Ecological Factors Driving Aspen (*Populus tremuloides* Michx.) Stand Stability in a Semi-Arid Montane Region of the Intermountain West

A Dissertation
Presented in Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy
with a
Major in Natural Resources
in the
College of Graduate Studies
University of Idaho
by
Benjamin A. Spei

Approved by:
Major Professor: P. Charles Goebel, Ph.D.
Committee Members: Eva K. Strand, Ph.D.; Grant Harley, Ph.D.; Daniel M. Kashian, Ph.D.
Department Administrator: P. Charles Goebel, Ph.D.

August 2022

Abstract

In the western United States, *Populus tremuloides* Michx. (hereafter referred to as aspen) is considered an important forest cover type because of its contribution to local and regional biodiversity, as well as its use for habitat and food for a variety of large and small mammals, songbirds, and game birds. These critical ecosystem services provided by aspen have made many ecologists and resource managers concerned with recent trends of aspen decline across the western U.S. For decades, many ecologists have predicted a trajectory of disappearance while more recently others have suggested that aspen persistence is contingent on local disturbance regimes, management, and or geographical location. The greatest threats to aspen persistence in the West have been identified by researchers as competition with succeeding conifers, browsing by ungulates, and acute drought. In this dissertation, I investigate the relationship of aspen persistence and regeneration ecology with these identified threats focusing primarily on drought and soil moisture, while also accounting for successional conifer density and browsing pressure. My efforts focus on aspen stands in the Caribou-Targhee National Forest (CNF), and specifically focus on: 1) the importance of precipitation as a proxy for potential soil moisture limitation on aspen growth across the CNF using dendrochronological methods; 2) the relationship between soil moisture availability and aspen stand persistence by comparing the structure, composition, and regeneration densities of nine aspen stands with nine paired upland aspen stands; 3) .

In terms of determining the importance of precipitation as a proxy for potential soil moisture limitation on aspen growth (Chapter 2), I applied a new dendrochronological technique, Blue Intensity (BI), for extraction of a climate signal in aspen latewood to explore the importance of mid-summer precipitation on aspen growth. Blue intensity is an effective and inexpensive proxy for wood density that has been found to correlate more accurately with climate factors (precipitation, temperature) than ring width, especially in latewood. The results of this analysis showed a positive correlation between mid-summer precipitation and latewood density that supports my hypotheses that

1) aspen growing on sites with expected soil moisture limitations will produce a climate signal that correlates with precipitation and 2) that this signal reveals latewood development is driven by and dependent on soil moisture availability based on precipitation. From these results I conclude that late season growth for aspen on exposed high elevation sites is dependent on precipitation and growth is thus limited by soil moisture availability.

Following the results of the dendroecological analyses and the numerous studies published over the last two decades that suggest drought being the main inciting factor of aspen decline on xeric sites, I hypothesized that the proximity to perennial streams will lead to higher soil moisture availability and thus increase the probability of aspen persistence on the CNF landscape (Chapter 3). My results partially supported this hypothesis that riparian areas support aspen stands that show evidence of a higher probability of persistence relative to upland aspen stands. While there were few differences in terms conifer encroachment and ungulate densities between the riparian and upland aspen stands, the most compelling evidence comes from the significantly higher aspen regeneration densities at the seedling layer (< 1m- height) on average in riparian areas relative to upland aspen stands. Specifically, the riparian stands on average had 849 ± 317 seedlings ha^{-1} while the upland aspen stands had on average 249 ± 74 seedlings ha^{-1}. In the sapling layer (≥ 1 m height) aspen regeneration densities were still considerably higher in the riparian areas than in the upland areas (1159 ± 286 and 810 ± 110 seedlings ha^{-1}, respectively), but this difference was not statistically significant ($p < 0.05$). It is important to note that in this study I did not differentiate between aspen regeneration originating from seed versus regeneration originating from suckering.

Building off of the finding of significant differences in the density of aspen in the regeneration layer between riparian and upland aspen stands, I examined directly the relative impacts and interactions of physiographic and ecological factors that affect aspen regeneration densities using a model path analysis using structural equation modeling (SEM). Before any analysis of the direct factors influencing soil moisture availability on aspen regeneration, however, it was important to characterize the clonal diversity of each site as clonal diversity has recently been suggested to be an important factor in aspen resilience to drought. To do this, I used a traditional approach based upon leaf morphology using modern techniques to estimate differences in the clonal diversity on each site (Chapter 4). Overall, I found that there were differences in mean clonal diversity between riparian and upland aspen stands, with riparian stands tending to have higher mean clonal diversity (include means). When included with the results of the SEM analysis (Chapter 5), there was support for my hypothesis that factors affecting soil moisture availability have the strongest effect on aspen regeneration. Specifically, the exogenous factor with the strongest direct effect on aspen regeneration was incident radiation (heatload; -0.661), and the endogenous factor with the strongest direct effect on regeneration was percent cover of competitive plant species (-0.952).

Overall, the results of these analyses support my global hypothesis that factors reducing soil moisture availability and increase site susceptibility to drought have a negative effect on aspen growth and regeneration. Additionally, these results demonstrate that aspen persistence and resilience to increasing drought in semi-arid montane regions of Idaho and the northern Rockies is determined largely by physiographic and ecological context. Consequently, proper assessment of aspen vulnerability in the West requires analyses at multiple scales that can incorporate the relative weights and interactions of elements influencing aspen persistence. Based upon these results, it does appear that riparian areas may provide an important habitat for the persistence of aspen through increased soil moisture availability.

Acknowledgments

I would like to thank my field assistance for their help in obtaining data important to my research even in less than ideal conditions. I would also like to acknowledge my major professor and committee members who each helped guide me through this process and were willing to sit down and discuss my results whenever requested.

Dedication

This work is dedicated to Elizabeth, Biscuit, and Babette.

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Chapter 1: Introduction

In the western United States, *Populus tremuloides* Michx., hereafter referred to as aspen, is considered an important forest cover type because of its contribution to local and regional biodiversity (Chong et al. 2001; Kuhn et al., 2011), as well as its use for habitat and food for a variety of large and small mammals, songbirds, and game birds (DeByle, 1985; Finch and Ruggiero, 1993; Oaten & Larsen, 2008; Perala,1985). These critical ecosystem services provided by aspen have made many ecologists and resource managers concerned with recent trends of aspen decline across the western U.S. Many ecologists have for decades predicted a trajectory of disappearance (Bartos and Campbell 1998; Campbell and Bartos, 2001; Packard, 1942) while more recently others have suggested that aspen persistence is contingent on local disturbance regimes, management, and/or geographical location (Kashian et al., 2007; Shinneman et al., 2013; Stevens-Rumann et al., 2018)

Aspen primarily regenerates vegetatively via suckering from the root stock, forming large clones of genetically identical stems. Damage of the mature stem physiologically signals the production of suckers when the flow of sprout-suppressing hormones from the crown to the roots is interrupted (Schier, 1976). Sucker production frequently occurs in the absence of disturbance, however, and many stands in the West have been identified as self-replacing or “stable”, in that stands contain aspen at all vertical strata and regeneration occurs without overstory mortality. This form of reproduction can result in large clones that cover dozens and even over 100 acres (Kemperman & Barnes, 1976), although some aspen stands consists of multiple clones (Zeigenfuss et al. 2008). In the absence of disturbance, aspen sprouting is initiated by warmth, moisture, and available open space (Schier 1976). The number of aspen sprouts varies among stands and particularly among clones, and this variability appears to be controlled by both abiotic (e.g., aspect, slope) and biological (e.g., competition) ecological factors. Understanding the potential for aspen persistence therefore depends heavily upon understanding the ecological context of a given aspen stand, including the governing factors potentially limiting regeneration and growth and their interactions.

The greatest threats to aspen persistence in the West have been identified by researchers as competition with shade tolerant conifers, browsing by ungulates, and acute drought (Baker et al., 1997; Crawford et al., 1998; Worrall et al., 2008). The magnitude and variability of these disturbances across large landscapes have been affected greatly by humans via land use and climate change. For example, wildfire across the West was historically frequent enough to suppress conifer expansion and stimulate aspen sprouting. Nearly a century of active fire suppression has increased the return interval, reducing aspen regeneration and allowing conifers to invade. Notably, conifer expansion into aspen stands has in many places always had a successional and cyclical relationship with aspen dominance. Further, there is evidence that aspen facilitates the expansion of conifers into meadows by altering the site conditions through increased shade and soil moisture, that eventually leads to decline of aspen dominance as the newly established conifers begin to outcompete and suppress aspen regeneration (Buck & St. Clair, 2012; Calder & St. Clair, 2012). Similarly, ungulate browsing of aspen regeneration has, in some areas, exceeded natural pre-EuroAmerican levels due to reduction of large carnivores, increases in cattle grazing, and ungulate hunting restrictions (Binkley et al., 2006). Finally, increases in the average and maximum temperatures and the incidence of drought over the past century are projected to continue into the future (Crowley, 2000; Hegerl et al., 2019; Krinner et al., 2013), likely hindering aspen growth, regeneration, and persistence.

In this dissertation, I investigate the relationship of aspen persistence and regeneration in the presence of various threats in the Caribou-Targhee National Forest (CNF), focusing primarily on drought and soil moisture, but accounting for conifer invasion and browsing pressure. The CNF is a heterogeneous landscape with aspen stands that occur on a variety of sites from low to high elevation, southern to northern aspects, and in association with an array of different tree and shrub species.

Many relatively large (> 1 ha) aspen stands are located within riparian areas, making the CNF an ideal area to investigate how different ecological factors affect aspen stand structure and regeneration.

In Chapter 2, I explore the importance of moisture availability during the growing season on aspen growth. To investigate this relationship, I examine the potential of precipitation as a proxy for soil moisture limitation on aspen growth across the CNF using dendrochronological methods. Specifically, I apply a new technique, Blue Intensity (BI), for extraction of a climate signal in aspen latewood. Blue Intensity uses the minimum blue light reflectance from high-resolution scans of the wood surface to quantify wood density. Latewood density and consequently BI analyses are most commonly used for historical temperature reconstructions by sampling conifer species growing on cold sites (e.g. high latitudes >50º or high elevations >3000 m) (Barber et al., 2004; Briffa et al., 2002; Davi, 2003; Heeter et al., 2020). Considering its application in extracting temperature signals from conifer tree species growing in cold environments, I expected a precipitation signal could be derived from a broad-leaf deciduous species growing on dry sites, such as aspen growing at mid- to high-elevations on exposed southern aspects. I also investigate the timing of this correlation on monthly and daily scales to estimate at what point in the growing season latewood development is initiated. These results may provide insight into how the timing and severity of drought directly affects aspen growth in areas where soil moisture is limited.

In Chapter 3, I investigate the relationship between soil moisture availability and aspen stand persistence by comparing the structure, composition, and regeneration densities of nine riparian aspen stands with nine upland aspen stands. My hypothesis is that the proximity to perennial-flowing streams in riparian areas will lead to higher soil moisture availability, supporting the persistence of aspen in these areas of the CNF landscape. As such, riparian areas would provide a potential refuge for aspen in the future in areas threatened with increased incidence of drought. This hypothesis is based on the several studies that suggest that drought is the predominant cause of aspen decline in the arid to semi-arid regions of the Intermountain West (Anderegg et al., 2013; Chen et al., 2017; Hogg et al., 2008; Krasnow & Stephens, 2015; Worrall et al., 2015).

Chapter 4 focuses on identifying the number of aspen clones present in riparian vs. upland aspen stands of the CNF based upon leaf morphology and digital morphometric techniques. Prior to the development and availability of genetic analysis, phenological differences in leaf and branch morphology, bark color, growth form, spring leaf flush, and fall leaf color were used as indicators for clone delineation in aspen stands (Barnes, 1975; Barnes, 1969). Morphometric techniques have been shown to be a reliable and accurate proxy for genetic analysis (Jelínková et al., 2014), and are considerably less expensive than traditional genetic analysis yet quicker and simpler than ocular estimation of morphology. The results of may analyses in this chapter were used in a larger study investigating aspen ecology and stability in the Intermountain West (Chapter 5).

Chapter 5 is an exploration of the relative weight and interaction of ecological factors that influence aspen regeneration with a path analysis using Structural Equation Modeling (SEM). SEM is especially useful for the examination of ecological systems because it can incorporate a hierarchical structure that allows for the investigation of the relationship between factors that directly and indirectly effect a response via other nested and interacting factors. For example, physiography influences the structure of a forest overstory, and the forest overstory in turn influences the structure of the understory and groundcover. SEM can model these complex relationships and calculate the relative strength of the direct effect of physiography on the lower understory and groundcover layer but also its indirect effects on the understory layer via its impact on the upper overstory layer. When combined with *a priori* analysis and theoretical understanding of a system, SEM can reveal cause-and-effect relationships with higher confidence than most traditional analyses (Grace et al., 2009).

Finally, in Chapter 6 I explore the results of these related studies of aspen as they relate to the persistence of aspen in the West. Further, I discuss how the results of my analyses support my hypotheses that soil moisture availability affects the factors supporting or inhibiting aspen persistence for each study. I also consider how forest and natural resource management can help promote aspen; and describe the future directions I can take in analyzing new questions that arose from uncertainties in my results.

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Chapter 2: The Application of Blue Intensity on a Diffuse Porous Species (*Populus tremuloides* Michx.) Reveals a Mid-Summer Precipitation Signal in a Semi-Arid Montane Region

Abstract

Dendrochronology studies use annual tree ring width and wood density to investigate the relationships between site (disturbance) and climate factors (precipitation, temperature), and growth. Maximum latewood density, specifically, has been identified as having the strongest correlations with climate factors. Blue intensity (BI) is a relatively new technique that is becoming widely accepted as an inexpensive surrogate for maximum latewood density (MXD). Many studies have investigated the formation of MXD in several conifer species and have identified significant correlations of latewood development with air temperature averages and extremes. This relationship has been used to reconstruct historical climate trends and monitor the effects of current increasing temperature on local and regional tree growth. Fewer studies have used wood density to investigate the relationship of precipitation and drought on growth. This study tests the efficacy of BI analysis for extracting a climate signal that correlates with wood density in a moisture-sensitive species, trembling aspen (*Populus tremuloides*). Optimum parameters for blue intensity were determined by comparing mean correlation coefficients and expressed population signal for latewood, full ring, and the difference between latewood and earlywood density at varying window depths, widths, offsets, and percent blue extraction. The strongest and most consistent climate correlations occurred between aspen latewood and July precipitation of the current year ($r = 0.40 – 0.47$). Aspen tree ring width also showed a significant correlation with May maximum temperature of the current year ($r = 0.4 – 0.55$). These results suggest that blue intensity is a viable method for investigation of aspen's relationship with changing climate factors that dictate moisture availability.

Introduction

Over the past several decades most climate studies have focused on predicting how increasing temperatures and changes to hydrologic cycles will affect ecosystems. For example, climate studies utilizing multiple proxy climate indicators (e.g., coral, ice cores, pollen samples, tree rings) have identified global trends of increasing variability in temperatures throughout the 20th and into the 21st century, and in many instances these temperatures are higher than experienced for at least the last 500 years (Flannery et al., 2017; Luterbacher et al., 2004; Mann et al., 1998; Masson-Delmotte et al., 2006; Ruiz-Labourdette et al., 2014; Salzer & Kipfmueller, 2005). Anthropogenic activities of the nineteenth and twentieth centuries have been accepted as the cause of these increases (Crowley, 2000; IPCC, 2022; Hegerl et al., 2019) and models predict that average and maximum temperatures will continue to increase over the next century (Almazroui et al., 2020; Cook et al., 2015; Feng et al., 2014).

In North America, dendroclimatological studies have used tree-ring proxies for historical temperature and precipitation reconstructions at regional and local scales that show similar trends to previous global studies (Cook et al., 1999; Fritts et al., 1965; Gray et al., 2004; Holmes et al., 1986; Salzer et al., 2009; Stahle et al., 2000). Wood density, specifically, the maximum density of annual latewood growth was discovered to produce a stronger relationship with climate than ring width (Conkey, 1979). In the late 1970s, the development of maximum latewood density (MXD) methods fallowed for the extraction of high-resolution signals that correlate with mean and maximum summer temperatures for trees at higher latitudes (> 50º) or high elevations (> 3000 m) where temperature limits growth (Barber et al., 2004; Briffa et al., 2002; Cook et al., 1998). In turn, isolation of a temperature signal allowed for historical reconstructions of summer temperature variability and extremes. Wood density in late-season wood was originally determined with x-ray film densitometry, a relatively expensive and labor-intensive process. In contrast, blue intensity (BI) was recently developed to detect wood density and is quickly being accepted as an inexpensive and practical surrogate for x-ray densitometry.

Blue intensity uses the minimum blue light reflectance from high-resolution scans of the wood surface to quantify wood density. Lignin in the cell walls of the vascular tissue absorbs blue light, such that the reflectance is an inverted signal of the density (i.e., higher cell wall to cell interior ratio). McCarroll et al. (2002) found an almost perfect correlation ($r = -0.976$) of minimum blue light reflectance and maximum latewood density. Since this discovery, BI has been used in multiple temperature reconstruction studies across the world with high certainty (Campbell et al., 2007; Wilson et al., 2014). Initially, blue light research like most MXD studies was primarily applied to conifers at high latitudes. More recently, this technique has been applied to disjunct, high-elevation conifer populations in the southern Appalachian and Rocky Mountains (Harley et al., 2021; Heeter et al., 2020, 2021). MXD and now BI provide a more robust and consistent climate signal than ring-width variation, and as a result, temperature reconstructions for North America have now been extended by centuries.

Because conifers typically feature strong relationships between late-wood density and climate, their use in identifying summer temperature variations is a primary application of dendroclimatology. Fewer studies, however, have used MXD or BI to retrieve a climate signal for hardwood species or broadleaf deciduous tree species. Indeed, there is a paucity of research showing a robust and consistent moisture signal that correlates significantly with wood density. One example, Meinardus et al. (2012) found a significant positive correlation between MXD and precipitation during mid-summer in European beech (*Fagus sylvatica* L.) growing on xeric sites. This is likely because these sites were moisture-limited and growth was dependent on, or limited by, the influence of climatic forces including precipitation.

Populus tremuloides Michx. (trembling aspen, hereafter aspen) in the western United States has been theorized to be most affected by climate factors that influence site moisture availability, especially drought (Elliott & Baker, 2004; Fairweather et al., 2007; Rehfeldt et al., 2009; Worrall et al., 2015). Evidence of aspen die-back and reduced growth following instances of acute drought has been reported in the Intermountain West and the Canadian Parklands in aspen stands growing of semi-arid to arid sites (Anderegg et al., 2013; Guyon & Hofman, 2011; Hogg et al., 2008; Worrall et al., 2008). Multiple dendrochronological studies of aspen in the western United States have used ring-width to estimate the sensitivity of aspen to changing environmental conditions and disturbances (Elliott & Baker, 2004; Hogg et al., 2008; Lapointe-Garant et al., 2010; Romme et al., 2000). The results from these studies consistently show that TRW is reduced during periods of drought as indicated by high Palmer Drought Severity Index (PDSI) scores or vapor pressure deficit (VPD) values (i.e. climate driven moisture stress). Severe cases of acute drought have been implicated as the main inciting events causing sudden aspen decline (SAD), a disease that causes rapid aspen mortality across large patches of aspen stands in the western United States and Canada (Hanna & Kulakowski, 2012; Singer et al., 2019; Worrall et al., 2013, 2015).

The objective of this study is to test the efficacy of BI analysis on aspen for climatological analysis. If western aspen exhibits reduced growth during periods of acute drought on sites that are inherently water limited, then BI analysis may be useful to extract a climate signal from the variation in wood density that accompanies moisture stress (e.g., precipitation, VPD, PDSI). Such a relationship would provide researchers with an option for tracking changes in site water availability in areas where aspen is present. Further, this technique could aid in the investigation of the threshold between moisture stress and aspen mortality more accurately than ring-width alone. With the growing concern of aspen's response to future climate stressors this may become even more valuable in predicting aspen decline.

Methods

Study Site and Sample Collection

During the summer of 2019, I sampled aspen stems across the Caribou National Forest (CNF) in Bear Lake County, Idaho. The CNF is within the semi-arid M331D ecological sub-region (McNab et al., 2007) that features average annual precipitation of approximately 36.5 cm, which is less than 50 percent of the national average (NOAA, 2022), mid- to high-elevations (1400-3100 m), and clay-rich soils. Predictive models of the effects of climate change on the geographical range of aspen have shown this area to be especially vulnerable to increasing temperatures and altered moisture regimes (Rehfeldt et al., 2009).

A total of 60 aspen cores were extracted at approximately 30 cm above the ground and parallel to the slope of the landscape from 30 dominant aspen stems (2 cores per stem taken at 180º of each other) with a 5.0 mm diameter Haglöf increment borer. The 30 stems chosen for sampling were from the largest (diameter at breast height) and oldest estimated aspen stems found within four stands. All stands sampled were located within a 5-km radius of each other. Any stems with visible signs of infection, excessively discolored heartwood, or heart-rot were avoided during sampling. Sampled stands were restricted to near peaks or ridgelines with southern- to southwestern-facing aspects and at elevations of 2100 - 2500 m.

As there have been few studies investigating MXD and virtually none investigating the application of BI in broadleaf angiosperms, we deemed it appropriate to include a comparative analysis (i.e., as a proxy control) with a conifer species to increase confidence in the execution of the methods and thus validity of the results discovered. Engelmann spruce (*Picea* engelmannii Parry ex Engelm.) was chosen as the conifer species for comparative analysis because (1) Engelmann spruce stands are commonly found adjacent to aspen stands in this region and (2) Engelmann spruce has been identified as an ideal species for BI studies and climate reconstruction in the Rocky Mountain region (Heeter et al., 2020). Engelmann spruce cores were collected using the same methods for aspen, and stems were chosen from sites at relatively high elevation (>2600 m) for this area and on flatter and less exposed peaks and slope than for aspen. A total of 62 cores were also collected from 31 Engelmann spruce stems growing in monospecific stands located within the same 5-km radius of the four aspen stands.

Sample Preparation and Chronology Development

All core samples were stored in paper straws and allowed to dry completely before mounting on beveled wooden core mounts. Cores were sanded with progressively finer grit sandpaper from 400, 600 to 1000 grit, and finished by hand with 1500 grit sandpaper and 9 µm film. Following sanding, all core samples were examined with a dissecting microscope to check for insect damage and discoloration, as wood discoloration has been identified as a primary source of error due to distortion of the brightness signal in BI analysis (Sheppard et al., 1996). All Engelmann spruce cores showed no discoloration due to resins or heartwood/sapwood differences and thus were not treated for pigment extraction. However, most of the collected aspen cores did show varying levels of discoloration, and thus required pigmentation extraction. Aspen cores were initially soaked in 95% ethanol (ETOH) solution for 72 hours, allowed to dry for 24 hours, then soaked in acetone for 36 hours. Once the aspen cores dried for 24 hours, they were treated with a 50/50 solution of sodium bicarbonate and 3% hydrogen peroxide, again allowed to dry for 24 hours, and finally hand sanded again with 9 µm film to a high polish.

Following core sample preparation, all cores were individually scanned at 3200 dpi on an Epson Expression XL 12000 scanner fitted with an IT8.7/2 calibration card. The high-resolution photos were then uploaded into CooRecorder 9.6 (Larsson, 2014) for growth ring boundary delineation to develop TRW values to the nearest 0.001 mm. Due to the diffuse porous structure of aspen wood, ring boundaries required manual detection. In contrast, Engelmann spruce cores had distinct early wood and late wood boundaries, such that ring widths were determined using the automated collection feature with little need for correction.

Dating accuracy of TRW measurements were validated for aspen and Engelmann spruce using the software COFECHA (Holmes, 1983). Any aspen cores that showed a cross-correlation of less than 0.3 were removed and any Engelmann spruce cores that showed a cross-correlation below 0.4 were removed from any subsequent analysis. Once measurements and collections were validated, BI measurements were determined in CooRecorder. Optimal CI parameters have not yet been determined for aspen, so a variety of window widths (w), offsets (f), depths (d), and %-blue reflectance were tested for collection of aspen LWB, delta blue intensity (difference of latewood blue and earlywood blue; ΔBI), and full-ring blue intensity (FRB). Optimum settings for BI measurements were then determined and applied using the highest RBAR, EPS, and inter-series-correlations for LWB, ΔBI, and FRB (Table 1). For Engelmann spruce, only latewood blue intensity (LWB) measurements were collected using previously determined optimal color-intensity (CI) parameters (Heeter et al., n.d.). Once blue-intensity measurement collections were created, they were again cross-correlated in COFECHA and any cores with blue-intensity measurement correlations less than or equal to zero were removed from the analysis. Once the optimal BI collections were developed, multiple series detrending methods were tested in ARSTAN (Cook and Holmes 1996) until ultimately a 2/3 spline was used to de-trend all TRW and BI collections. Following detrending, chronologies were developed and standardized from the residuals using an auto-regressive model. A total of six chronologies were developed: four for aspen (TRW, LWB, ΔBI, and FRB) and two for Engelmann spruce (TRW and LWB).

Climate Response

Chronologies were analyzed for spatial correlations with climate factors (e.g., precipitation, temperature, etc.) obtained from the Royal Netherlands Meteorological Institute (KNMI) Climate

Explorer using Pearson's correlation coefficient. When a significant correlation of climate factors with a chronology were found, datasets from the Parameter-elevation Relationships on Independent Slopes Model (PRISM) for monthly and daily maximum temperature and precipitation were retrieved. Each chronology was further tested for signal strength and stability using a static, moving, and evolving interval analysis; and for temporal correlations with climate factors using Pearson's correlation analysis with the TreeClim package in R 4.2.0 (Zang and Biondi, 2015). The dendroTools package in R was used to examine daily correlations of chronologies with climate factors.

Results

Tree Ring Width

Only 24 of the original 60 aspen cores cross-correlated well enough to give a series inter-correlation greater than 4.0 and an EPS value above 0.84. Spatial analysis with KNMI climate explorer showed a positive correlation ($r > 0.4$) of aspen TRW with maximum temperatures (Tmax) for the month of May during the period from 1974-2018 (Fig.1; A). Because the resulting aspen LWB chronology only covers 42 years (1975 – 2017) a 35-year window was chosen for correlation analysis. Correlation analysis across an 18-month period reveals a strong and consistent relationship of Tmax with TRW during May of the current year ($0.4 < r < 0.55$; Fig. 2; A). Additionally, aspen has a weak negative correlation between TRW and Tmax during the previous year's November. A trace-plot of correlations over time shows the coefficients for correlations of Tmax with TRW during the month of May fluctuate between 4.0 and 5.5 for the entire time series analyzed (Fig. 2; B), suggesting that the trend is consistent across the entire sampling period.

Fifty-four of the 62 Engelmann spruce cores cross-correlated well with a series inter-correlation of 0.601 and an EPS value of 0.934. The 35-year moving correlation analysis across an 18-month period showed an initial negative correlation for TRW with Tmax for the month of August for the previous year, until the end of the 20th century when September became the highest negatively correlated month (Fig. 3; A). The switch of strongest monthly correlation coefficients is more noticeable in the trace-plot that shows coefficients fluctuating between -0.5 and -0.3 (Fig. 3; B).

Blue Intensity

For the aspen BI collection, the strongest signal based on RBAR, EPS, mean sensitivity, and series inter-correlation values was produced using window frame specifications of 160 width (w), 5 offset (f), and depth (d) of 50, 300, and 500 for LWB, ΔBI, and FRB respectively, and 15% use of dark/light wood for color data (Table 1). Following BI collection at these specifications, several additional cores were removed to increase series inter-correlation and RBAR resulting in chronologies of 9, 10, and 11 for LWB, FRB, and ΔBI, respectively. Spatial correlations of FRB with climate factors showed a similar but weaker signal with Tmax for the month of May than TRW (Fig. 1; B); and showed no strong correlations with precipitation or drought (VPD, PDSI). Spatial correlations of LWB, and ΔBI showed similar results to one another with no significant correlations to Tmax at any month but both with a significant correlation with precipitation during the month of July across the time period of 1974-2018 (Fig. 4; A and B). The correlation with LWB was stronger and covered a slightly larger area than FRB, thus LWB was used in further analysis. The highest correlating coordinates for LWB with precipitation were between -111.25, 42.25, and -111.25, 42.75 longitude and latitude ($r = 0.371$), thus the PRISM time series data for precipitation was utilized from the interpolated grid value surrounding the point -111.25, 42.50 longitude and latitude at a 4 km resolution.

Analysis of the aspen LWB chronology with the PRISM precipitation data set demonstrates a consistently significant positive correlation with precipitation during the month of July for the current year using a 35-year evolving window (Fig. 5; A). There were few other significant monthly relationships and no consistent monthly signal across the 18-month series. The correlation coefficients for the month of July appear to fluctuate between 0.40 and 0.50 with one point falling just below 0.40 between the evolving periods ending in 2015 and 2016 (Fig. 5; B). Daily analysis of the PRISM daily precipitation dataset revealed the strongest response to daily precipitation values occurred with approximately a 30-day window during mid-summer of the current year for the period 1981 – 2016 (Fig. 6; A). Further analysis with a fixed 30-day window showed the strongest correlation of LWB with precipitation between June 28 and July 27 with the highest correlation occurring on Day 179 (end of June) of the current year (r = 0.56) (Fig. 6; B).

The Engelmann spruce LWB chronology was constructed using the best correlating cores in the series, as with the aspen chronology, resulting in a chronology consisting of 43 Engelmann spruce cores. The spatial analysis in KNMI explorer revealed the strongest correlations of Engelmann spruce with Tmax during the month of August (Fig. 7). The strongest correlation nearest the sampling point occurred at -111.25, 41.75, longitude and latitude (r = 0.524). This point was used to collect the monthly and daily Tmax data from PRISM for further analysis. The strongest and most consistent correlations of Engelmann spruce LWB with Tmax occurred during the month of August for the current year with a 35-year moving window. A trace plot of the data shows that coefficients over time fluctuate between 0.5 and 0.75 throughout the majority of the series (Fig. 8; A and B). Using varying window widths for the period 1981-2016, the greatest correlations between Engelmann spruce LWB and daily Tmax occur near the end of summer of the current year and with a 50-day window. Analysis with the 50-day fixed window showed a significant correlation of Engelmann spruce LWB and Tmax throughout the summer months with the peak correlation coefficient occurring on day 212 [(early August; r = 0.692), (Fig. 9].

Discussion

My results provide compelling evidence that BI analysis is useful in extracting a climate signal – in particular, a signal for mid-summer moisture availability (precipitation) - from tree cores obtained from aspen on xeric sites. The relationship of aspen latewood BI with precipitation was more robust and consistent than for aspen ring width, which only showed relationships with temperature. A caveat to these results is the relatively low sample size (n = 9) and EPS value (0.498) of the aspen LWB chronology, which may have caused an overestimation of the correlation strength of precipitation with latewood growth. Despite these limitations, however, the relatively robust signal for mid-summer precipitation that I identified corroborates the prevailing theory that aspen growth is limited by water availability in this area and late-season growth is thus driven by and dependent on precipitation.

When radial cell growth switches from earlywood to latewood, cell development changes its investment in growth from cell elongation to cell wall thickening. The signal for cells to switch from earlywood to latewood is based on daylength; and the rate of growth (elongation in earlywood, cell wall thickening in latewood) is primarily controlled by climate factors such as temperature or precipitation (Friend, 2020). Increasing temperatures at the end of the growing season in conifers growing at high latitudes (>50º) and elevations (>3000 m) translates into a stacking of more and thicker walled cells (i.e., higher density in the latewood). Further, the development of latewood appears less affected by site variability such as micro-climate and low-level disturbance or “noise” that can corrupt the signal (Conkey, 1979; D’Arrigo, 1992). This is why latewood density can produce a more robust climate signal than ring-width alone especially when applied to particular species growing at sites that highlights the driving factor on growth.

Engelmann spruce chronologies of the study area were constructed and used as a proxy for a control to help increase confidence in the results retrieved from the collection of aspen cores. The relationship between Engelmann spruce LWB and climate was strongest and most consistent with Tmax in the late summer months of the current growing season, primarily in August. These results are consistent with other studies in the southern Rocky Mountains that identified the strongest correlations of Engelmann spruce LWB with August and September maximum temperatures of the current year (Heeter, 2020). Most studies utilizing MXD and BI methods to extract climate signals are applied almost exclusively to conifer species and suggest that temperature is the driving factor of growth in mid and late season. The increase in latewood density is interpreted as a response to increasing temperatures near the end of the growing season that means temperature is driving growth.

BI is a relatively new technique and its application in identifying climate factors that affect growth is still being developed. Most studies that use MXD or BI focus on the temperature signal derived from the timing and intensity of latewood development in conifers by focusing on locations (e.g., near treeline ecotones of subalpine forests) that are temperature limited. Few MXD and BI studies of conifers investigate the relationship of the changing water availability on forested ecosystems except in cases where the extremes of precipitation or drought are severe enough to impose a signal in the latewood. Even in these studies, however, the water availability signal is often confounded by the temperature signal, showing correlations with cold-wet and warm-dry years.

Aspen stands develop and even persist on a wide variety of sites throughout its range, especially in western North America where aspen stands can be found on low to high elevation, wet to dry, and nutrient-rich to nutrient-poor sites when competition is low. In the last few decades multiple studies have shown that drought is the main factor associated with increasing aspen mortality across the West, especially on stands that are vulnerable to water limitations (Rehfeldt et al., 2009). Thus, aspen growing at water-limited sites (e.g., southern aspects, mid-high elevations) was expected to show a signal identifying precipitation as the driver of latewood growth. Indeed, the results show latewood growth is dependent on precipitation during the month of July which is the hottest and driest month of the year in this region (U.S. Climate Data). This means the specific time period when growth is most limited by water availability can be tracked; and if drought-induced mortality becomes an issue in these sites than thresholds of water availability on aspen mortality can be identified.

Currently, the CNF does not show evidence of drought-induced decline but is theorized to be vulnerable in the future.

Sudden aspen decline is a syndrome that affects western aspen populations and has presented as rapid die-back of large patches of overstory aspen stems in stands that were considered to be historically stable in Utah, Colorado, and the Canadian parklands (Bartos and Shepperd, 2010; Worrall et al., 2008). Sudden aspen decline has been theorized to be the result of a combination of factors (e.g., drought, pathogens, age, etc.) but the most prevalent theory is that drought is the main inciting factor of aspen mortality (Singer et al., 2019). For example, Hanna and Kulakowski (2012) showed a reduction in TRW for at least 5 years prior to mortality in SAD affected stands of Colorado and Wyoming and attributed the reduced growth and ultimate death to drought. In such studies that attempt to separate the factors leading to reduced growth and mortality, blue intensity may be a useful method for tracking changes in water availability and their long-term effect on growth.

To the best of my knowledge there is no research in the literature that has used maximum latewood density or BI analysis for extraction of a climate signal in aspen. This is presumably because traditional methods for maximum latewood density required the use of expensive and labor-intensive x-ray densitometry. Blue intensity is a relatively new method, and it is much less expensive and more accessible than x-ray densitometry. My results suggest that BI may be a practical and more efficient method for tracking aspen growth, or conversely lack of growth, in sites that are susceptible to increasing drought stress than traditional ring width analysis

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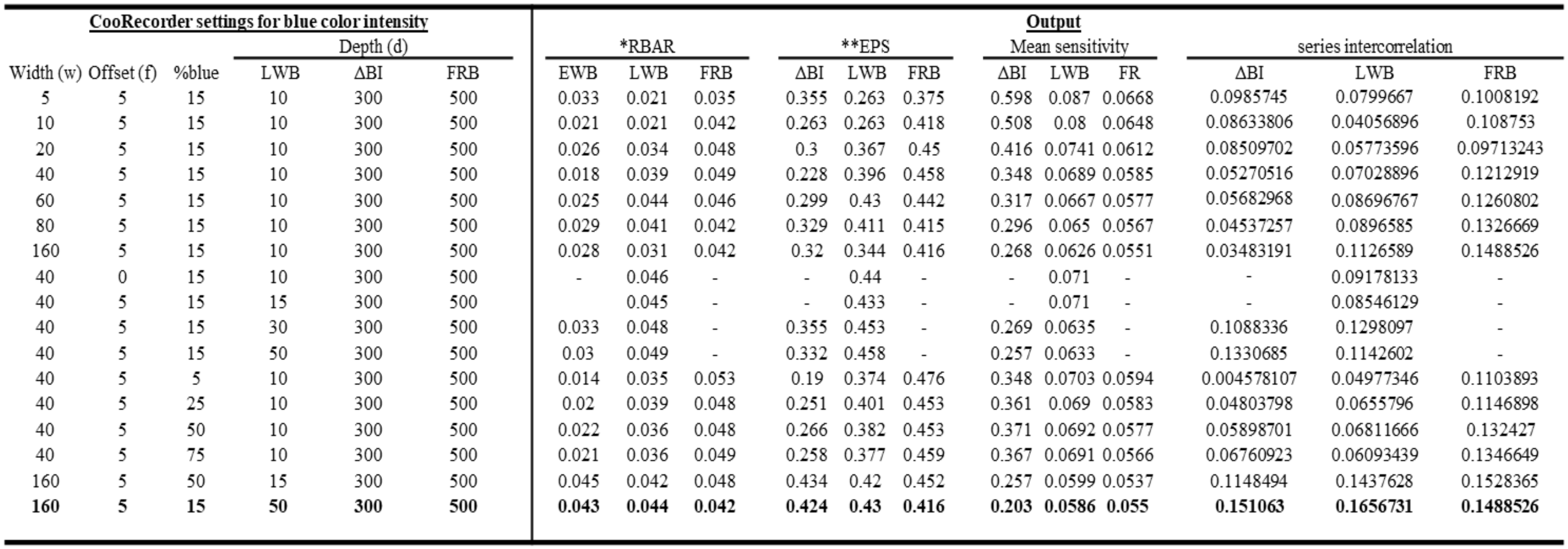
Figures and Tables



CooRecorder settings for blue color intensity						**Output**											
			Depth (d)			*RBAR			**EPS			Mean sensitivity			series intercorrelation		
Width (w)	Offset (f)	%blue	LWB	ΔBI	FRB	EWB	LWB	FRB	ΔBI	LWB	FRB	ΔBI	LWB	FR	ΔBI	LWB	FRB
5	5	15	10	300	500	0.033	0.021	0.035	0.355	0.263	0.375	0.598	0.087	0.0668	0.0985745	0.0799667	0.1008192
10	5	15	10	300	500	0.021	0.021	0.042	0.263	0.263	0.418	0.508	0.08	0.0648	0.08633806	0.04056896	0.108753
20	5	15	10	300	500	0.026	0.034	0.048	0.3	0.367	0.45	0.416	0.0741	0.0612	0.08509702	0.05773596	0.09713243
40	5	15	10	300	500	0.018	0.039	0.049	0.228	0.396	0.458	0.348	0.0689	0.0585	0.05270516	0.07028896	0.1212919
60	5	15	10	300	500	0.025	0.044	0.046	0.299	0.43	0.442	0.317	0.0667	0.0577	0.05682968	0.08696767	0.1260802
80	5	15	10	300	500	0.029	0.041	0.042	0.329	0.411	0.415	0.296	0.065	0.0567	0.04537257	0.0896585	0.1326669
160	5	15	10	300	500	0.028	0.031	0.042	0.32	0.344	0.416	0.268	0.0626	0.0551	0.03483191	0.1126589	0.1488526
40	0	15	10	300	500	-	0.046	-	-	0.44	-	-	0.071	-	-	0.09178133	-
40	5	15	15	300	500		0.045	-	-	0.433	-	-	0.071	-	-	0.08546129	-
40	5	15	30	300	500	0.033	0.048	-	0.355	0.453	-	0.269	0.0635	-	0.1088336	0.1298097	-
40	5	15	50	300	500	0.03	0.049	-	0.332	0.458	-	0.257	0.0633	-	0.1330685	0.1142602	-
40	5	5	10	300	500	0.014	0.035	0.053	0.19	0.374	0.476	0.348	0.0703	0.0594	0.004578107	0.04977346	0.1103893
40	5	25	10	300	500	0.02	0.039	0.048	0.251	0.401	0.453	0.361	0.069	0.0583	0.04803798	0.0655796	0.1146898
40	5	50	10	300	500	0.022	0.036	0.048	0.266	0.382	0.453	0.371	0.0692	0.0577	0.05898701	0.06811666	0.132427
40	5	75	10	300	500	0.021	0.036	0.049	0.258	0.377	0.459	0.367	0.0691	0.0566	0.06760923	0.06093439	0.1346649
160	5	50	15	300	500	0.045	0.042	0.048	0.434	0.42	0.452	0.257	0.0599	0.0537	0.1148494	0.1437628	0.1528365
160	**5**	**15**	**50**	**300**	**500**	**0.043**	**0.044**	**0.042**	**0.424**	**0.43**	**0.416**	**0.203**	**0.0586**	**0.055**	**0.151063**	**0.1656731**	**0.1488526**

Table 2-1 Table 1: Window parameter settings and output values for blue color intensity collection for latewood blue intensity (LWB), delta blue intensity (ΔBI), and full ring blue intensity (FRB). Parameters used in final analysis are shown in bold.

*RBAR: mean correlation coefficient
**EPS: expressed population signal

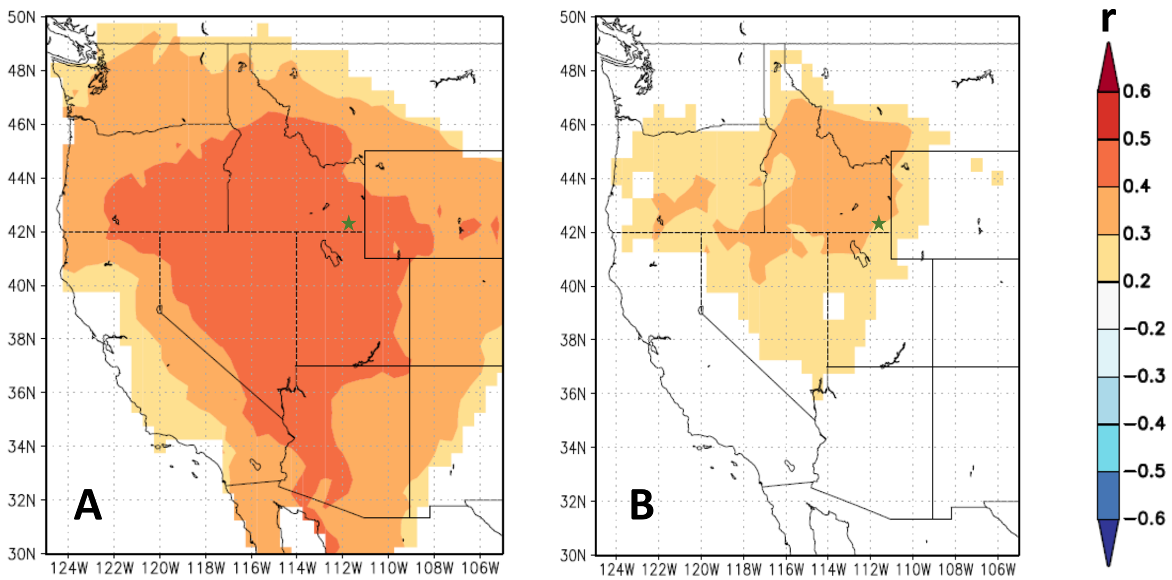


Figure 2-1 Spatial correlation (Pearson's correlation coefficient; α = 0.10) map of CRU TS 4.05 May maximum temperature (0.5° resolution) with (A) aspen TRW chronology during the period of 1961-2018 and with (B) aspen LWB during the period of 1974 - 2018. Green star indicates sampling area. Maps were created in KNMI climate explorer with geo-referenced climate data sets.

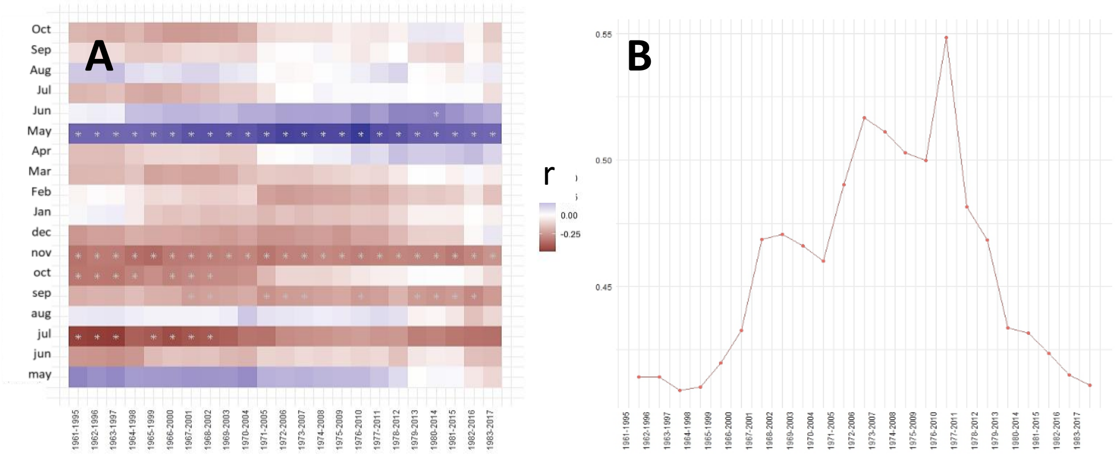


Figure 2-2 (A) Correlation plot of Tmax with aspen TRW chronology analyzed with a 35-year moving window across an 18-month series. Months beginning with a lower-case letter represent the previous year. Months marked with an asterisk are significant (Pearson's correlation coefficient (r); α = 0.05). (B) Trace-plot of correlation coefficients between May monthly maximum temperature of the current year and aspen TRW.

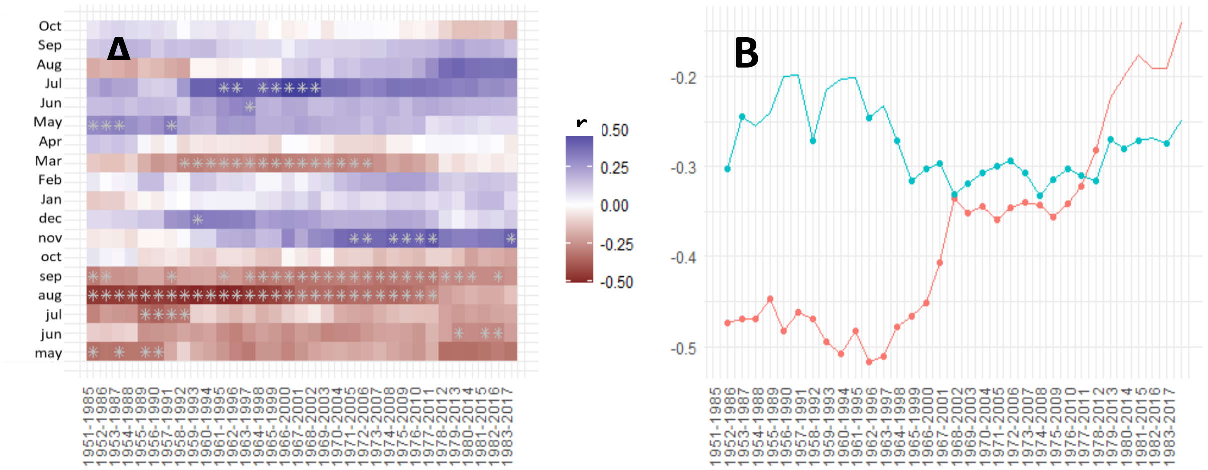


Figure 2-3 (A) Correlation plot of Tmax with Engelmann spruce TRW chronology analyzed with a 35-year moving window across an 18-month series. Months beginning with a lower-case letter represent the previous year. Months marked with an asterisk are significant (Pearson's correlation coefficient (r); α = 0.05). (B) Trace-plot of correlation coefficients for August (red) and September (blue) monthly maximum temperature of the previous year with E. spruce TRW. All series marked with a point are significant (Pearson's correlation coefficient; α = 0.05).

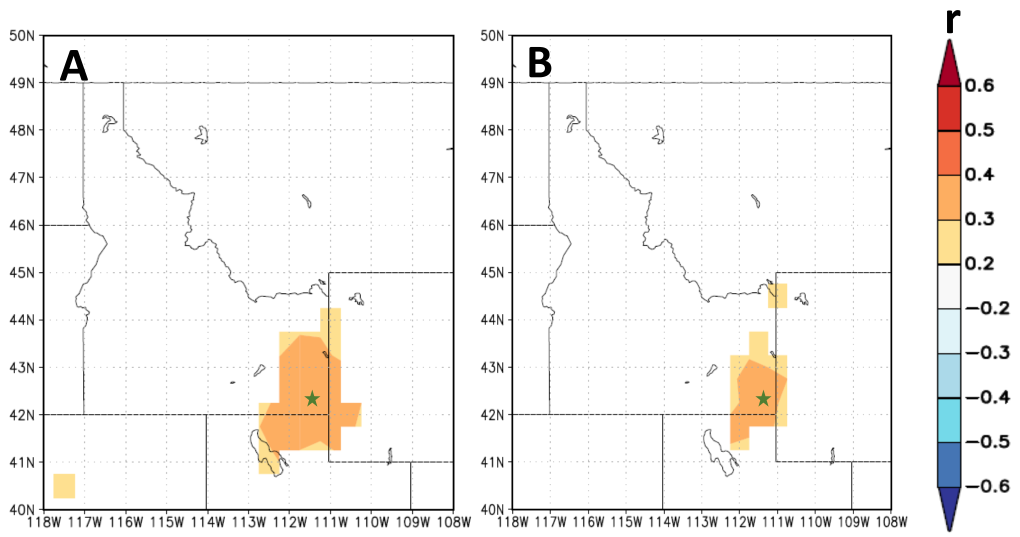


Figure 2-4 Spatial correlation (Pearson's correlation coefficient; α = 0.10) map of CRU TS 4.05 precipitation (0.5° resolution) with aspen (A) LWB chronology and (B) ΔBI for the month of July during the period of 1974-2018. Green star indicates sampling area. Maps were created in KNMI climate explorer with geo-referenced climate data sets.

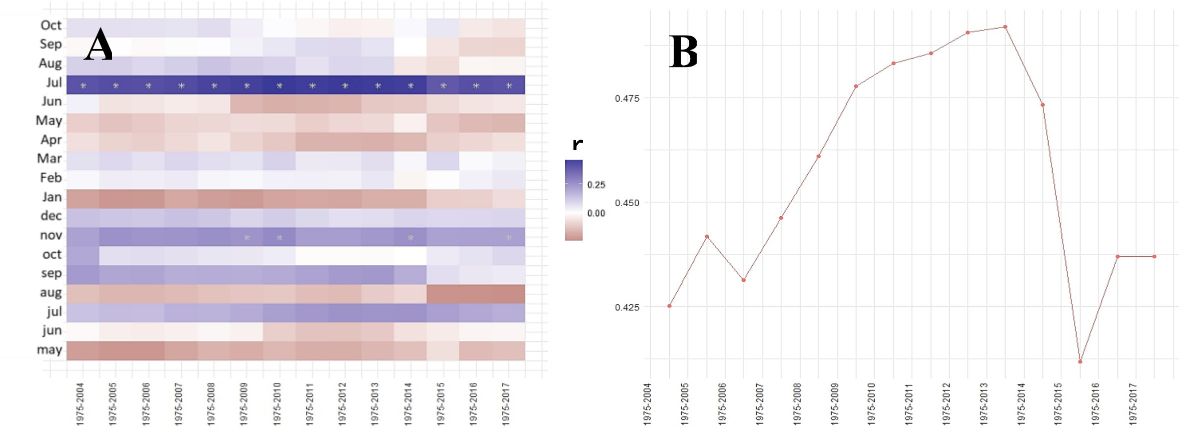


Figure 2-5 (A) Correlation plot of precipitation with aspen LWB chronology analyzed with a 35-year evolving window across an 18-month series. Months beginning with a lower-case letter represent the previous year. Months marked with an asterisk are significant (Pearson’s correlation coefficient (r); α = 0.05). (B) Trace-plot of correlation coefficients for the July monthly precipitation values with aspen LWB across the entire time series. All series marked with a point are significant (Pearson’s correlation coefficient (r); α = 0.05).

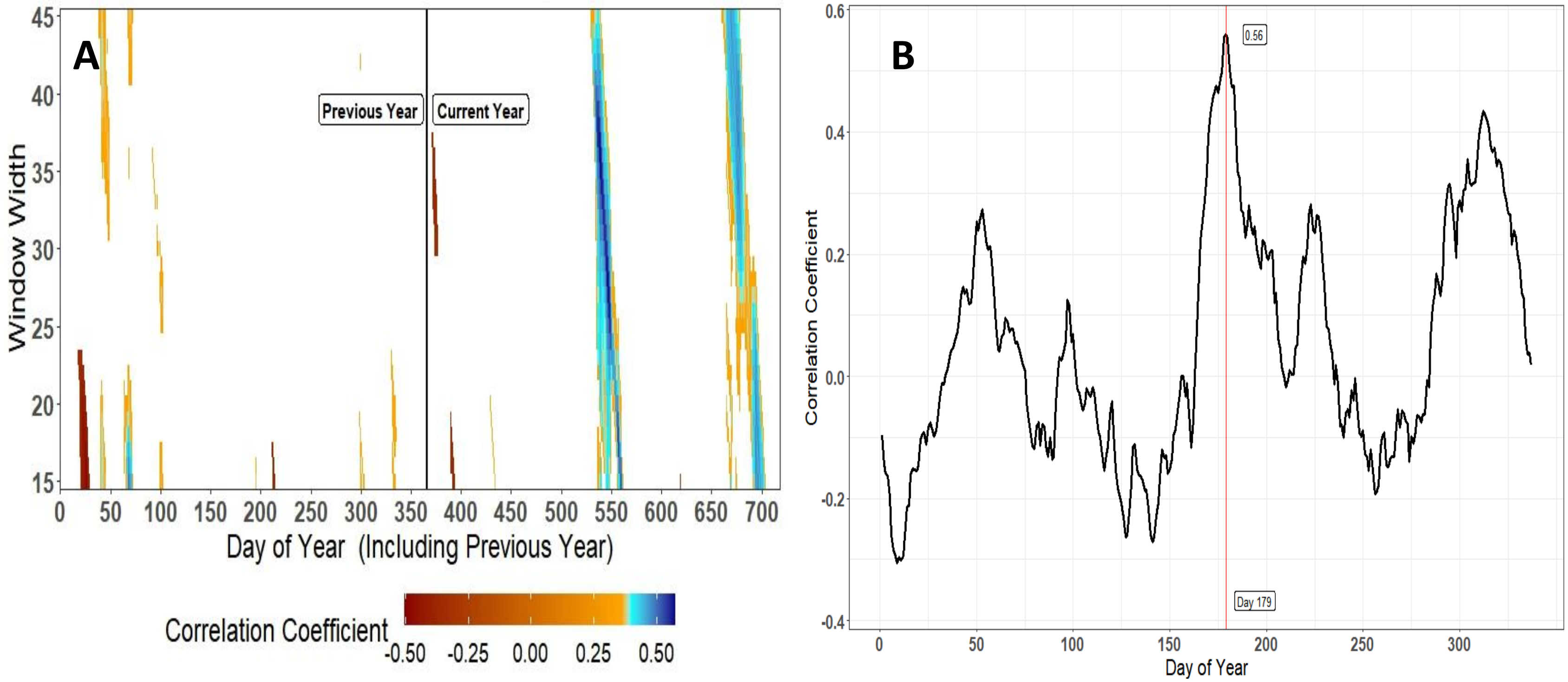


Figure 2-6 Fig. 6: (A) Heatmap of highest correlating window size for daily precipitation with aspen LWB chronology for the period 1984 – 2016 (Pearson’s correlation coefficient (r); α = 0.05). The highest correlation occurred with a 30-day window. (B) Plot of correlation coefficients (Pearson’s r) analyzed with a 30-day window for daily precipitation with aspen LWB. The optimum period occurs between late June and the end of July. The highest correlation occurs on day 179 (r = 0.56).

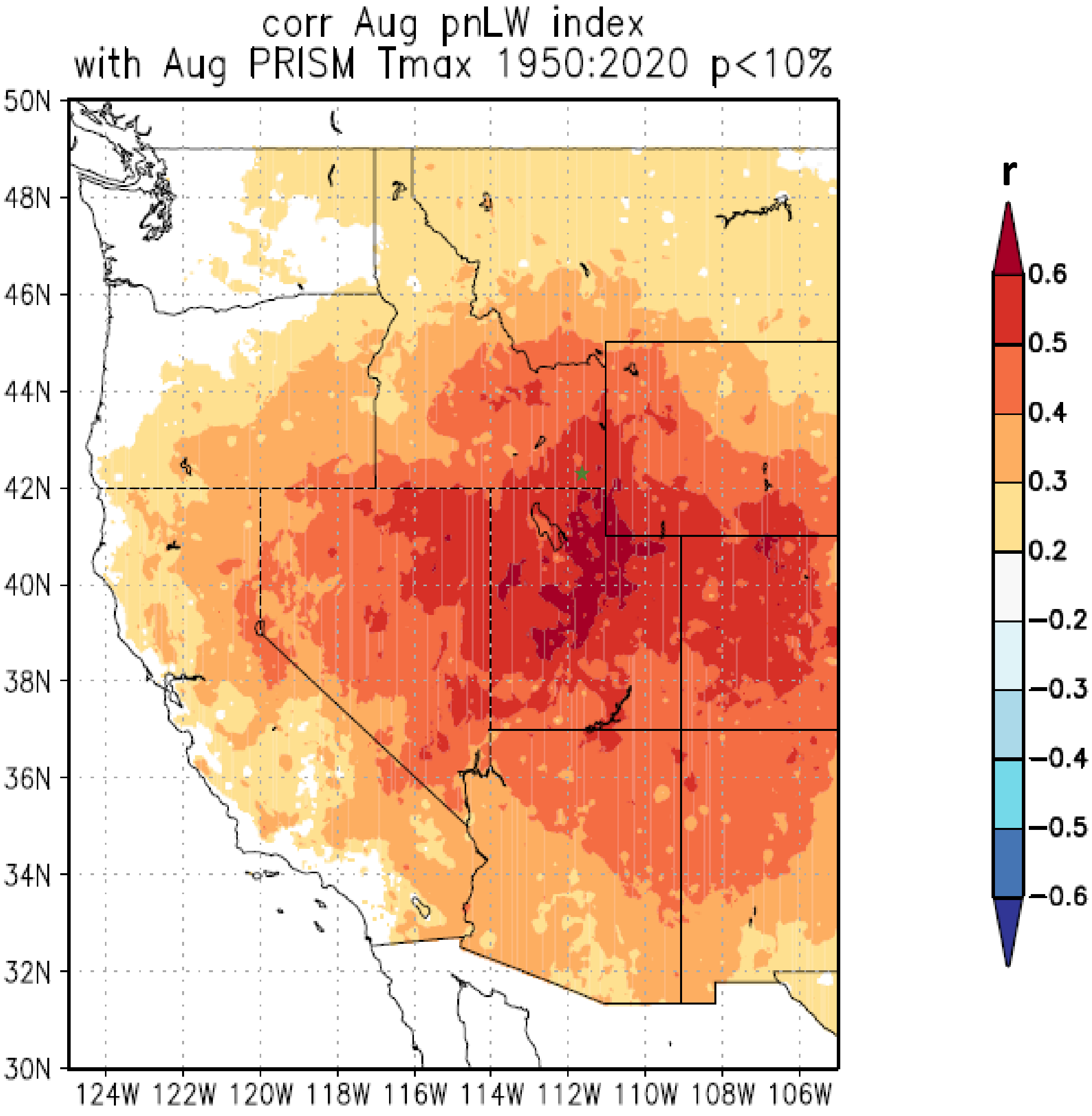


Figure 2-7 Spatial correlation (Pearson's correlation coefficient; α = 0.10) map of 4K PRISM maximum temperature (0.5º resolution) with Engelmann spruce FRB chronology for the month of August during the period of 1950-2018. Green star indicates sampling area.

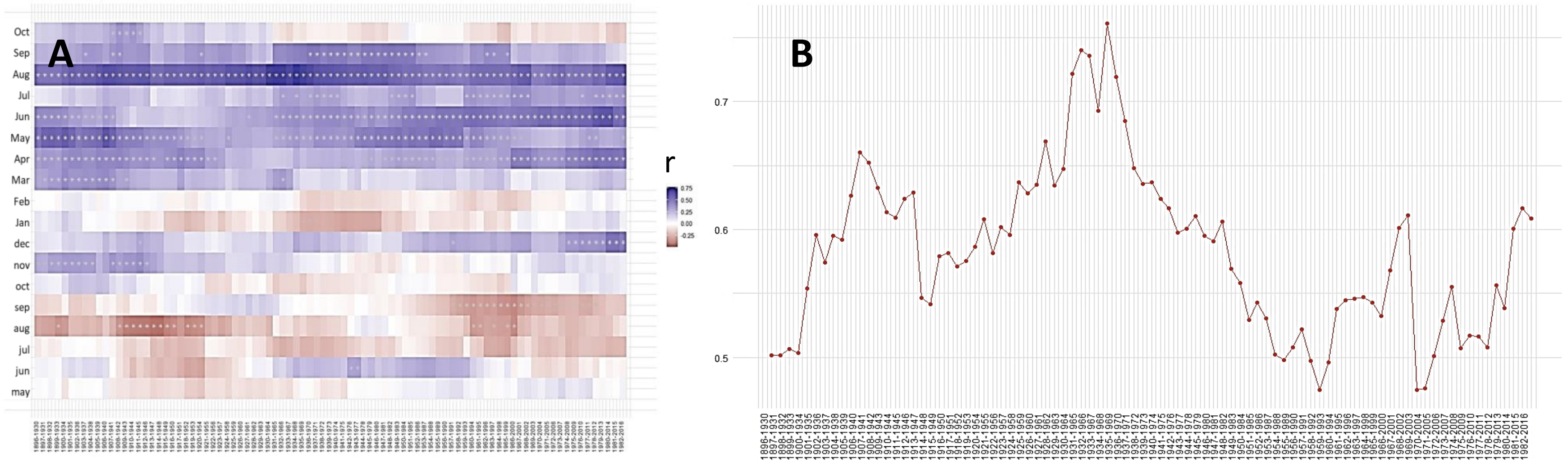


Figure 2-8: (A) Correlation plot of Tmax with Engelmann spruce LWB chronology analyzed with a 35-year moving window across an 18-month series. Months beginning with a lower-case letter represent the previous year. Months marked with an asterisk are significant (Pearson’s correlation coefficient (r); α = 0.05). (B) Trace-plot of 35-year moving window correlation coefficients for the August monthly maximum temperature of the current year with E. spruce LWB. All series are significant (Pearson’s correlation coefficient (r); α = 0.05).

Chapter 3: The Relative Potential for Stand Stability and Resistance to Drought between Riparian and Upland Aspen Stands of the Caribou-National-Forest

Introduction

Quaking aspen (*Populus tremuloides* Michx.), hereafter referred to as aspen, has the largest geographic and ecological range of any native deciduous tree species in North America (Perala, 1985; Peterson, 1996). Aspen contributes to local and regional biodiversity across many of the conifer-dominated landscapes of the western United States (Chong et al. 2001; Kuhn et al., 2011) providing habitat and food for a variety of large and small mammals, songbirds, and game birds (DeByle, 1985; Finch and Ruggiero, 1993; Oaten & Larsen, 2008; Perala,1985). These critical ecosystem services provided by aspen have made many ecologists and resource managers concerned with recent trends of aspen decline in the West. Many ecologists have for decades predicted a trajectory of disappearance (Bartos and Campbell 1998; Campbell and Bartos, 2001; Packard, 1942) while more recently others have suggested that aspen persistence is contingent on local disturbance regimes, management, and or geographical location (Kashian et al., 2007; Shinneman et al., 2013; Stevens-Rumann et al., 2018)

Aspen stands have long been divided into two functional groups: stable and seral (Kemperman and Barnes, 1976; Reed, 1971; Warner and Harper 1972). In the West, Shinneman et al. (2013) further subdivided these groups based on region and landscape factors and has broadly classified aspen of the northern Rocky Mountain region as seral. Generally, seral aspen stands of the northern Rocky Mountains require disturbances such as fire to regenerate and persist. Disturbances such as fire are considered necessary in these stands for removal of competitive shade-tolerant conifers, and to aid in the stimulation of clonal sprouting from the root systems via interruption of the flow of hormones from crown to root system (Debyle & Winokur, 1985; Peterson et al., 1996; Schier, 1975). Decades of fire suppression, however, have led to decreased fire frequency that some researchers propose has resulted in overstocked, senescing stands and aspen decline in areas where fire was considered necessary (Hessl & Graumlich, 2002; Rogers, 2002). For example, Strand et al. (2009) showed how the suppression of fire in the last century may be responsible for aspen decline in the Owyhee Plateau of southwestern Idaho by using state-and-transition models with historic fire return intervals of 70 – 80 years, much shorter than the current fire rotation of 340-450 years. Their results suggested the current fire return interval is too long to ensure aspen persistence in the Owyhee Plateau.

Conversely, aspen stands are often considered stable if they self-perpetuate in the absence of disturbance and are not at threat of conversion to non-aspen from competitive shade tolerant conifers and shrubs. Using this basic understanding of the factors that are considered important in determining aspen stability, Bartos & Campbell (1998) suggested that aspen stands that exhibit one or more of the following conditions are likely to have a higher potential for decadence or conversion from aspen: (1) conifer understory and overstory exceeding 25%; (2) dominant aspen trees greater than 100 years of age; (3) aspen regeneration less than 1,250 stems per hectare (at two size classes); and (4) sagebrush (*Artemisia* spp.) cover greater than 10%.

Other than altered fire regimes and conifer encroachment, excessive ungulate browsing of aspen regeneration has also been suspected as a cause for suppression of aspen regeneration that can effect both stable and seral stands depending on the ecological context (Eisenberg et al., 2013; Fairweather et al., 2007; Kaye et al., 2005). While some browsing may stimulate suckering in aspen by reducing competition and disturbing the roots just below the soil surface, excessive over-grazing can deplete the suckering capacity of the root stock (Schier, 1976).

Finally, drought, which affects all aspen stands, has been implicated as an important factor in aspen persistence and the driving factor of sudden aspen decline (SAD), especially in light of increasing temperatures and altered precipitation patterns associated with global climate change (Rehfeldt et al., 2009; Worrall et al., 2015). Aspect appears to be an important co-variate with elevation (especially lower elevations) in determining aspen vulnerability during drought with more southern aspects at lower elevations being most vulnerable. For example, Fairweather et al. (2007) found the highest rates of aspen decline occurred on xeric sites at low elevations during periods of long-term drought in the Coconino National Forest of Arizona. Rhodes et al. (2017) found similar trends in aspen decline, however, aspen regeneration and recruitment on southern aspects was higher than on northern aspects at high elevations.

If drought is a major inciting factor of aspen decline across the West, then more mesic ecosystems, such as riparian areas, may ameliorate the negative effects associated with global climate change. As climate change progresses, upland aspen-dominated ecosystems are likely to experience less water availability and drier soils due to loss of snowpack earlier in the growing season in southwestern Idaho (Kretchun et al. 2020; Soderquist et al., 2018). Moreover, predictive models of the effects of climate change on aspen’s geographical range have shown the Northern Rocky Mountain Region to be especially vulnerable to increasing temperatures (Rehfeldt et al., 2009; Worrall et al., 2013). Further, in the broad classification of aspen as seral in the Northern Rocky Mountain Region proposed by Shinneman et al. (2013), riparian aspen was listed as one sub-type with potential for stability in this area. If riparian areas have a higher potential for stability and a higher potential for escaping projected future climate extremes, then they may provide a refuge where aspen can avoid multiple risks to its survival and persistence.

The objective of this study is to assess the potential of riparian areas to support stable aspen stands in semi-arid montane zones. Specifically, we examined how conifer encroachment, stand structure, regeneration, and community composition, and browsing pressure differ between aspen stands growing in riparian areas and aspen stands growing in adjacent upland areas. Our hypothesis is that aspen stands in riparian areas have a higher potential for persistence relative to upland stands based on regeneration, and will have lower competitive conifer and shrub encroachment, stand structure that trends towards smaller and younger stems, and more variability in community composition. These results are expected due to the expected higher water availability throughout the growing season in riparian areas relative to upland areas.

Methods

Study Area

In the Intermountain West, some of the largest contiguous stands of aspen are in the Caribou-Targhee National Forest [CNF, (Fig. 1)]. The CNF is located within the semi-arid M331D ecological sub-region (McNab et al., 2007) characterized by average annual precipitation of approximately 36.5 cm which is less than 50 percent of the national average (NOAA, 2022), mid- to high-elevations (1400-3100 m), and clay-rich soils.

Using locations and classification of aspen stands of the CNF provided by the U.S. Forest Service (USFS), nine riparian aspen stands were selected and sampled during the summer of 2019. Riparian stands were deemed appropriate for sampling if they were aspen dominated (>50% basal area), were at least one hectare (ha) in area, were located along perennial streams, and were within three traversable km of a road or trail. Riparian aspen stands were paired with proximal (within 2 km) upland/non-riparian aspen stands by aspect, soil type, and ecological sub-section (Fig. 1).

Sampling design

Within riparian aspen stands, transects were established parallel to streamflow for the entirety of the stand, with the first transect run within 15 m of the stream's edge to compensate for stream meandering and beginning at least 15 m from stands edge to avoid edge effects. Successive parallel transects were established every 20–40 m away from the stream depending on stand structure and valley morphology. Upland aspen stands were sampled with the same methods, with the first transect run perpendicular to slope at the stand's lowest elevation and at least 15 m from the stand edge with each successive transect increasing in elevation. Circular 0.01-ha plots (100-m^2, r = 5.65 m) were sampled every 25 m along each transect.

Data collection

Within each 100-m^2 plot, all stems ≥ 3 cm diameter at breast height (DBH) were measured to the nearest 0.1 cm DBH and identified to species to characterize the overstory. Each stem was also classified into one of five crown position classes: dominant (D), co-dominant (C), intermediate (I), overtopped (O), and open-grown (G) based on relative height, modified from Bechtold (2003). An ocular estimate of aspen stem health (e.g., fungus) and percent crown dieback was also recorded following methods described in Schomaker (2007). Within each plot a minimum of four aspen cores (one from each crown position) were extracted approximately 30 cm above ground parallel to slope to estimate stand average and maximum age. Increment core samples were stored in paper straws for a minimum of 48 hours before being mounted on beveled wooden core-mounts and sanded with progressively finer grit sandpaper until annual rings were visible. All cores were individually scanned at 1600 dpi on an Epson Expression XL 12000 scanner fitted with an IT8.7/2 calibration card. The high-resolution photos were then uploaded into CooRecorder 9.6 (Larsson, 2014) for growth ring boundary delineation to estimate age for each stem.

The regeneration layer was quantified by identifying all woody species < 3 cm DBH (seedlings, saplings, shrubs) in each 100-m^2 plot. Aspen suckers were and grouped into two height classes (seedlings, < 1.0 m; saplings, ≥ 1.0 m), as sucker height has been found to be a better representation of canopy recruitment potential than age in aspen (Baker et al., 1997). Each plot was further sub-divided into four 1.0-m^2 quadrats placed at the center of each plot and the termini of three 5.65 m sub-transects run at 90º, 240º, and 300º of the central transect. Percent cover of plant functional groups (forbs, graminoids, shrubs, bryophytes and fungi) and bare ground was estimated for each quadrat.

To characterize browsing pressure, ungulate droppings (cow, moose, elk, deer) fallen within 1 m of either side of the central transect were also tallied within each 100-m^2 plot to assess relative presence and potential ungulate browsing using methods as described by Neff (1968). These methods have been utilized in aspen stands with varying levels of browsing pressure and have shown significant inverse relationships with aspen regeneration growth and recruitment (Rhodes et al., 2017).

Aspect at the center of each 100-m^2 plot was recorded as azimuth in the downhill direction and averaged for each stand. Aspect was cosine transformed following a 45° shift to give a value that ranges between -1 and 1 for southwest and northeast, respectively (Beers et al., 1966). Slope was recoded for each plot using a Nikon Forestry Pro II hypsometer 6x Rangefinder by aiming the laser upslope from the lowest point of the plot to a point of equal height as the observer's eye determined on flat ground. Degree of slope was averaged across all plots for each stand. Latitude, longitude and elevation were recorded from approximate stand centers using a handheld Garmin GPSMAP 66S.

Data Analysis

Prior to analysis, basal area and density of all overstory trees, and density of seedlings and saplings, were calculated and converted to a per hectare basis. Relative dominance was calculated by summing the total basal area per stand per species and dividing by the total basal area of all species per stand. Relative density for each species was similarly calculated using number of stems per stand of each species divided by total number of stems for all species. Relative frequency for each species was calculated by the number of plots per stand where the species was present divided by the total number of plots sampled. Importance values (IV; average of relative dominance, density and frequency) for all species were calculated for each stand and separated into a canopy layer (dominant and codominant) and a subcanopy layer (intermediate and overtopped).

Overstory compositional differences between riparian and upland stands were analyzed using total IVs of the canopy (all dominant and co-dominant trees) and subcanopy (all intermediate and overtopped trees) layer combined with non-metric multidimensional scaling (NMDS). Dimensionality of the NMDS was determined with a scree plot of stress vs. number of dimensions and the lowest number of dimensions with a stress less than 2.0 following criteria suggested by McCune and Grace (2002). Goodness-of-fit of the model with the chosen number of dimensions was cross-referenced with a stress plot of the residuals. Differences in mean conifer IV's for the canopy and subcanopy layer were tested for significance with a paired t-test.

To evaluate differences in the composition of the regeneration layer, we examined the total number of stems per hectare of each species for each stand with NMDS. Additionally, we compared aspen regeneration densities between riparian and upland stands for the seedling and sapling layer with a paired t-test (sapling) or a Wilcoxon signed rank test (seedling) if assumptions of normality or equal variances were violated following a Shapiro-Wilk's test and a Levene's test. To explore species diversity in the regeneration layer, we also calculated Shannon-Weiner and a Simpson's diversity index of both the seedling and sapling layers combined for each stand.

Finally, to explore differences in the ground layer functional group composition between riparian and upland aspen stands, we used a Multi-Response Permutation Procedure (MRPP) with a Bray-Curtis dissimilarity index. Pairwise differences were checked for significance with a Mann-Whitney U test with a Bonferroni correction ($\alpha = 0.01$).

Results

Differences in overstory composition and structure

Overall, NMDS analysis of importance values (IV) showed riparian and upland aspen stands have similar overstory composition, distributed across a strong elevation gradient along the first NMDS axis and slope percent along the second NMDS axis (Fig. 2). As expected, aspen dominated both the riparian and upland stands, with a mean (± SE) IV of 85.0 ± 2.1 and 81.4 ± 3.9, respectively (Table 1). Douglas-fir (*Psuedotsuga menzesii* (Mirb.) Franco) was found in both stand types, with an IV of 9.2 ± 2.3 in the riparian stands and 10.0 ± 1.7 in the upland stands (Table 1). Lodgepole pine (*Pinus contorta* Douglas ex Loudon) was also present in both stand types, however, it was more dominant in the upland stands (IV = 6.9 ± 3.5) than the riparian stands (IV = 3.4 ± 1.9) (Table 1). Some species only appeared in riparian areas such as grand fir (*Abies grandis* (Douglas ex D. Don) Lindl.) (0.78 ± 0.73), bigtooth maple (*Acer grandidentatum* Nutt.) (IV = 0.56 ± 0.53), Utah juniper (*Juniperus osteosperma* (Torr.) Little) (0.44 ± 0.43), and choke cherry (*Prunus virginiana* L.) (0.44 ± 0.43), while few appeared only in the upland sites such as subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) (0.5 ± 0.43), and Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) (1.11 ± 0.7). Additionally, the NMDS suggests that riparian stands tend to be more variable in terms of overstory composition than upland stands (Fig. 2), however, these differences were not significant (p = 0.292). Riparian stands included a higher number of species in the overstory (7) compared to those in adjacent uplands (5), and several species occurred exclusively in riparian or upland areas (Table. 1).

Based on relative IV of all combined conifer species, upland stands had higher mean (± SE) conifer encroachment in the canopy layer compared to riparian stands (7.4% ± 1.8 and 4.7% ± 1.2 respectively; t = 1.417; p = 0.1941), and about the same level of conifer encroachment in the subcanopy layer (11.8% ± 2.8 and 14.0% ± 3.7, respectively; t = 0.003; p = 0.9997) (Table 2). Corresponding to the observed higher variability in riparian overstory composition of the NMDS, we found that the 6.7% of the subcanopy of the riparian stands were dominated by other non-conifer species while only 2.0% of the subcanopy of the upland stands were dominated by other non-conifer species (Table 2).

Overall, the overstory structure of aspen in riparian stands exhibited an even size and age distribution, while upland aspen stands were characterized by larger and older stems (Table 3; Fig. 3).

Specifically, the (± SE) age of riparian aspen stands was 47.0 ± 4.1 years and 60.2 ± 5.8 years for the upland stands (t = -1.96; p = 0.0856), mean maximum age of riparian aspen stands was 87.3 ± 7.4 years and 106.8 ± 8.8 years for the upland stands (t = -2.01; p = 0.0793), and quadratic mean diameter (Qm) was 11.6 ± 0.9 cm and 13.7 ± 0.9 cm the for the upland stands (t = -2.1293; p = 0.0659) (Table 3). While these differences in size and age were not significant at $\alpha = 0.05$, they are considerable, and the trends are near significant.

Differences in regeneration layer

The composition of the regeneration layer between riparian and upland aspen stands also appears to be similar based upon the NMDS and distributed across the first NMDS axis by elevation [$p < 0.05$; (Fig. 4)]. Overall, we observed a total of 25 woody species in the regeneration layer, with aspen having the highest overall mean (± SE) density for both the riparian 2058.7 ± 259.4 stems/ha and upland stands 1016.67 ± 125.1 stems/ha (Table 4). Other species were also common to both the riparian and upland stands, including Saskatoon serviceberry (*Amelanchier alnifolia* Nutt.) with densities of 385.7 ± 48.6 stems/ha in the riparian areas and 728.79 ± 89.7 stems/ha in the uplands, choke cherry (*P. virginiana*) with densities of 493.7 ± 62.2 stems/ha in the riparian areas and 793.9 ± 97.7 stems/ha in the uplands, and western huckleberry (*Vaccinium membranaceum* Douglas ex Torr.) with densities of 2212.7 ± 278.8 stems/ha in the riparian areas and 5257.6 ± 647.2 stems/ha in the uplands. Sagebrush (*A. tridentata* Nutt.) densities were considerably higher in the uplands 939.4 ± 115.6 stems/ha than in the riparian areas 185.7 ± 23.4 stems/ha Species found only in the riparian stands included species that are typically consider mesic or wet-mesic, including alders (*Alnus sp.*), Rocky Mountain maple (*Acer glabrum* Torr.), and red-osier dogwood (*Cornus sericea* L) (Table 4; Fig. 4). As observed with the overstory, riparian stands showed a higher variability in the composition and relative abundances of woody species when compared to the upland stands (Table 4).

Mean (± SE) woody species richness in the regeneration layer was 8.1 ± 0.5 for the riparian aspen stands and 8.4 ± 0.5 for the upland aspen stands. When the regeneration layer was divided into saplings and seedlings, we found that aspen sapling and seedling density was higher in the riparian stands 2058.73 ± 1491.70 than in the upland stands 1016.67 ± 447.07 (Fig. 5). These differences were significant for the seedling layer determined by a Wilcoxon signed rank test [$p = 0.0391$; (Fig. 5)]. The difference in mean biodiversity between the riparian areas ($H = 1.4 \pm 0.1$; $S = 8.1 \pm 0.5$) and the upland areas ($H = 1.48 \pm 0.1$; $S = 8.4 \pm 0.5$) were not significant when compared with values from Shannon-Weiner and Simpson's biodiversity indices (Fig. 6).

Differences in the groundcover composition

Composition of groundcover lifeform guilds differed significantly between riparian and upland aspen stands following analysis with an MRPP ($p = 0.001$, $A = 0.03809$, delta = 0.501). The groups contributing to the difference in community compositon were graminoids ($p < 0.001$), forbs ($p < 0.001$), and shrubs ($p < 0.001$); bare soil, and bryophytes and fungi did not differ between groups (Fig. 7).

Discussion

The objective of our study was to investigate the difference in potential for aspen persistence between riparian and upland aspen stands in a semi-arid, montane region. As many researchers have suggested drought as the main inciting factor of aspen overstory mortality and stand senescence we grouped aspen stands based on ecosystem types with the greatest expected difference in water availability (Anderegg et al., 2013; Hogg et al., 2008; Singer et al., 2019; Soderquist et al., 2018; Worrall et al., 2013, 2015). Our results provide some insight on the relationship of ecosystem type on aspen regeneration in the absence of excessive overstory mortality or SAD. Specifically, we found higher aspen seedling and sapling densities in riparian stands located along perennial streams than in nearby upland stands. No stands, riparian or upland, showed evidence of SAD affliction or rapid decline. Given the presence of many mesic and wet-mesic species in the riparian areas; and the higher relative presence of xeric species in the upland areas we believe this relationship is driven by the higher water availability in the riparian areas. There are multiple site factors, however, that are inherently different in riparian areas (e.g., elevation, soil) that warrant further investigation as drivers of aspen regeneration.

Drought induced senescence has been found in stands both affected and unaffected by SAD. Hanna & Kulakowski (2012) found that overstory mortality of aspen in SAD-affected stands was preceded by multiple years of reduced growth, and the frequency of mortality was closely associated with multiple years of drought. Hogg et al. (2008) found aspen mortality and dieback were best correlated with the climate moisture index in northwestern Alberta, Canada, and concluded aspen health was most sensitive to moisture limitations. While few studies have quantified the effects of drought on aspen regeneration, some have found a relationship between drought induced overstory mortality and reduced regeneration densities (Worrall et al., 2015). This suggests that drought may have a direct effect on aspen regeneration by decreasing sucker and seedling production or an indirect effect on regeneration by increasing overstory mortality in a way that does not stimulate sucker production.

While aspen regeneration in the absence of disturbance is the primary factor determining stand stability, it is not the only factor. Encroachment of competitive conifers and shrubs, and excessive browsing from ungulates have also been implicated as major factors influencing aspen stand stability. Higher encroachment of competitive species can lead to reduced regeneration and recruitment of aspen (Kaye et al., 2005). Conifer encroachment appears to be occurring in both stand types and was not found to differ significantly between riparian and upland stands with around 10-13% of the subcanopy of both stand types dominated by conifers. There is a trend, however, of higher conifer encroachment in the canopy layer of upland aspen stands but this may be indicative of longer fire return intervals in the uplands. This increase of conifer encroachment in the canopy of upland stands may be at least one explanation of several interacting factors driving the suppression of aspen regeneration in these stands.

Stand structure and composition have also been found to be indicators of the potential for aspen stand stability. Older stands made up of larger stems have been found to be more susceptible to the negative effects of climate extremes (e.g. drought) and conifer encroachment; and stand age has been found to correlate negatively with sucker production (Bell et al., 2014; Schier, 1975). Neither the composition nor the structure of the riparian and upland aspen stands differed significantly, although the upland sites generally included older and larger stems. Again, while statistical analyses could not detect differences in these characteristics that indicate regeneration and stability, they may be interacting in a way that reduces aspen in the regeneration layer. More specifically, it is possible that while the riparian aspen stands are producing regeneration densities high enough, based on the criteria set by Bartos and Campbell (1998) to ameliorate suppression from browsing and encroachment, it may only be a matter of time before these stands reach an age and structure that is no longer conducive to producing regeneration in high enough quantities to escape these stressors.

In the regeneration and groundcover layer, composition differed significantly between the riparian and upland stands for woody species and for life-form guilds. Woody species in the riparian zones showed higher variability in their distributions and occurrence; and riparian aspen groundcover was dominated mostly by graminoids and forbs while upland aspen groundcover was dominated mostly by shrubs. Mueggler (1989) found a weak but positive relationship between graminoids and sucker production, but admits these results were highly variable and that herbaceous groundcover itself was a poor indicator of sucker production potential. It is more likely that graminoids and sucker production were both being positively influenced by higher moisture availability.

Overall, our results suggest that riparian aspen stands have a trend of smaller and younger canopy structure, higher aspen regeneration densities, and a different groundcover composition relative to adjacent non-riparian stands. The trend of smaller and younger stems, and the higher regeneration densities in the riparian areas may suggest the higher potential for aspen persistence in relative to upland stands. Riparian aspen stands, however, are still vulnerable to conifer encroachment. Three riparian areas have >25% conifer encroachment in the subcanopy and if conditions allow for recruitment of these conifers into the upper canopy, senescence is likely inevitable without treatment or fire.

In the western U.S., it is not uncommon for a single clone to occupy several acres (Kemperman & Barnes, 1976), and the capacity for suckering can vary greatly among genotypes (Barnes, 1966). Schier (1976) has proposed that temperature and water availability are the primary drivers of suckering initiation even if the clone has the genetic capacity for suckering. If temperatures are warm enough and water availability is sufficient in the early stages of the growing season prior to and immediately following leaf bud burst and flushing, then suckering can occur. One caveat to this is study is the uncertainty of genetic diversity and clone age for each stand sampled. The current study, however, is an initial description and study of the general differences in aspen stand function based on ecosystem type (riparian vs. upland). Considering our results support the classification of Shinneman (2013) and suggest there is evidence of a difference in aspen regeneration based on ecosystem type, the next step will be a deeper investigation into the other factors not explored in this study, including genetic diversity, to aid in a deeper understanding of aspen ecology in semi-arid montane zones of the West.

Aspen suckering requires heat and moisture to initiate from the root stock. In a future that threatens higher temperatures and drier conditions, ecosystems with the highest potential for suckering will be those with higher water availability, such as those in riparian areas. Furthermore, the best strategy to ensure aspen's presence on the landscape is to keep a persistent seed source for sexual reproduction that helps increase genetic diversity during a threat of changing conditions. Considering the recent evidence that sexual reproduction is more common for aspen than previously thought (Long & Mock, 2012), riparian areas dominated by aspen may provide an opportunity for coevolution of western aspen with predicted climate changes.

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Figures and Tables

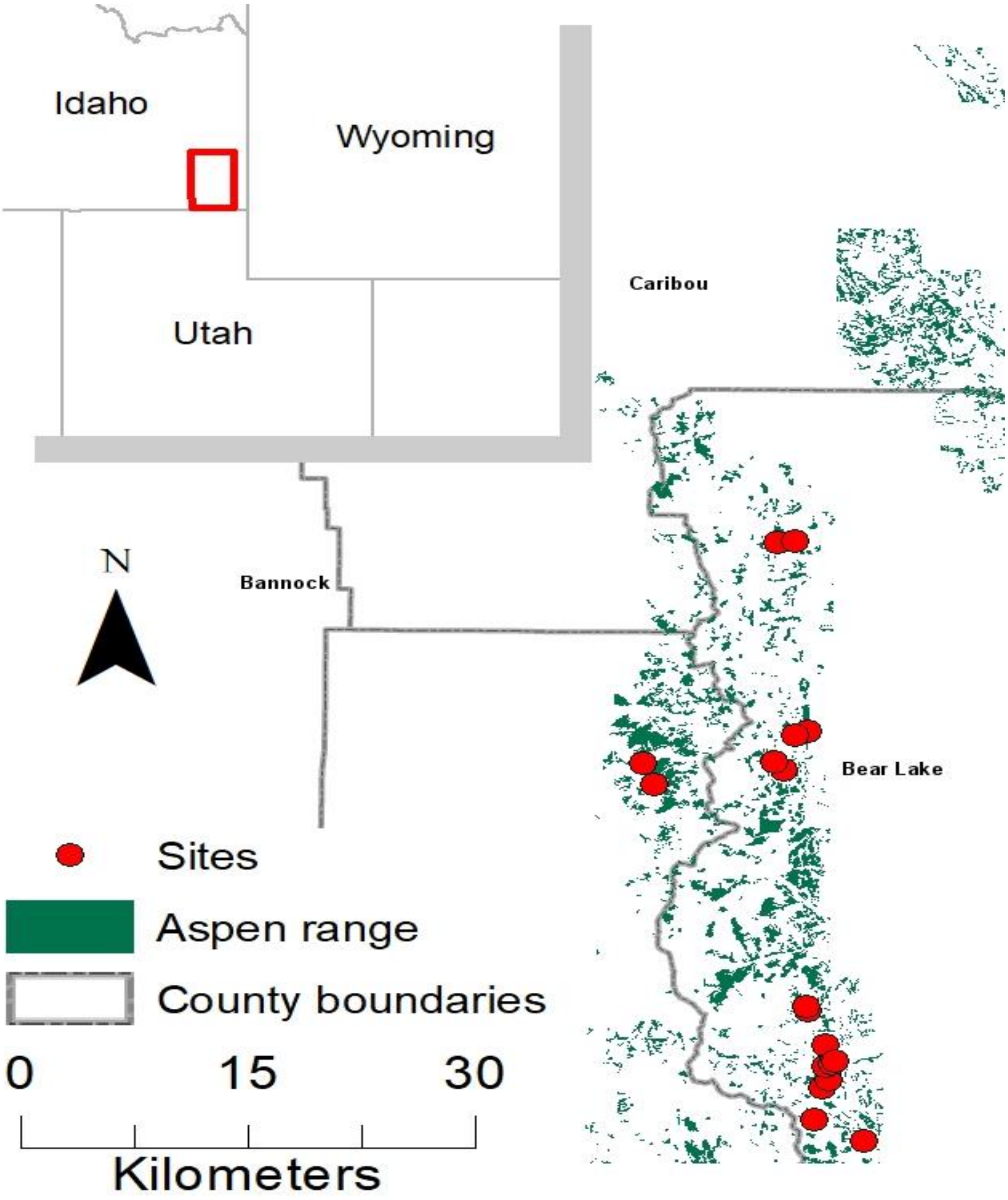


Figure 3-3-1 Map of eighteen sites total (9 riparian and 9 upland) were sampled. Riparian stands chosen occupied at least 1 hectare, were dominated by aspen, and did not contain grazing exclusion. Upland sites were paired with riparian sites by eco-region sub-section, proximity, and aspect.

Species	Symbol	Riparian	Upland
Populus tremuloides	Potr	85 (2.1)	81.44 (3.93)
Abies grandis	Abgr	0.78 (0.73)	0
Abies lasiocarpa	Abla	0	0.5 (0.43)
Acer grandidentatum	Acgr	0.56 (0.53)	0
Juniperus osteosperma	Juos	0.44 (0.43)	0
Pinus contorta	Pico	3.44 (1.93)	6.89 (3.53)
Pinus engelmannii	Pien	0	1.11 (0.7)
Prunus virginia	Prvi	0.44 (0.43)	0
Pseudotsuga menziesii	Psme	9.22 (2.33)	10 (1.67)

Table 3-3-1 Mean values with standard errors in parentheses of all (canopy and subcanopy combined) IV values by site type and symbols used in NMDS.

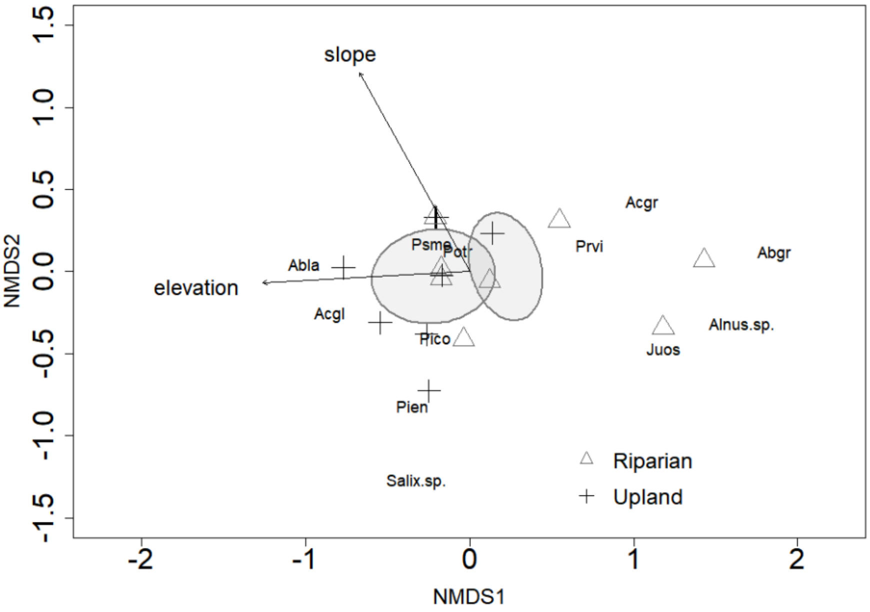


Figure 3-2 NMDS of overstory importance values of all canopy tree species found within aspen stands, analyzed by site type (riparian or upland). Riparian stands appear to support a higher variability of species. Gray ellipses represent a 95% confidence interval.

		Canopy			Subcanopy		
	Site	**Conifer**	**Aspen**	**Other spp.**	**Conifer**	**Aspen**	**Other spp.**
Riparian	R006	0	100	0	25	75	0
	R4421	0	100	0	9.09	84.85	6.06
	RFH	9.38	90.63	0	13.95	86.05	0
	R MB	3.23	96.77	0	26.19	73.81	0
	R minne 1	4.55	93.94	1.52	5	75	20
	R minne 2	8.51	89.36	2.13	4	64	32
	R minne 3	2.63	97.37	0	0	97.73	2.27
	R361	9.09	90.91	0	10.81	89.19	0
	RIP 11	4.48	95.52	0	31.48	68.52	0
	means	4.65 (1.22)	94.94 (1.33)	0.41 (0.27)	13.95 (3.70)	79.35 (3.60)	6.70 (3.84)
Upland	Up 442	2.04	97.96	0	0	91.3	8.7
	U 373	3.85	96.15	0	6.12	85.71	8.16
	UGCF 138	9.76	90.24	0	17.95	82.05	0
	UP 312	0	100	0	14.29	85.71	0
	UP 373	3.7	96.3	0	6.67	93.33	0
	UP minne 1	7.69	92.31	0	4.55	95.45	0
	UP RIP11	15.38	84.62	0	27.18	71.84	0.97
	UPFH	10.94	89.06	0	12.82	87.18	0
	UPMB	13.04	86.96	0	16.67	83.33	0
	means	7.38 (1.76)	92.62 (1.76)	0.00	11.81 (2.78)	86.21 (2.34)	1.98 (1.22)

Table 3-2 Importance values by site for the canopy and subcanopy. Importance values were calculated as the average of relative frequency, relative dominance, and relative density to represent percentages. Standard error of means represented in parentheses.

				Aspen structure			Aspen regeneration		
	Site	aspect	elevation (m)	Qm*	mean age	max age	Seedling (stems/ha)	Sapling (stems/ha)	total
Riparian	R361	N	1981	8.2 (0.51)	29.3 (3.2)	60	167 (131)	1717 (554)	1883 (682)
	Rip 442	N	2042	13 (0.79)	58.2 (6.3)	110	50 (14)	133 (47)	185 (47)
	Rip 6	SE	2286	15.7 (1.17)	63.0 (7.6)	118	2083 (1097)	2500 (782)	4583 (1863)
	Rip FH	N	2073	9 (0.56)	39.6 (3.0)	77	138 (84)	913 (216)	1050 (276)
	Rip MB	SE	2073	14.5 (0.88)	50.3 (5.5)	118	250 (106)	733 (212)	983 (224)
	Rip Minne1	SE	1951	10.3 (0.37)	36.1 (2.3)	71	575 (158)	388 (125)	963 (224)
	Rip Minne2	S	1935	12.1 (0.62)	44.7 (2.1)	73	667 (219)	783 (274)	1450 (438)
	Rip11	N	2438	12.5 (0.31)	62.7 (2.6)	85	2750 (563)	825 (275)	3575 (572)
	Rminne3	SE	1966	9.1 (0.45)	39.4 (2.0)	73	978 (202)	2444 (427)	3422 (621)
Upland	Up 312	S	2301	13.8 (0.90)	57 (5.7)	100	367 (176)	1400 (307)	1767 (305)
	Up 373	NW	2988	15.1 (0.77)	56.4 (5.2)	98	38 (26)	638 (153)	675 (152)
	Up 442	S	2316	15 (0.78)	85.7 (7.0)	131	38 (26)	650 (153)	688 (154)
	Up FH	N	2438	9.5 (0.34)	36.9 (1.4)	45	716 (560)	583 (164)	1300 (695)
	Up GCF	N	2309	16 (0.91)	70 (6.3)	118	329 (81)	1042 (146)	1371 (220)
	UP MB	SE	2332	15.9 (0.49)	81 (7.6)	118	160 (112)	900 (247)	1060 (254)
	Up minne1	NW	2393	9.8 (0.49)	40.3 (3.0)	100	233 (100)	1033 (245)	1267 (282)
	Up rip11	SE	2393	11.2 (0.62)	46.4 (4.4)	126	338 (121)	788 (227)	1125 (288)
	Up 137	S	2319	16.7 (1.01)	68 (5.9)	125	22 (15)	256 (91)	278 (98)

Table 3-3 Aspen structure characteristics by site for riparian and upland stands. Upland aspen stands were on average dominated by larger and older stems. Riparian aspen stands supported higher average aspen regeneration. Values in parentheses represent standard error.
*Quadratic mean of mature aspen stem diameters.

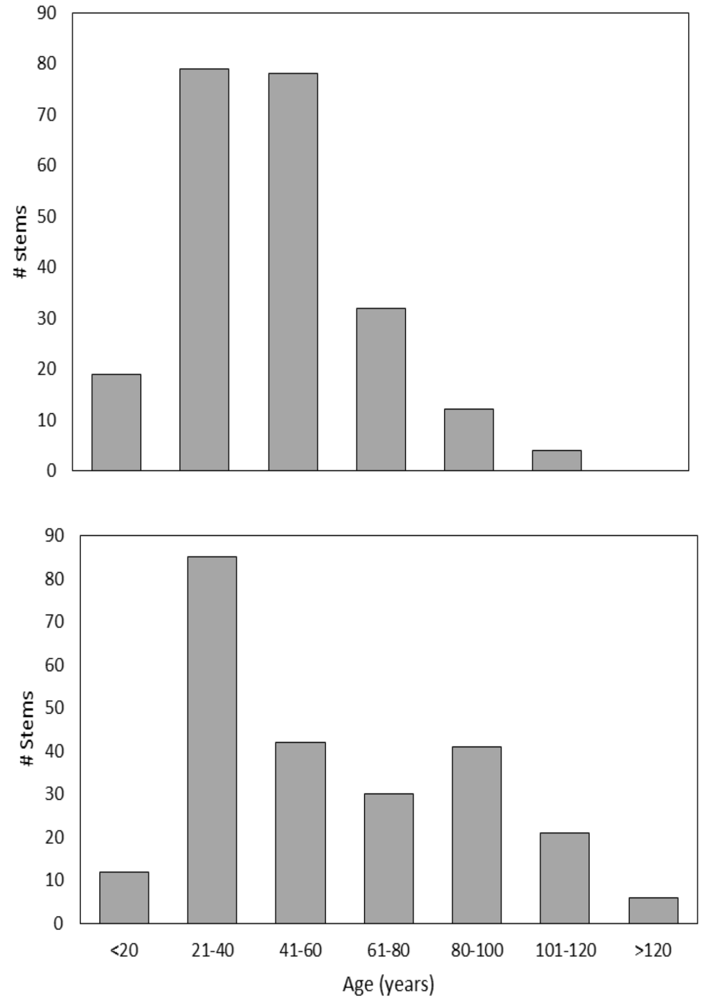
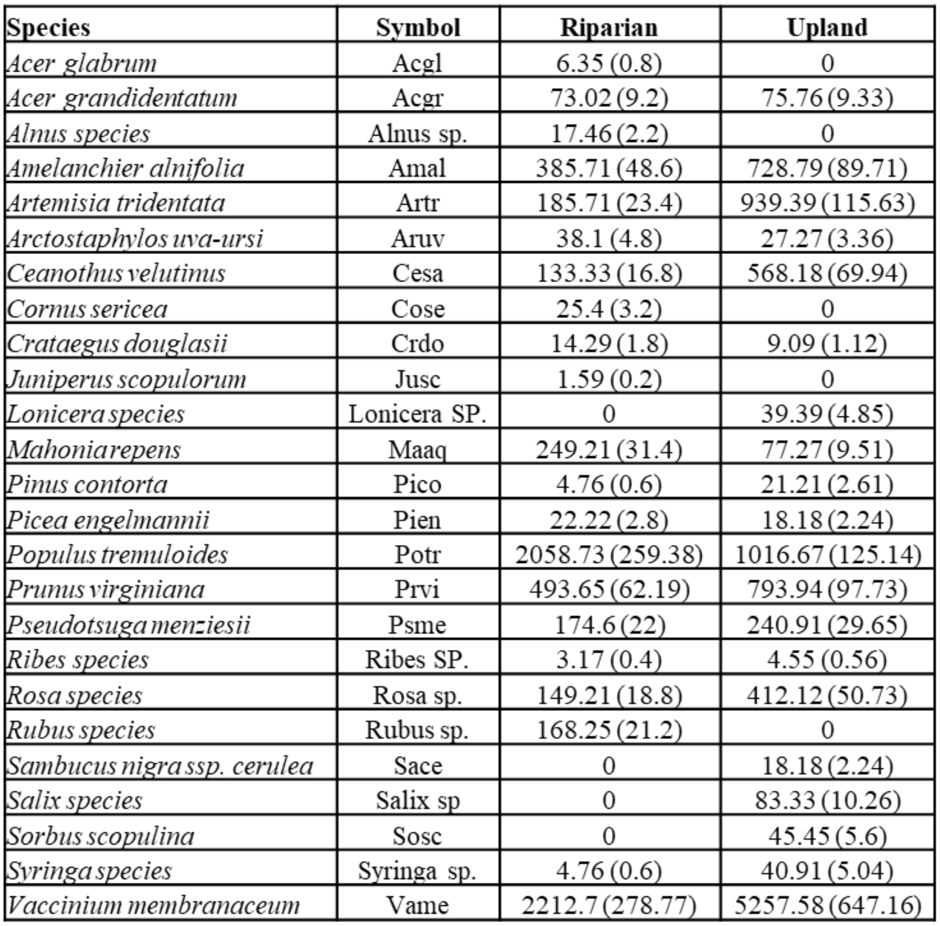


Figure 3-3 Age distributions for all aspen stems sampled across all riparian (above) and all non-riparian (below) aspen stands.



Species	**Symbol**	**Riparian**	**Upland**
Acer glabrum	Acgl	6.35 (0.8)	0
Acer grandidentatum	Acgr	73.02 (9.2)	75.76 (9.33)
Alnus species	Alnus sp.	17.46 (2.2)	0
Amelanchier alnifolia	Amal	385.71 (48.6)	728.79 (89.71)
Artemisia tridentata	Artr	185.71 (23.4)	939.39 (115.63)
Arctostaphylos uva-ursi	Aruv	38.1 (4.8)	27.27 (3.36)
Ceanothus velutinus	Cesa	133.33 (16.8)	568.18 (69.94)
Cornus sericea	Cose	25.4 (3.2)	0
Crataegus douglasii	Crdo	14.29 (1.8)	9.09 (1.12)
Juniperus scopulorum	Jusc	1.59 (0.2)	0
Lonicera species	Lonicera SP.	0	39.39 (4.85)
Mahonia repens	Maaq	249.21 (31.4)	77.27 (9.51)
Pinus contorta	Pico	4.76 (0.6)	21.21 (2.61)
Picea engelmannii	Pien	22.22 (2.8)	18.18 (2.24)
Populus tremuloides	Potr	2058.73 (259.38)	1016.67 (125.14)
Prunus virginiana	Prvi	493.65 (62.19)	793.94 (97.73)
Pseudotsuga menziesii	Psme	174.6 (22)	240.91 (29.65)
Ribes species	Ribes SP.	3.17 (0.4)	4.55 (0.56)
Rosa species	Rosa sp.	149.21 (18.8)	412.12 (50.73)
Rubus species	Rubus sp.	168.25 (21.2)	0
Sambucus nigra ssp. cerulea	Sace	0	18.18 (2.24)
Salix species	Salix sp	0	83.33 (10.26)
Sorbus scopulina	Sosc	0	45.45 (5.6)
Syringa species	Syringa sp.	4.76 (0.6)	40.91 (5.04)
Vaccinium membranaceum	Vame	2212.7 (278.77)	5257.58 (647.16)

Table 3-4 List of woody species found in the regeneration layers. Values represent mean number of stems per hectare by site type.

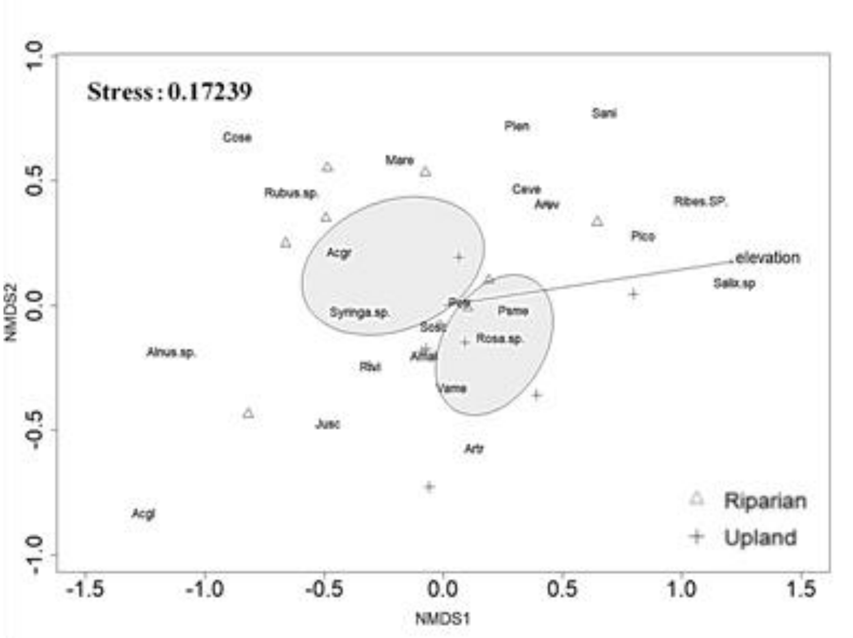


Figure 3-4 NMDS of regeneration layer within aspen stands, analyzed by site type (riparian or upland). Composition of woody regeneration differs significantly ($p < 0.05$). Gray ellipses represent a 95% confidence interval.

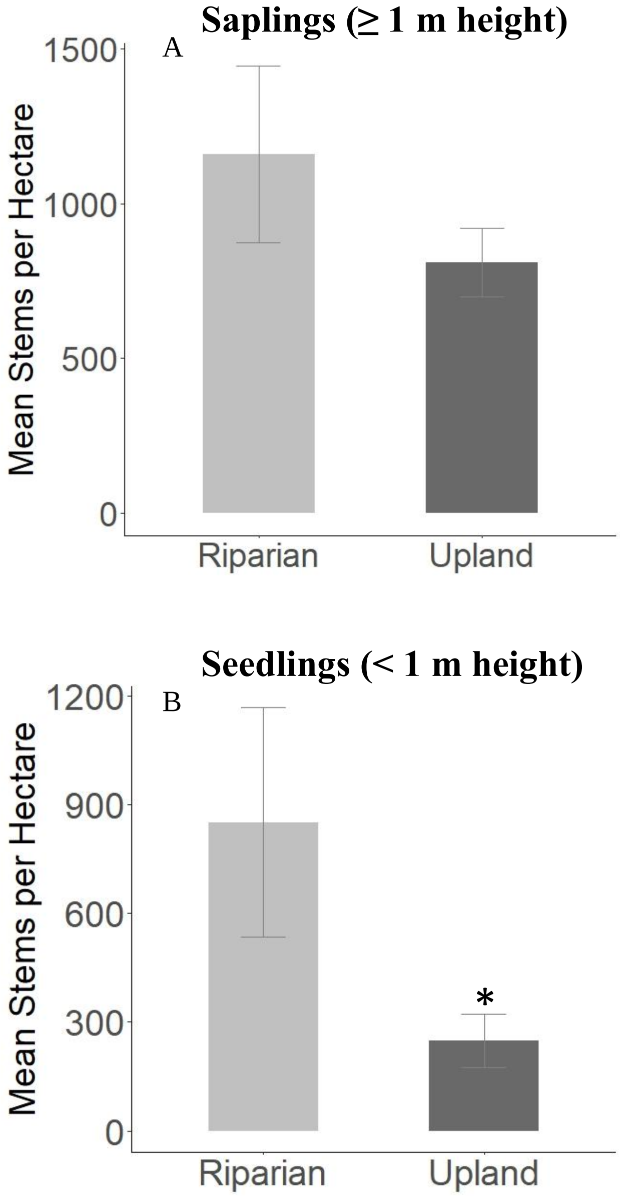


Figure 3-5 Mean stems per hectare of aspen regeneration seedlings (A) and saplings (B) for riparian and upland aspen stands. Error bars represent the standard error. * Significant at the $p < 0.05$ level following a Wilcoxon signed rank test.

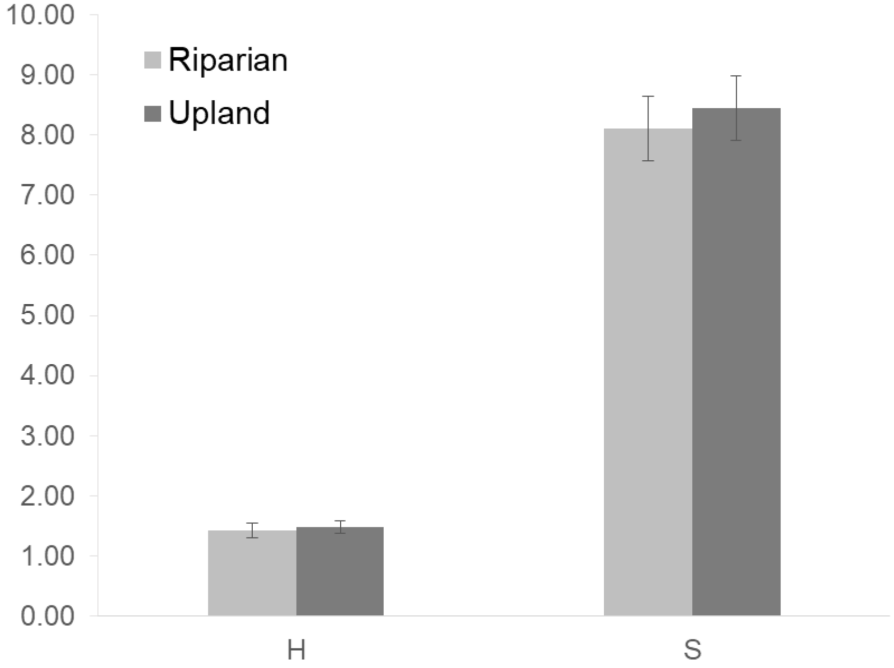


Figure 3-6 Mean Shannon-Weiner (H) and Simpson's (S) diversity indices compared between riparian and upland aspen stands for the regeneration layer.

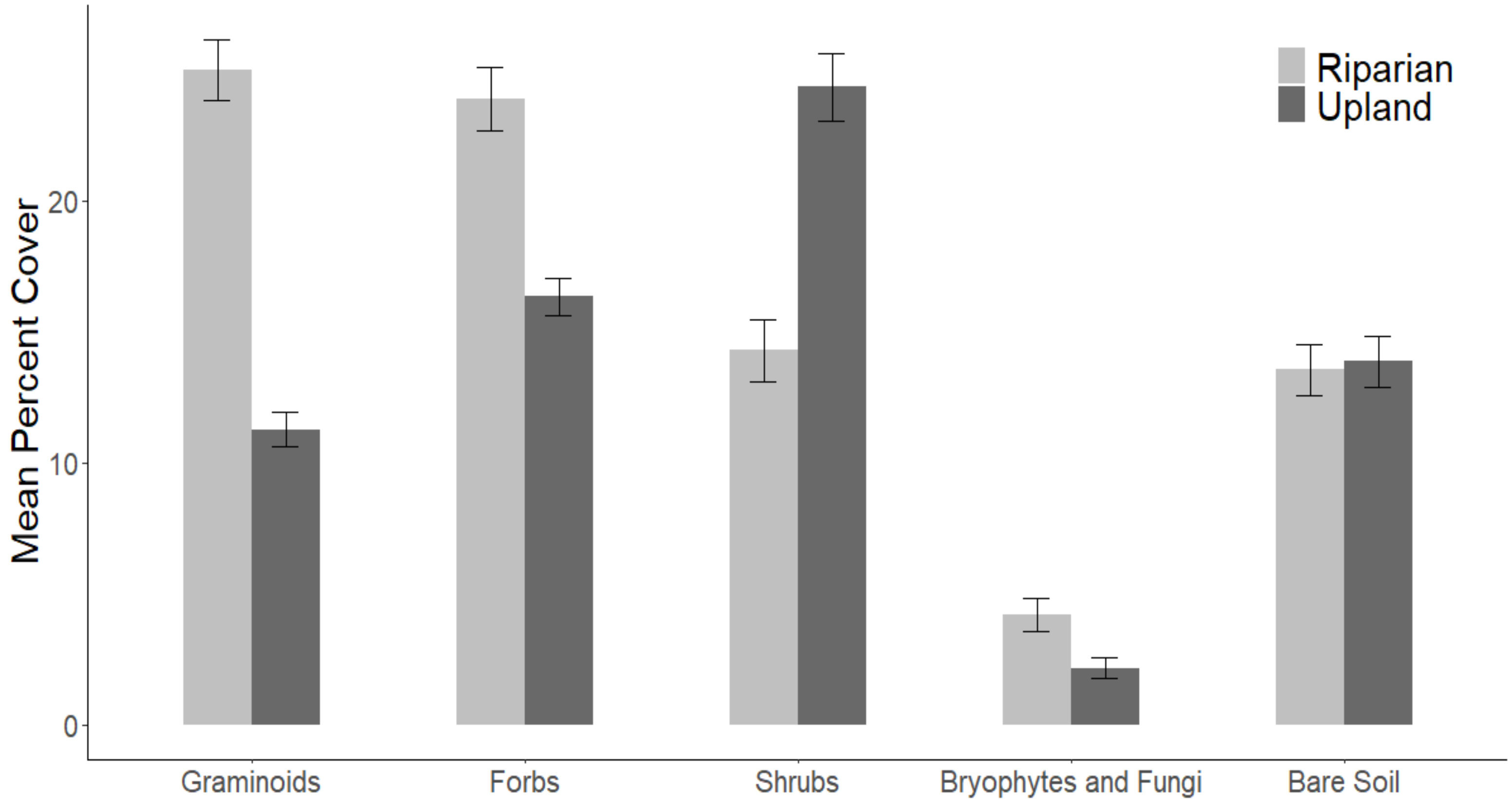


Figure 3-7 Mean percent cover of ground flora by lifeform guild by site type. Graminoids, forbs, and shrubs differed significantly following a Mann-Whitney U ($p < 0.001$). Overall composition of ground flora differed significantly following and MRPP ($p = 0.001$, $A = 0.03809$, $\delta = 0.501$).

Chapter 4: Delineation of *Populus tremuloides* Michx. (Aspen) Clones Using Digital Morphometric Analysis on Leaf Shape

Introduction

Conventional theory suggests that western aspen stands of the Rocky Mountains and Intermountain West are made up of large clones and maintain little to no genetic diversity. This convention has begun to shift over the past several decades, however, as several studies have documented the presence of multiple genets along the boundaries and sometimes disbursed as single stems within the stand that can appear as one uniform clone (Long & Mock, 2012; Mock et al., 2008; Zeigenfuss et al., 2008; Cheliak & Pitel, 1984). Perhaps the best example of this shift followed the discovery of multiple clones as part of the Pando complex by Mock et al. (2008). Prior to this study the Pando complex was believed to be one large genet made of hundreds to thousands ramets. The genetic analysis carried out by Mock et al. (2008) revealed the complex to consist primarily of one clone, but few other multi-ramet clones and even several genetically unique single stems were found around the boundaries of the stand.

While direct genetic testing has become the standard for analysis of aspen genetic diversity, these techniques are both expensive and time-consuming. Prior to the development and availability of genetic analysis, phenological differences in leaf and branch morphology, bark color, growth form, spring leaf flush, and fall leaf color were used as indicators for clone delineation in aspen stands ((Barnes, 1975; Barnes, 1969). Specifically, leaf shape has been most useful and reliable in differentiating aspen clones and with the advancement of digital morphometric analysis software, techniques have been developed that have shown agreement as high as 93% in assigning ramets into groups when compared with molecular analysis (Flesher et al., 2016; Jelínková et al., 2014).

Here, we describe the efforts to describe and delineate clones (genets) for use in a larger study investigating aspen ecology and stability in the Intermountain West. Additionally, we compare the relative genetic diversity based upon leaf morphology between 14 riparian and upland aspen stands located on the Caribou National Forest.

Methods

Leaf collection and digital morphometric analysis were conducted using a modified version of the methods described by Jelínková et al. (2014). A total of 14 aspen stands (7 riparian and 7 upland) within the Caribou National Forest (Bear Lake County, Idaho) were sampled. Transects were drawn along the long axis of the stand and at least 15 meters into its interior from its edge to allow for the establishment of circular sampling plots of 0.01 ha (100-m^2, $r = 5.65$ m) every 25 m that did not overlap. Successive transects were established in parallel every 20 m for the entirety of the stand. A minimum of two trees were sampled from each plot unless a plot was qualitatively estimated to contain more than one patch/genet based on bark color, leaf morphology, form, or clustering of stems in which case an additional two stems were sampled. Leaves were collected from trees that received full sun to the majority of the crown. For consistency, leaves were collected from the mid-crown position on the south side of the stem for all trees sampled. Along each shoot sampled leaves were taken from the mid-shoot position to avoid late and early leaf differences in morphology (Barnes, 1969). A minimum of 16 leaves were collected from two branches for each stem. The total number of stems sampled for each stand varied with stand size and availability of quality leaves. Any leaves with deformations from fungal infection or deterioration from insect herbivory were avoided and this resulted in a range of 6 – 30 stems sampled for each stand. Leaves were pressed flat with an herbarium press and allowed to dry for a minimum of 48 hours. After leaves were pressed and dried, petioles were removed, and blades were scanned on a white background with an Epson Expression XL 12000 scanner at 300 dpi resolution.

Images were uploaded into and analyzed with the software package SHAPE version 1.3 (Iwata 2006). Leaves were grouped by stem and analyzed using elliptic Fourier coefficients with 80 harmonics as recommended by Jelínková et al. (2014) to help delineate minor variations in leaf morphology. All elliptic Fourier descriptors (EFDs) for each leaf were normalized manually to remove the size component from the analysis. To further ensure the analysis was dependent on shape and not influenced by size, only components of the symmetrical features were used. Employing these components also allowed for the use of leaf length and width ratios. The SHAPE software package allows for the creation of a variance-covariance matrix of the Normalized EFDs (nEFDs). The resulting variance-covariance was summarized and analyzed with principal component analysis (PCA) to reduce the variables extracted from the chain code. Variation in leaf shape accounted for by the first two principal components (PC1 and PC2) for each stem were re-drawn with an inverse Fourier transformation to allow for visualization of mean shape +/- 2 standard deviations (Fig. 1). Averages of principal component scores from the first and second principal component axes thus represent mean leaf shape of each stem and were plotted for aid in estimating groups for cluster analysis. (Fig. 2 and 3).

Cluster analysis was carried out for each collection of leaves for each stand with the ‘stats’ package in R 4.2.0. Prior to cluster analysis, PC scores were scaled and converted into a distance matrix using mean Euclidean distance. Estimation of acceptable number of clusters for each stand was determined by plotting the ratio of the between groups sum of squares and the total sum of squares against varying values of clusters (K-means = 1-10). The point on the K-means estimation plot that showed the greatest change in slope (i.e., the elbow) was cross-referenced visually with plots of PC scores and again visualized with dendrograms created with the Ward’s minimum variance method that is an interpretation of the unweighted pair-group method of averages [(UPGMA), (Fig 4 and 5).] Trees that stood alone in the cluster analysis as “singletons” were investigated for validity by comparing means +/- 2 standard deviations (i.e., the 95% confidence interval) of the PC scores and checked for overlap to determine significance in differences.

Results

A range of 2-5 estimated genets were found across the 14 stands sampled with an mean (± SE) of approximately 3.7 ± 0.76 genets per stand for the riparian group and 3.1 ± 0.69 for the upland group. The distribution of estimated ramet values for each group followed a normal distribution as confirmed by a Shapiro-Wilk normality test (riparian: W = 0.840, p = 0.099; upland: W = 0.833, p = 0.086). The difference in means between the riparian group and the upland group was also determined to be statistically significant following a paired T-test [(t = 2.828, df = 6, p =0.030); (Fig. 6)].

Discussion

Aspen regeneration ecology has traditionally held the theory that genetic diversity within western aspen stands is extremely low, and that sexual reproduction is rare. Over the past few decades multiple studies have found considerably higher genetic diversity within stands historically assumed to be a single genet (Mock et al., 2008; Cheliak & Pitel, 1984). Following these discoveries, and the increased availability of genetic analysis, other studies of western aspen have found similar results (Long & Mock, 2012; Zeigenfuss et al., 2008). The mapping of genetic diversity in aspen stands of Utah and Colorado suggests that it is common for large stands that appear to be one clone by ocular estimate are actually made up of few to several dominant genets and the occurrence of ortets (singletons) near the periphery of a stand is common. Further, non-contiguous patches of ramets from a given genet are not uncommon, such that one or more genets may have meandering genets and interdigitating clusters throughout a stand, especially in older stands (Namroud et al., 2005).

While clone delineation by digital morphometrics is not a perfect surrogate for genetic analysis, it is arguably a suitable substitute for estimation when molecular analysis is unavailable. The results of 2-5 genets per stand is a reasonable estimate considering its similarity to the results of previous studies in western aspen. Moreover, studies that compare the use of leaf morphology to genetic analysis for clone delineation found agreement in assignment to groups as high as 94% (Flesher et al., 2016; Jelínková et al., 2014).

The significant difference in genet number between riparian and upland aspen is an interesting and somewhat intuitive result. It is a reasonable assumption that sites with higher moisture availability throughout the growing season (i.e., riparian areas) would facilitate an increase in germination and establishment leading to higher genetic diversity. When considering predicted future climate changes, higher genetic diversity will likely be important for aspen's ability to resist novel climate extremes. Given the relatively low sample size, however, these results should be interpreted with caution and not generalized to other aspen stands in the region. These results pose an interesting and testable hypothesis for potential further investigation.

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Figures

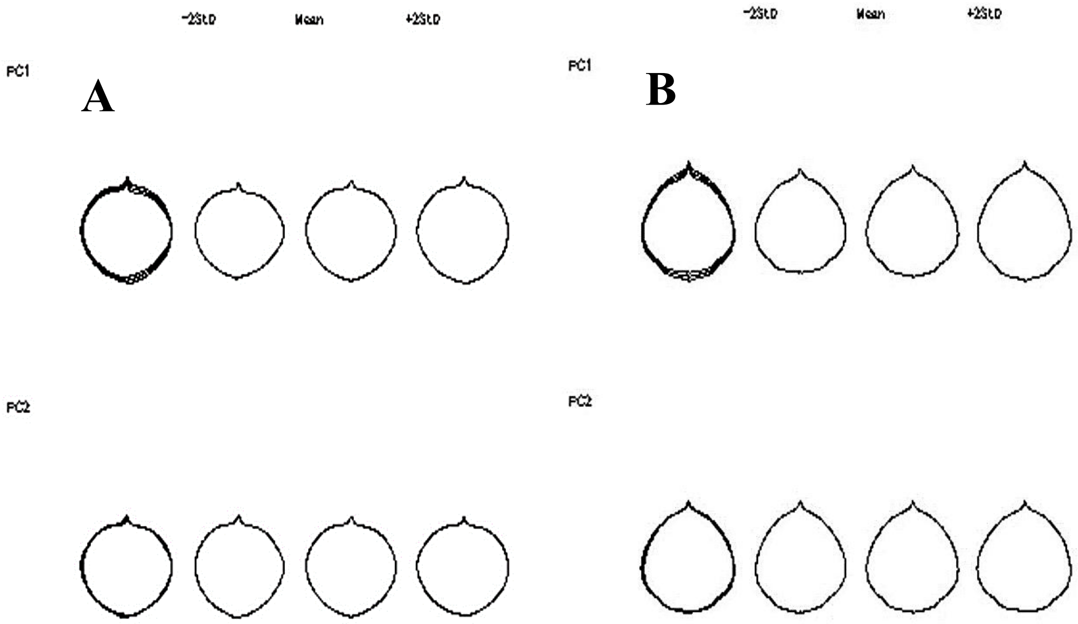

Figure 4-1 Variation in leaf shape based on principal components 1 and 2 (PC1 and PC2) from two stems (A and B) identified as separate genet groups growing in the same stand.

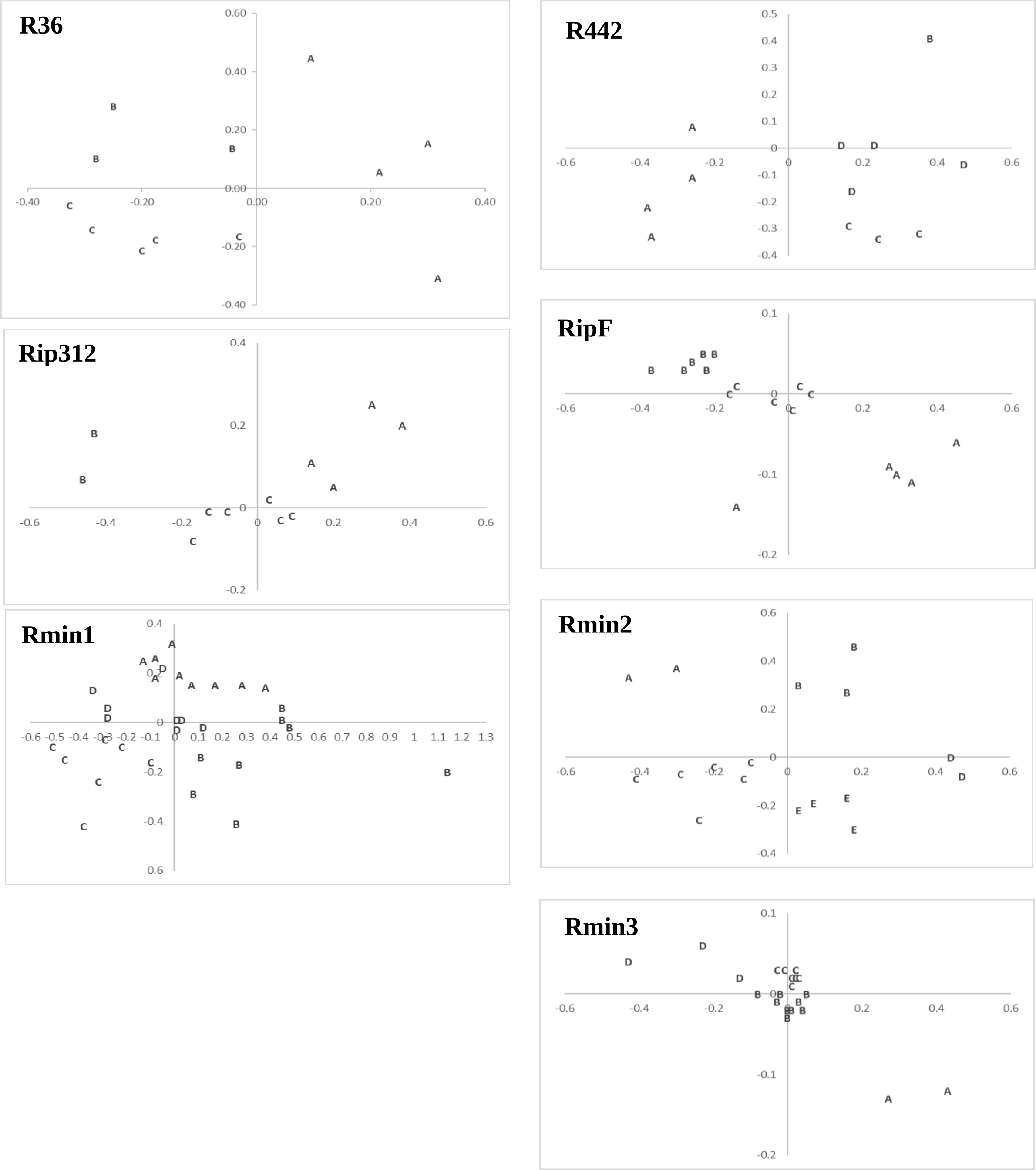

Figure 4-2 Plots of mean principal component scores for seven riparian aspen stands. The x-axis represents the first principal component (PC1) and the y-axis represents the second principal component (PC2). Letters represent genet group for each individual stem. Note: Difference in axis scale to accommodate visibility.

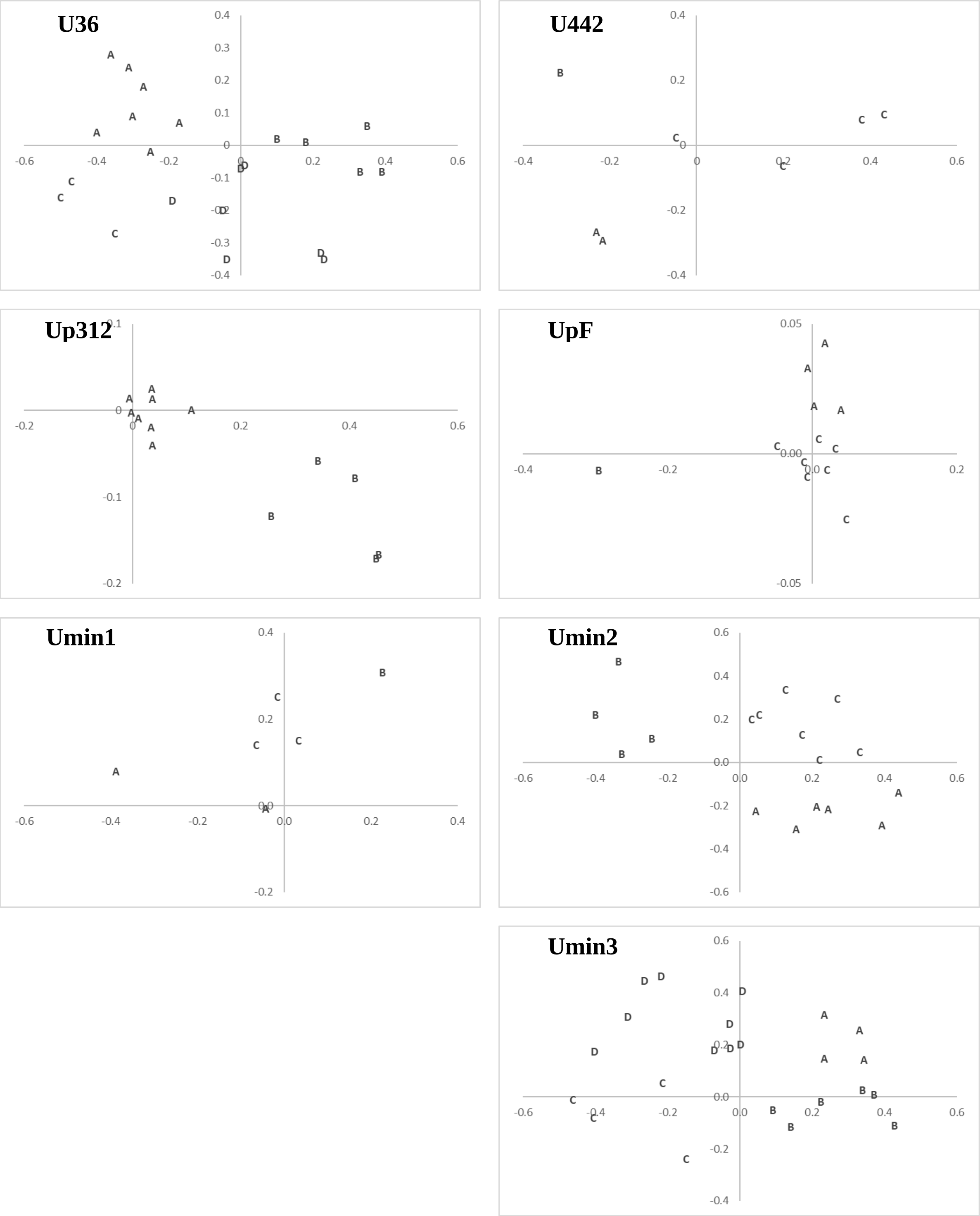

Figure 4-3 Plots of mean principal component scores for seven upland aspen stands. The x-axis represents the first principal component (PC1) and the y-axis represents the second principal component (PC2). Letters represent genet group for each individual stem. Note: Difference in axis scale to accommodate visibility.

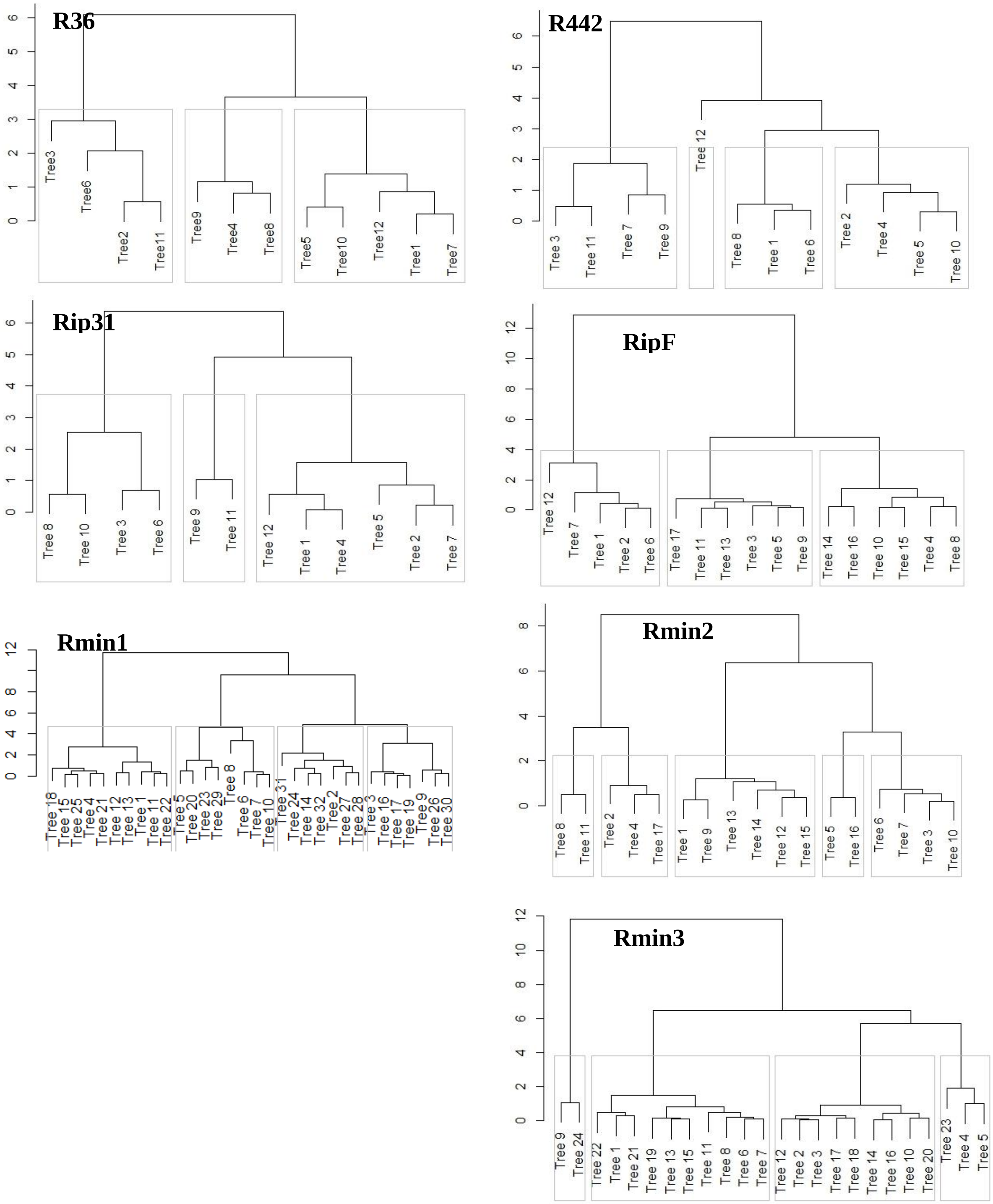

Figure 4-4 Dendrograms of seven riparian zones created from cluster analysis of mean Euclidean distance. Distance matrix was calculated from principal components of normalized elliptic Fourier descriptors. Gray rectangles represent assigned genet group for each individual stem. X-axis represents mean distance between clusters

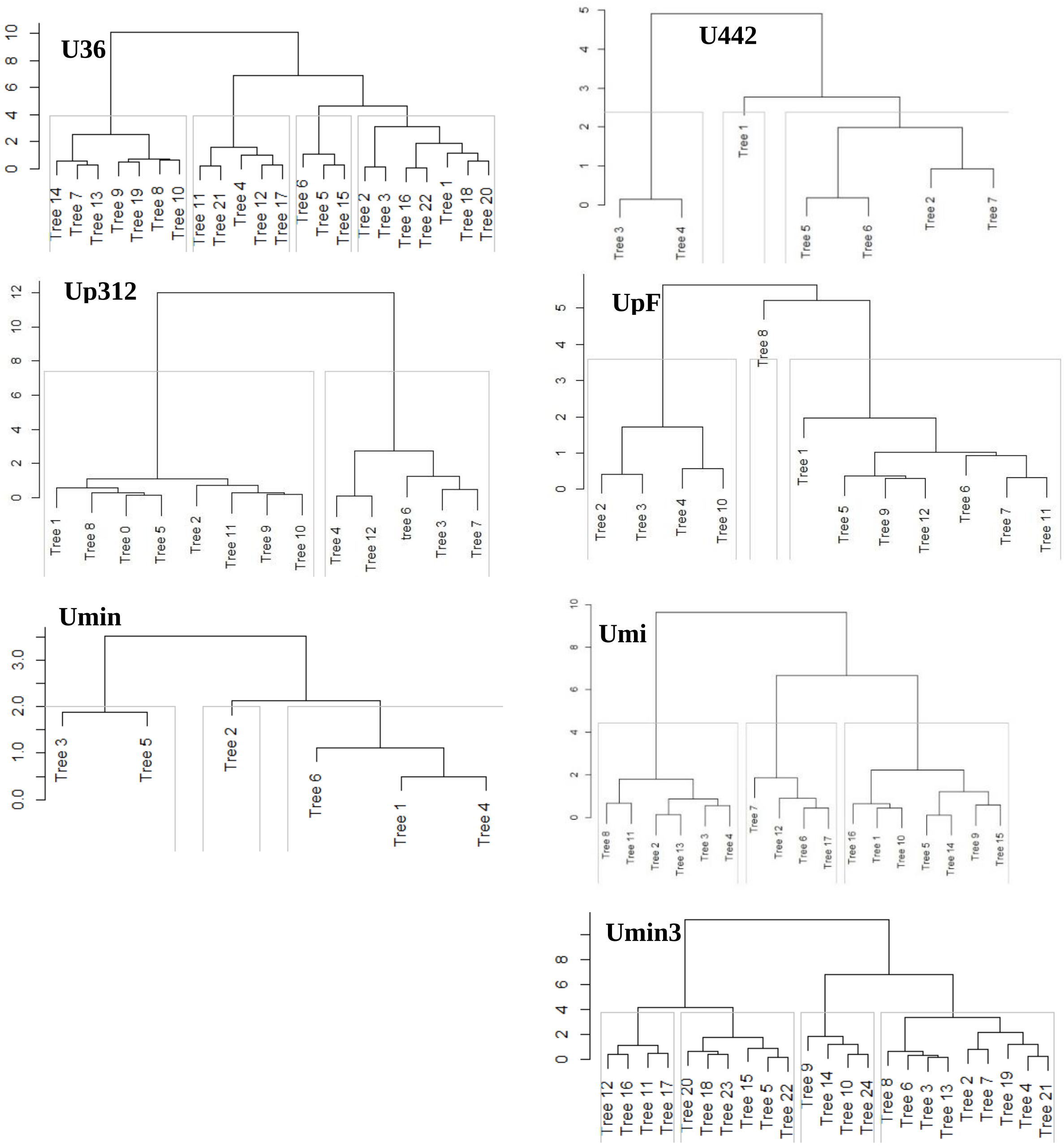

Figure 4-5 Dendrograms of seven upland zones created from cluster analysis of mean Euclidean distance. Distance matrix was calculated from principal components of normalized elliptic Fourier descriptors. Gray rectangles represent assigned genet group for each individual stem. X-axis represents mean distance between clusters

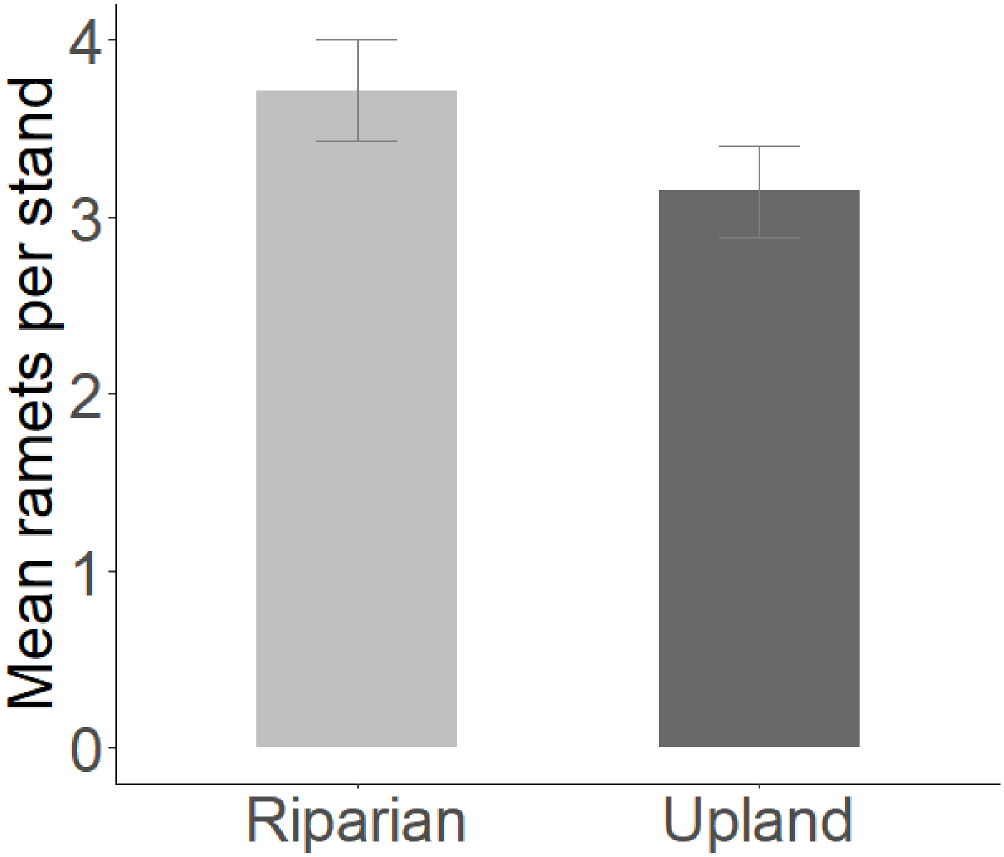

Figure 4-6 Mean number of ramets per stand for Riparian and upland aspen. The difference was statistically significant following a paired T-test (t = 2.828, df = 6, p =0.030). Stands were paired based on aspect and proximity.

Chapter 5: Disentangling Factors Influencing Aspen (*Populus tremuloides* Michx.) Regeneration in a Semi-Arid Montane Region Using Structural Equation Modeling

Abstract

Many studies of aspen across the West have investigated the factors limiting aspen regeneration at among various sites. Depending on their context, altered fire regimes, conifer invasion, browsing, and drought have all been identified as factors that may negatively affect aspen regeneration. Few studies, however, have discerned the relative weight of each factor on regeneration, nor have they been able to incorporate the interaction and covariance of each factor. The objective of this study is to investigate the individual and interacting factors that regulate aspen regeneration. Our global hypothesis is that the most important drivers or suppressors of aspen regeneration are factors affecting competition and soil moisture. We investigated the relationship between soil moisture availability and aspen regeneration using 14 aspen stands growing in riparian and upland areas in the Caribou-Targhee National Forest. and a structural equation model (SEM) with path analysis. We fit a SEM that explained 84% of the variation in aspen regeneration. The strongest direct effects on aspen regeneration were associated with percent groundcover of competitive plants (-0.952%, $p < 0.001$), incident radiation (-0.661, $p < 0.001$), time since conifer encroachment (TSCE) (-0.457, $p = 0.003$), and aspen overstory basal area (0.424, $p = 0.006$). The most influential indirect effects on aspen regeneration were incident radiation (0.672), mostly accounted for by its negative relationship with groundcover (-0.77, $p = 0.007$); and percent sand (- 0.604), mostly accounted for by its significant relationship with TSCE (0.913, $p < 0.001$). These results support our hypothesis that the strongest direct effect on aspen regeneration were related to soil moisture availability (competition, incident radiation), but also highlights the relative importance of other factors, as well as their indirect relationships. This study emphasizes that multiple ecological factors interact at multiple levels and

magnitudes, and proper assessment of aspen persistence requires the understanding of both primary and secondary limiting factors to persistence, as their interactions may mitigate or compound negative effects.

Introduction

Quaking aspen (*Populus tremuloides* Michx.), hereafter referred to as aspen, has the largest geographic and ecological range of any native deciduous tree species in North America (Perala, 1985; Peterson, 1996). Aspen provides important local and regional biodiversity across conifer-dominated landscapes of the western United States (Chong et al. 2001; Kuhn et al., 2011), providing habitat and food for a variety of large and small mammals, songbirds, and game birds (DeByle, 1985; Finch and Ruggiero, 1993; Oaten & Larsen, 2008; Perala,1985). The critical ecosystem services provided by aspen have concerned many ecologists and land managers given recent trends of aspen decline across the West. Many ecologists have for decades predicted a trajectory of decline (Bartos and Campbell 1998; Campbell and Bartos, 2001; Packard, 1942), while more recently others have suggested that aspen persistence is contingent on local disturbance regimes, management, and/or geographical location (Kashian et al., 2007; Shinneman et al., 2013; Stevens-Rumann et al., 2018; Strand et al., 2009).

Aspen primarily regenerates vegetatively via suckering from the root stock, as damage and mortality of the mature stem signals the production of suckers by interrupting the flow of hormones from the crown to the roots (Schier, 1976). Sucker production frequently occurs in the absence of disturbance, however, and many aspen stands in the West have been identified as self-replacing or “stable”, indicating that while disturbance is important for aspen establishment, it is not necessary for continued propagation and self-persistence. This form of reproduction can lead to large clonal stands that cover dozens or even more than 100 acres (Kemperman & Barnes, 1976). Aspen sucker production in the absence of disturbance requires heat, moisture, and available above- and belowground open space to initiate (Schier 1976). Aspen clones form with the first stem establishing from seed, then as the roots spread suckers produce new stems (ramets) that continue to spread and develop more ramets. Over time ramet mortality can lead to patches of ramets that are no longer attached by the root stock (Barnes, 1966). While the density of sucker production often varies between clones, this variability is believed to be at least partially regulated by interacting ecological factors, both abiotic (e.g., aspect, slope) and biotic (competition). For example, Johnston (2001) compared regeneration of 90 previously clear-cut aspen stands and found that poorly regenerating aspen stands were, most often, affected by two or more interacting negative factors such as seasonally high water tables on gentle slopes, moderate to heavy browsing, or soils with thin organic layers and limited nutrients. To better understand aspen regeneration, it is therefore critical to consider multiple, interacting factors that limit regeneration and growth at local scales (i.e., environmental context).

Many aspen studies across the West have examined the factors limiting regeneration among various sites. Depending on their context, altered fire regimes, conifer invasion, browsing, and drought have all been identified as factors that may negatively affect aspen regeneration (Bartos & Mueggler, 1979; Bartos & Campbell, 1998; Romme et al., 1995; Smith et al., 2011; Worrall et al., 2010). Most studies are designed to identify the most influential factor affecting regeneration among different sites and analyze the direct effects of each variable independently. For example, in a previous study we found that aspen regeneration was significantly higher in riparian areas compared to upland areas but did not provide an ecological explanation for such differences (see Chapter 3). Few studies have discerned the relative weight of each factor on regeneration, and even fewer have examined the interactions among factors. Furthermore, many factors that affect sucker development and growth (e.g., conifer encroachment) are themselves influenced by many of the same factors directly impacting regeneration (e.g., physiography).

The objective of this study is to investigate the relationships among factors regulating aspen regeneration. Our global hypothesis is that factors affecting competition and soil moisture are the most important drivers or constraints of aspen regeneration. We also expect that many factors affecting soil moisture availability will not only promote aspen regeneration but will also interact with competition from other tree species. Acute drought has been implicated as the main inciting factor for reduced aspen growth, productivity, and mortality in the West (Chen et al., 2017; Hogg et al., 2008; Krasnow & Stephens, 2015; Worrall et al., 2013, 2015), and thus our study focuses on the negative relationship of drought and aspen growth and persistence.

Methods

Study Area

Study sites were located in the montane region of the Caribou-Targhee National Forest (CNF). The CNF is located within the semi-arid M331D ecological sub-region (McNab et al., 2007) characterized by an average annual precipitation of approximately 36.5 cm, which is less than 50 percent of the national average (NOAA, 2022), mid- to high-elevations (1400-3100 m), and clay-rich soils. The CNF is a heterogeneous landscape that supports many dominant aspen stands on a variety of sites from low to high elevation, southern to northern aspects, and in association with an array of different species. This diversity of stand characteristics and conditions makes it an ideal area to investigate how different ecological factors and their interactions may drive aspen regeneration.

Given our hypothesis that soil moisture is a driving factor of aspen regeneration, we incorporated proximity to perennial streams as factor influencing regeneration. Using locations and classification of aspen stands of the CNF provided by the U.S. Forest Service (USFS), we sampled seven riparian and seven nearby upland aspen stands during the summer of 2019. Riparian stands were deemed appropriate for sampling if they were aspen dominated (>50% basal area), were at least one ha in size, were located along perennial streams, and were within three traversable km of a road or trail. Riparian aspen stands were paired with proximal (within 2 km) upland/non-riparian aspen stands by aspect, soil type, and ecological sub-section (Fig. 1).

Sampling design

Within riparian aspen stands, transects were established parallel to streamflow for the entirety of the stand, with the first transect run within 15 m of the stream's edge to compensate for stream meandering and beginning at least 15 m from the stand's edge to avoid edge effects. Successive transects were established every 20–40 m in parallel depending on stand structure and valley morphology. Upland aspen stands were sampled with the same design, except the first transect ran perpendicular to slope at the stand's lowest elevation and at least 15 m from the stand edge with each successive transect increasing in elevation. Circular 0.01 ha plots (100 m^2, r = 5.65 m) were sampled every 25 m along each transect. Thus, the center of each plot was 25 m from the center of the next plot.

Data collection

At the center of each 100-m^2 plot aspect was recorded as azimuth in the downhill direction and averaged for each stand. Slope was recoded for each plot using a Nikon Forestry Pro II hypsometer 6x Rangefinder by aiming the laser upslope from the lowest point of the plot to a point of equal height as the observer's eye determined on flat ground. Degree of slope was averaged across all plots for each stand. Latitude, longitude and elevation were recorded from approximate stand centers using a handheld Garmin GPSMAP 66S. Values for latitude, aspect, and slope were converted to radians and aspect was further transformed by 'folding' the aspect about the NE-SW line using the equation: Folded aspect = $| \pi - |\text{aspect} - (5\pi/4)| |$ taken from McCune & Keon (2002).

Soil samples were collected from the approximate center of a randomly chosen plot for each transect with a 10 cm diameter soil auger to a depth of 1 m. Organic matter was removed from soil surface prior to collection. Soil samples were held in a polyethylene-low density bags until being homogenized and analyzed for texture using a hydrometer separation method (Huluka & Miller, 2014). Soil analysis was carried out by the Analytical Sciences Laboratory at the University of Idaho.

Within each 100-m^2 plot all stems ≥ 3 cm at breast height (DBH) were measured to the nearest 0.1-cm DBH and identified to species to characterize the overstory. Each stem was also classified into one of five crown position classes: dominant (D), co-dominant (C), intermediate (I), and overtopped (O) based on relative height, modified from Bechtold (2003). Within each plot, increment cores from all aspen ≥ 10 cm and any conifers ≥ 3 cm present were extracted approximately 30 cm above the ground, parallel to the slope to estimate stand (ramet) maximum age and maximum age of encroaching conifers. Core samples were stored in paper straws for a minimum of 48 hours before being mounted on beveled wooden core mounts and sanded with progressively finer grit sandpaper until annual rings were visible. All cores were individually scanned at 1600 dpi on an Epson Expression XL 12000 scanner fitted with an IT8.7/2 calibration card. The high-resolution photos were then uploaded into CooRecorder 9.6 (Larsson, 2014) for growth ring boundary delineation to estimate age for each stem.

A minimum of two stems per plot were also sampled for clone delineation unless a plot was qualitatively estimated to contain more than one clone based on bark color, form, leaf morphology, or clustering of stems. Only stems from the dominant and co-dominant layer that received full sun to the majority of the crown were sampled. For consistency, all stems were sampled from the mid-crown position on the southern side. A minimum of 16 leaves were collected from the mid to outer position of two branches and pressed for a minimum of 48 hours with an herbarium press. Once dried, petioles were removed, and blades were scanned on a white background with an Epson Expression XL 12000 scanner at 300 dpi resolution. Digital morphometric analysis was conducted with the software package SHAPE version 1.3 (Iwata 2006), and clone delineation was carried out using methods describe by Jelínková et al. (2014), (see Chapter 3 for full description of methods).

Aspen regeneration density was quantified by tallying all stems < 3 cm DBH in each 100-m^2 plot and expressing on a per-hectare basis. Each plot was further sub-divided into four 1.0-m^2 quadrats placed at the center of each plot and the termini of three 5.65 m sub-transects run at 90º, 240º, and 300º of the central transect. Percent cover of all competitive (non-aspen) plants (herbaceous and woody species) and bare ground was estimated for each quadrat. To estimate browsing pressure, ungulate droppings (cow, moose, elk, deer) fallen within 1 m belts of the central transect were tallied within each 100-m^2 plot using methods described by Neff (1968).

Data preparation for model parameters

Prior to analysis all aspen stems ≥ 10 cm were converted to basal area for each stand to quantify aspen overstory. Conifer encroachment was represented by importance value (IV; average of relative dominance, density and frequency) of all conifer stems occupying the upper canopy (dominant and co-dominant position). Time since conifer encroachment (TSCE) was estimated as the oldest sampled conifer stem from each stand. Maximum ramet age (Max age) of each aspen stand was estimated as the age of the oldest sampled aspen stem from each stand. Aspen regeneration was converted to stems per hectare and square root transformed to adjust for the non-linear relationship with indicator variables (i.e., $x^2 = y$) following criteria suggested by McCune and Grace (2002). Percent coverage of groundcover was averaged for each stand across all quadrats for all competitive plant species. Ungulate droppings were converted to droppings per hectare to represent browsing pressure.

Prior to analysis, several environmental variables were also converted. Direct incident radiation and estimated heat load for each stand were calculated using latitude, slope, and folded aspect with the equation from McCune & Keon (2002):

$$\ln(X) = -1.467 + 1.582 * COS(L) * COS(S) - 1.5 * COS(A) * SIN(S) * SIN(L) - 0.262 * SIN(L) * SIN(S) + 0.607 * SIN(A) * SIN(S)$$

where (L) is latitude, (S) is slope, and (A) is folded aspect all in radians, and (X) is potential direct radiation that was represented as “Radiation” in the model. Values for incident radiation range between 0 – 1 and were multiplied by 100 to better represent the linear relationship with other variables and to increase model fit. Distance to stream was estimated as the linear distance from the approximate stand center to the closest perennial riparian area and relativized by dividing the value by the column maximum following criteria suggested by McCune and Grace (2002). The percent sand soil component showed the greatest variance between stands and gave the best model fit (see Results) and was used to explain the variance in aspen regeneration as related to soil texture.

SEM Model development

We used a structural equation model to examine the differences and strengths in the factors influencing the variability in aspen regeneration. Structural equation modeling is a versatile analysis that uses maximum likelihood and path analysis to describe relationships between factors within a multivariate context. It allows for the inclusion of correlation and covariate analysis between indicator variables and estimates the strength of their relative effects on a response variable. When combined with *a priori* analysis and theoretical understanding of a system it can support cause and effect relationships with greater confidence then most traditional analyses (Grace et al., 2009). Structural equation modeling is especially useful for the examination of ecological systems because it can incorporate a hierarchical structure that allows for the investigation of the relationship between factors that directly and indirectly effect a response via other nested and interacting factors. Finally, models constructed in SEM can be tested for fitness with a chi-square goodness of fit analysis, relative fitness between models can be evaluated with Akaike information criterion (AIC), and test of normality of variables can be assessed with skewness and kurtosis.

To analyze the relationship between parameters, we utilized a SEM path analysis, using SPSS AMOS version 25 software (SPSS Inc., Chicago, IL). Maximum aspen age, elevation, distance to stream, radiation, percent sand, and ungulate browsing were used as exogenous (external) indicator variables, while aspen basal area, number of clones per stand, percent groundcover, conifer encroachment, and TSCE were input as endogenous (internal) indicator variables as their quantities are directly influenced by the exogenous variables. Percent sand was the only soil factor used as it had the highest variability among sites, and it followed a linear relationship with the response variable that gave the highest model fitness. Further, increasing percent sand is likely to have the strongest inverse relationship with soil moisture.

Correlation and covariance paths were drawn between elevation, distance to stream, radiation, and percent sand. Maximum aspen age was expected to correlate with radiation and percent sand, and these relationships were also drawn and accounted for with the initial SEM model. Relative browsing pressure was not hypothesized to be correlated with any other exogenous factors, as browsing pressure is likely not influenced by these factors, and prior analysis indicated browsing pressure did not differ significantly between these stands (see Chapter 2).

Results

Relative to upland areas, riparian stands tended to have higher aspen densities (mean ± SE) in the regeneration layer (riparian: 1934 ± 583 stems/ha; upland: 1050 ± 195 stems/ha). This trend was also found within different strata of the regeneration layer, including both in the seedling (< 1 m height; riparian: 665 ± 268 stems/ha; upland: 249± 95 stems/ha) and sapling (≥ 1 m height; riparian: 1268 ± 363 stems/ha; upland: 800 ± 144 stems/ha) layer (Fig. 2). These differences, however, were not statistically significant following a Wilcoxon signed rank test (seedlings: W = 24, p = 0.109; saplings: W = 22, p = 0.219; Total: W = 22, p = 0.219).

Differences in predictor variable means showed a range of magnitudes when compared between riparian areas and upland areas (Table 1). As expected, the greatest difference was for mean

(± SE) stand center's distance to stream between riparian stands (26.4 ± 7.0 m) and upland stands (1106.4 ± 204.4 m). Riparian areas also showed higher mean values in several other predictor values such as percent sand with a mean (± SE) of 39.4 ± 3.8% for riparian stands and 32.0 ± 2.8% for upland stands, percent groundcover with a mean of 86.4 ± 2.2% for riparian stands and 83.8 ± 1.8% for upland stands, and number of clones with a mean of 3.7 ± 0.3 in the riparian stands and 3.1 ± 0.2 in upland stands. All other predictor values showed higher mean values in the upland stands relative to the riparian areas, including incident radiation with means of 0.8 ± 0.04 Rad in the upland stands and 0.7 ± 0.03 Rad in the riparian stands; overstory aspen basal area with means of 17.4 ± 2.5 m^2/ha in the upland areas and 9.6 ± 2.1 m^2/ha in the riparian stands; maximum aspen stand age (estimated by oldest sample stem) with mean values of 102.4 ± 10.0 years in the upland stands and 83.1 ± 8.26 years in the riparian stands; overstory conifer importance value with means of 5.5 ± 1.4 in the upland stands and 5.1 ± 1.7 in the riparian stands; and time since conifer encroachment (estimated with the oldest conifer stem in each stand) with means of 77.1 ± 13.8 yrs in the upland stands and 70.3 ±19.2 years in the riparian stands (Table 1).

Using all available variables, we fit a SEM that explained 84% of the variation in aspen regeneration, 55% of the variance in time since conifer encroachment, 51% of the variance in canopy conifer encroachment, 51% of the variance in aspen basal area in the overstory, and 50% of the variation in number of clones per stand ($\chi^2 = 30.87$, $p = 0.126$, df = 23; Fig. 3). The strongest direct effect on aspen regeneration was associated with percent groundcover (-0.952%, $p < 0.001$). Incident radiation had the next strongest relationship with aspen regeneration (-0.661, $p < 0.001$), followed by time since conifer encroachment (-0.457, $p = 0.003$), and aspen overstory basal area (0.424, $p = 0.006$). Indirect effects of incident radiation showed a positive relationship with aspen regeneration (0.672), mostly accounted for by its significantly negative relationship with percent groundcover (-0.77, $p = 0.007$). Similarly, the indirect effect of percent sand on aspen regeneration showed a strong negative relationship (- 0.604), mostly accounted for by its significant effect and positive relationship with time since conifer encroachment (0.913, p < 0.001).

Maximum observed aspen age for each stand showed a relatively weak and non-significant direct effect on aspen regeneration (-0.146, p = 0.322). Maximum aspen age did, however, show a significant negative relationship with conifer overstory IV (-0.523, p = 0.033), and a significant positive relationship with aspen overstory basal area (0.64, p =0.008). Elevation had a weak, non-significant, negative direct effect on aspen regeneration (-0.146, p = 0.596). The indirect effect of elevation on aspen regeneration showed an even more negative relationship (-0.331). Elevation only showed a significant relationship with clone number (- 0.829, p = 0.044). See Appendix for table of regression weights and significance values.

Discussion

The objective of this study was to identify how variability in ecological factors may explain the variability observed in regeneration densities in aspen-dominated stands of the Caribou-Targhee National Forest. Aspen regeneration was most strongly affected directly by percent groundcover of competitive plants, incident radiation, time since conifer encroachment, and aspen overstory basal area.

The high negative correlation of percent groundcover as a predictor of aspen regeneration is likely a better representation of the reciprocal nature of the data set than an actual driver of aspen regeneration. One might expect higher aspen regeneration densities at more productive sites, but higher non-aspen groundcover also indicates higher levels of competition with aspen in the regeneration layer. For example, Donaldson et al. (2006) found a reduction in relative growth rates of aspen from competition with grass. Further, Donaldson et al. (2006) found that when combined, low-nutrient soil and competition together compounded these effects, and they observed a 45% reduction in growth rate relative to their control. Thus, there is presumably a window between higher levels of site nutrient availability and lower levels of competition that allows aspen regeneration to thrive, but this would be dependent on several contextual factors such as disturbance regimes and associated competitive plant species.

The negative relationship between incident radiation and aspen regeneration is consistent with other studies that examine soil moisture availability as an important driver of aspen regeneration (Debyle & Winokur, 1985; Frey et al., 2004; Schier, 1976; Worrall et al., 2015). The equation used for incident radiation is also an estimate of heat load determined by the combination of physiographic features and geographic location (McCune & Keon, 2002). While heat and solar radiation is necessary for seedling germination, sucker production, and plant growth, an excessive heat load can impose an increase in evapotranspiration that can exacerbate the effects of acute drought, especially on dry sites. Indirectly, however, incident radiation showed a positive relationship with aspen regeneration. Considering the significant negative relationship of incident radiation on percent groundcover of competitive plant species this likely means there is also a trade-off between soil moisture availability and the density of competition.

The negative relationship of time since conifer encroachment but the weak relationship of conifer cover in the overstory is an interesting and somewhat expected result as it suggests that the age of the dominant conifers is more important than the relative representation of conifer stems in the overstory. Some of these conifers are as old as, or older than the oldest sampled aspen stems in these stands meaning it is possible that these conifers established at a similar time as some of these aspen stands. In many studies, conifer presence on its own is a poor predictor of aspen regeneration. For example, Kurzel et al. (2007) found the majority of stands they observed with conifer invasion in western Colorado still showed regeneration and recruitment levels that suggested self-replacement. It appears the amount of time of ongoing competition with conifers is a better predictor of the negative effects of conifer encroachment on aspen's ability to self-replace than quantity of conifer stems or percent cover. Again, however, this is not alone a strong predictor and should be taken as one component of a multiple factor approach of aspen persistence assessment.

Aspen overstory basal area showed a significant positive relationship with aspen regeneration but it was a relatively weak relationship when compared to other significant factors. Aspen basal area was also positively and significantly correlated with maximum aspen age meaning that in this model stands with older stems had higher basal area and higher basal area was indicative of higher regeneration densities. While some studies in the West have found higher aspen age and basal area can correlate with lower regeneration densities (Binkley et al., 2014; Mueggler, 1989) these were usually in senescing stands that reported multiple stems > 150 years. In the stands sampled in this study the average (± SE) maximum age was relatively low, 83.1 ± 8.3 in riparian stands and 102.4 ± 10.0 in upland stands. Considering the primary mode of aspen regeneration in the West is vegetative, it is intuitive that more stems that have developed over longer periods of time can lead to higher regeneration densities unless a multitude of interacting factors limits its ability to sprout fresh suckers (e.g., heat load, moisture availability) or recruit saplings into the overstory (e.g., browse).

Our model was unable to account for 16% of the variance in aspen regeneration, possibly because we lacked sufficient data to describe disturbances other than browsing, including past land use and fire history. Anecdotally, we observed wildlife other than ungulates in the sampled stands, such as beavers and pocket gophers, but their relative frequency of occurrence was not quantified. The effect of these animals on regeneration densities has been shown to be minimal in some cases (Baker et al., 1997; Runyon et al., 2014), but may have had varying levels of effect in the stands sampled in this study especially considering that half of the stands were growing near streams. Finally, the genetic diversity of each stand was not directly calculated, although our methods used for clone delineation have been shown to have accuracy up to 94% (Jelínková et al., 2014) but this level of accuracy can vary based on stand structure and ramet development. The unknown level of error in the methods used for clone delineation may have influenced the outcome of the model but likely only ion the direct and indirect effects of the clone component. These effects were relatively small compared to other predictor variables.

This study emphasizes that multiple ecological factors interact at multiple levels and at different magnitudes. Proper assessment of aspen stand persistence therefore requires understanding the limiting factor for persistence, but also to the secondary and tertiary factors and their interactions. In our study, if incident radiation is high enough to suppress competitive species such that suckers are able to proliferate, aspen regeneration is favored. When incident radiation is too high, aspen sucker longevity will likely be reduced due to lower water availability, resulting in a negative relationship. Similarly, overstory conifer cover only appeared to negatively affect aspen regeneration when older conifers were present suggesting that some “seral” stands may still self-replace and persist for multiple generations.

Overall, our results corroborate other studies that documented that physiographic factors that decrease soil moisture availability, such as incident radiation, can suppress aspen regeneration. In our study, incident radiation was the most influential exogenous factor affecting regeneration, and time since conifer establishment was the most influential endogenous factor. It is important to note that these results come from a semi-arid montane region with limited ungulate browsing relative to other studies of aspen in the West. These results should therefore not be generalized to other regions, but we recommend the application of similar models that can help estimate the relative effect of factors influencing aspen regeneration.

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Figures and Tables

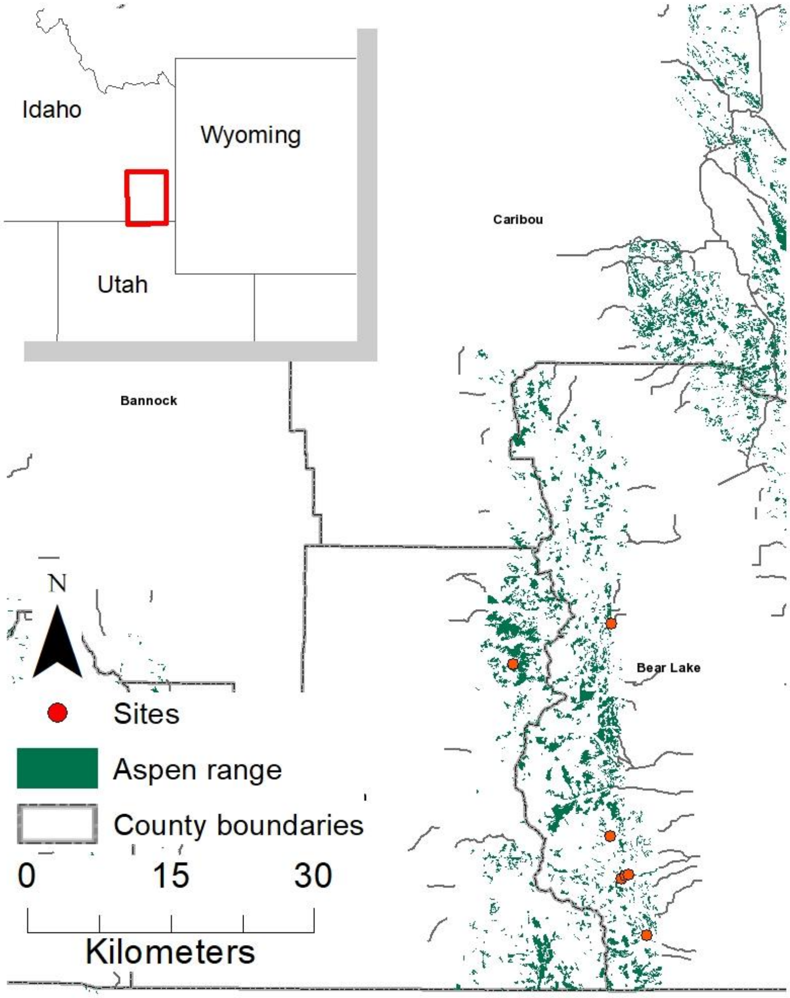
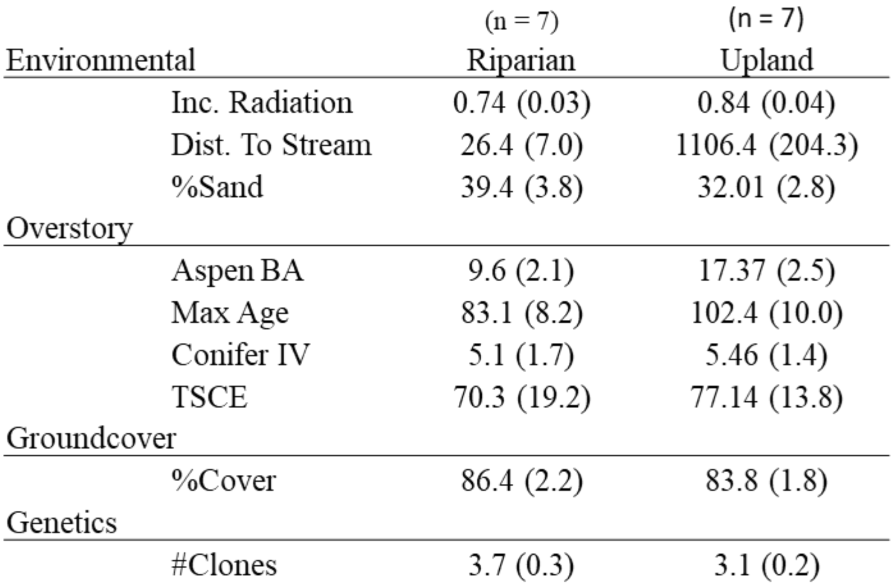


Figure 5-1 Map of fourteen aspen dominated sites (7 riparian and 7paired upland) sampled in the Caribou-Targhee National Forest. Stands were considered appropriate for sampling if they occupied at least 1 hectare and were dominated (> 50% basal area) by aspen. *Note: only locations of riparian zones are shown for visibility as the proximity of paired upland sites are within 2 Km.



		(n = 7)	(n = 7)
Environmental		Riparian	Upland
	Inc. Radiation	0.74 (0.03)	0.84 (0.04)
	Dist. To Stream	26.4 (7.0)	1106.4 (204.3)
	%Sand	39.4 (3.8)	32.01 (2.8)
Overstory			
	Aspen BA	9.6 (2.1)	17.37 (2.5)
	Max Age	83.1 (8.2)	102.4 (10.0)
	Conifer IV	5.1 (1.7)	5.46 (1.4)
	TSCE	70.3 (19.2)	77.14 (13.8)
Groundcover			
	%Cover	86.4 (2.2)	83.8 (1.8)
Genetics			
	#Clones	3.7 (0.3)	3.1 (0.2)

Table 5-1 Mean values of each indicator variable used in the structural equation model. Values in parentheses represent standard error.

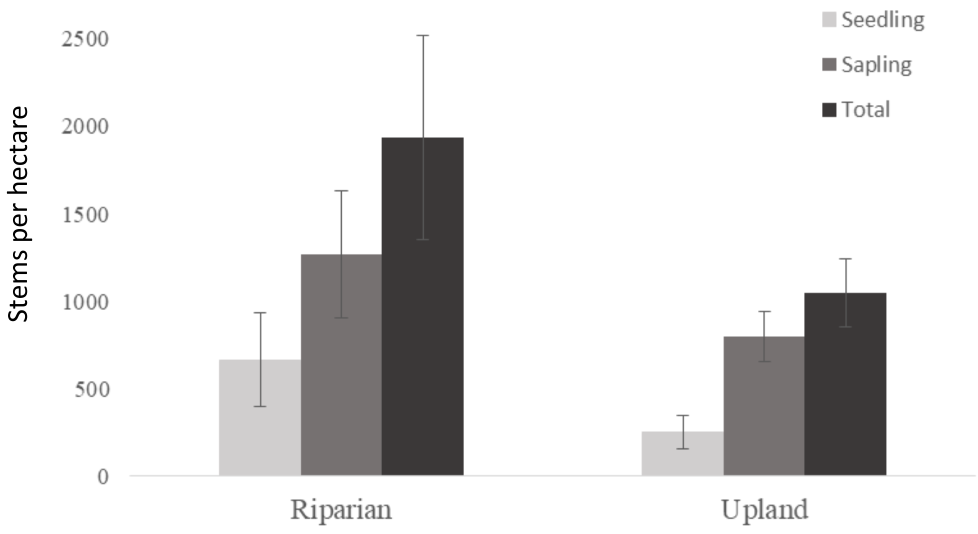


Figure 5-2 Mean values of aspen regeneration densities (stems/ha) for the seedling (<1 m), sapling (≥1 m) and total layers, separated by site type.

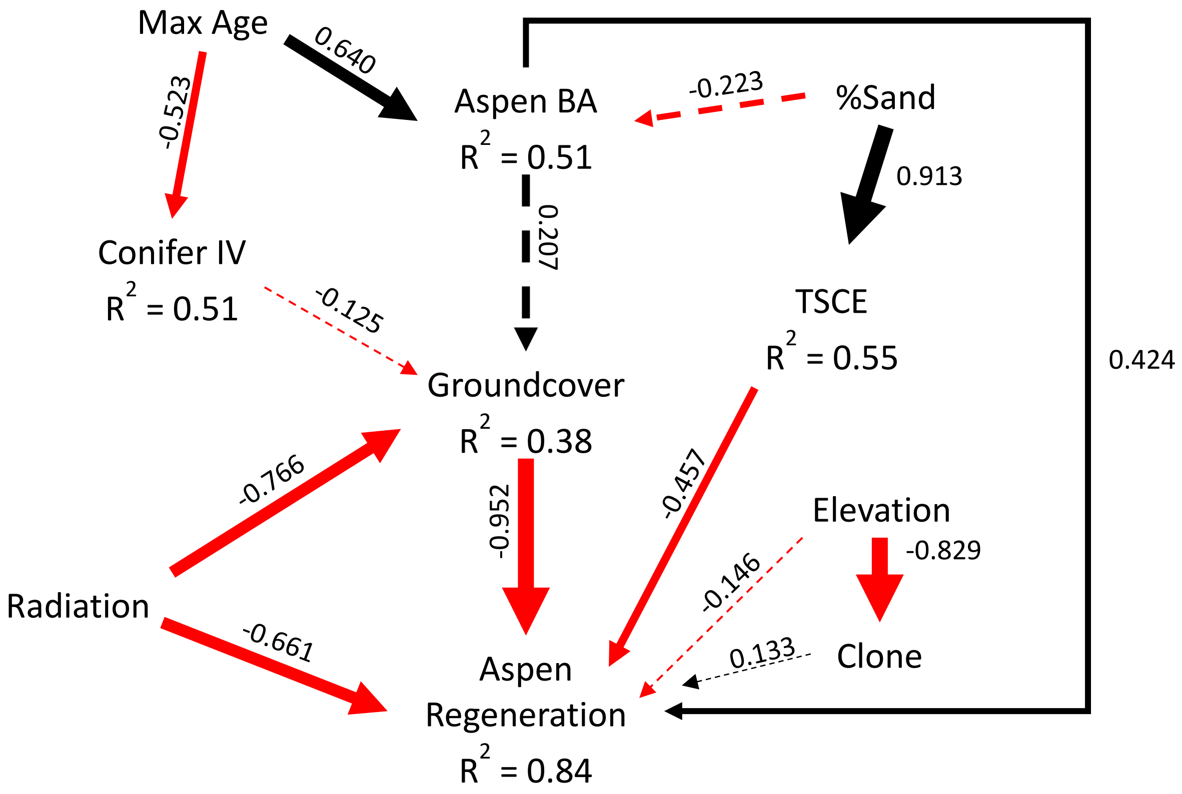


Figure 5-3 Structural equation model of factors influencing aspen regeneration. Arrow thickness represents relative significance level. Arrows represented with a dashed line are non-significant but considered important for interpretation and total effect. Negative relationships are represented in red, and positive relationships are represented in black.

Chapter 6: Conclusions

Projections of future climate change are that average and maximum temperatures will continue to increase globally, increasing the frequency of drought in some areas. These changes would impose varying levels of impact on forest communities. In the case of aspen in the western United States, these factors are expected to have a negative effect on aspen's range and persistence, and model projections predict a loss of habitat suitable for aspen establishment between 46-94% by 2090 (Rehfeldt et al., 2009). Many researchers have seized on this estimate to predict potential "doomsday" scenarios for aspen in the future (Michaelian et al., 2011; Worrall et al., 2013, 2015). Aspen has a broad ecological range, however, and sweeping generalizations about its decline based on expected changes to regional precipitation and dryness indices, while compelling, may underestimate the variability of local site conditions and the tolerance of aspen to moisture stress. In fact, aspen is common across the West on historically droughty sites at high elevations on rocky and coarse soils as well as on moister sites along draws and drainages at mid- and low-elevations when browsing pressures are not severe (Binkley, 2008; Kashian et al., 2007; Lieffers et al., 2001). Although drought has been implicated as the main factor driving sudden aspen decline (SAD), characterized by unprecedented levels of rapid and widespread die-back of mature stems, mortality and decline is ultimately caused by secondary agents (insects, disease) in many cases (Marchetti et al., 2011; Singer et al., 2019). Thus, interpreting aspen vulnerability to drought-related decline should consider multiple site factors at multiple ecological scales. In this dissertation I assessed aspen sensitivity to factors influencing soil moisture variability in the Caribou-Targhee National Forest across multiple scales, including regional (Chapter 2), local (Chapter 3), and site-specific (Chapters 4 and 5).

In Chapter 2, I applied a new dendrochronological technique, Blue Intensity (BI), for extraction of a climate signal from aspen latewood to explore the importance of mid-summer precipitation on aspen growth. Blue intensity is an effective and inexpensive proxy for wood density that has been found to more accurately indicate precipitation and temperature patterns than ring width, especially in latewood. This technique has been to reconstruct historical temperature, typically from conifer species growing at temperature limited sites (e.g., high latitudes >50º or high elevations >3000 m) (Barber et al., 2004; Briffa et al., 2002; Davi, 2003; Heeter et al., 2020). I hypothesized that BI could be used to extract a precipitation signal from aspen because it is sensitive to soil moisture limitations on dry sites such as mid to high elevations on exposed southern facing slopes. I found a positive correlation between mid-summer precipitation and latewood density that supports my hypothesis. From these results I conclude that late season growth for aspen on exposed high elevation sites is dependent on precipitation and growth is thus limited by soil moisture availability.

As part of my dendroecological study, I used Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) as a comparison species because its use as a temperature proxy is well documented and it is a common cover type in the Caribou-Targhee National Forest. Engelmann spruce stems were sampled at similar site types as the aspen stems (high elevation) but differed slightly in aspect; aspen stems sampled were on exposed southern aspects while Engelmann spruce stems were sampled at or near flatter peaks. This site difference emphasized the difference in temperature versus precipitation limitations between Engelmann spruce and aspen, but the dependence of latewood growth on mid-summer precipitation in aspen is consistent with studies that found reduced overall growth based on tree ring width during periods of drought or increased temperatures with stable precipitation patterns (Cahoon et al., 2018; Hanna & Kulakowski, 2012). These studies, however, found inconsistencies in the relationship of reduced growth and climate variables over time and between stems growing in the same sites. For example, Cahoon et al. (2018) found a positive relationship between aspen ring width and the previous growing season's July precipitation in Alaska, but the signal was not consistent and degraded over time because of invasion by the aspen leaf miner (*Phyllocnistis populiella* Cham.). The benefit of maximum latewood density and BI analysis is its ability to isolate the climate signal even with variability in site factors and disturbance.

In Chapter 3, I investigated the relationship between soil moisture availability (using landscape position as a proxy) and aspen persistence by comparing the structure, composition, and regeneration density of nine aspen stands with nine paired upland aspen stands. Considering the numerous studies published in the last two decades that implicate drought as the main driver of aspen decline, I hypothesized that closer proximity to perennial streams will result in higher soil moisture availability, such that the probability of aspen persistence is likely higher on riparian areas on the Caribou-Targhee National Forest landscape. My results for this study partially supported my hypothesis, with the most compelling evidence being the significantly higher aspen seedling densities (< 1m height) in riparian areas relative to upland aspen stands.

The differences in composition and abundance of ground cover species found in the riparian and upland areas suggest that soil moisture availability is higher in riparian areas. For example, Rocky Mountain maple (*Acer glabrum* Torr.), alder (*Alnus spp.*), and red-osier dogwood (*Cornus sericea* L.) are indicators of wet-mesic to mesic soils and were exclusively found in the riparian areas. Likewise, big sagebrush (*Artemisia tridentata* Nutt.), a xeric species, was found considerably more frequently in the uplands than in the riparian areas. Groundcover community composition was significantly different between riparian and upland areas in a way that suggests high soil moisture availability in the riparian areas, with graminoids and forbs dominating in the riparian areas and woody shrubs dominating in the uplands.

Upland aspen stands contained older and larger stems than in riparian aspen stands, but these values were not significant. These differences in structure , however, may be indicative of unaccounted for disturbances in the riparian areas that were not present or as frequent in the uplands. For example, I observed a beaver lodge in one riparian site and very active pocket gopher communities in several riparian stands that appeared much less frequently in the upland stands. Cantor & Whitham (1989) showed that overstory aspen mortality increases significantly with increasing gopher activity, which could affect the mean age of the surviving ramets and stimulate suckering from sections of the newly severed root stock.

Although no upland or riparian aspen stands showed evidence of imminent decline, higher regeneration densities in the riparian areas suggest that they have higher potential for self-replacement relative to upland stands. The proximity to perennial streams and the difference in associate woody, and groundcover species in the riparian areas suggest that soil moisture availability is at least one important factor in predicting aspen regeneration, and as such riparian areas may serve as critical refugia for aspen as the incidence of drought increases.

In Chapter 5, I developed a model path analysis using structural equation modeling (SEM) to explore the relative impacts and interactions of ecological factors that affect aspen regeneration density. This analysis was performed on a subset of the data collected in Chapters 2 and 3 to better interpret site factors driving aspen regeneration other than simply riparian vs. upland. Some site factors are expected to be inherently different between riparian and upland stands (e.g., elevation, soil texture) and have some level of effect on aspen regeneration. Based on my results from Chapters 2 and 3, I hypothesized that factors influencing soil moisture availability (e.g., incident radiation, soil texture, competition) would have the strongest effect on aspen regeneration variability among sites. It was important to characterize the genetic diversity of each stand before attempting to interpret the influences of site factors on aspen regeneration. To do this, in Chapter 4 I used a traditional approach – variation in leaf morphology - with modern techniques to determine the number of clones in each stand sampled for use in the SEM. Overall, I found that there were moderate differences in the mean number of clones within riparian vs. upland aspen stands, with riparian stands having more.

The results of the SEM analysis (Chapter 5) suggest that factors affecting soil moisture availability have the strongest effect on regeneration, supporting my hypothesis. The exogenous factor with the strongest direct effect on aspen regeneration was incident radiation (heat load), and the endogenous factor with the strongest direct effect was percent cover of non-aspen plant species. Unsurprisingly, ground cover had the highest overall correlation with aspen regeneration. More abundant ground cover likely increases the competition with aspen suckers for soil moisture, but also for available nutrients and growing space. The high correlation of the competing ground cover with aspen regeneration is in part due to the reciprocal nature of the data set (i.e., more non-aspen cover directly correlates negatively with aspen cover).

Percent sand in the soil showed a weak but positive direct effect on aspen regeneration. The increase in sand negatively affects aspen regeneration because it increases soil drainage, but its effect on soil moisture availability is context dependent. For example, it appears this can be explained by its much higher negative correlation with elevation (-0.623). Elevation in turn has an even higher positive correlation with distance to stream (0.743), meaning that the riparian areas have a higher percentage of sand, probably because they frequently experience fluvial disturbances and aggradation/degradation processes that deposit coarse-textured particles. Moreover, sandy soils are preferred by burrowing mammals such as pocket gophers, which by disturbing the root stock may increase aspen regeneration as observed at some riparian sites (Cantor & Whitham, 1989; Coggins & Conover, 2005).

Overall, the results of each chapter support my global hypothesis that factors reducing soil moisture availability and increasing site susceptibility to drought have a negative effect on aspen growth and regeneration. My results , however, also emphasize that context is important in determining the collective effect of these factors. Thus, proper assessment of aspen vulnerability in the West requires analyses at multiple scales that can incorporate the relative weights and interactions of all elements influencing persistence. My results are consistent with many other studies of western aspen susceptibility to drought, but the novelty of my study is in its approach. The methods utilized in this dissertation can aid in investigating thresholds of aspen overstory growth and regeneration. Understanding these thresholds and more site-specific factors influencing aspen regeneration can be useful to natural resource managers hoping to maintain and promote aspen in the western U.S.

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