fit using the GLIMMIX procedure in SAS V9.2 (SAS Institute Inc., Cary, NC, USA), with the exception of the Gehan's test on censored data for survival analyses, which was processed with program R V 2.14.0 (R Development Core Team, Vienna, Austria) using the package 'gtools' (Warnes 2012). Gehan's test was run at 5000 permutations to get a large-sample approximation for our lower-tail *p*-value.

Models included the fixed effects of burning, severing, and time. Watershed was used as the whole-plot experimental unit within burning treatments. The split-plot experimental unit was defined as each stem within the corresponding watershed by sever treatment. The split-split-plot incorporated the factor of time and the experimental unit was stem by all factors listed above. Denominator degrees freedom and standard errors were approximated using the Kenward-Roger's estimation and Bonferroni's adjustment was used for all multiple comparisons. The model structure was evaluated using Akaike's Information Criteria, which decreases as model fit improves. A_{max} and λ, were both log-transformed in order to meet assumptions of normality.

Results:

Over the 2011 growing season, 58% of rhizome-severed ramets died within the three months that we monitored this experiment, with half of these mortality events occurring within the first month following severing (June 4 through July 10). A higher percentage of these mortality events were observed within the non-burned watersheds (78.3%) in comparison to the burned watersheds (34.8%; $Z = -4.57$, $p < 0.001$). The intact control ramets, however, showed 0% mortality over the growing season.

ANOVA results (Table 3.1) showed that the effects of severing and the interaction of burning and atmospheric CO_2 concentrations (C_a, µmol CO_2 mol^{-1} air) had the greatest influence on the maximum rate of photosynthesis (Fig. 3.1). Mid-summer maximum photosynthetic rates were ~46% greater (Bonferroni adjusted $p < 0.0001$) for intact ramets (7.81 ± 1.56 µmol CO_2 m^{-2}s^{-1}; ± 1 SE) than severed ramets (5.46 ± 1.13 µmol CO_2 m^{-2}s^{-1}). Higher levels of atmospheric CO_2 concentrations were generally reflected in higher photosynthetic rates across all burning treatments as well, though there were no significant differences in the main effects of burning at each C_a level ($p > 0.05$). Within the burning treatments, the difference in A_{max} between sub-ambient and ambient C_a was ~22% ($p < 0.0001$), with 1.73 ± 0.27 μmol CO_2 $m^{-2}s^{-1}$ and 9.33 ± 2.01 μmol CO2 $m^{-2}s^{-1}$ in the sub-ambient and ambient treatments respectively. The difference in A_{max} between ambient and super-ambient C_a in the burned treatments was ~59 % ($p = 0.0038$), with 16.17 ± 2.08 μmol CO_2 $m^{-2}s^{-1}$ in the super-ambient treatment. In the non-burned treatments, the difference in A_{max} from sub-ambient to ambient was ~34 % ($p < 0.0001$), with 1.28 ± 0.19 μmol CO_2 $m^{-2}s^{-1}$ and 5.27 ± 1.58 μmol CO_2 $m^{-2}s^{-1}$ in the sub-ambient and ambient treatments respectively. The difference between ambient and super-ambient C_a was not significant ($p = 0.65$), with an A_{max} at super-ambient of 3.72 ± 1.34 μmol CO_2 $m^{-2}s^{-1}$.

Severing was also a major factor influencing predawn water potential ($df = 1, 61.22$, $F = 7.68$, $p = 0.0017$). Seasonal patterns of predawn water potential showed that severed ramets experienced greater water stress throughout the growing season, with a seasonal mean of -1.39 ± 0.13 MPa, compared to intact ramets with -1.14 ± 0.13 MPa. Severed ramets also showed greater seasonal declines in midday water potential ($df = 2, 159.1$, $F = 3.70$, $p < 0.0269$), becoming increasingly water stressed as the growing season progressed into the midsummer drought periods, where air temperatures were high and soil water moisture was low (Fig. 3.2). The difference between intact and severed ramets was observed shortly after severing, with a mean difference of 0.59 ± 0.09 MPa ($p < 0.0001$) in June and 0.40 ± 0.09 MPa ($p < 0.0001$) in July. At the end of the growing season this difference became more pronounced with a mean difference of 0.77 ± 0.11 MPa ($p < 0.0001$) in August. Midday water potential was also influenced by the effects of fire throughout the growing season ($df = 2, 162.4$, $F = 5.15$, $p < 0.0068$). In burned watersheds, water potential decreased through the growing season from June through August (-1.34 ± 0.12 MPa, -1.92 ± 0.10 MPa, and -2.88 ± 0.11 MPa for June, July, and August respectively). A similar trend was observed in the non-burned watersheds as well (-1.47 ± 0.10

MPa, -1.87 ± 0.12 MPa, and -2.55 ± 0.13 MPa for June, July, and August respectively). The only significant difference between burned and non-burned watersheds was in August, with a difference of 0.33 ± 0.17 MPa ($p = 0.05$).

The two way interactions of rhizome severing by C_a and burn treatment by C_a were significant drivers of instantaneous WUE (Table 3.1). WUE was generally higher in the intact ramets in comparison to the severed ramets with significant differences at ambient CO_2 concentrations (1.38 ± 0.36 mm CO_2 mol^{-1} H_2O, $p = 0.0005$) and at super-ambient CO_2 concentrations (1.38 ± 0.27 mm CO_2 mol^{-1} H_2O, $p < 0.0001$; Fig. 3.3). The only significant difference in WUE between burned and non-burned ramets was at super-ambient CO_2 concentrations, with a mean difference of 1.11± 0.37 mm CO_2 mol^{-1} H_2O ($p = 0.0714$). For intrinsic WUE, severing was once again a major influencing factor, as was sampling date (Table 3.1). Carbon isotope discrimination (Δ) was higher in intact ramets versus severed ramets, with a difference of 0.58 ± 0.22‰ ($p = 0.0113$). The severed ramets had higher (less negative) values of foliar $\delta^{13}C$, with a composition of -26.79 ± 0.24 ‰ versus the intact control ramets, which had -27.36 ± 0.24 ‰. This relationship between Δ and foliar $\delta^{13}C$ translated to higher iWUE for severed ramets over the course of the growing season (Fig. 3.3, Table 3.1). We also observed an increasing trend in iWUE across all treatments from the beginning of the growing season through the end of the growing season, with a seasonal difference of 0.09 ± 0.04 mmol CO_2 mol^{-1} H_20 ($p = 0.0248$).

There was a lagged effect in the response of leaf demography, with no change in λ for the first 18 days (Fig. 3.4). Severed ramets had uniformly lower λ values than the intact control ramets throughout the growing season, with λ values ≤ 1. A similar trend was observed in the non-burned treatments, which generally had lower λ values throughout the growing season compared to the burned ramets. Table 3.2 shows the ANOVA results for the main effects of severing and burning versus time. At 36 days post-severing, the severed ramets had a mean λ value of 0.92 (95% CI: 0.81-1.04), while the intact control ramets had a mean λ value of 1.49 (95% CI: 1.34-1.65). At 108 days post-severing, the severed ramets had a mean λ value of 0.83 (95% CI: 0.72-0.94), while the intact control ramets had a mean λ value of 0.99 (95% CI: 0.91-1.09). With the non-burned treatments, the mean λ value at 36 days post-severing was 0.91 (95% CI: 0.80-1.03), and the mean λ value for burned ramets was 1.50 (95% CI: 1.35-1.68). With the non-burned treatments, the mean λ value at 108 days post-severing was 0.80 (95% CI: 0.70-0.92), and the mean λ value for burned ramets was 1.02 (95% CI: 0.92-1.14).

Total above ground biomass also had a similar relationship with burning and severing ($df = 1, 4$, $F = 12.48$, $p = 0.0242$), showing a general increase over time ($df = 5, 897$, $F = 32.36$, $p < 0.0001$). In the burned watersheds, intact ramets had 3.24 g m^{-2} (95% CI: 1.76-5.98, $p = 0.006$) greater biomass production than severed ramets. However, in the non-burned watersheds no significant difference in above ground biomass production was observed ($p > 0.05$). Ramet height was most affected by the severing and burning treatments as well, with a three-way interaction between burning, severing, and time (Table 3.2). The most notable differences in height were in the burned watersheds, where the mean difference in height between severed and intact ramets at the beginning of the growing season was not significantly different (0.09 ± 3.0 cm, $p = 1.0$), but at the end of the growing season intact raments were significantly higher than severed ramets (66.9 ± 8.06 cm vs. 47.19 ± 8.21 cm respectively) with a difference of 19.75 ± 3.40 cm ($p < 0.0001$). Intact ramets generally grew at a greater rate than the severed ramets in both burned and non-burned watersheds, though the differences were smaller for the non-burned watersheds (Fig. 3.5).

Discussion:

Congruent with the findings of Heisler *et al.* (2004) and Ratajczak *et al.* (2011) burning and water relations appeared to be two of the greatest factors influencing the growth dynamics of *C. drummondii*. Our results indicate that the clonal integration of ramets has a profound effect on the survivorship of daughter ramets following fire. Increased soil temperatures following fire disturbance may be a significant factor influencing the higher survivorship of ramets in the burned watersheds, facilitating growth and the mobilization of nutrients (McCarron and Knapp 2003). These ramets are also able to take advantage of the transient period of greater resource availability following fire, exhibiting a positive response in growth and survivorship (McCarron and Knapp 2003).

The cover of *C. drummondii* at the Konza Prairie has increased in a nearly linear fashion over the last 26 years, becoming a major vegetation component (Knapp *et al.* 1993; Briggs *et al.* 2002; Ratajczak *et al.* 2011). Woody encroachment has traditionally been linked to anthropogenically altered disturbance regimes (Briggs *et al.* 2002; McCarron and Knapp 2003; Buitenwerf *et al.* 2012), but there is increasing evidence for implicating atmospheric CO_2 concentration for the increase of woody cover (Bond and Midgley 2000; Buitenwerf *et al.* 2012). The ability to store carbon below-ground and up-regulate photosynthesis under increased atmospheric CO_2 concentrations facilitates the development and release of above-ground ramets from the fire trap of successive top-killing (Paula and Pausas 2006; Barney *et al.* 2010; Buitenwerf *et al.* 2012). Greater atmospheric CO_2 concentrations also contribute to increased water-use efficiency, reducing the limiting factor of water availability (Schlesinger *et al.* 1990; Scholes and Archer 1997; Bond 2008; Ratajczak *et al.* 2011; Bond and Midgley 2012).

Under high water stress, due to seasonal deficits associated with mid-summer drought, the incidence of xylem embolisms and ramet dieback are higher with the daughter ramets that were severed from their clonal parents. Unable to access the deeper water sources from the rhizomatous connection to parent stems, severed *C. drummondii* daughter ramets became increasingly water stressed as the growing season progressed (Fig. 3.2). This was exemplified in the xylem water potential of severed ramets, which had much lower xylem water potentials than the intact control ramets throughout the season, with these differences in water potential increasing towards the end of the growing season.

As a C_3 shrub, leaf level photosynthesis was likely constrained by the limited water availability for the uptake of CO_2, as shown by lowered values of A_{max}. Stomatal conductance became a stronger limiting factor at ambient and super-ambient CO_2 concentrations, where the decrease in water potential resulted in much lower rates of photosynthesis in severed ramets. Burning also contributed to this trend, as ramets in burned watersheds had higher rates of photosynthesis than the non-burned ramets, which may be attributed to greater foliar nitrogen concentrations and higher growth demands (McCarron and Knapp 2003). The higher photosynthetic rates in burned ramets were counteracted by the decrease in stomatal conductance for severed ramets, thus contributing to higher WUE. For severed ramets, the lowered rates of photosynthesis were maintained throughout the growing season and led to overall higher seasonal water-use efficiency, especially as photosynthesis equalized with the lower partial pressure of intercellular CO_2 and transpiration rates. The $\delta^{13}C$ values, which were more negative in the intact ramets, indicated reduced stomatal regulation to maximize carbon assimilation throughout the growing season, even when water stressed towards the end of July and August. The reduced water availability and lowered photosynthetic rates of severed ramets further contributed to decreased biomass production and negative finite rates of increase for nodal leaf populations.

Our results are the first to quantitatively show the reliance of intraclonal *C. drummondii* ramets on parent stems and how the physiological integration provided by the connective rhizome is related to the spatial and temporal heterogeneity of water availability and effects of fire disturbance. The effects of water availability and responses to fire are integrated over the entirety of the clonal genet, reducing the strength of recruitment filters and potential demographic bottle-necks related to competition and fire induced top-kill. This integration in intraclonal ramets increases the performance of daughter ramets, increasing the likelihood of successful establishment and long-term survival in burned habitats. With greater survival rates and reduced effects of competition, clonal integration may facilitate further clonal expansion, conferring persistence to woody species under disturbance (Hartnett and Bazzaz 1985; Hartnett 1993; Bond and Midgley 2000; Ratajczak *et al.* 2011). Even though clonal reproduction may cause a decrease in genet performance when individual ramets exert a strong resource sink (Schmid and Bazzaz 1987; Abrahamson *et al.* 1991), the continual iteration of clonal ramets may allow for the eventual escape from fire traps of successive top-killing as they fundamentally alter the effects of fire behavior (Barney *et al.* 2010) by competitively excluding herbaceous vegetation (Oborny *et al.* 2000; Lett and Knapp 2003).

With atmospheric CO_2 concentrations continually rising, it is possible that restoring historic disturbance regimes will not be sufficient to circumvent woody encroachment, as the positive bio-feedback systems that facilitate woody encroachment are augmented by the effects of elevated CO_2. Once established, returning to a graminoid dominated stable state may be near impossible without intensive mechanical removal, and even then, many of these species leave a lasting legacy effect on the community dynamics (Lett and Knapp 2005). With greater temperature extremes and alterations in precipitation regimes associated with climate change, woody species may become even more pronounced in grasslands (Knapp *et al.* 2002), with clonal woody species driving the majority of this conversion (Ratajczak *et al.* 2011).

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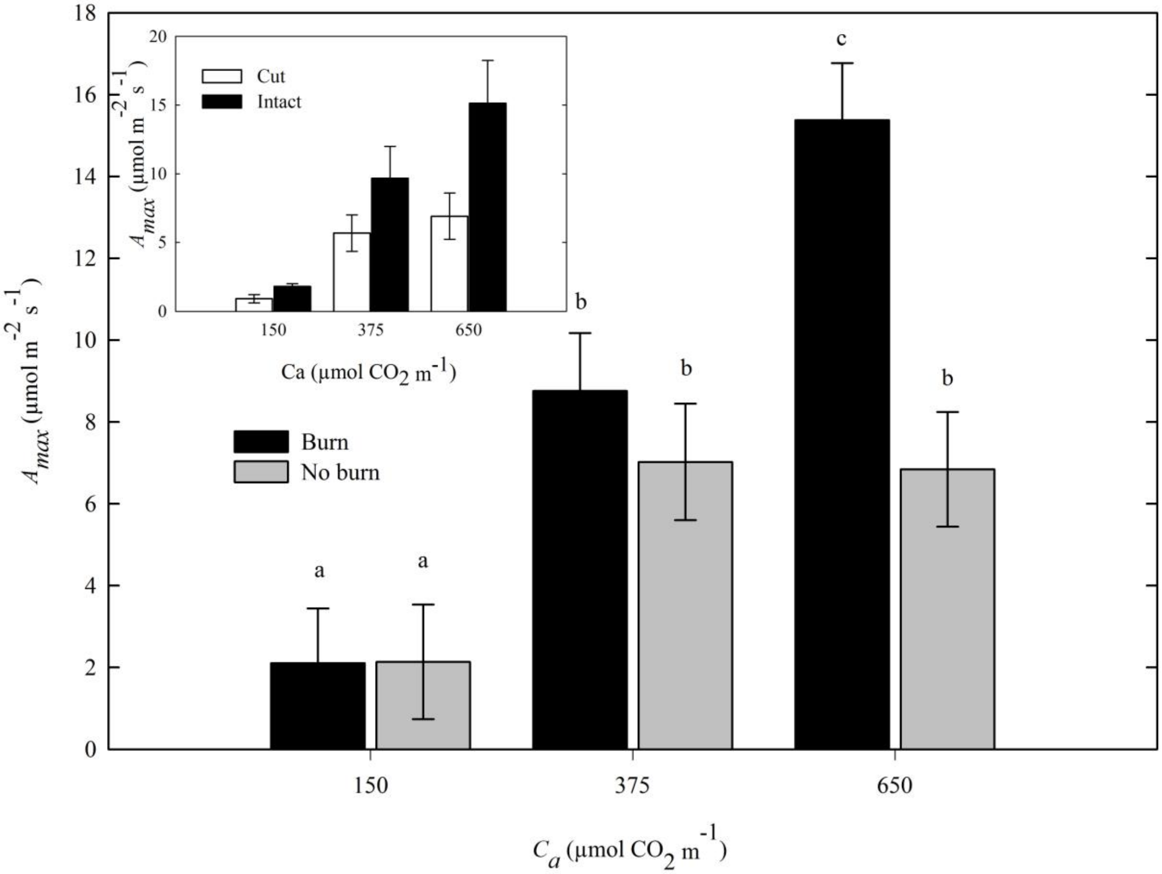

Fig. 3.1. Maximum photosynthetic rates (± 1 SE) before transformation for each of the burning treatments. Letters indicate significant differences among burning treatments between C_a levels (Bonferroni adjusted $p < 0.05$). Insert: Maximum rates of photosynthesis (± 1 SE) before transformation for ramets with severed and intact rhizomes. The difference in A_{max} between ramets with severed rhizomes and ramets with intact rhizomes in the pairwise comparison was 0.46 µmol m^{-2} s^{-1} (95% CI: 0.33-0.63, $p < 0.0001$).

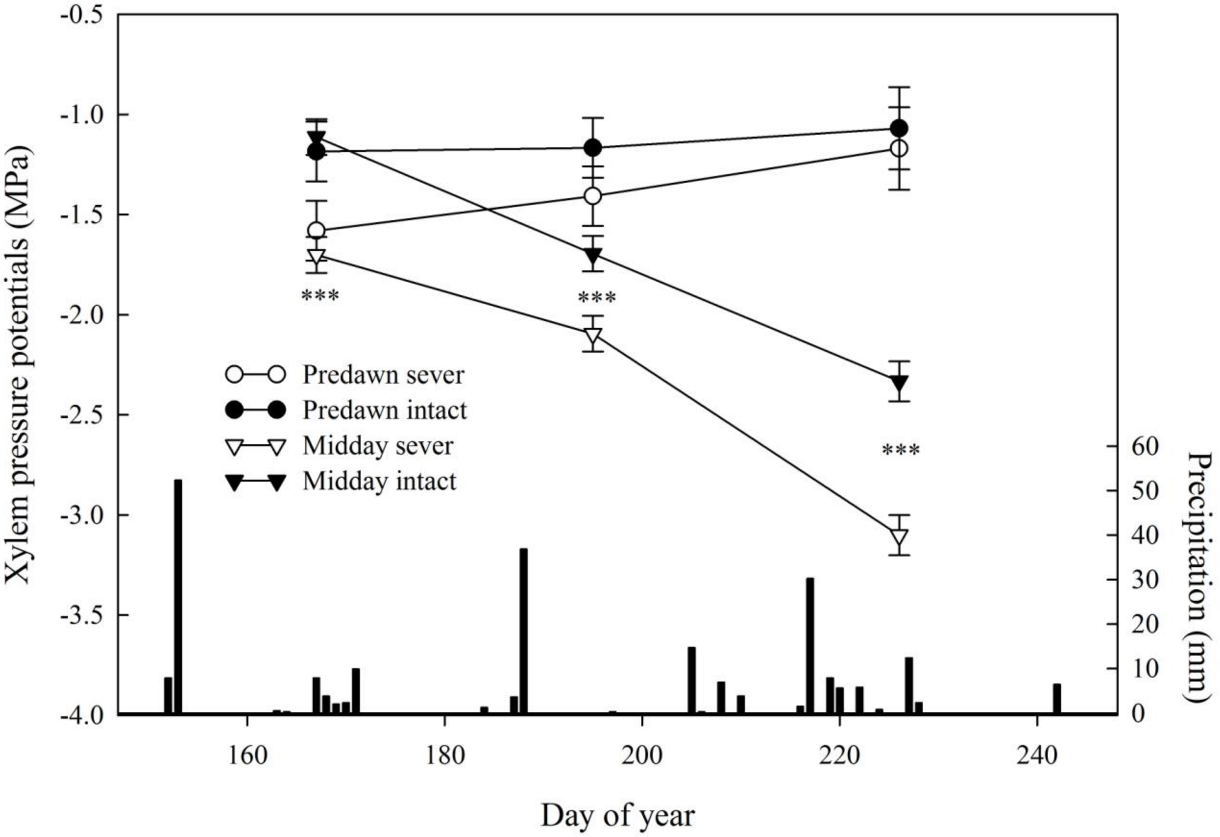

Fig. 3.2. Predawn and midday xylem water potential for severed and intact ramets throughout the growing season. The difference in severed versus intact ramets in predawn water potentials was 0.25 (± 0.08, $p = 0.0017$). Asterisks represent significant differences in midday water potential for each date ($p < 0.0001$). KPBS received 466 mm precipitation during the 2011 growing season (April through September).

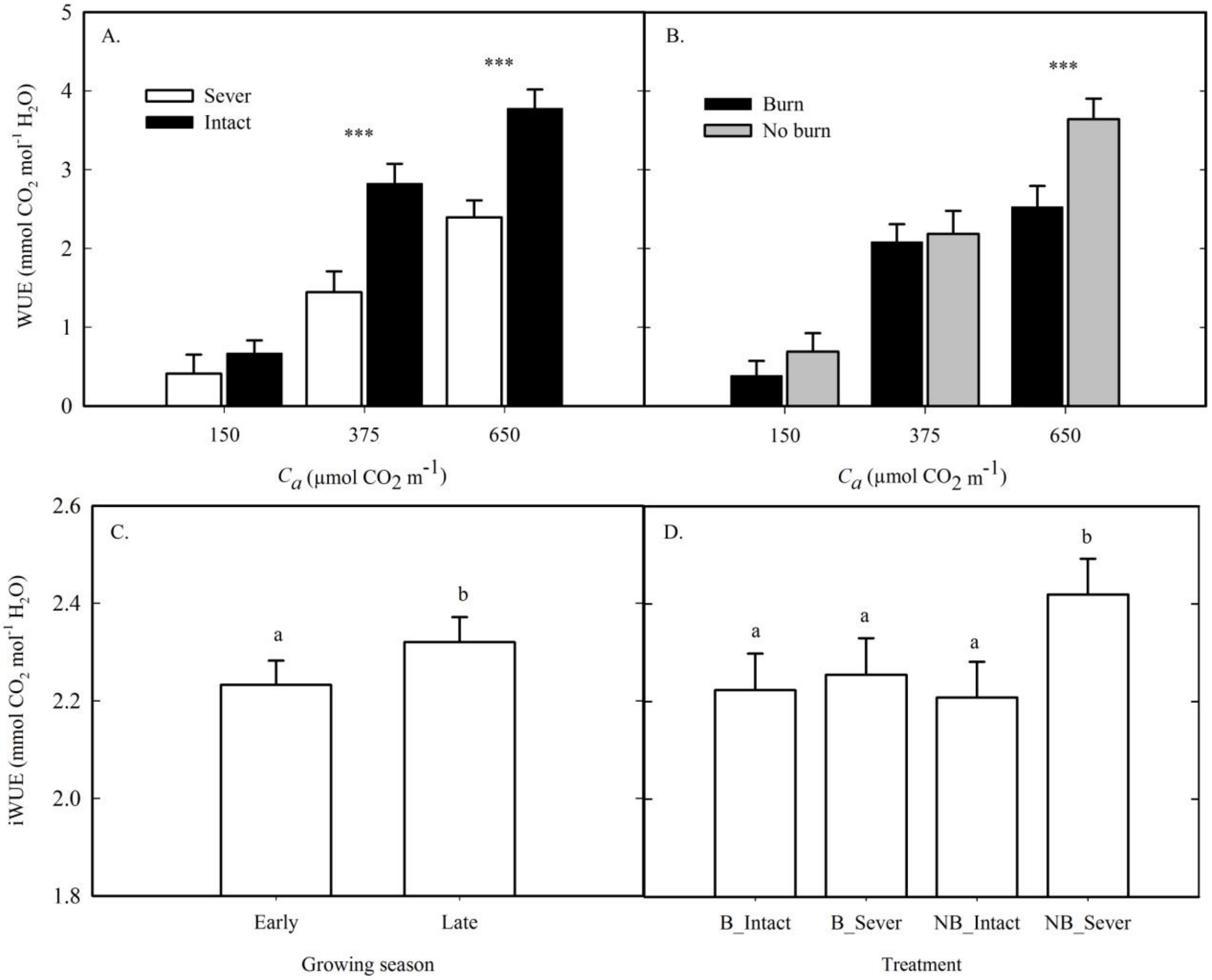

Fig. 3.3. Top panels. Instantaneous water use efficiency for severed and intact ramets (A.) and burned and non-burned ramets (B.) at each atmospheric CO_2 concentration levels (C_a). Asterisks represent significant differences between severed and intact ramets at each C_a level and differences in burned versus non-burned ramets at each C_a level (Bonferroni adjusted $p < 0.05$). Bottom panels. Intrinsic WUE over the growing season (C.), where early season refers to samples obtained in June and late growing season refers to samples obtained in August. Intrinsic WUE with severing within the burned (B_*) and non-burned (NB_*) watersheds (D.). Letters indicate significant differences between treatments (Bonferroni adjusted $p < 0.05$).

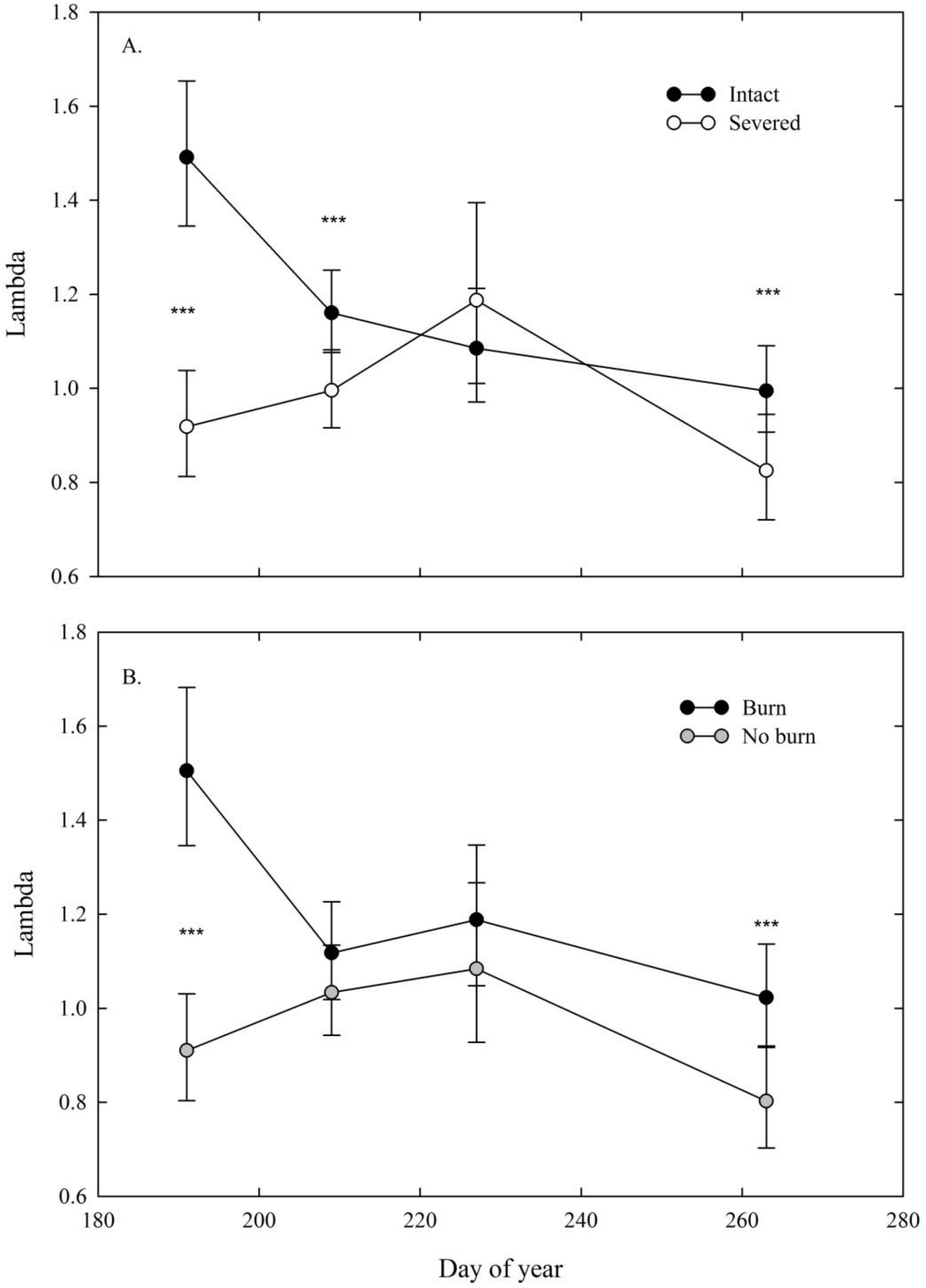

Fig. 3.4. A. Seasonal lambda values, indicating the finite rate of increase for nodal leaf populations on ramets with severed vs. intact ramets. B. Seasonal lambda values for nodal leaf populations on ramets in burned and non-burned watersheds.

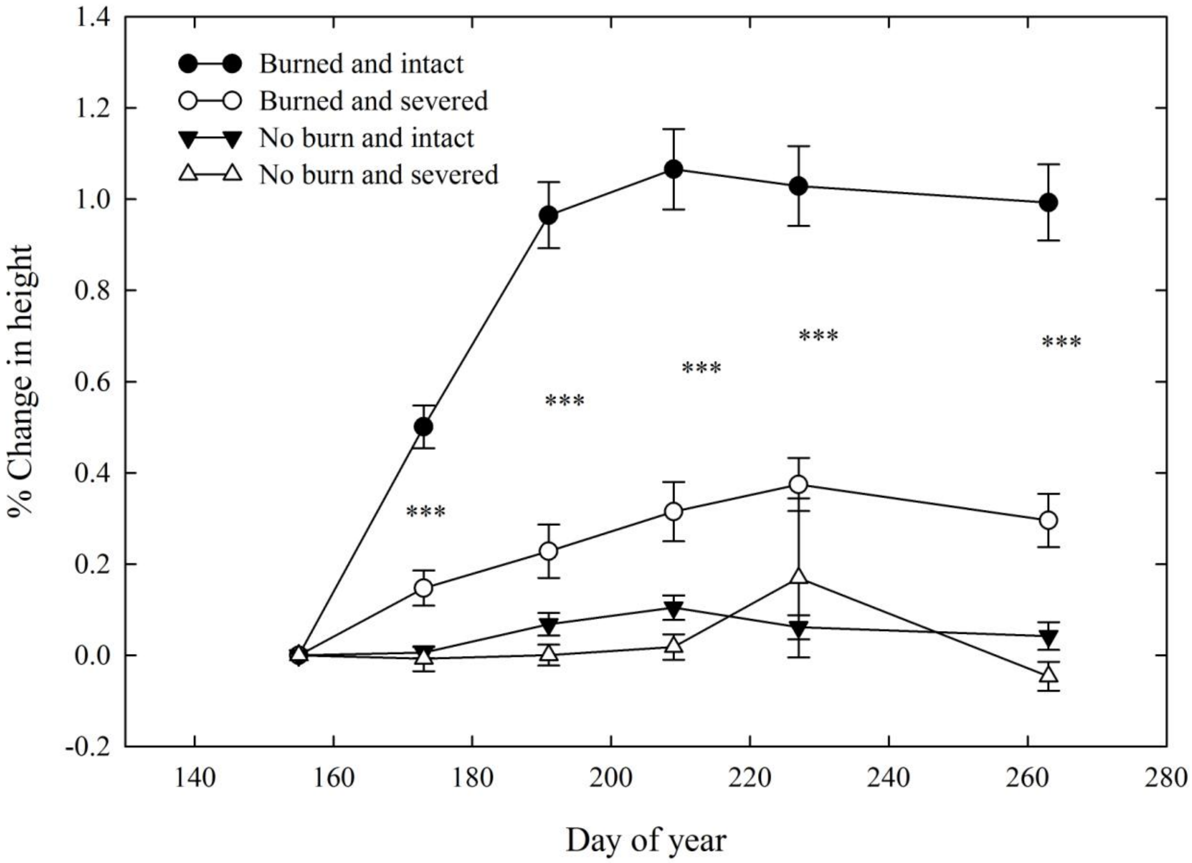

Fig. 3.5. Changes in ramets height (cm) over the growing season for severed and intact ramets within each of the burn treatments over the growing season. Error bars are ± 1 SE, and asterisks indicate significant differences between severed and intact ramets within the burned treatment ($p < 0.05$). Overall ramet height was influenced by the three-way interaction of burning, severing, and time ($df = 5, 903$, $F = 2.93$, $p = 0.0124$).

Table 3.1. Type III tests for fixed effects in each of the *Cornus drummondii* physiology ANOVA analyses. Fixed effects include atmospheric CO_2 concentrations (C_a), instantaneous water-use efficiency (WUE), and intrinsic water-use efficiency (iWUE). Order of fixed effects also indicates split-plot model structure for ANOVA analysis.

Model	*df.*	*F*	*P*
Photosynthesis			
Burn	1, 3.53	0.74	0.4435
Sever	1, 18.34	26.89	< 0.0001
Burn*Sever	1, 18.34	1.12	0.3035
C_a	2, 19.3	75.79	< 0.0001
Burn*C_a	2, 19.3	5.49	0.0129
Sever* C_a	2, 30.43	0.10	0.9089
Burn*Sever* C_a	2, 30.43	1.41	0.2586
WUE			
Burn	1, 1.77	3.80	0.2068
Sever	1, 19.44	22.56	0.0001
Burn*Sever	1, 19.44	0.68	0.4185
C_a	2, 29.88	75.11	< 0.0001
Burn* C_a	2, 29.88	3.78	0.0343
Sever* C_a	2, 35.33	5.94	0.0060
Burn*Sever* C_a	2, 35.33	0.34	0.7123
iWUE			
Burn	1, 3.86	0.64	0.4709
Sever	1, 76.58	6.73	0.0113
Burn*Sever	1, 76.58	3.69	0.0586
Date	1, 73.63	5.25	0.0248
Burn*Date	1, 73.63	2.42	0.1241
Sever*Date	1, 74.22	0.17	0.6839
Burn*Sever*Date	1, 74.22	0.43	0.5123

Table 3.2. Type III tests for fixed effects in each of the Cornus drummondii ANOVA analyses on ramet growth and nodal leaf population demographics. Order of fixed effects also indicates split-plot model structure for ANOVA analysis.

Model	*df*	*F*	*P*
Biomass production			
Burn	1, 4	36.23	0.0038
Sever	1, 4	13.63	0.0210
Burn*Sever	1, 4	12.48	0.0208
Time	5, 897	32.36	< 0.0001
Burn*Time	5, 897	0.65	0.6613
Sever*Time	5, 897	1.59	0.1599
Burn*Sever*Time	5, 897	2.00	0.0770
Height			
Burn	1, 4.00	14.45	0.0191
Sever	1, 903	73.58	< 0.0001
Burn*Sever	1, 903	41.29	< 0.0001
Time	5, 903	24.55	< 0.0001
Burn*Time	5, 903	11.96	< 0.0001
Sever*Time	5, 903	5.21	0.0001
Burn*Sever*Time	5, 903	2.93	0.0124
Leaf demography			
Burn	1, 5.20	19.78	0.0061
Sever	1, 6.67	16.26	0.0055
Burn*Sever	1, 6.67	0.49	0.5089
Time	3, 275.1	9.21	< 0.0001
Burn*Time	3, 275.1	9.35	<0.0001
Sever*Time	3, 275.1	8.76	<0.0001
Burn*Sever*Time	3, 275.1	0.63	0.5941

Chapter 4 - Conceptual model of woody encroachment

Fire and clonal integration are two of the greatest factors driving the encroachment of the C_3 shrub *Cornus drummondii* in the tallgrass prairie. Fires of high intensity and high frequency can act as strong recruitment filters for *C. drummondii* seedlings, but once established, the effects of fire promote shrub expansion (Briggs *et al.* 2002; Heisler *et al.* 2003). Increased soil temperatures following fire facilitate the mobilization of nutrients and the growth of belowground meristems (McCarron and Knapp 2003). This leads to a positive response in growth and survivorship in clonal ramets following fire, as they are able to take advantage of the transient period of higher light and nitrogen resource availability (McCarron and Knapp 2003). The acropetal transport of nutrients and water from parent to daughter ramets also provides daughter ramets with a greater competitive ability through the integration of environmental heterogeneity across the clonal architecture (Hartnett and Bazzaz 1985; Hartnett 1993), and at a critical size class will lead to the exclusion of herbaceous vegetation in the understory (Heisler *et al.* 2003). This decrease in herbaceous vegetation further diminishes the behavior and effects of fire, destabilizing fire propagation and consequently decreasing fire intensity and ramet mortality (Finney *et al.* 2010; Pearce *et al.* 2010). Fig. 4.1 illustrates this process through the life history stages in a *C. drummondii* invasion and the corresponding vegetation and fire dynamics.

Woody encroachment has traditionally been linked to anthropogenically altered disturbance regimes (Briggs *et al.* 2002; McCarron and Knapp 2003; Buitenwerf *et al.* 2012), but atmospheric CO_2 concentration appears to be strongly correlated with the increase in woody cover (Bond and Midgley 2012; Buitenwerf *et al.* 2012) and may favor the development and expansion of clonal shrubs (Bond *et al.* 2003). The ability to store carbon gains below-ground and up-regulate photosynthesis under increased atmospheric CO_2 concentrations facilitates the developmental growth and release of above-ground ramets from the fire trap of successive top-killing (Paula and Pausas 2006; Barney *et al.* 2010; Buitenwerf 2012). This is accomplished by allocating carbon to biomass production in the aboveground structures that shade out herbaceous vegetation in the understory (Heisler *et al.* 2004), leading to reduced fire intensity and heat flux at each ramet within the shrub island (Fig 2.1, Fig. 2.2). In addition to increased photosynthetic rates, greater atmospheric CO_2 concentrations also contribute to increased water-use efficiency, reducing the limiting factor of water availability (Schlesinger *et al.* 1990; Scholes and Archer 1997; Bond 2008; Ratajczak *et al.* 2011; Bond and Midgley 2012).

The degree to which daughter ramets rely on the physiological integration of intraclonal ramets was evident from the increased incidence of xylem embolisms and ramet dieback in severed ramets. These severed ramets were more susceptible to the higher water stress associated with mid-summer drought (Fig 3.2), as they were unable to access the deeper water sources available through the rhizomatous connection to parent ramets, becoming increasingly water stressed as the growing season progressed, eventually leading to higher incidence of mortality. As a C_3 shrub, leaf level photosynthesis was also likely reduced by the limited water availability for the uptake of CO_2, as shown by lowered photosynthetic rates for severed ramets (Fig. 3.1). Stomatal conductance was likely a stronger limiting factor for the more water stressed severed ramets, which had overall lower rates of photosynthesis and higher seasonal water-use efficiency (Fig. 3.3). The reduced water availability and lowered photosynthetic rates of severed ramets further contributed to decreased biomass production and negative finite rates of increase for nodal leaf populations (Fig. 3.4).

With climate models indicating an ever increasing trend in atmospheric carbon dioxide enrichment (IPCC 2001), it is possible that restoring historic disturbance regimes will not be sufficient to thwart woody encroachment or suppress the rate of spread, as the positive bio-feedback systems that facilitate woody encroachment are augmented by the effects of elevated atmospheric CO_2 (Ratajczak *et al.* 2011; Bond and Midgley 2012). Once *C. drummondii* has established, returning to a graminoid dominated stable state may be near impossible without intensive mechanical removal, and even then, many of these species leave a lasting legacy effect on the community dynamics (Lett and Knapp 2005). With greater temperature extremes and alterations in precipitation regimes associated with climate change, woody species may become even more pronounced in the grassland vegetation structure (Knapp *et al.* 2002), with clonal woody species driving the majority of this conversion (Ratajczak *et al.* 2011).

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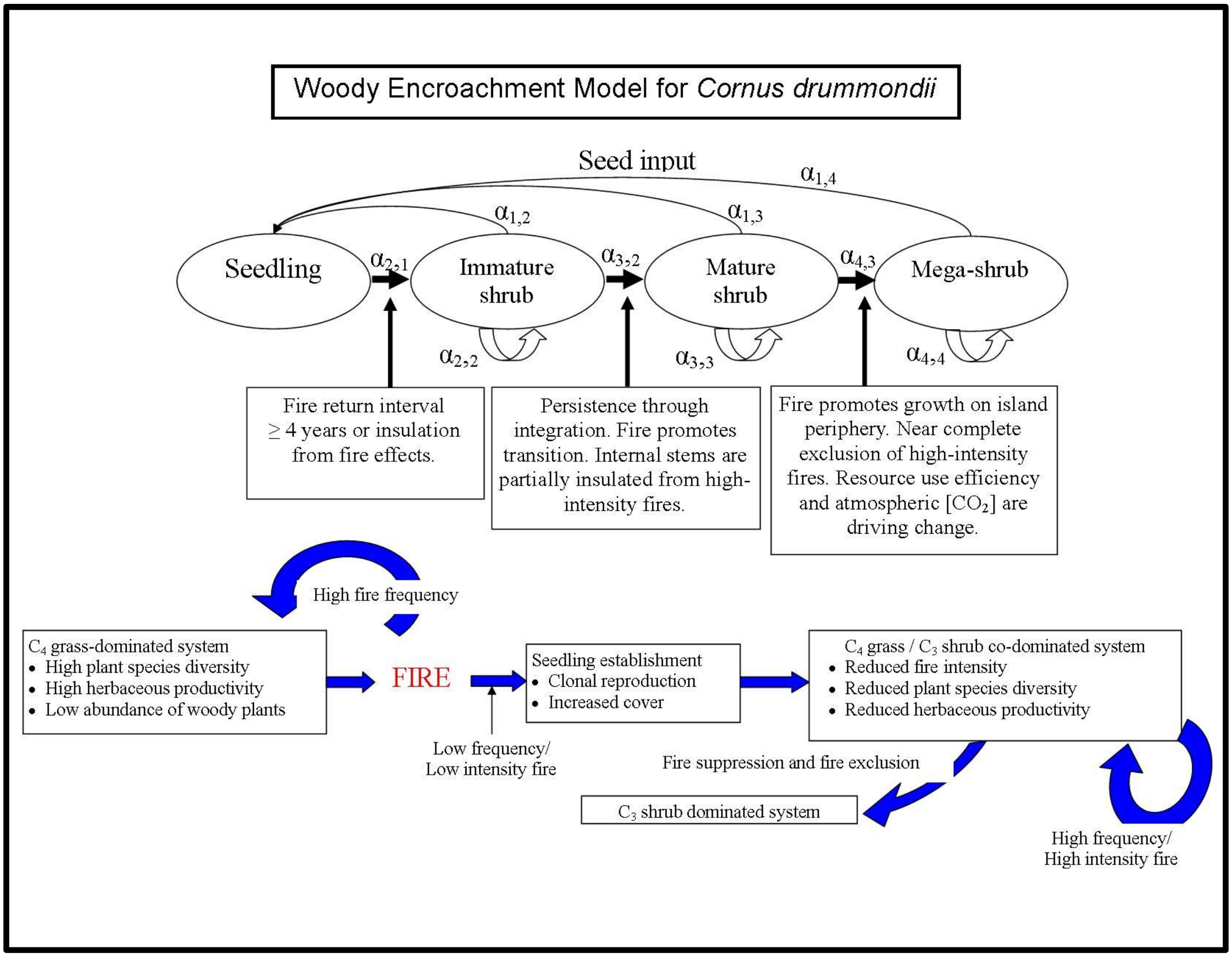

Fig. 4.1. Woody encroachment model for Cornus drummondii in the tallgrass prairie, showing the life cycle graph for the C_3 shrub Cornus drummondii as it transitions from the seedling stage to that of a large clonal population and the corresponding vegetation and fire dynamics. The life cycle has four identified stage-classes (1-4, where class 1 is the seedling stage). Within the life cycle the values of $\alpha_{1,x}$ are fecundities from seed input, values $\alpha_{x,x}$ are the rates of survival for shrubs that maintain their current stage but are reproducing clonally, and values of $\alpha_{x+1,\ x}$ are the rates at which the shrub transitions to the next stage class. One of the most important consequences predicted by this model is that a change in fire frequency will alter the ratio of seed to vegetative reproduction, influencing genetic variability and perhaps adaptive potential.