Predisposing and contributing factors associated with aspen mortality and the effects of Sudden Aspen Decline on ecological attributes, southwestern Colorado, USA

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Introduction

Aspen (*Populus tremuloides*) is one of the most broadly distributed tree species in North America with populations ranging from Alaska in the North and Newfoundland in the East, to Mexico in the South (Little 1971). Aspen is the only upland hardwood species in the central Rocky Mountains (Stohlgren et al. 1997) and represents the third largest forest type in Colorado covering approximately 1.32 million ha (www.fs.fed.us/rm/ogden/annual_tables/co_annual_tables.htm). Aspen forests are one of the most biologically diverse ecosystems in the West (Kay 1997), exceeded only by riparian zones (White et al. 1998). Biological diversity in aspen forests is partially due to the multilayered structure of these forests, which allows variable light to reach the forest floor resulting in variations in plant understory diversity and productivity due to unique microhabitats (Mueggler 1985). Specifically, under open aspen canopy there is higher sunlight penetration and decomposition rates than under closed aspen canopy and therefore increased plant productivity (Mills et al. 2000). Many organisms depend on this unique and varied habitat within aspen forests which provides critical wildlife habitat and valuable grazing resources for livestock and native ungulates (Bartos 2001) and supports high levels of bird, butterfly and mammal diversity (DeByle 1985; Rumble et al. 2001).

Temperate forest ecosystems are characterized by complex histories of natural and human disturbances. Disturbances when combined tend to have complex influences on vegetation community dynamics (Kulaknowski et al. 2012). Disturbances that cause tree mortality create biological legacies through new forest species associations that may alter flora and fauna diversity and ecosystem processes and services (Anderegg et al. 2012). Sudden aspen decline (SAD), the sudden dieback of branches, crown loss and rapid mortality of aspen stems, is a relatively new phenomenon since 2004 in the Southwest; a similar phenomenon has been observed in many parts of North America (Frey et al. 2004; Worrall et al. 2008, Michaelian et al. 2011). SAD is distinct from age-induced dieback, as it is a sudden, landscape-scale event that can lead to loss of most of an aspen stand (Worrall et al. 2008). The average time frame for naturally occurring dieback for aspen is approximately 100 years while the average time frame for SAD induced dieback is between 3 to 6 years (Frey et al. 2004). In the Southwest, SAD has been detected in

western Colorado, northern Arizona and southern Utah (Fairweather et al. 2008; Rehfeldt et al. 2009; Worrall et at 2010; Marchetti et al. 2011; Huang and Anderegg 2012; Zegler et al. 2012). As of 2006, 220,000 ha of aspen in Colorado had been afflicted with SAD with the largest occurrence on the San Juan National Forest Dolores-Mancos District (Worrall et al. 2008). More recent remote sensing research in conjunction with field surveys in 2010 suggest approximately 58% of aspen stands on the San Juan National Forest have been afflicted by intermediate or high SAD (Huang and Anderegg 2012).

SAD is the result of three interacting factors: predisposing, inciting, and contributing factors (Manion and LaChance 1992; Worrall et al. 2010). Long-term predisposing factors of SAD in southwestern Colorado include static or slow changing factors such as low elevation, south or southwestern aspects, low stand density and physiological maturity (Worrall et al. 2010). The inciting factor of SAD in southwestern Colorado, which resulted in short-term severe stress, was a multi-year, severe drought that peaked in 2002 combined with above average growing season temperatures considered by Breshears and others (2005) as a global-change-type drought. Finally, contributing factors of SAD in southwestern Colorado include secondary insects and diseases that attack trees stressed by predisposing and inciting factors such as aspen bark beetles (*Trypophloeus populi* and *Procryphalus mucronatus*), poplar borer beetles (*Saperda calcarata*), bronze poplar borer beetles (*Agrilus liragus*), and Cytospora canker (usually caused by *Valsa sordida*, the sexual fruiting stage of the fungus) (Worrall et al. 2010; Marchetti, et al. 2011). Aspen bark beetles were previously not considered a threat in southwestern Colorado until recent studies conducted by Worrall and others (2008). Research suggests that both bark beetles are secondary SAD insects because they do not attack vigorous trees under normal circumstances (Worrall et al. 2010). *Trypophloeus populi*, however, is considered the more harmful beetle because it is more prevalent on dying trees with live tissue (Petty 1977). *Saperda calcarata* and *Agrilus liragus* only attack stressed trees (Jones et al. 1985). *Saperda calcarata* larvae can cause mortality in small trees and severe damage in large trees. Multiple borer attacks on adult trees result in large numbers of tunnels, decreasing the tree's stability and making it more susceptible to wind breakage. *Agrilus liragus* larvae feed on the cambium and phloem. Multiple attacks cause tree girdling, which leads to direct mortality (Jones et al. 1985).

Stressed aspen are susceptible to *Valsa sordida* that causes Cytospora canker (Hinds 1985). Trees are killed when the fungus grows into the phloem and xylem girdling the tree (Guyon et al. 1996). Other pathogens associated with aspen mortality but not directly linked to SAD include white trunk rot (caused by *Phellinus tremulae*), black canker (caused by *Ceratocystis fimbriata*), sooty-bark canker (caused by *Encoelia pruinosa*) and Armillaria root rot (caused by *Armillaria* spp.) (Worrall et al. 2010). Recent research in 2010 by Anderegg and others (2012) suggested that hydraulic impairment of distal branches and roots is the primary causal mechanism of SAD and that root mortality most likely precedes canopy mortality; however, the authors recommend further research to better understand the relationship between hydraulic and carbon stress and its influence on rapid aspen mortality.

Aspen is a clonal species that typically regenerates through vegetative reproduction following disturbances such as fire, geomorphic events, wind-throw, tree harvesting or avalanches (Schier et al. 1985). Aspen vegetative reproduction is controlled primarily by the hormones auxin and cytokinins. When an individual aspen stem dies, auxin, a hormone suppressing root suckering, is no longer transported to the roots allowing cytokinins in the roots to stimulate lateral roots to grow new ramets through root suckering (Schier et al. 1985). Aspen also reproduces sexually but conditions in the Southwest generally are not favorable to seed germination because of low soil moisture levels and a specific combination of necessary conditions to initiate seed germination (Shepperd and Engelby 1983; McDonough 1985). Aspen that do germinate from seed, however, are able to graft into an existing aspen clone (DesRochers and Lieffers 2001). Aspen clonal dynamics allow an entire stand to be affected by root deterioration from disease or stress (Epstein 1978) and therefore concern for the rapid decline of aspen is not only because of high stem mortality but also because of a lack of aspen suckering (Worrall et al. 2008). Generally, once branch dieback initiates, aspen stands often deteriorate rapidly resulting in tree death and stand breakup. Seemingly healthy stands of mature aspen have been shown to be reduced to only a few dying stems in less than six years (Shields and Bockheim 1981). Aspen dieback paired with a loss of suckering magnifies the negative effects of aspen decline at the landscape scale (Frey et al. 2004)

with many afflicted stands likely converting to either conifer dominated vegetation types (Shepperd et al. 2001) or shrublands (Worrall et al. 2010) due to root systems no longer being able to regenerate stands. At a finer scale, large changes in forest floor microclimate due to high crown loss and synchronous branch dieback from SAD may have significant effects on plant understory productivity and diversity (Kudo et al. 2008; Soo et al. 2009). Powell and Bork (2007) found that aspen leaf cover reduced photosynthetically active radiation (PAR) and air temperature during the day and increased relative humidity at night on the forest floor. In addition, they found that tree canopy cover buffers understory vegetation from temperature extremes in the summer and may enhance soil moisture by reducing evaporation (Powell and Bork 2007). Carlson and Groot (1995) found that decreases in aspen canopy cover increased PAR at the forest floor resulting in amplified plant productivity. In addition, Strong and La Roi (1983) found that plant cover and productivity increased when aspen root growth decreased because of reduced competition for resources (Strong and La Roi 1983). Plant understory communities offer a useful lens to quantify the effects of SAD on ecosystem dynamics because they provide the majority of biological diversity in aspen forests.

The goal of our research study was twofold: 1) to quantify predisposing and contributing factors associated with aspen mortality: stand structure, site variables, and secondary insects and diseases; and 2) quantify the effects of SAD on a diverse suite of ecological attributes: microclimate, arbuscular and ecto-mycorrhizae, root mortality and regeneration, and understory vegetation.

Methods

Study Area

Our study is located within the Dolores Ranger District of the San Juan National Forest (SJNF) (N37° 28-30', W108° 10-15') in the southwestern-most extent of the San Juan Mountains where land managers are conducting harvest and burn treatments to try and assist aspen regeneration (Figure 1). The study area is bordered to the east by the La Plata Mountains and is topographically composed of mid-elevation (2200 m to 3300 m) foothills that transition into extensive mesas that create conditions favorable for aspen (~53,000 ha within the Dolores Ranger District). The study area is dominated by pure aspen stands

intermixed at a landscape scale with warm-dry mixed conifer species such as Douglas-fir (*Pseudotsuga menziesii*), white fir (*Abies concolor*), and ponderosa pine (*Pinus ponderosa*). The understory is diverse and comprised of species including snowberry (*Symphoricarpos albus*), peavine (*Lathyrus latifolius*), monkshood (*Aconitum columbianum*), wild strawberry (*Fragaria virginiana*), and muttongrass (*Poa fendleriana*). The majority of precipitation occurs during the winter months as snow with June having the least precipitation. The majority of soils are of the Behanco-Powderhorn family complex, the Fly-Foidel complex, and the Teedown-Nordicol complex. Historical and current management activities include cattle grazing, recreation, and timber harvesting. Timber harvests over the past thirty years have produced a patch mosaic of aspen habitat consisting of mature stands bordered by immature regenerating stands and/or open meadows.

Experimental design

We initially selected stands with overstories composed of ≥95% aspen by number of stems. We used a stratified random sampling design by controlling for aspen age, slope, aspect, and elevation (2600 m to 3000 m range) during aspen stand selection. We defined an aspen stand as any area of contiguous aspen forest with similarities in tree species composition, height and density. Sixty sample points were randomly located within aspen that met the defined criteria including a range of healthy to damaged stands. Any sample points that were <50 m from stand perimeters to reduce edge habitat effects were discarded. We defined edge as any transition into stands that did not have similar stand characteristics; therefore, transitions to immature aspen stands were considered edge. We classified all sample point locations into one of three SAD levels based upon the mean percent of recent crown loss (RCL) for each plot: 1) low SAD (0-25%), 2) moderate SAD (25.1-50%), and 3) high SAD (50-100%). We followed similar methods to Worrall and others (2008; 2010) for quantifying RCL as the percentage of foliage lost from recent crown thinning or twig and branch dieback for an individual stem. Dead branches that had <50% of bark intact in the RCL estimates were not included. Dead aspen trees that had recent dead branches were included in RCL estimates as 100% crown loss (Worrall et al. 2010). Our SAD levels for

designating healthy versus damaged stands are consistent with the SAD literature (Worrall et al. 2010; Anderegg et al. 2012; Huang and Anderegg 2012). We had 20 sample points for each level.

Forest stand and site field methods

We collected overstory forest data on all sixty plots during the summer of 2009 and 2010. In the summer of 2011, a random subset of plots (7 plots/SAD level) was remeasured to determine if SAD plot categorization was consistent among the three years of sampling. None of the plots changed SAD category over time and therefore only data from 2010 is presented since this is the year that additional ecological measurements were taken. We established two circular plots at each point (hereafter referred to as plot center). An outer 201 m^2 (8 m radius) plot was used to gather data on all trees ≥ 12 cm dbh (diameter breast height), >1.37 m height). Data included: 1) species, 2) condition (living or snag/log classes [Thomas et al. 1979]) 1 = live, 2 = declining, 3 = recent snag, 4 = loose bark snag, 5 = clean snag, 6 = broken above breast height, 7 = broken below breast height, 3) DBH (stems ≥ 12 cm DBH), 4) tree canopy height (m) (measured with a digital laser rangefinder/hypsometer), and 5) RCL [assessed by ocular estimation of the percentage of RCL for an individual stem, which was categorized into a 1-9 scale (0 = 0-9% loss,…9 =90-99% loss)]. Finally, for each aspen stem the presence of any secondary insects and diseases associated with SAD was recorded. We calculated secondary insect and diseases incidence as the frequency of stems within a plot identified with a specific agent or signs of agent damage (Marchetti et al. 2011). We recorded the following secondary insects and diseases based on their physical presence or agent damage (e.g., insect gallery): aspen bark beetles, poplar borer, bronze poplar borer and Cytospora canker. The two aspen bark beetles present in the study site were not identified to species because their tree markings, tiny pinholes, are similar for both species and species can only be identified using a microscope. In addition, we recorded white trunk rot (caused by *Phellinus tremulae*), black canker (caused by *Ceratocystis fimbriata*), sooty-bark canker (caused by *Encoelia pruinosa*) and Armillaria root rot (caused by *Armillaria* spp.). Secondary insects and diseases on recent dead aspen are easy to detect on stems with little bark (Ostry et al. 1989). A concentric, 40.7 m^2 (3.6 m radius) plot was used to quantify aspen regeneration (trees ≤ 1.37 m) and categorized regeneration by condition, height

class (<40 cm; 40.1–80 cm; 80.1–137 cm), and ungulate browse status (browse or no browse); cattle and native ungulate browsing were not separated. We recorded tree canopy cover using a vertical projection densiometer every 3 m along a permanently marked 50-m line transect oriented upslope through the plot center. Dead woody biomass and forest floor (litter and duff) depth was measured on a 15.2-m planar transect in a random direction from plot center (Brown 1974). Additionally, at each plot center we recorded aspect, slope (measured with a hypsometer), latitude, longitude, and elevation (measured with a Garmin 360 SCX GPS unit, Garmin International, Inc., Olathe, KS, USA). We took two photographs; one from plot center facing NE and one facing E. Each sample point was permanently monumented with a wooden stake, and a reference tree with sample point location information.

Understory vegetation field and lab methods

We randomly chose 10 plots/SAD level to sample understory vegetation in 2010 and 2011. At each plot center we established a modified Whittaker plot (Korb et al. 2007) consisting of a 50 x 20 m plot with four 1 m^2 subplots (0.5 x 2.0 m^2) that were placed along the 50 m transect at 0-2 m, 17-19 m, 30-32 m, and 48-50 meters. We recorded all species present in the larger 1000 m^2 plot. In the four 1 m^2 subplots, we identified all species and recorded abundance for each species using the ocular cover method to the nearest quarter percent (Korb et al. 2003a). In addition, a 50 m line intercept transect was used to quantify shrub abundance by species. We recorded shrub cover as the number of meters intercepted by each shrub species along the 50 m transect and then calculated shrub cover for individual species by dividing the number of meters intercepted for each species by 50 m.

We collected understory vegetation standing biomass within seven of the ten modified-Whittaker plots/SAD category in 2011. All standing understory and shrub vegetation in a 0.5 x 2.0 m^2 subplot adjacent to the 17-19 m and 30-32 subplots where understory composition and abundance data was recorded was clipped. We clipped live plant biomass during peak biomass in mid-late July at the soil surface and divided biomass by species' functional groups (forb, grass, and shrub). Total standing biomass was calculated as the sum of forb, grass, and shrub biomass for each 1 m^2 plot. We dried clipped

vegetation within 24 hours of clipping in a forced air oven at 65º C for 48 hours and weighed dried biomass (Cornelissen et al. 2003).

Mycorrhizae field and laboratory methods

We used the same 10 plots/SAD category used to sample understory vegetation in 2010 and 2011 for mycorrhizal sampling. Only 2011 data is presented since there were no significant differences in mycorrhizae between years. We collected soil samples for arbuscular mycorrhizae (AM) corn bioassays in the same 0.5 x 2.0 m^2 subplots along the 50 m transect at 17-19 m and 30-32 m used to quantify understory species composition and abundance (N=2/subplot). Soil was collected to a depth of 10 cm and immediately placed into a 4 x 20-cm diameter deep Conetainer (Stuewe and Sons Inc., Corvallis, Oregon USA) for bait-plant bioassays (Brundrett & Abbott 1994). We took samples to this depth because AM fungal propapule densities are generally highest in the surface 15 cm (Smith and Read 1997). Bait-plant bioassays are designed to detect all types of viable mycorrhizal fungal propagules including spores, fragments of mycorrhizal roots and extraradical hyphae. This method quantifies total mycorrhizal fungi more accurately than direct counts of sporocarps, spores or colonized root lengths (Brundrett and Abbott 1994; Johnson et al. 1999). We used a corn *Zea mays* L. bioassay to determine the relative amount of infective propagules of AM fungi. Corn is mycotrophic with many species of AM fungi and grows rapidly and uniformly; these advantages outweigh the disadvantage of not using a native host-plant (Johnson et al. 1999). Corn plants were grown from seed in a greenhouse and watered every 3 days until they were harvested at 6 weeks. Roots were carefully washed free from the soil. We prepared corn roots for AM analysis by cutting roots into 2·5-cm segments, taking a random subsample of the cut roots of a known mass, clearing roots in 5% potassium hydroxide, and then staining with Chorazal black E (Koske and Gemma 1990). The gridline intersect method with a dissecting microscope was used to measure the proportion of root length containing AM fungal structures: arbuscules, vesicles, coils, internal mycorrhizal hyphae and external mycorrhizal hyphae (Giovannetti and Mosse 1980). We collected aspen root samples from two randomly selected ramets per plot to quantify ectomycorrhizae (EM). We randomly cut ten root segments from the main root within 10 cm from the aspen ramet and immediately

wrapped the root segments in a wet paper towel, placed segments in a zip-loc bag and stored roots in a refrigerator until laboratory analysis. We examined root tips using a dissecting microscope to quantify the proportion of root tips colonized with EM fungi; tips were classified as an EM tip or non-mycorrhizal tip (Gehring and Whitham 1991).

Microclimate field methods

We collected microclimate data during the growing season from mid-June until mid-September in same subset of 21 plots (7 plots/SAD category) surveyed for understory biomass. We positioned two Thermochron I-button temperature data loggers (Embedded Data Systems, Lawerenceberg, KY) in the 17-19 m Modified, modified Whittaker subplot used for understory vegetation; one button was placed at the soil surface and the second 3 cm directly beneath the soil surface (28 plots x 2 loggers = 56 total loggers). We placed small, opaque plastic shields over the surface temperature data loggers to prevent direct solar radiation skewing temperature data. The loggers collected data at 30-minute intervals for the growing season. A Hobo Micro Station Data Logger (Onset Computer Corporation, Pocasset, MA) with two sensors to collect soil moisture data within the 17-19 m subplots was established in three randomly chosen plots/SAD category for a total of 9 data loggers, 18 soil moisture sensors, and 9 photosynthetic active radiation (PAR) sensors.

Aspen root health field methods

We quantified aspen root health and mass in the 7 plots/SAD category surveyed for microclimate and plant biomass. Methods established by Shepperd and others (2001) were used to quantify root mass in each plot. We randomly located and dug a trench just outside the overstory outer 201 m^2 (8 m radius) circle plot to a depth of 20 cm and a length of 3 m. The trench was just wide enough to determine root health and diameter. Root health was observed on one side of the trench using a binary classification (0=living, 1=dead). We recorded every root with a diameter of $\geq$ 4mm and we calculated root length per unit area of soil surface using Worrall and others (2010) modification of van Wagner's (1968) formula for wood volume using a transect.

Statistical analyses

We assessed differences among the three SAD levels in stand structure, site, vegetation and other variables using Kruskal-Wallis one-way analysis of variance (ANOVA) in SPSS version 18 (SPSS 2009). When statistically significant ($\alpha = 0.05$) differences were noted, we used Mann–Whitney tests to analyze rank differences among SAD levels and adjusted alpha levels by the number of pairwise comparisons using a Bonferroni correction (Kuehl 1994). We used simple linear regression to compare the relationship between RCL (the dependent variable) and numerous stand and site attributes. Stepwise-forward multiple linear regression was utilized to explore the multivariate relationship of RCL with stand and site attributes; this method looks at numerous possible explanatory attributes and isolates the attributes that contribute most to explaining RCL. We followed this exploratory analysis with non-metric multidimensional scaling (NMS) to examine differences in insect and pathogen communities among SAD levels and to examine differences in understory vegetation communities among SAD levels (Clark 1993). NMS analyses were conducted using PC-ORD software (version 6, McCune and Mefford 2011). We ran the NMS ordinations using a Bray–Curtis distance measure, random starting configurations, 50 runs with real data, a maximum of 200 iterations per run and a stability criterion of 0.00001. The stress value of the final solution was compared to 50 random solutions using a Monte Carlo test. Any understory vegetation species that did not occur on a minimum of 5% of the plots were filtered out (McCune and Grace 2002). We did not filter out any insect or pathogen species since they met the minimum %5 criterium. We determined the relationships between stand structure and abiotic variables and ordination axes using the main matrix Pearson's r correlations (McCune and Grace 2002). We considered variables with the highest correlation coefficients to have the strongest weight on the axes. Differences among the three SAD levels for insect and pathogen frequency and understory vegetation was examined using a permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001; McArdle and Anderson 2001). PERMANOVA uses common ecological distance measures (Bray–Curtis for this study) to examine multivariate datasets and calculates P-values using permutations, rather than tabled P-values that assume normality. We used a one fixed factor (SAD level) as our main effect (PC-ORD software version 6, McCune and Mefford, 2011). Species that were present in a minimum of 5% of the plots as

recommended by McCune and Grace (2002) were analyzed. We used indicator-species analysis (McCune and Grace 2002), which uses species richness and associated abundance values of species, to identify species that were particularly consistent indicators for a particular SAD level. A comparison between the maximum indicator value (0–100) and random trials for occurrence of a given species (1000 Monte Carlo randomizations) provided an approximate P-value (McCune and Grace 2002). Species with $P \leq 0.05$ and indicator values (INDVAL) > 25 (INDVAL = relative abundance x relative frequency; INDVAL ranges from 0 to 100) were accepted as indicator species for a particular time period (Dufrene and Legendre 1997).

Results

Stand structure, site variables, and secondary insects and diseases

Stand structure

Stand structure varied among the three different SAD levels. Basal area was significantly higher in low SAD stands than moderate (P=0.005) or high (P=0.001) SAD stands with no differences between moderate and high SAD levels (Table 1). Regression of RCL illustrated a significant negative relationship with basal area (P<0.000, R^2 =-0.22). Average aspen DBH , for aspen ≥ 12 cm, was significantly higher (P=0.007) in high SAD stands in comparison to low SAD stands (Table 1). Regression of RCL illustrated a significant positive relationship with DBH (P<0.039, R^2 =0.072), however DBH explained little variation in RCL. There was no difference in average tree height among SAD levels (Table 1) or relationship (P=0.61, R^2=0.005) between mean tree height and RCL. Tree slenderness (ratio of height to diameter) (Wang et al. 1998) was significantly lower (P=0.005) in low SAD stands than moderate or high SAD stands and had a significant negative relationship (P=0.002, R^2=0.15) between tree slenderness and RCL. Stand density was significantly (P<0.000) higher in low SAD stands than moderate or high SAD stands (Table 1). Regression of RCL illustrated a significant inverse relationship with stand density (P<0.000, R^2=-0.30). As expected due to high collinearity with RCL, tree canopy cover (P<0.000) and tree status (P<0.000) were different among all three SAD levels with tree canopy cover significantly lower in high SAD stands and most of the trees categorized as recent

snags (Table 1). Our multiple regression model for RCL explained 68.7% of the variation based on the adjusted R^2 ($_{F3,36}$=44.08, P≤0.000). Recent crown loss increased with increasing bronze poplar borer incidence, poplar borer incidence and elevation (Table 2).

Site variables

Elevation was significantly higher in low than in moderate (P=0.032) and high (P<0.000) SAD stands (Table 1). Regression of RCL with elevation showed a significant negative relationship (P<0.000, R^2=0.377). There were no differences in aspect among the three SAD levels with all stands having a general southwestern aspect. Slope was not significantly different among SAD levels however high SAD areas were slightly flatter. Mean slope and RCL were unrelated (P=0.28, R^2=0.02).

Insects and diseases

All eight insects and diseases monitored for this study were present in all three SAD levels (Table 3). In low SAD stands, Cytospora canker had the highest (44%) incidence, followed by poplar borer (13%), bronze poplar borer (10%) and bark beetles (7%) (Table 3). Cytospora canker also had the highest incidence (56%) in moderate SAD, follow by poplar borer (30%), bronze poplar borer (29%), and bark beetles (22%). Finally, in high SAD stands, Cytospora canker had the highest incidence (76%) followed by bronze poplar borer (73%), poplar borer (68%), and bark beetles (52%) (Table 3).

Aspen bark beetle incidence was significantly lower in low SAD stands than moderate (P=0.05) or high (P<0.000) SAD stands (Table 3). Bark beetles illustrated a significant positive relationship with RCL (P<0.000, R^2=0.442). Bronze poplar borer incidence was also significantly lower in low SAD stands than moderate (P=0.004) or high (P<0.000) SAD stands (Table 3). There was a strong significant positive relationship between bronze poplar borers and tree RCL (P<0.000, R^2=0.614). Poplar borer incidence was significantly lower in low SAD stands than moderate (P=0.05) or high (P<0.000) SAD stands (Table 3). Poplar borers showed a strong positive relationship with RCL (P<0.000, R^2=0.611). Cytospera canker incidence was significantly lower in low than high SAD stands (P=0.012) with no difference between low and moderate or moderate and high SAD stands (Table 3). Cytospera canker illustrated a positive relationship with RCL (P<0.000, R^2=0.270). White trunk rot incidence was significantly lower

in moderate than high SAD stands (P=0.045) with no differences between low and moderate or low and high SAD stands (Table 3). There were no differences among SAD levels for Armillaria root rot, black canker, and sooty-bark canker incidence or relationships for Armillaria root rot, black canker, sooty-bark canker or white trunk rot with RCL (Table 3).

Bronze poplar borer, bark beetles, Cytospora canker and poplar borer were commonly found together in stands afflicted by SAD (Table 4). White root rot was also significantly correlated with the bronze poplar borer and poplar borer but not as strongly. Sooty-bark canker was only weakly associated with white root rot and Armillaria root rot was only weakly associated with Cytospora canker. There were no associates between black canker and other damage agents (Table 4).

Bronze poplar borer, bark beetles, Cytospora canker and poplar borer were all strongly positively correlated with RCL, root mortality and DBH and were strongly negatively correlated with tree density and litter and duff (Table 5). Bronze poplar borer, bark beetles and poplar borer were also strongly negatively correlated with elevation and basal area (Table 5). Bronze poplar borer, Cytospora canker, poplar borer were strongly negatively associated with tree slenderness and Armillaria root rot and sooty-bark canker were weakly negatively associated with tree slenderness (Table 5). Sooty-bark canker was also negatively correlated with litter/duff, shrub cover, and tree height (Table 5). White root rot was negatively correlated with litter/duff (Table 5).

There was no difference in insect and disease species richness among SAD levels (Table 6). In contrast, there was a significant difference in the Shannon-Weiner diversity index between low and moderate (P=0.01) and low and high (P=0.00) SAD stands (Table 6). Overall insect and disease communities among SAD levels were significantly different (F=13.16, P=0.0002) (Table 7). Non-metric dimensional scaling ordination of insect and disease communities showed strong separation between low and high SAD stands with moderate insect and disease communities overlapping low and high SAD communities (Figure 2). Axis 1 for the NMS ordination showed positive Pearson's r correlations for DBH and negative correlations for tree canopy cover, trees/ha^{-1}, elevation, litter/duff and basal area/ha^{-1} (Table 8, Figure 2).

Indicator species analysis detected insects and diseases that were particularly consistent indicators for SAD levels (Table 9). Bronze polar borer, aspen bark beetles, poplar borer and Cytospera canker were all strong indicator species for high SAD levels. There were no indicator insects or pathogens for low or moderate SAD levels (Table 9).

Effects of SAD on Aspen Stand Attributes

Microclimate

Day (700-1859 hrs) surface temperatures were significantly higher in high SAD stands in comparison to low SAD stands (P=0.014) (Figure 3). Similarly, day subsurface temperatures were significantly higher in high SAD stands in comparison to low SAD stands (P=0.008) (Figure 3). There was a strong negative relationship between day surface temperature and RCL (P=0.004, R^2=-0.617) and day subsurface temperature and RCL (P=0.04, R^2=-0.405). There were no differences for night (1900-659 hrs) surface temperatures among SAD levels; however, there was a significant difference for night subsurface temperatures between moderate and high SAD stands (P=0.034) (Figure 3). Night subsurface temperatures illustrated a strong negative relationship with RCL (P=0.015, R^2=-0.472).

Surface minimum temperatures were significantly higher (P=0.002) in low SAD versus high SAD stands (Figure 4). In contrast, subsurface minimum temperatures were significantly lower in low SAD stands in comparison to moderate (P=0.05) and high (P=0.004) SAD stands (Figure 4). There was a strong, negative relationship between surface maximum temperature and RCL (P<0.000, R^2=-0.552) and positive relationship between subsurface temperature and RCL (P=0.001, R^2=-0.474). Surface maximum temperatures were higher in high SAD stands but there were no significant differences due to high variability of maximum temperatures. Subsurface maximum temperatures were higher in high SAD stands versus low SAD stands (P=0.004) and there was a strong, positive relationship between subsurface maximum temperatures and RCL (P<0.000, R^2=-0.568) (Figure 4).

Mean soil water content (P=0.014) and PAR (P=0.032) was significantly lower in high SAD stands than low SAD stands with no differences among other SAD stands (Figures 5 & 6, respectively). There was a

negative relationship between mean soil water content and RCL (P=0.038, R^2=-0.531) and between PAR and RCL (P=0.05, R=-0.237).

Arbuscular and ecto-mycorrhizae

The mean root length colonized by arbuscular mycorrhizae was significantly higher (P=0.018) in low SAD stands than high SAD stands with no other differences among SAD levels (Figure 7). There was a strong, positive significant relationship (P<0.000; R^2=0.56) between arbuscular mycorrhizae and RCL. Arbuscular mycorrhizae was positively associated with DBH and dead aspen root length and numerous microclimate variables including mean diurnal surface temperature, surface minimum temperature, subsurface minimum and maximum temperature and PAR (Table 10). In contrast, there were no differences in the mean root length colonized by ecto-mycorrhizae among SAD levels even though ectomycorrhize root length was greater in low versus high SAD stands due to high variability; RCL and ecto-mycorrhizae were unrelated (Figure 8). Ectomycorrhizae had a positive correlation with trees/ha^{-1} and a negative correlation with numerous variables including dead root length, tree DBH, PAR, surface minimum and maximum temperatures and diurnal surface temperature (Table 10).

Roots and regeneration

Mean live aspen root length was significantly higher in low SAD stands in comparison to moderate (P = 0.05) or high (P =0.038) SAD stands and mean dead aspen root length was significantly lower in low SAD versus high SAD stands (P =0.026) (Figure 9). There was a significant negative relationship (P=0.018, R^2=0.45) between live aspen root length and RCL and a significant positive relationship (P=0.021, R^2=0.48) between dead aspen root length and RCL.

Live aspen regeneration was significantly higher than dead aspen regeneration for all SAD levels; however, there was extremely high variability in live aspen regeneration across all SAD levels with a few plots having high regeneration (> 5000 stems/ha^{-1}) and many plots having no aspen regeneration (Figure 10). As a result, there was no difference in total live regeneration (Figure 10) or live regeneration separated by browse categories (Table 11). In contrast, total dead regeneration was significantly higher in low SAD than moderate SAD stands (P=0.05) and high (P=0.02) SAD stands (Figure 10). Specifically,

dead regeneration was different for dead regeneration with no signs of browsing between low and moderate (P=0.01) SAD stands and low and high (P=0.008) SAD stands (Table 11).

Understory vegetation

There were no differences in total understory plant biomass between the three SAD levels or for shrub or forb biomass (Figures 10 and 11, respectively). Grass biomass was significantly higher (P=0.033) in high SAD stands than low SAD stands (Figure 11). Grass biomass was strongly positively correlated with PAR, diurnal surface temperature, subsurface maximum temperature, and bare soil and negatively correlated with RCL (Table 12). Forb biomass was strongly positively correlated with total dead aspen regeneration and litter cover. Shrub biomass, dominated primarily by snowberry (*Symphoricarpos albus*), and trees/ha^{-1} had a positive correlation (Table 12).

There was no difference in understory species richness or Shannon-Weiner diversity among SAD levels (Table 13). Similarly, overall understory vegetation communities among SAD levels were not different (F=1.534, P=0.1080) (Table 14) and non-metric dimensional scaling did not show any strong separation of understory vegetation communities among SAD levels. Indicator species analysis detected understory species that were particularly consistent indicators for SAD levels (Table 14). *Osmorrhiza occidentalis* was an indicator for low SAD stands and *Mahonia repens* and *Campanula rotundifolia* were indicator species for high SAD stands. There were no indicator understory species for moderate SAD levels (Table 14).

Discussion

Stand structure, site variables, and secondary insects and diseases

Stand structure

Our study showed that within a relatively small geographic region, ~50 km^2, with a similar climate there can be a diversity of aspen RCL with relatively healthy stands directly adjacent to stands with high mortality illustrating the importance of understanding predisposing and causal factors associated with SAD. Huang and Anderegg (2012) also observed a patchy pattern of aspen tree mortality at a larger landscape scale in the San Juan National Forest where our study occurred and suggested that complex

interactions of stand, site, secondary insects and diseases and genotypes were responsible for the spatial patterns. We detected significant differences in overstory stand structure among the three levels of SAD. Specifically, our study illustrated that stands afflicted with high SAD tended to have low basal area and stand density and high DBH (stems ≥ 12 cm DBH) and tree slenderness in comparison to low SAD stands. Stand density and basal area had the highest negative relationships with RCL. Worrall and others (2010) found a similar pattern between healthy and damaged aspen stands for basal area and tree slenderness and stand density (2008); however the association between basal and RCL was weak. Worrall and others (2008) did not find significant differences in DBH (stems ≥ 12 cm DBH) but their results did show aspen mortality was skewed towards larger trees and that as variation in DBH increased, RCL decreased (2010). Zegler and others (2012) did not find any significant relationships of aspen stand structure with mortality; however, conifer BA was significantly positively associated with aspen mortality. In our study, we only investigated stands afflicted with SAD that were composed of at least 95% aspen accounting for the differences between the two studies. Contrary to suggestions by Frey and others (2004), tree slenderness was negatively associated with RCL; trees most afflicted by SAD were large and tall and found in open stands that are susceptible to drying due to increased sunlight and wind further exasperating climatic warm, dry conditions and increasing tree susceptibility to heat stress. Large, tall trees were more afflicted with signs of SAD likely due to decreased stress tolerance associated with physiological maturity and decreased hydraulic conductivity (Frey et al. 2004). The open structure found in our high SAD stands was further intensified by a high number of felled snags (tree status), which decreased tree canopy cover and increased forest floor woody debris in high SAD stands (data not shown).

Site variables

We standardized aspect, Southwest, and slope, < 7°, in our study in order to quantify stand variables and damage agents associated with SAD. Low elevation was the only site variable significantly associated with RCL, which was similar to the majority of findings in other studies in the Southwest, which is near aspen's southern realized niche (Worrall et al. 2008, 2010; Marchetti et al. 2011; Zegler et al. 2012). In

contrast, Huang and Anderegg (2012) using remoting sensing found no correlation between aspen mortality and elevation in the same study region and suggested that mortality might have moved upslope in recent years. Our field-based study in the same study region did not show this trend possibly illustrating some limits to remotely sensed data. The mean difference in elevation between low and high SAD stands was 122 m in our study. Rehfeldt and others (2009) suggest that the lower elevation limit for aspen in the Southern Rocky Mountains will increase by 250 m by 2030, which is twice the elevation difference between our healthy and damaged plots. Using this model, Worrall and others (2010) showed that 92% of aspen stands on the San Juan National Forest, where our study occurred, will be outside suitable aspen habitat by 2060. While there were no significant differences in slope among SAD levels, flatter areas were more common with SAD supporting findings by Worrall and others (2008) that suggested rooting is shallow in flat areas that normally have high moisture and therefore are more sensitive to drought during periods of drying.

Insects and diseases

Cytospora canker, bronze poplar borer, poplar borer and aspen bark beetles, secondary damaging agents, all showed a strong association with high SAD stands and were strongly correlated with one another illustrating their influence in stands afflicted with SAD. Our findings are consistent with general findings of Worrall and others (2008) for dominant secondary agents associated with SAD in the Southwest but differ from a more detailed study by Marchetti and others (2011) where poplar borer was not found to have a significant association with SAD. Cytospora canker, bronze poplar borer and aspen bark beetles all damage the phloem and vascular cambium of aspen which alters nutrient transport, resulting in rapid dieback and mortality of aspen and significant population increases of these damaging agents further exasperating SAD (Marchetti et al. 2011). In contrast, poplar borers mainly attack the xylem of aspen which is more resistant to insect attacks and therefore doesn't result in rapid dieback (Marchetti et al. 2011). Egg-laying female poplar borers favor large trees with partially-shaded or unshaded boles in open stands, which is consistent with our results showing poplar borer positively associated with DBH (trees ≥ 12 cm DBH) and negatively associated with tree density and basal area (Jones et al. 1985). Poplar borers

weaken trees making them more susceptible to wood rotting diseases and wind damage and rarely result in tree death (Jones et al. 1985). We found a strong, positive association between poplar borer and white root rot, an important wood rooting disease in the western US. While Marchetti and others (2011) did not find a positive association with poplar borer and RCL and root mortality, we did find this pattern indicating the weakening effect of poplar borer in combination with the presence of more aggressive agents such as the bronze poplar borer and bark beetles results in severe branch dieback and tree mortality. The bronze poplar borer generally attacks weakened aspen that have undergone heavy elk defoliation, poplar borer infestation or canker infection that results in girdling and tree mortality (Solomon 1995), which supports the strong association of bronze poplar borers with poplar borers, aspen bark beetles, Cytospora canker and white root rot in our study. Similar to the bronze poplar borer, aspen bark beetles attack trees undergoing stress feeding on the phloem and vascular cambium resulting in girdling and tree mortality (Petty 1977). While we did not identify the two bark beetles (*Trypophloeus populi* and *Procryphalus mucronatus*) to species, Marchetti and others (2011) found that *T. populi* had significantly higher incidence than *P. mucronatus* in SAD stands in southwestern Colorado and that *T. populi* is generally found in stressed, live trees.

Zegler and others (2012) divided damaging agents into categories such as canker diseases and wood-boring insects but noted Cytospora canker, sooty-bark canker, bronze poplar borer, and poplar borer were widespread and common; aspen bark beetles were present but not frequent. Our findings in association with other studies in the Southwest (Worrall et al. 2008, 2010; Marchetti et al. 2011; Zegler et al. 2012) show that a few damaging agents tend to be most prominent in stands with SAD but the specific agents may vary. Cytospora canker in our study was one of the most dominant damaging agents in all three levels of SAD; however, the variation in incidence of Cytospora canker between low and high SAD stands was low compared to all of the other damaging agents. As a result, while Cytospora canker is strongly associated with SAD, it is not an indicator species, extremely faithful species, with high SAD stands in our study unlike the bronze poplar borer, poplar borer and aspen bark beetles. Cytospora canker was not associated with elevation or basal area and only weakly positively associated with tree density

and negatively associated with root mortality in comparison to the bronze poplar borer, poplar borer and aspen bark beetles, which were all strongly associated with these four stand variables. Similar to Marchetti and others (2011), we found strong, positive correlations with RCL and strong, negative correlations with tree slenderness for Cytospora canker, bronze poplar borer, and aspen bark beetles. Cytospora canker's strong positive association with RCL and weak negative association with root mortality suggest that Cytospora can rapidly infect moisture stressed trees in both healthy and damaged stands that will result in stem dieback but doesn't always result in immediate tree death. These findings support other studies that illustrate inoculum is not a limiting factor in the spread of Cytospora canker and is available to any susceptible trees due to drought stress (Hinds 1985; Marchetti et al. 2011).

Effects of SAD on aspen stand attributes

Microclimate

Microclimate varied significantly among three levels of SAD. Specifically, mean day surface and subsurface temperatures, mean night subsurface temperatures and minimum and maximum subsurface temperatures were significantly warmer in high SAD stands. Our findings are consistent with research by Powell and Bork (2007) that showed increased maximum temperatures and decreased minimum temperatures as aspen canopy cover declined and research by Breshears and others (1998) where subsurface soil temperatures were consistently warmer in intercanopy locations than underneath full canopies. Low SAD stands had the highest minimum surface temperatures, indicating that tree canopy cover assisted in regulating the understory microclimate. Photosynthetic Active Radiation (PAR) was higher in high SAD stands supporting findings by Powell and Bork (2007) that higher PAR can reach understory vegetation in areas with excessive aspen leaf loss. In contrast, soil moisture decreased as RCL increased most likely due to increased surface and subsurface mean daily temperatures resulting in a drying effect and possibly from decreased water uptake of aspen roots which generally occurs within the top 20 cm of soil because of root mortality and lower stand densities in high SAD areas (Powell and Bork 2007).

Anderegg and other (2012) illustrated that increased temperature and water stress on tree physiology was strongly associated with SAD. Our microclimate data shows the potential for positive feedbacks in stands afflicted by high SAD where warmer temperatures and decreased soil moisture, consequences of SAD, can make surviving trees within SAD stands more stressed and therefore more susceptible to secondary insects and diseases leading to increased branch dieback and tree mortality and thus altering microclimate even further resulting in conditions more favorable to SAD.

Arbuscular and ecto-mycorrhizae

Mycorrhizae are a critical link between above-ground plants and the soil system, playing an important role in plant nutrition, nutrient cycling and the development of soil structure (Allen 1991). Many tree species are highly dependent on ectomycorrhizal (EM) fungi, including aspen which has over 60 species of EM (Cripps 2001) and most herbaceous plants are associated with arbuscular mycorrhizae (AM) fungi (Smith et al. 1998). Members of the poplar family have been associated with AM but aspen is rarely infected (Cripps 2001).

Arbuscular mycorrhizae was significantly higher in high SAD stands than low SAD stands and was correlated with numerous surface and subsurface temperature variables and PAR indicating that arbuscular mycorrhizae propagule densities respond favorably to warmer surface and subsurface soil temperatures. This finding is congruent with research in ponderosa pine restoration treatments in the Southwest where AM responded rapidly to decreased tree canopy cover due to mechanical harvesting, increasing by ~20 percent , (Korb et al. 2003b) which is similar to AM increases in our research. A study by Kovacic and others (1984) found that AM hosts and AM fungal abundance were significantly higher in mountain pine beetle *Dendroctonus ponderosae* killed ponderosa pine stands than under live ponderosa pine stands in northern Colorado. Other studies in different ecosystems have also shown changes in AM propagule densities with changes in tree densities. A successional study of AM propagule densities across a grassland to forest chronosequence showed AM inoculum potential increased with increasing grass cover and decreased in later successional sites with EM trees (Johnson et al. 1991). Similarly, a study by Benjamin and others (1989) illustrated that herbaceous plants had lower AM colonization as tree density

and shading increased, possibly because these plants had insufficient photosynthetic capability to support AM infection.

Contrary to our expectations, we found no significant differences in EM between the three levels of SAD even though EM was lower in high SAD plots. This finding illustrates that even though EM host-plant (aspen) density was significantly reduced in high SAD stands, EM fungi are able to maintain viable propagules following aspen root and tree mortality. A study by Visser and others (1998) showed pre-disturbance population densities of EM fungi were maintained for 2 years after clear-cutting an aspen stand. It is possible that if the organic layer where EM mycelia are concentrated remains largely undamaged that EM fungal propagule densities will not be reduced. Higher densities of EM propagules in low SAD stands make plants more drought tolerant by having increased uptake of water and nutrients and therefore less susceptible to insects and diseases and more resilient to climate induced dieback and mortality (Cripps 2001). More research is needed to see if EM propagule densities remain unaltered by SAD or if there is a delay time between tree mortality and EM densities decreasing, which will have impacts on aspen tree health and tree regeneration.

Roots and regeneration

In our study, we found significant differences in aspen root health among SAD levels with live roots lower and dead roots higher in high SAD stands. Our findings are consistent with findings by Worrall and others (2010) that showed a significant correlation of root mortality with RCL. Recent studies have suggested that root mortality precedes extensive canopy loss and that positive feedbacks begin to interact below and above-ground as tree physiological stress increases (Worrall et al. 2010; Anderegg et al. 2012). The absence of new ramets in stands afflicted by SAD suggests a low amount of live roots (Shepperd et al. 2001). In our study, there was high variability and no significant differences in live aspen regeneration among the three SAD levels; overall mean regeneration was low ($\leq$ 1250 stems/ha^{-1}), which is consistent with Worrall and others (2010) small size class regeneration and Zegler and others (2012) short regeneration density in SAD stands. Typical aspen regeneration following disturbance is higher, up to 70,000 plus stems/ha^{-1}, in southwestern Colorado (Crouch 1983). There is no consistent regeneration

density for maintaining aspen stands following disturbance in the literature but regeneration found in our study is at or below all suggested densities. For example, Mueggler (1989) suggests ≥1235 stems/ha^{-1} in the small (0.3-1.4 m) size class and Kurzel and others (2007) suggest ≥2500 stems/ha^{-1} in the small size class as the minimum regeneration necessary to sustain aspen. In addition, these numbers don't include the percentage of small regeneration surviving to maturity, which would be lower due to interspecific competition, browsing, and secondary insects and diseases (Worrall et al. 2010).

Regeneration mortality was low for all sites but was significantly higher in low SAD stands; Zegler and others (2012) also found low mortality in short regeneration with ungulate browsing as the only damage agent. We did not find any differences in browsing on regeneration due to high variability; however, browsing was higher in high SAD stands with approximately 50% of live regeneration being browsed in comparison to approximately 30% of live regeneration in low SAD stands. In addition, we could have missed signs from ungulate browsing if the entire stems were eaten. Numerous studies have illustrated the importance of ungulate browsing in impeding successful aspen regeneration (Fitzgerald and Bailey 1984; Bailey et al. 1990; Rumble et al. 1996; Binkley et al. 2006; Fairweather et al. 2008). Shepperd (1996) recommended excluding cattle from areas recovering from disturbance until aspen have reached ≥ 2 m height, which is 0.6 m smaller then our regeneration.

Understory vegetation

Microhabitats, areas within an environment that exhibit unique features, are an integral part of understanding understory plant dynamics. Forest floor microhabitat heterogeneity is important for maintaining forest shrub and understory species diversity because it plays an important role in plant germination and establishment (Hartgerink and Bazzaz 1984; Oswald and Neuenschwander 1993). Tree canopy cover creates microhabitat variability by modifying microclimate, which is important in the Southwest where water is the main limiting resource to plant growth (Vetaas 1992). Specifically, trees affect microclimate through the interception of solar radiation thus lowering soil temperature and evapotranspiration and increasing soil moisture (Belskey et al. 1989; Vetaas 1992). Tree cover also increases litter on the forest floor which further reduces soil temperature and evaporation while increasing

infiltration rates (Grunow et al. 1980). In our study, low and moderate SAD created microhabitats with fine scale spatial heterogeneity due to gaps in the tree canopy. In contrast, high SAD stands were more homogenous with no or little overstory canopy. Despite variations in microhabitats among SAD levels, there were no significant differences in total mean understory biomass due to high plot variability even though moderate SAD stands had 1/3 more biomass, 1000 kg/ha, then low or high SAD stands. Low SAD stands in our study had lower PAR and mean day surface and subsurface temperatures than moderate SAD stands providing conditions favorable to shade-tolerant species. In contrast, high SAD plots had low soil moisture, high PAR and high mean day surface and subsurface temperatures that provide microclimate conditions favorable to species that prefer open, dry microhabitats. We found significantly higher grass biomass in high SAD stands illustrating the ability of grasses to take advantage of this microhabitat and increases in arbuscular mycorrhizae. While not significant, shrub cover and shrub biomass was higher in moderate SAD stands than low or high SAD stands. This conflicts with results by Worrall and others (2008, 2010) that showed shrub cover increasing with higher RCL. Moderate SAD stands provide a mixture of microclimates due to fine-scale spatial heterogeneity in forest canopy cover creating shaded, moist and open, dry microhabitats for understory plant growth, which can result in increased diversity and abundance (Frey et al. 2004; Richardson et al. 2012). Another possible reason for no significant trends in total understory biomass or species diversity was due to cattle grazing. Grazing impacts are difficult to interpret because they can be affected by numerous factors such as seasonality, stocking rate, timing, and length of grazing (Keeley et al. 2003). Biomass data was collected during peak season, mid-July, however cattle were present during data collection. Ungulate browsing on aspen regeneration was higher in high SAD stands and therefore browsing of understory palatable species was most likely higher in high SAD stands as well. Future studies investigating the effects of SAD on understory biomass, community composition and diversity need to include areas with no cattle grazing to tease out confounding effects of multiple disturbances (e.g., SAD and cattle grazing). In addition, long-term monitoring is needed to determine if there is a lag-time in understory composition and diversity responses to SAD.

Conclusion

Research by Rehfeldt and others (2009) and Worrall and others (2008, 2010) has clearly illustrated that an extremely warm drought in 2002 was the inciting factor associated with SAD in the southwestern U.S and that aspen's lower elevation limit will increase due to continued climatic warming. Further research by Huang and Anderegg (2012) suggested that aspen in the San Juan National Forest is suffering the most severe biomass loss from drought-induced mortality for any vegetation type in North America illustrating the extreme magnitude of aspen decline in southwestern Colorado. Aspen are unique from other dominant trees in western forests because they reproduce almost exclusively by root suckering initiated from disturbance. If aspen is unable to regenerate in stands afflicted by SAD, aspen will likely be lost because aspen rarely establishes by seed and if it does, it is not consistent enough to reestablish aspen when clone mortality occurs (Romme et al. 2005). Aspen loss from SAD has numerous biological and ecosystem function consequences including, but not limited to, shifts in vegetation composition and structure (Worrall et al. 2010, Zegler et al. 2012) altered avian diversity (Bombaci and Korb 2012), changed animal-plant disease relationships (Lehmer et al. 2012) and decreased carbon sequestration (Huang and Anderegg 2012). We recommend land managers focus aspen regeneration treatments such as prescribed fire and/or coppice harvesting in moderate SAD stands because most high SAD stands have already crossed an ecological threshold where aspen regeneration probability is low because it is at the lower elevation climate envelope for aspen, high root mortality, altered microclimate, and the high abundance of secondary insects and diseases. We also recommend that cattle should be excluded from areas experiencing or being treated with SAD to minimize additional stresses to the ecosystem and to promote aspen regeneration (Rumble et al. 1996; Binkley et al. 2006; Fairweather et al. 2008).

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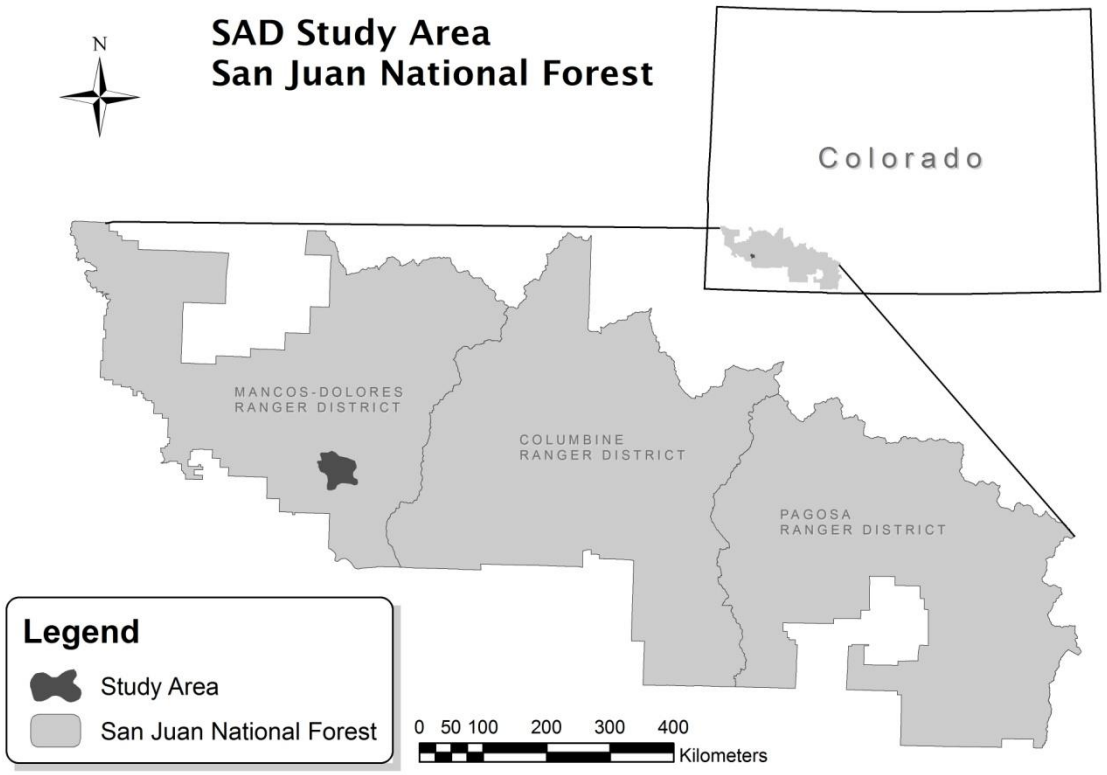

Figure 1. Study location within the Mancos-Dolores Ranger District of the San Juan National Forest, Montezuma County, Colorado. Map by Sara Bombaci.

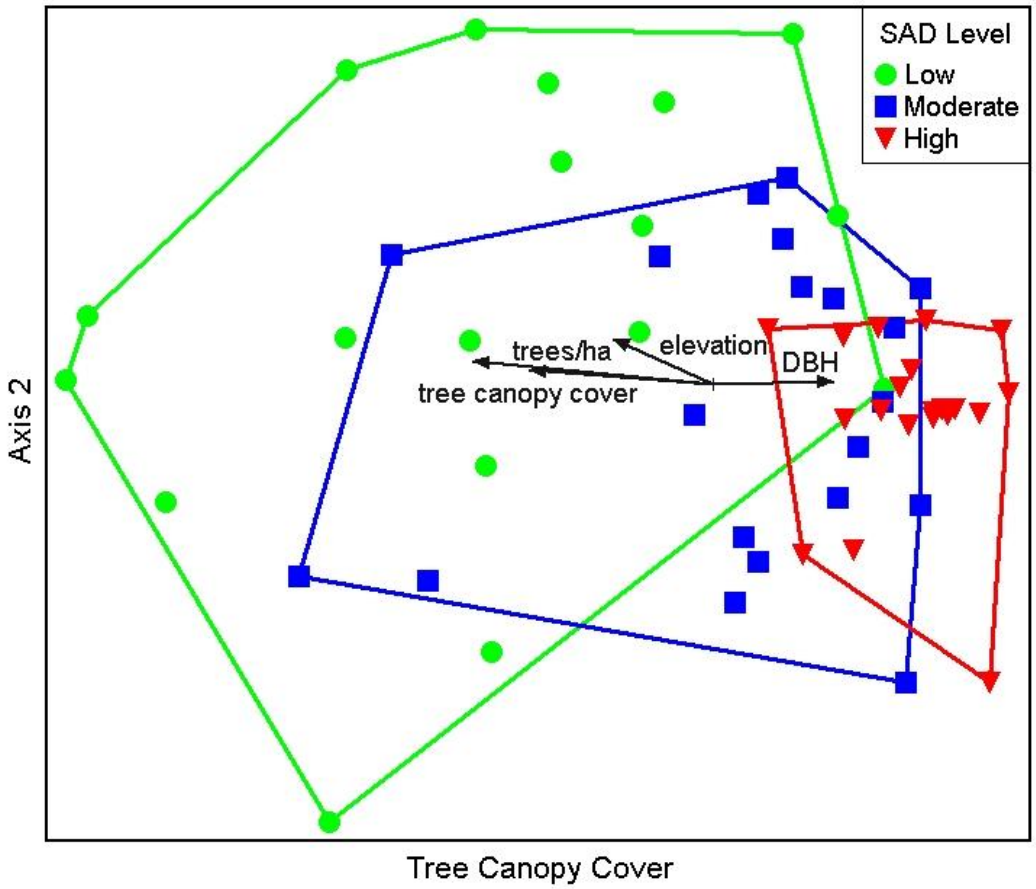

Figure 2. Non-metric multidimensional scaling ordination of frequency relativized to maximum transformed species data for three SAD levels (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%). Each symbol represents one SAD plot (N = 20/SAD level). Vector lengths are proportional to correlations with community composition. The final solution had three dimensions, stress = 15.09 and P = 0.02.

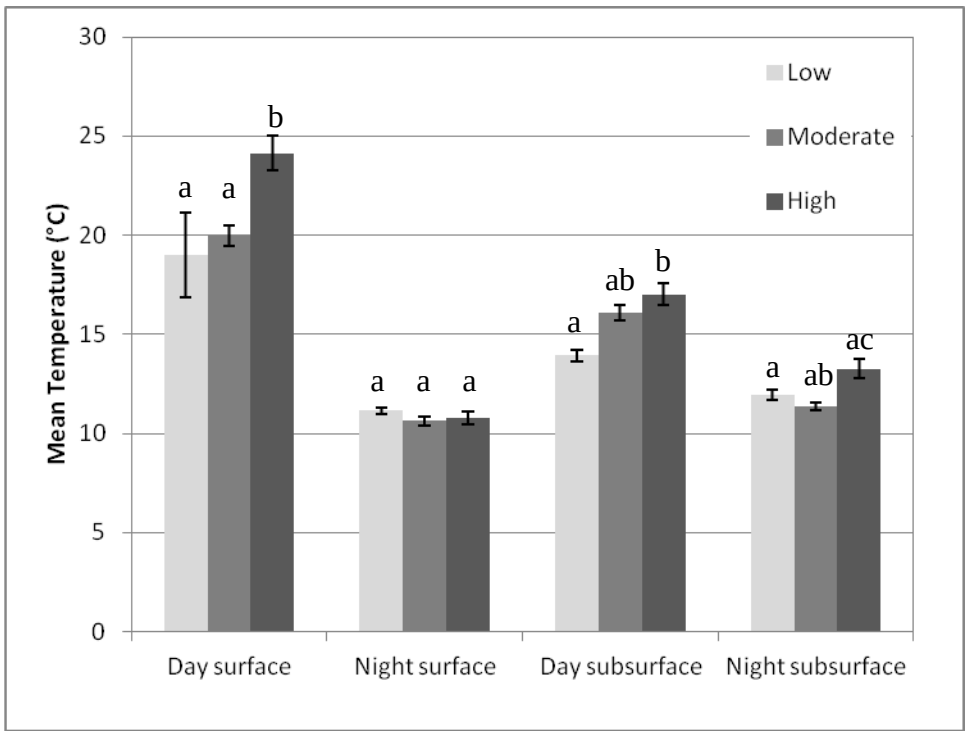

Figure 3. Average day (700-1859 hrs) and night minimum (1900-659 hrs) soil surface and soil subsurface (3 cm mineral soil) temperatures (C°) for three SAD levels (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%). Error bars represent ± SEM. Different letters indicate significance at $p \leq 0.05$ determined by a Kruskal Wallis test followed by Mann–Whitney tests to analyze rank differences among SAD levels for specific surface or subsurface temperatures. Alpha levels were adjusted by the number of pairwise comparisons using a Bonferroni correction. N=7/SAD level.

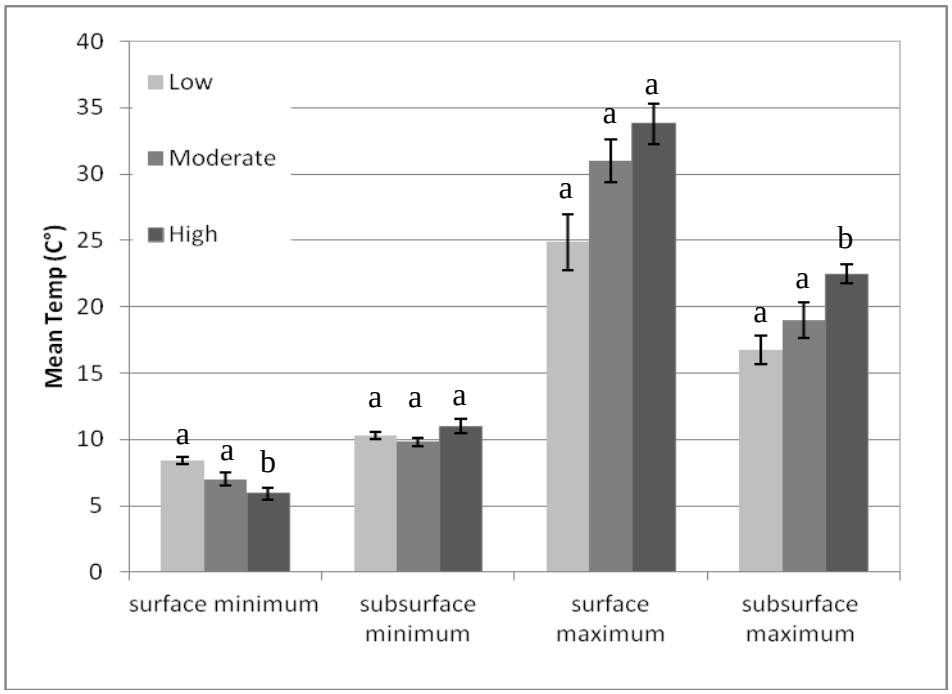

Figure 4. Average minimum and maximum soil surface and soil subsurface (3 cm mineral soil) temperatures (C°) for three SAD levels (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%). Error bars represent ± SEM. Different letters indicate significance at $p \leq 0.05$ determined by a Kruskal Wallis test followed by Mann–Whitney tests to analyze rank differences among SAD levels for specific surface or subsurface temperatures. Alpha levels were adjusted by the number of pairwise comparisons using a Bonferroni correction. N=7/SAD level.

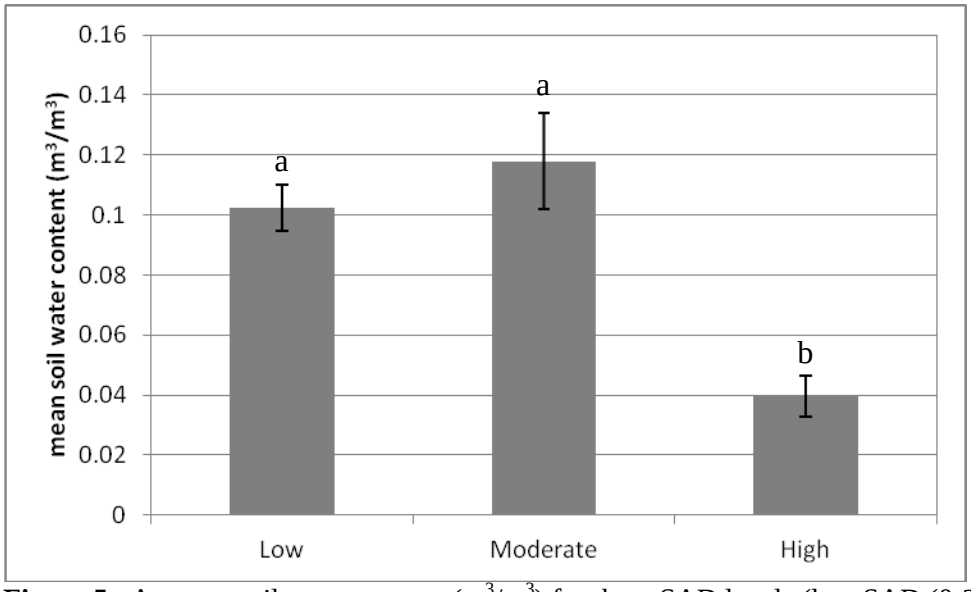

Figure 5. Average soil water content (m^3/m^3) for three SAD levels (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%) and an aspen harvest treatment. Error bars represent ± SEM. Different letters indicate significance at $p \leq 0.05$ determined by a Kruskal Wallis test followed by Mann–Whitney tests to analyze rank differences among SAD levels. Alpha levels were adjusted by the number of pairwise comparisons using a Bonferroni correction. N=3/SAD level.

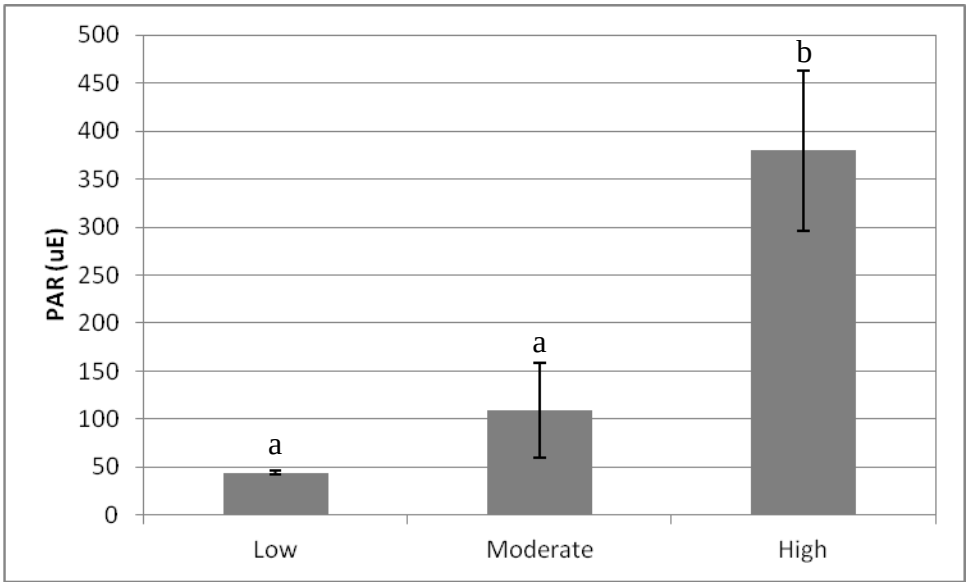

Figure 6. Average PAR (μE) for three SAD levels (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%). Error bars represent ± SEM. Different letters indicate significance at $p \leq 0.05$ determined by a Kruskal Wallis test followed by Mann–Whitney tests to analyze rank differences among SAD levels. Alpha levels were adjusted by the number of pairwise comparisons using a Bonferroni correction. N=3/SAD level.

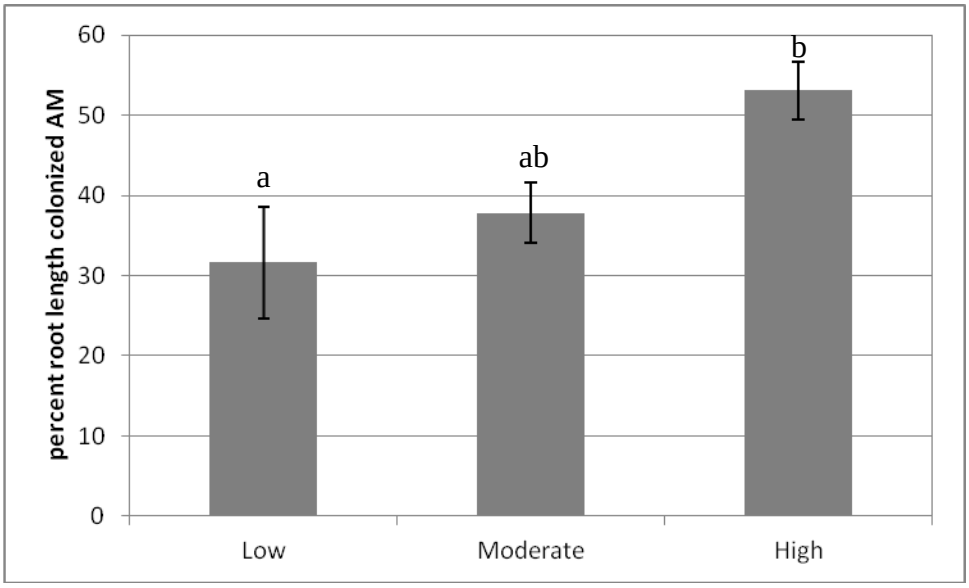

Figure 7. Average percent root length colonized of arbuscular mycorrhizae for three SAD levels (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%). Error bars represent ± SEM. Different letters indicate significance at $p \leq 0.05$ determined by a Kruskal Wallis test followed by Mann–Whitney tests to analyze rank differences among SAD levels. Alpha levels were adjusted by the number of pairwise comparisons using a Bonferroni correction. N=10/SAD level. There were no significant differences between years for individual SAD levels.

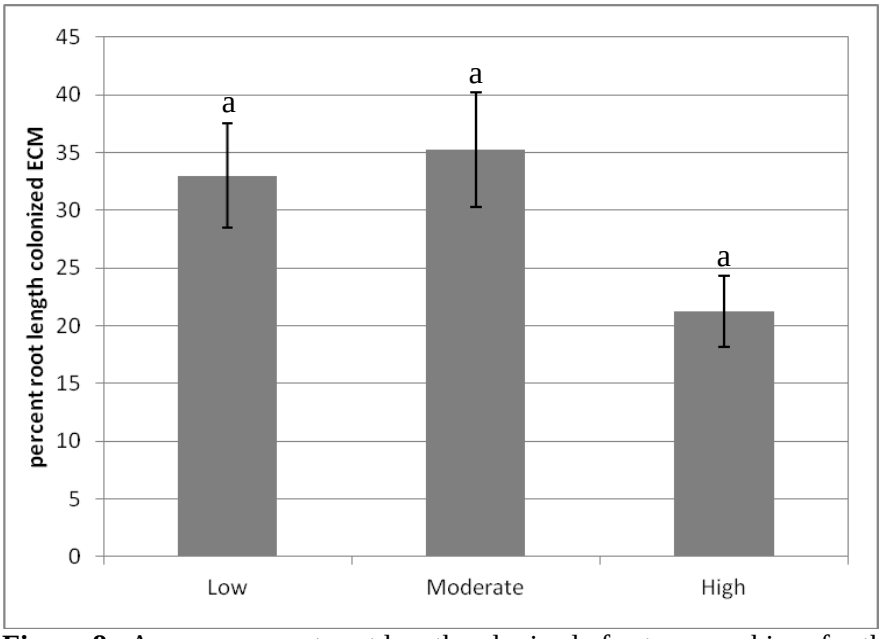

Figure 8. Average percent root length colonized of ectomycorrhizae for three SAD levels (low mortality (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%). Error bars represent ± SEM. Different letters indicate significance at $p \leq 0.05$ determined by a Kruskal Wallis test followed by Mann–Whitney tests to analyze rank differences among SAD levels. Alpha levels were adjusted by the number of pairwise comparisons using a Bonferroni correction. N=10/SAD level. There were no significant differences between years for individual SAD levels.

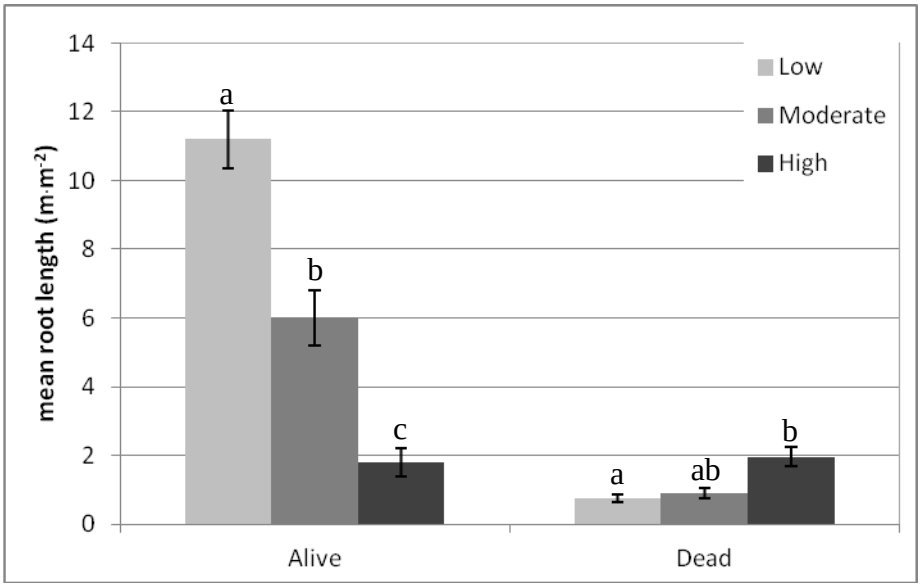

Figure 9. Average alive and dead aspen root lengths associated with three SAD levels (low mortality (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%). Error bars represent ± SEM. Different letters indicate significance at $p \leq 0.05$ determined by a Kruskal Wallis test followed by Mann–Whitney tests to analyze rank differences among SAD levels for specific root (alive/dead) categories. Alpha levels were adjusted by the number of pairwise comparisons using a Bonferroni correction. N=7/SAD level.

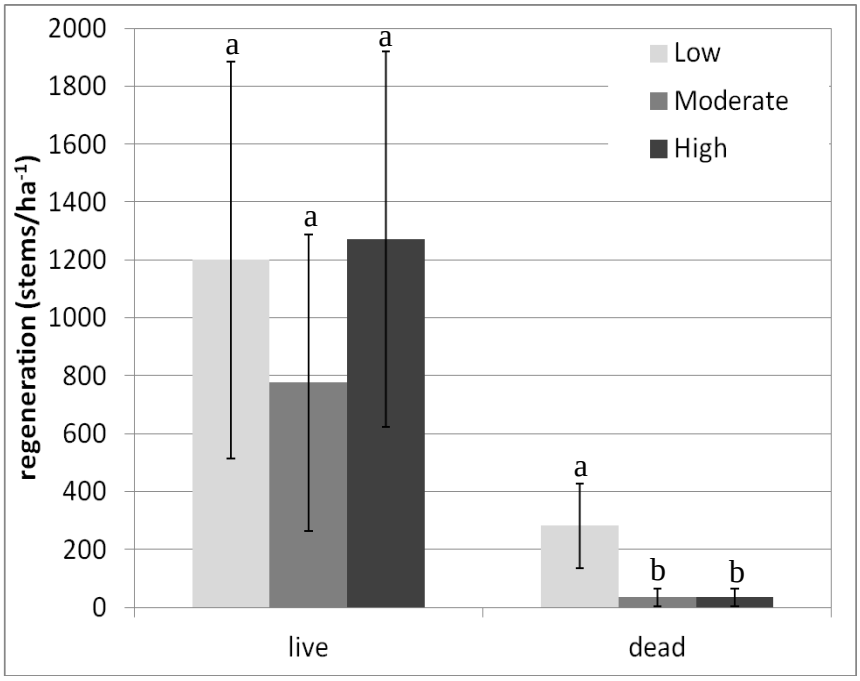

Figure 10. Average live and dead aspen (≥ 30.5 cm tall and <12.7 cm DBH) regeneration (stems/ha^{-1}) for three SAD levels (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%). Error bars represent ± SEM. Different letters indicate significance at $p \leq 0.05$ determined by a Kruskal Wallis test followed by Mann–Whitney tests to analyze rank differences among SAD levels for specific (live/dead) categories. Alpha levels were adjusted by the number of pairwise comparisons using a Bonferroni correction. N=7/SAD level.

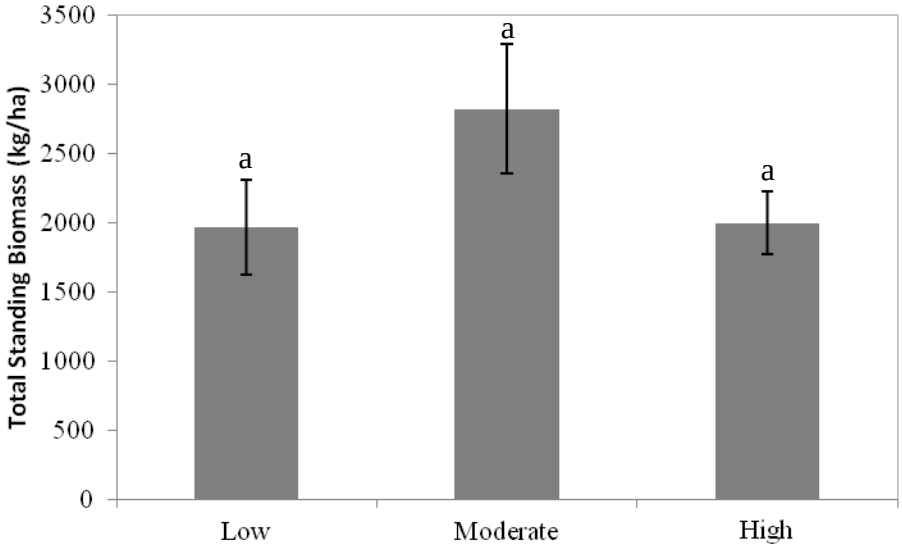

Figure 11. Total standing understory plant biomass for three SAD levels (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%). Error bars represent ± SEM. Different letters indicate significance at $p \leq 0.05$ determined by a Kruskal Wallis test followed by Mann–Whitney tests to analyze rank differences among SAD levels. Alpha levels were adjusted by the number of pairwise comparisons using a Bonferroni correction. N=7/SAD level.

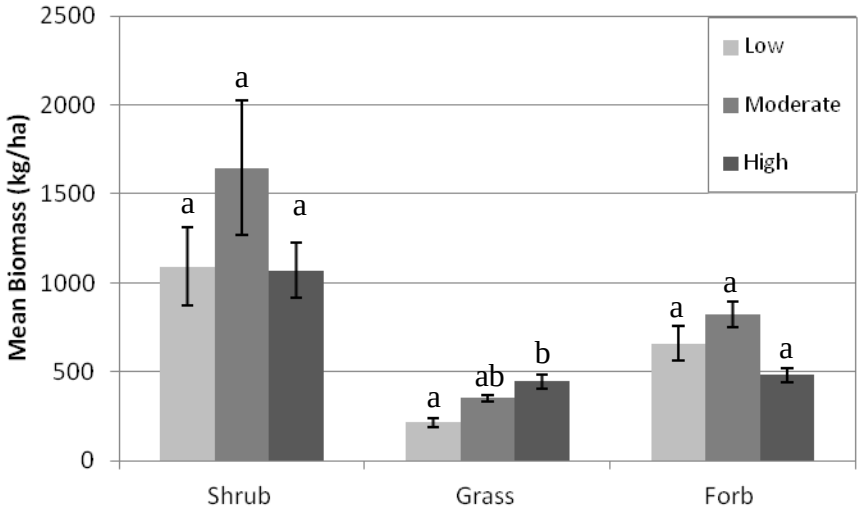

Figure 12. Average plant biomass by species functional groups for three SAD levels (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%). Error bars represent ± SEM. Different letters indicate significance at $p \leq 0.05$ determined by a Kruskal Wallis test followed by Mann–Whitney tests to analyze rank differences among SAD levels for specific functional groups. Alpha levels were adjusted by the number of pairwise comparisons using a Bonferroni correction. N=7/SAD level.

Table 1. Mean stand and abiotic variables for three SAD levels (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%). Error bars represent ± SEM. Different letters indicate significance at $p \leq 0.05$ determined by a Kruskal Wallis test followed by Mann–Whitney tests to analyze rank differences among SAD levels. Alpha levels were adjusted by the number of pairwise comparisons using a Bonferroni correction. N=20/ level. Tree status evaluated based on the US Forest Service tree status scale (1 = live, 2 = declining, 3 = recent snag, 4 = loose bark snag, 5 = clean snag, 6 = broken above breast height, 7 = broken below breast height).

	Low SAD	Moderate SAD	High SAD
Stand Variables			
Basal area (m^2/ha^{-1})	54.01 ± 5.05^{a}	39.35 ± 2.32^{b}	41.32 ± 3.15^{b}
DBH (centimeters)	17.19 ± 0.97^{a}	18.55 ± 0.93^{ab}	21.74 ± 0.97^{b}
Litter/duff (cm)	2.7 ± 0.33^{a}	2.3 ± 0.26^{ab}	1.7 ± 0.20^{b}
Shrub cover (%)	29.45 ± 4.12^{a}	32.33 ± 4.72^{a}	27.26 ± 3.43^{a}
Stand density (stems/ha^{-1})	1887.26 ± 136.69^{a}	1248.27 ± 130.59^{b}	1069.94 ± 84.91^{b}
Tree canopy cover (%)	81.54 ± 2.00^{a}	59.54 ± 5.00^{b}	33.10 ± 4.00^{c}
Tree height (meters)	11.96 ± 0.52^{a}	11.78 ± 0.50^{a}	13.45 ± 0.80^{a}
Tree status	1.46 ± 0.05^{a}	2.43 ± 0.08^{b}	3.51 ± 0.08^{c}
Site Variables			
Elevation (m)	2857.9 ± 8.52^{a}	2803.5 ± 20.91^{b}	2735.21 ± 11.2^{c}
Slope (%)	5.53 ± 0.67^{a}	5.42 ± 0.96^{a}	4.41 ± 0.58^{a}

Table 2. Multiple regression model for RCL (recent crown loss). N=60; R^2=68.7% (^{F3,36}=44.08, $P \leq 0.000$).

Factor	**Parameter estimate**	**Standard error**	**t-ratio**	**P-value**
Bronze poplar borer	0.243	0.95	2.562	0.013
Poplar borer	0.288	0.94	3.071	0.003
Elevation	-0.018	0.008	-2.329	0.023

Table 3. Insect and disease incidence for three SAD levels (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%). Error bars represent ± SEM. Different letters indicate significance at $p \leq 0.05$ determined by a Kruskal Wallis test followed by Mann–Whitney tests to analyze rank differences among SAD levels. Alpha levels were adjusted by the number of pairwise comparisons using a Bonferroni correction. N=20/ level.

	Low SAD	Moderate SAD	High SAD
Armillaria root rot	2.19 ± 1.26^{a}	4.33 ± 1.39^{a}	3.23 ± 1.29^{a}
Bark beetles	6.91 ± 2.0^{a}	21.76 ± 4.07^{b}	52.14 ± 6.59^{c}
Black canker	0.63 ± 0.39^{a}	2.5 ± 2.5^{a}	0.58 ± 0.40^{a}
Bronze poplar borer	9.69 ± 3.64^{a}	29.35 ± 5.33^{b}	73.32 ± 3.12^{c}
Cytospora canker	44.43 ± 7.44^{ab}	56.09 ± 8.74^{b}	75.89 ± 5.87^{bc}
Poplar borer	13.33 ± 3.74^{a}	30.09 ± 5.15^{b}	68.25 ± 5.41^{c}
Sooty-bark canker	2.03 ± 0.87^{a}	3.86 ± 1.67^{a}	2.71 ± 1.10^{a}
White trunk rot	2.63 ± 0.96^{ab}	0.78 ± 0.44^{a}	6.43 ± 2.54^{b}

Table 4. Pearson correlation coefficients of insect and disease incidence among each other. There were no significant correlations of black canker with any damage agents and therefore it is not listed. *=P≤0.05, **=P≤0.01, ***=P≤0.001.

	Bronze borer	Cytospora canker	Poplar borer	White root rot
Cytospora canker	0.438***			
Poplar borer	0.818***	0.612***		
Bark beetles	0.762***	0.411**	0.624***	
White root rot	0.272*		0.317*	
Armilliaria root rot		0.299*		
Sooty-bark canker				0.255*

Table 5. Significant Pearson correlation coefficients of insects and diseases with forest stand and site variables. *=P≤0.05, **=P≤0.01, ***=P≤0.001.

Variable	Armillaria root rot	Bronze poplar	Bark beetles	Cytospora canker	Poplar borer	Sooty-bark canker	White root rot
Aspect					-0.241*		
Basal area/ha^{-1}		-0.426***	-0.385**		-0.401*		
DBH		0.379**	0.370**	0.354**	0.316*		
Elevation		-0.587***	-0.452**		-0.526***		
Litter/duff		-0.401***	-0.347**	-0.370**	-0.414***	-0.292*	-0.272*
Recent crown loss		0.784***	0.665***	0.520***	0.781***		
Root mortality		0.412***	0.328**	0.286*	0.408***		
Shrub cover						-0.300*	
Tree density/ha^{-1}		-0.569***	-0.519***	-0.278*	-0.525***		
Tree slenderness	-0.248*	-0.399**		-0.407***	-0.381**	-0.249*	
Tree height						-0.330**	

Table 6. Average insect and disease species richness and Shannon Weiner Diversity Index for three SAD levels (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%). Error bars represent ± SEM. Different letters indicate significance at $p \leq 0.05$ determined by a Kruskal Wallis test followed by Mann–Whitney tests to analyze rank differences among SAD levels. Alpha levels were adjusted by the number of pairwise comparisons using a Bonferroni correction. N=20/ level.

	Species Richness	**Shannon Diversity**
Low	4.1 ± 0.35^{a}	1.015 ± 0.11^{a}
Moderate	4.8 ± 0.17^{a}	1.325 ± 0.04^{b}
High	4.8 ± 0.27^{a}	1.408 ± 0.04^{b}

Table 7. PERMANOVA based on Bray-Curtis dissimilarities of insect/pathogen frequency data associated with three SAD levels (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%).

Source	**df**	**MS**	**F**	**P (perm)**
SAD Level	2	1.409	13.159	0.0002
Residual	57	0.107		
Total	59			

Table 8. Pearson's r correlation coefficients of stand structure and abiotic variables from the main matrix for insect/pathogens along NMS ordination axes. There were no strong correlation coefficients with Axis 2.

	Attribute	**Correlation Coefficient**
Axis 1 (SAD level)		
Positive	DBH (cm)	0.481
Negative	Tree canopy cover	-0.743
	Trees/ha^{-1}	-0.647
	Elevation	-0.511
	Litter/duff	-0.463
	Basal area/ha^{-1}	-0.371

Table 9. Indicator insects and diseases associated with three SAD levels (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%). There were no indicator insects/pathogens for low or moderate SAD levels.

SAD Level	**Species**	**Indicator value**	**P value**
High	Bronze poplar borer	62.2	0.0002
High	Bark beetles	59.1	0.0002
High	Poplar borer	55.7	0.0002
High	Cytospera canker	40.6	0.0440

Table 10. Significant Pearson correlation coefficients of arbuscular and ecto-mycorrhizae with forest stand and site variables. *=P≤0.05, **=P≤0.01, ***=P≤0.001.

Variable	Arbuscular mycorrhizae	Ectomycorrhizae
Diurnal surface temperature	0.489*	-0.441*
Surface minimum temperature	0.470*	-0.470*
Surface maximum temperature		-0.458*
Subsurface minimum temperature	0.556**	
Subsurface maximum temperature	0.647***	
PAR	0.821***	-0.567**
Trees/ha^{-1}		0.546*
DBH	0.493**	-0.616**
Dead root length	0.821***	-0.567**

Table 11. Average live and dead aspen (≥ 30.5 cm tall and <12 cm DBH) regeneration categorized into no browse or browse (stems/ha^{-1}) for three SAD levels (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%). Error bars represent ± SEM. Different letters indicate significance at p≤0.05 determined by a Kruskal Wallis test followed by Mann–Whitney tests to analyze rank differences among SAD levels for a specific regeneration category. Alpha levels were adjusted by the number of pairwise comparisons using a Bonferroni correction. N=7/ level.

	Live/no browse	**Live/browse**	**Dead/no browse**	**Dead/browse**
Low	353.1 ± 177.7^{a}	847.2 ± 554.8^{a}	105.9 ±73.5^{a}	176.5 ± 88.8^{a}
Moderate	317.7 ± 317.7^{a}	458.9 ± 325.5^{a}	0 ± 0^{b}	35.2 ± 35.2^{a}
High	635.4 ± 410.2^{a}	635.4 ± 340.4^{a}	0 ± 0^{b}	0 ± 0^{a}

Table 12. Significant Pearson correlation coefficients of functional group understory biomass with forest stand and site variables. *=P≤0.05, **=P≤0.01, ***=P≤0.001.

Variable	Grass Biomass	Forb Biomass	Shrub Biomass
Diurnal surface temperature	0.521**		
Subsurface maximum temperature	0.531**		
PAR	0.541**		
RCL	-0.453*		
Bare soil cover	0.521*		
Total dead aspen regeneration		0.619**	
Litter cover		0.664***	
Trees/ha^{-1}			0.411*

Table 13. Average understory vegetation species richness and Shannon Weiner Diversity Index for three SAD levels (low SAD (0-25%), moderate SAD (25.1-50%) and high SAD (50.1-100%). Error bars represent ± SEM. Different letters indicate significance at $p \leq 0.05$ determined by a Kruskal Wallis test followed by Mann–Whitney tests to analyze rank differences among SAD levels. Alpha levels were adjusted by the number of pairwise comparisons using a Bonferroni correction. N=20/ level.

	Species Richness	**Shannon Diversity**
Low	21.4 ± 1.88^{a}	2.091 ± 0.146^{a}
Moderate	19.3 ± 0.51^{a}	1.906 ± 0.173^{a}
High	19.6 ± 0.89^{a}	1.907 ± 0.115^{a}

Table 14. Understory vegetation indicator species associated with three SAD levels (low mortality (0-29.9%), moderate mortality (30-69.9%) and high mortality (70-100%). There were no indicator species for moderate SAD levels.

SAD Level	**Species**	**Indicator value**	**P value**
Low	*Osmorrhiza occidentalis*	49.6	0.002
High	*Mahonia repens*	78.8	0.001
High	*Campanula rotundifolia*	57.1	0.017

Table 15. PERMANOVA based on Bray-Curtis dissimilarities of understory vegetation associated with three SAD levels ((low mortality (0-29.9%), moderate mortality (30-69.9%) and high mortality (70-100%).

Source	**df**	**MS**	**F**	**P (perm)**
SAD Level	2	0.252	1.534	0.1080
Residual	18	0.164		
Total	20			