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Original Contribution

The Interplay of Plant and Animal Disease in a Changing Landscape: The Role of Sudden Aspen Decline in Moderating Sin Nombre Virus Prevalence in Natural Deer Mouse Populations

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Abstract: We examined how climate-mediated forest dieback regulates zoonotic disease prevalence using the relationship between sudden aspen decline (SAD) and Sin Nombre virus (SNV) as a model system. We compared understory plant community structure, small mammal community composition, and SNV prevalence on 12 study sites within aspen forests experiencing levels of SAD ranging from $<10.0\%$ crown fade to $>95.0\%$ crown fade. Our results show that sites with the highest levels of SAD had reduced canopy cover, stand density, and basal area, and these differences were reflected by reductions in understory vegetation cover. Conversely, sites with the highest levels of SAD had greater understory standing biomass, suggesting that vegetation on these sites was highly clustered. Changes in forest and understory vegetation structure likely resulted in shifts in small mammal community composition across the SAD gradient, as we found reduced species diversity and higher densities of deer mice, the primary host for SNV, on sites with the highest levels of SAD. Sites with the highest levels of SAD also had significantly greater SNV prevalence compared to sites with lower levels of SAD, which is likely a result of their abundance of deer mice. Collectively, results of our research provide strong evidence to show SAD has considerable impacts on vegetation community structure, small mammal density and biodiversity and the prevalence of SNV.

Keywords: hantavirus, Sin Nombre virus, sudden aspen decline, deer mice, *Peromyscus maniculatus*, forest dieback, climate change

Introduction

Since the middle of the twentieth century, the incidence of human infection with newly emerging infectious diseases has increased dramatically, and disturbance associated with climate change is considered to be a primary driver of this pattern (Jones et al. 2008). This increased incidence of disease has been coincident with a measurable decline in global forests over the same time period. Increased tree mortality has occurred in woodland and savanna ecosystems worldwide, including southern Europe, Africa, Asia, Australia, South America, Central America, and North America and is thought to result from the combined impacts of higher temperatures and reduced precipitation

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(see Allen et al. 2010 for review). Within western North America, forest dieback associated with sudden aspen decline (SAD) is of particular concern. SAD first appeared as a phenomenon in Canada in the early 1990s (Peterson and Peterson 1992) and was reported Arizona and southern Utah as early as 2002 (Ohms 2003; Fairweather 2008). By definition, SAD is the death of a mature aspen (*Populus tremuloides*) overstory without subsequent regeneration and is differentiated from normal successional patterns because of how quickly the decline occurs (Worrall et al. 2010). While the typical lifespan of a healthy aspen tree is about 110 years, SAD kills more than 90% of aspen in a stand, including young trees, within a 3- to 6-year period without subsequent regeneration (Peterson and Peterson 1992). Dieback with limited to no regeneration reduces the sustainability of the aspen clone as a whole, a phenomenon with potentially grave implications for the persistence of aspen on the landscape (Frey et al. 2004). The exact cause of SAD is unknown, but it is thought to be the result of long-term drought stress, coupled with secondary biological agents such as Cytospora canker, aspen bark beetles, poplar borers, and bronze poplar borers (van Mantgem et al. 2009; Worrall et al. 2008). The cumulative impact of these biotic and abiotic stressors results in rapid, synchronous branch dieback, crown thinning, and death of aspen stems at a landscape scale (Worrall et al. 2010). This rapid breakup of aspen cover has numerous ecological implications, as openings in the tree canopy alter microclimate by increasing wind exposure, sunlight penetration and evaporation stresses, resulting in further aspen dieback (Peterson and Peterson 1992). Death of aspen stems results in additional deposition of coarse woody debris on the forest floor, as well as shifts in species dominance in the understory vegetation community (Frey et al. 2004). Because aspen forests typically support a higher biodiversity of small mammals compared to other sympatric forest types (Holroyd and Van Tighem 1983; Oaten and Larsen 2008), changes in small mammal community composition associated with SAD are likely to be particularly severe.

A number of studies have documented substantial changes in community structure across trophic levels that result from forest dieback, demonstrating that rapid changes in forest canopy cover propagate fundamental changes in the composition of both plant and animal species that reside within the forest understory (Allen et al. 2010). Such changes in animal community dynamics could influence the prevalence of zoonotic diseases (i.e., diseases that can be transmitted from animals to humans). In natural host systems, the prevalence of zoonotic disease is regulated by complex community dynamics. As such, alterations in trophic structure are thought to have influenced patterns of host infection in a number of zoonotic disease systems. Terrestrial disease systems represent both "top-down" and "bottom-up" trophic cascades, in which shifts in the number of disease hosts alter vegetation structure, or shifts in vegetation structure alter the number of hosts, respectively (Collinge et al. 2005; Stapp 2007; Getz 2009). A "bottom-up" trophic cascade was used to explain the emergence of Sin Nombre virus (SNV), a North American hantavirus, among residents of the western US in the 1990s. In this scenario, increased precipitation associated with an El Niño Southern Oscillation (ENSO) event caused an increase in primary productivity, which was followed by increased rodent densities and an associated increase in SNV prevalence among deer mice (*Peromyscus maniculatus*), the primary host. This increased the likelihood of human encounters with infected mice and transmission of SNV to humans (Yates et al. 2002). Subsequent studies have supported these findings and have also shown that SNV prevalence is affected by temperature, precipitation, and vegetation structure (Calisher et al. 2002; Lehmer et al. 2008; Dearing et al. 2009). Collectively, the body of previous research demonstrates that SNV is a pathogen whose prevalence is highly regulated by perturbations in the natural environment of the host.

Transmission of SNV among deer mice is believed to occur through transfer of bodily fluids, likely resulting from aggressive interactions, such as biting, as evidenced by strong correlations between infection and external scarring (Calisher et al. 2007). In deer mice, SNV infections have an initial acute phase followed by a persistent phase that is maintained for the life of the animal (Botten et al. 2000). Although deer mice infected with SNV appear largely asymptomatic (Botten et al. 2003), SNV infections cause a chronic response of the immune system in that, once infected, deer mice produce detectable titers of neutralizing virus-specific antibodies for life (Herbst et al. 2001; Botten et al. 2003). Infected deer mice shed virus in urine, saliva, and possibly feces (Otteson et al. 1996), and SNV transmission to humans occurs most commonly via inhalation of aerosolized virus contained in deer mouse excrement (Doyle et al. 1998). The pattern of SNV infection in humans differs from that observed in deer mice, as infected humans can develop hantavirus pulmonary syndrome (HPS), a disease with exceptionally high (~38%) rates of mortality (Hjelle et al. 1994; Kilpatrick et al. 2004).

Humans that survive the infection clear the virus within a couple of months and are thus not considered reservoirs for SNV. Increased incidence of HPS in humans is correlated with high densities of deer mice both locally (in peridomestic settings) and regionally (Childs et al. 1997; Yates et al. 2002). HPS poses a particularly significant human health risk in the Four Corners region of the southwestern US, as this area has by far the highest reported number of HPS cases in North America (CDC 2009).

Our goal was to examine how forest dieback regulates zoonotic disease prevalence using the relationship between SNV and SAD as a model study system. Our primary objectives in this study were to (1) evaluate how SAD-induced changes in forest and understory vegetation structure alter small mammal population structure and demography and (2) determine how SAD-induced changes in small mammal biodiversity alter SNV prevalence.

Materials and Methods

Study Sites and Sampling Seasons

Our study sites were located in the San Juan National Forest (SJNF) in southwestern Colorado (Lat. = 37′30″N, Long. = 108′12″W; Fig. 1). SAD was first noted within our study area in 2004 (Worrall et al. 2008) and as of 2008 estimates, this portion of the SJNF had the largest percentage of aspen cover loss due to SAD (~10%) of any other location in Colorado (Worrall et al. 2010). Study sites were sampled from June 1 to July 15 in 2009 and 2010. Sampling periods were selected to coincide with the earliest dates that sites would be accessible due to snowpack and after spring green-up.

To identify appropriate study sites, we employed a stratified random sampling design by initially selecting 60

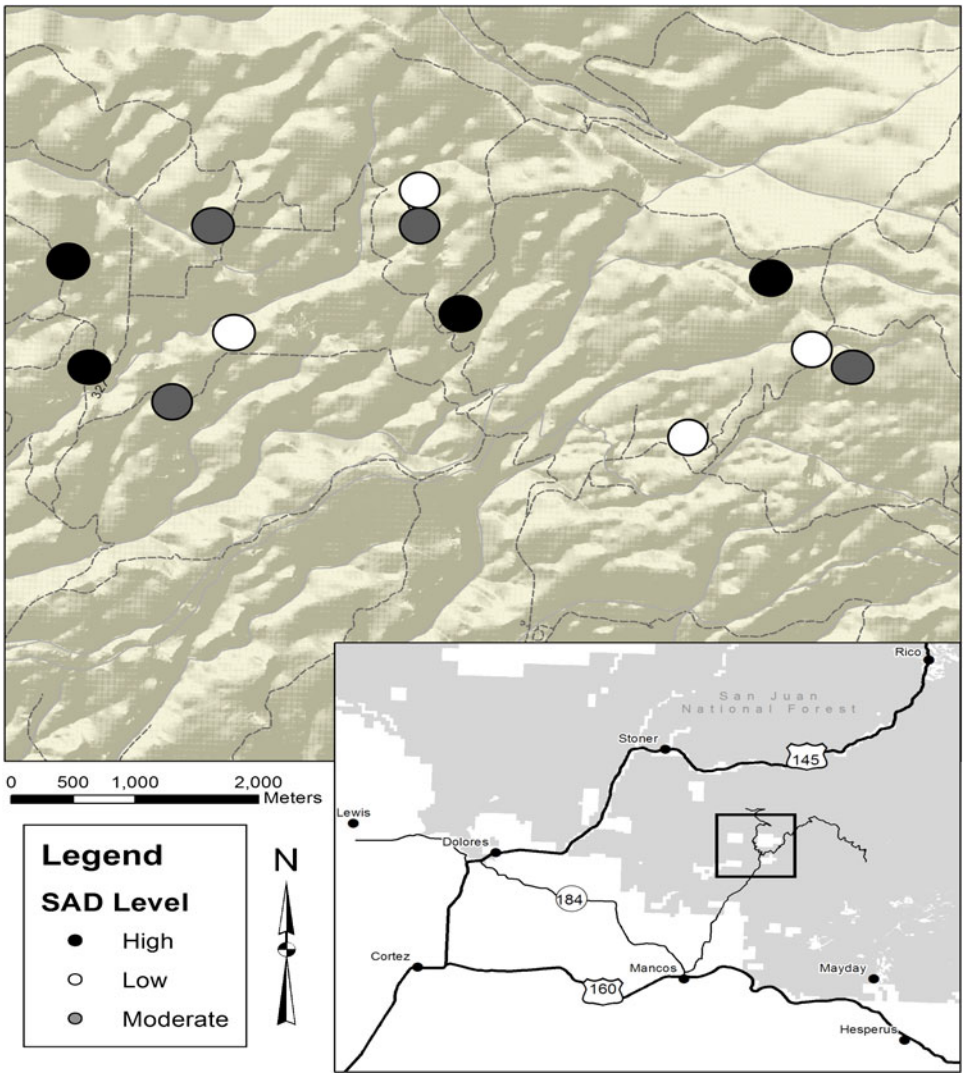

Figure 1. Study area. All study sites occurred within the Mancos-Dolores Ranger District of the San Juan National Forest (SJNF) in southwestern Colorado, USA.

stands composed of >95% aspen overstory of relatively similar age, as determined by stand structure, and to maintain homogeneity between stands we controlled for slope, aspect, and elevation (2,600–3,000 m). Stands were defined as any area of contiguous aspen forest with relative similarities in tree species composition and height. We then randomly located a total of 60 sample points within the stratified stands to establish a plot center. Each plot center was ≥100 m from the stand perimeter to reduce the influence of edge habitat on our results, defined as any transition into habitat that did not have similar characteristics to those that defined a stand. We considered transitions to immature aspen stands to be edge. We permanently monumented all plot centers and recorded geographic coordinates.

SAD Intensity and Forest Structure Assessment

SAD assessments involved establishing one (202 m^2 radius) circle plot within each of our 60 candidate stands to record forest overstory data at the plot center. In this process, we first assessed the percentage of SAD, defined as the average tree crown fade/plot, within each circle plot. We assessed crown fade by ocular estimation of the percentage of crown loss in individual trees. Based on crown fade for all trees in each circle plot, we classified each study site (average tree crown fade/plot) into one of three categories: (1) low SAD (0.0–29.9%), (2) moderate SAD (30.0–69.9%), and (3) high SAD (70.0–100.0%), based upon standards in the literature (Michaelian et al. 2011; Worrall et al. 2010). Following our assessment of SAD intensity, we measured tree diameter at breast height (cm) and tree height (m) within each circle plot. All stems with a height ≥1.37 m and with a diameter at breast height ≥5 cm were classified as trees (USFS 2007). We then used a 50-m transect intersecting the circle plot center to record tree canopy cover using a densiometer every 3 m along the transect for a total of 16 points. A subset of 12 of these 60 plots was randomly selected for intensive survey, including four replicates for low, moderate, and high SAD, based upon percentage of crown fade. These 12 study sites consisted of 10,000 m^2 sampling areas oriented around the plot centers and were used for understory vegetation sampling, biomass assessments, ambient temperature measurements, small mammal trapping, and SNV prevalence estimates.

Understory Vegetation Sampling

Cover of understory vegetation was assessed within each 10,000 m^2 study site in a standard grid pattern consisting of 100 sampling locations distributed across ten 100 m transects. In this process, we estimated the relative percent cover of forbs, graminoids, shrubs, litter, rock, and bare ground occurring within a 2 m × 0.5 m sampling frame using an ocular estimate to the nearest 1%. Total cover was estimated as the sum of forb, graminoid, shrub, and litter cover for each sampling frame. For each frame, total cover could not exceed 100%. Understory vegetation sampling was timed to coincide with small mammal trapping on each study site and was conducted prior to understory standing biomass assessments.

Understory Standing Biomass Assessments

From June 15 to June 30, 2010, we quantified understory vegetation standing biomass using traditional destructive oven-dried biomass clipping methodology (Cornelissen et al. 2003). On each study site, biomass assessments were conducted after both understory vegetation sampling and small mammal trapping had been completed. Briefly, we randomly selected 20 plots (0.5 m × 2.0 m) within each 10,000 m^2 study site and clipped all aboveground vegetation to ground level. Litter occurring at ground level was also collected. Vegetation was sorted into functional groups (forbs, grasses, shrubs, litter); standing dead material was grouped with living functional group counterparts. Total standing biomass was estimated as the sum of forb, grass, shrub, and litter biomass for each plot. Within 24 h of clipping, we dried vegetation in a forced air oven at 65°C for 48 h and weighed to quantify oven-dried biomass. Biomass is reported as kg/ha ± SE.

Ambient Temperature Measurements

To monitor the effect of SAD on microclimate, we conducted fine time scale measurements of air temperature on each of our 12 study sites. We installed four iButton temperature loggers (Maxim Integrated Products, Inc., Sunnyvale, CA), at the plot center of each study site; two loggers were placed in locations that were in full shade at soil surface at 12:00 pm and two loggers were placed in locations that were in full sunlight at soil surface at 12:00 pm. All loggers were equipped with plastic solar radiation shields. Loggers were programmed to record air temperatures every 30 min continuously from June 15 to July 30, 2010. We characterized daily mean, minimum, and maximum ambient air temperature values from average hourly data.

Small Mammal Trapping

At each of our 12 study sites, nocturnal small mammals were live-trapped (H.B. Sherman Traps, Tallahassee, FL) over multiple, consecutive nights that occurred between June 10 and June 30 during each year of the study using standard 10,000 m^2 grids consisting of 100 traps distributed across ten 100 m transects. Locations of the trapping grids within each study site did not change between 2009 and 2010. To ensure that the small mammal population at each site was exhaustively sampled, trapping continued during each year of the study until recapture rates exceeded 90%, which was never >4 nights. High recapture rates provide statistical confidence in estimating SNV prevalence on sites with few deer mice. The protocol for small mammal trapping involved setting baited traps in the evening and checking traps by 8:00 am the following morning. After capture, animals were anesthetized using isoflurane, identified to species, weighed, sexed, and marked with uniquely numbered ear tags (National Band and Tag Co., Newport, KY). We collected a small blood sample ($\sim$0.2 mL) from the retro-orbital sinus of all deer mice upon initial capture of each sampling season. Blood was immediately stored on ice for use in later SNV antibody assays. After processing, all animals were released to sites of capture. Personnel involved in trapping and handling of small mammals followed precautions for working with animals potentially infected with hantavirus (CDC 1995). General techniques for capturing and handling animals were approved by the Animal Care and Use Committee at Fort Lewis College. Small mammal capture data was used to estimate both small mammal species diversity and deer mouse density at each study site. Small mammal species diversity was estimated using the Gini-Simpson Index ($D = 1 - \sum p_i^2$; Gini 1912), which accounts for both species richness (number of species) and evenness (number of individuals within each species) in a population. The Gini-Simpson Index includes values of D that range from 0.0 (least diversity) to 1.0 (maximal diversity). Deer mouse densities were calculated based on capture data from a single trapping season on each study site and in each year of the study. Densities were estimated using the program DISTANCE (version 4.1), a software program designed to more accurately estimate animal densities in grid trapping designs compared to untransformed density values.

SNV Antibody Detection

In the Biosafety Level 2 Laboratory at Fort Lewis College, we performed enzyme linked immunoabsorbent assays (ELISA) to test deer mouse blood samples for antibodies (IgG) against SNV following the protocol described previously by Otteson et al. (1996) and Feldman et al. (1993). Because deer mice continue to produce virus-specific antibodies for life following SNV infection, the presence of SNV antibodies in serum is a reliable indicator of infection (Otteson et al. 1996). SNV prevalence was determined for each site in each season by dividing the total number of SNV-seropositive deer mice by the total number of deer mice captured.

Analyses

Data were checked for normality and preliminary examination showed that residual variation was normally distributed. Proportional data (e.g. SNV prevalence, canopy cover) did not approach upper or lower bounds of the potential distribution; thus, our data did not violate linear modeling assumptions. Differences in forest structure were estimated using separate Generalized Linear Mixed Models (GLMM) for each dependent variable of interest (percent canopy cover, stand density, diameter at breast height, and basal area). In these models, year was treated as a categorical fixed effect, percent crown fade was treated as a continuous fixed effect and site was treated as a random effect. Similar models were used to estimate differences in understory vegetation structure, with cover class variables coded as dependent factors. In these models, data from individual sampling frames were pooled and study sites were treated as experimental replicates. Differences in understory standing biomass across the gradient of SAD were estimated with General Linear Models (GLM), in which cover class variables were coded as dependent factors and crown fade and site were coded as independent factors. For these models, backward stepwise elimination (α to remove = 0.15) was used in an attempt to reduce general models to their most parsimonious version. For our analyses of understory standing biomass, data from individual sampling frames were pooled and study sites were treated as experimental replicates. Differences in ambient temperature across the SAD gradient were also estimated with GLM using backward stepwise elimination, with daily minimum, maximum, and mean air temperatures included as dependent factors and percentage crown fade and site coded as independent factors. As with vegetation data, temperature recordings from separate loggers on each site were pooled (sun and shade pooled separately) and study sites were treated as experimental replicates. Differences in

small mammal community composition and SNV prevalence were estimated using separate GLMMs for each dependent variable of interest (SNV prevalence, deer mouse density, density of infected deer mice, species diversity). In these models, independent factors included year (fixed effect), percentage crown fade (continuous fixed effect), and site (random effect). The relationships between SNV prevalence and both small mammal species diversity and deer mouse density (independent of SAD intensity) were also estimated with GLMM. In these models, SNV prevalence was treated as the dependent variable and independent factors included year (categorical fixed effect), site (random effect), and either species diversity or deer mouse density (continuous fixed effects). Unless otherwise stated, values are presented as mean ± SE of each variable and differences in these variables were considered to be statistically significant if $P \leq 0.05$.

Results

Forest Structure

Impacts of SAD on forest structure were evident across our study sites (Fig. 2). Aspen canopy cover declined with increasing SAD intensity (Fig. 3a; $F_{1,10} = 54.94$, $P < 0.01$) and also differed between the 2 years of the study ($F_{1,10} = 6.47$, $P = 0.03$). Both tree diameter at breast height ($F_{1,10} = 12.40$, $P < 0.01$) and basal area ($F_{1,10} = 6.24$, $P = 0.03$), defined as the area of the forest floor covered by tree stems, declined with increasing SAD intensity, but did not differ between years of the study (diameter at breast height = $F_{1,10} = 0.71$, $P = 0.42$; basal area = $F_{1,10} = 1.36$, $P = 0.27$). Forest stand density, defined as the number of trees/ha, was not influenced by SAD intensity ($F_{1,10} = 0.59$, $P = 0.46$) and did not differ between years of the study ($F_{1,10} = 2.34$, $P = 0.16$).

Understory Vegetation Community

Understory vegetation community structure was strongly influenced by SAD intensity but did not differ between years of the study for any functional group cover class. Grass ($F_{1,10} = 18.73, P < 0.01$), shrub ($F_{1,10} = 9.55, P = 0.01$), and total understory cover (Fig. 3b; $F_{1,10} = 10.75, P < 0.01$) were greatest on sites with the lowest levels of SAD. In contrast, bare ground was greatest on sites with the highest levels of SAD (Fig. 3c; $F_{1,10} = 5.44$, $P = 0.04$). Neither forb ($F_{1,10} = 0.43$, P 0.53) or litter ($F_{1,10} = 1.03, P = 0.33$) cover were influenced by SAD intensity. In addition to differences in vegetation cover across the gradient of SAD, we identified four plant species that were uniquely associated with low SAD ($<$29.9% crown fade) sites: *Ligusticum porteri, Goodyera oblongifolia, Viola adunca*, and *Osmorhiza occidentalis*. All of these species are associated with shaded/moist microsites. In contrast, early successional plant species that are adapted to open disturbed sites were uniquely associated with high SAD ($>$70.0% crown

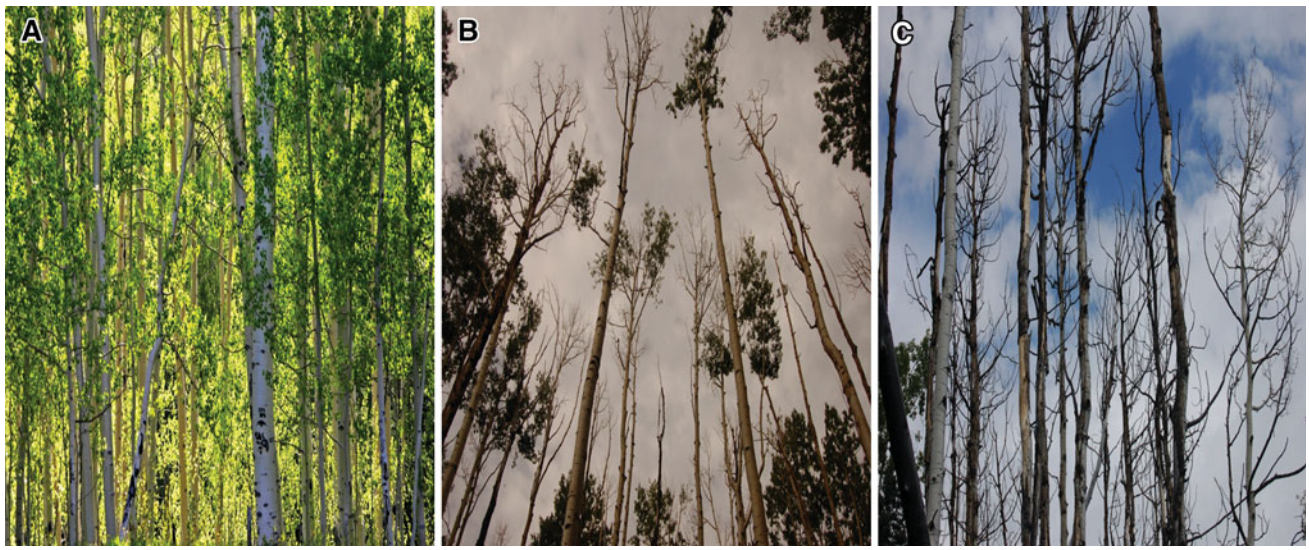

Figure 2. Canopy cover of representative study sites showing low (**a**), moderate (**b**), and high (**c**) levels of sudden aspen decline (SAD). Sites were located in the Mancos-Dolores Ranger District of the San Juan National Forest in the San Juan Mountains of southwestern Colorado, USA and were monitored from June 1 to July 15 in 2009 and 2010. Low SAD sites had 0.0–29.9% stand mortality, moderate SAD sites had 30.0–69.9% stand mortality and high SAD sites had 70.0–100.0% stand mortality.

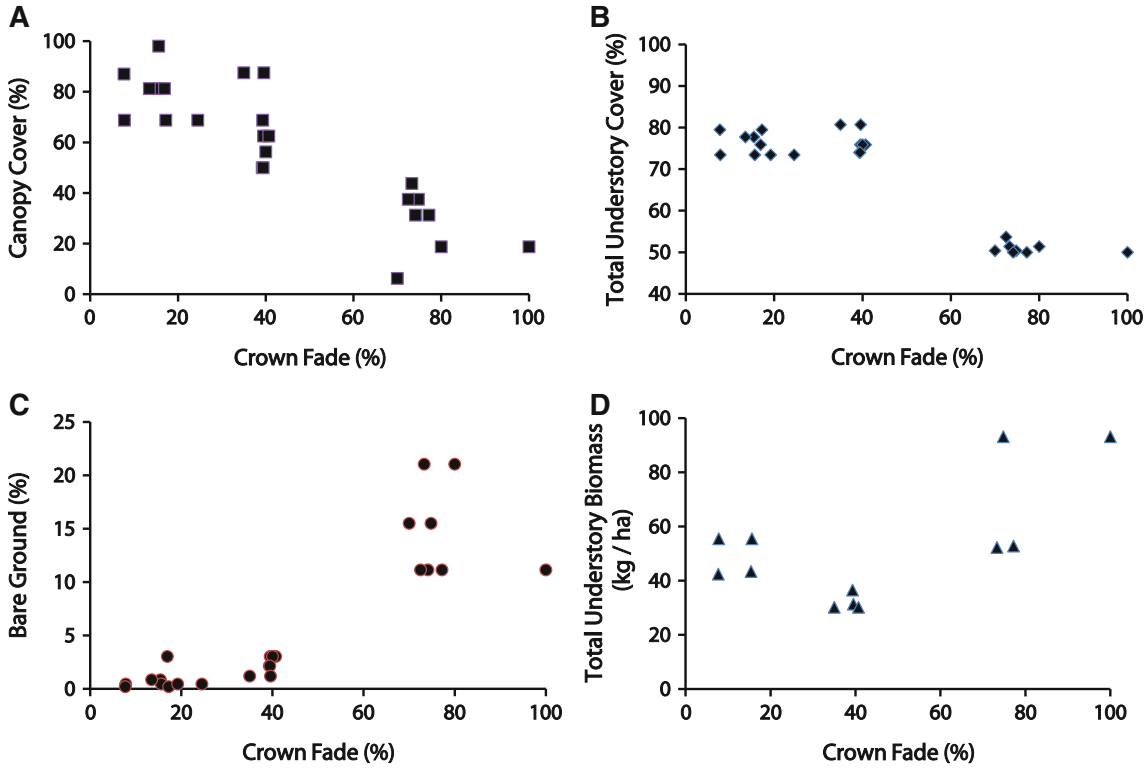

Figure 3. Differences in **a** canopy cover, **b** total understory cover, **c** percentage of bare ground, and **d** total understory biomass measured across a gradient of Sudden Aspen Death in the Mancos-Dolores Ranger District of the San Juan National Forest in the San Juan mountains of southwestern Colorado, USA. Sudden aspen death was estimated as the average percentage of crown fade on each study site (n = 12).

fade) sites: *Achillea lanulosa, Erigeron speciosa, Rosa woodsii, Vicia americana*, and *Thermopsis montana.*

Understory Standing Biomass

In 2010, we found distinct differences in standing understory biomass across the gradient of SAD, with biomass generally increasing for all functional group classes on sites with the highest levels of SAD. Biomass of grasses ($F_{1,10}$ = 8.45, P = 0.02), shrubs ($F_{1,10}$ = 19.92, $P < 0.01$), litter ($F_{1,10}$ = 3.09, P = 0.03), and total biomass (Fig. 3d; $F_{1,10}$ = 7.12, P = 0.02) were greater on sites with the highest levels of SAD. In contrast, biomass of forbs ($F_{1,10}$ = 2.17, P = 0.17) was not influenced by SAD intensity.

Ambient Temperatures

Both shade and sun ambient temperatures varied greatly across the SAD gradient. In both shade and sun locations, sites with the highest levels of SAD sites had greater daily mean temperatures (sun: $F_{1,10}$ = 5.12, $P < 0.01$; shade: $F_{1,10}$ = 3.13, P = 0.01), greater daily maximum temperatures (sun: $F_{1,10}$ = 4.86, $P < 0.01$; shade: $F_{1,10}$ = 5.01.41, $P < 0.01$) and lower daily minimum temperatures (sun: $F_{1,10}$ = 3.69, P = 0.01; shade: $F_{1,10}$ = 2.67 P = 0.04) compared to sites with lower levels of SAD.

Small Mammal Community Composition and SNV Prevalence

Small mammal species diversity varied across the SAD gradient (Fig. 4a; $F_{1,10}$ = 17.86, $P < 0.01$), with considerably lower species diversity on sites with the highest levels of SAD. Sites classified as low (<29.9% crown fade) and moderate (30.0–69.9% crown fade) SAD contained an average of five nocturnal small mammal species, versus an average of two nocturnal small mammal species on high SAD (>70.0% crown fade) sites. Species including deer mice, montane voles (*Microtus montanus)*, long-tailed voles (*M. longicaudus*), western harvest mice (*Reithrodotomys megalotis*), brush mice (*Peromyscus boylii*), montane shrews (*Sorex monticolus*), and dwarf shrews (*S. nanus*) were captured on low and moderate SAD sites, whereas montane voles, deer mice, and harvest mice were captured on high

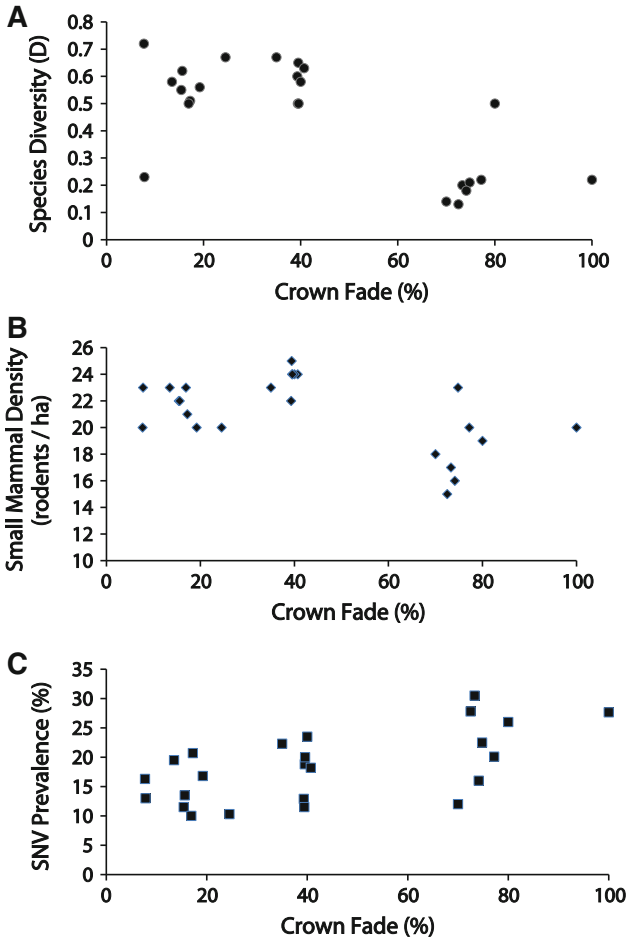

Figure 4. Differences in species diversity (**a**), small mammal density (**b**), and Sin Nombre Virus (SNV) prevalence (**c**) measured across a gradient of Sudden Aspen Death in the Mancos-Dolores Ranger District of the San Juan National Forest in the San Juan mountains of southwestern Colorado, USA. Sudden aspen death was estimated as the average percentage of crown fade on each study site ($n = 12$). Diversity was estimated using the Gini-Simpson Index ($D = 1 - \sum p_i^2$).

SAD sites. Montane voles, western harvest mice, deer mice, and montane shrews were the most abundant species on low and moderate SAD sites, whereas the most abundant species on high SAD sites were deer mice and montane voles. Species diversity did not change between 2009 and 2010 ($F_{1,10} = 0.24$, $P = 0.63$).

Similar to diversity, the density of the small mammal community differed across the SAD gradient (Fig. 4b; $F_{1,10} = 6.85$, $P = 0.03$), with the highest small mammal densities occurring on sites with the highest levels of SAD. Species diversity did not differ across years of the study ($F_{1,10} = 1.38$, $P = 0.27$). The density of deer mice also differed with SAD intensity ($F_{1,10} = 5.43$, $P = 0.04$), with greater numbers of deer mice occurring on sites with higher levels of SAD. It is also noteworthy that, across sites, deer mouse density declined by more than 20% from 2009 to 2010 (2009 mean = 12.8 (± 0.05) mice/ha vs 2010 mean = 10.09 (± 0.06) mice/ha); however, these differences were not statistically significant ($F_{1,14} = 2.36$, $P = 0.08$).

SNV prevalence differed across the gradient of SAD (Fig. 4c; $F_{1,10} = 24.45$, $P < 0.01$), with higher SNV prevalence occurring on sites with greater intensities of SAD. Across sites, SNV prevalence did not differ between 2009 and 2010 ($F_{1,10} = 1.57$, $P = 0.24$). The density of SNV infected deer mice followed a similar pattern ($F_{1,10} = 40.58$, $P < 0.01$), with a higher density of infected deer mice being found on sites with the greatest SAD intensities. The density of infected deer mice did not differ between 2009 and 2010 ($F_{1,10} = 1.02$, $P = 0.27$). SNV prevalence was not influenced by deer mouse density ($F_{1,10} = 0.52$, $P = 0.49$); however, there was a strong negative relationship between small mammal species diversity and SNV prevalence ($F_{1,10} = 15.46$ $P < 0.01$).

Discussion

Increased levels of SAD were associated with reduced canopy cover, stand density, and basal area. These differences in forest structure across the gradient of SAD were reflected in understory vegetation community structure, as plant cover was greatly reduced on sites with the highest levels of SAD. Shifts in understory vegetation structure that we observed were likely driven by reductions in canopy cover, as aspen stands with high levels of SAD have homogeneous, open canopy cover, whereas canopy cover is heterogeneous in stands with lower levels of SAD, with patches of open cover in areas between tree clusters and patches of closed cover within tree clusters. This heterogeneity in forest canopy cover creates diverse understory microsites, including shaded/moist and open/dry microhabitats for understory plant growth (Frey et al. 2004). Our results suggest that the abundance of diverse microsites on sites with low and moderate levels of SAD promoted greater understory species diversity and a greater number of specialist plant species. In contrast, early successional, generalist plant species that are adapted to open disturbed sites were associated with the highest levels of SAD. These early successional species generally form dense cover in disturbed environments and can preclude establishment of

less competitive, specialist plant species in disturbed areas (USDA 2011). In the context of the present study, these patterns are significant because an abundance of generalist understory plant species may preclude the persistence of specialist small mammals on sites where SAD intensity is elevated. Our results seem to support this trend, as sites with the highest levels of SAD had lower small mammal species diversity compared to sites with lower levels of SAD.

Loss of species diversity resulting from climate change is a commonly cited driver of the global trend toward increased pathogen prevalence (Jones et al. 2008; Ostfeld 2009). This assumption is based on the fact that several zoonotic pathogens are capable of infecting a relatively large number of host species, but are transmitted by only a small number of reservoir species. In these systems, high biological diversity can reduce disease spread by either decreasing the abundance of primary reservoirs, thus reducing density-dependent transmission events or by diverting pathogen transmission away from the primary reservoir species toward less competent hosts that act as ''pathogen sinks''. This phenomenon, termed the dilution effect, therefore predicts that high species diversity should be associated with reduced pathogen prevalence (Ostfeld and Keesing 2000a). A number of disease systems (e.g., Lyme disease, West Nile virus) follow predictions of the Dilution Effect hypothesis (Ostfeld and Keesing 2000a, b; Clay et al. 2009a; Gilbert et al. 2001; Keesing et al. 2006) and several studies have documented this pattern in New World hantaviruses. In Central America, for example, the generalist hosts for Choclo and Calabazo viruses, *Oligoryzomys fulvescens* and *Zygodontomys brevicauda*, become dominant in deforested areas where biodiversity is otherwise reduced (Suzan et al. 2008). Experimental removal of non-reservoir species from these systems resulted in increased hantaviral prevalence among competent hosts (Suzan et al. 2009). A negative relationship between biodiversity and infection prevalence also exists for the deer mouse-SNV system (Dizney and Ruedas 2009; Clay et al. 2009a, b) and results of prior studies suggest that as biodiversity is increased, the number of potential SNV-transmitting encounters between individuals in the population is reduced, a phenomenon referred to as ''encounter reduction'' (Suzan et al. 2008; Clay et al. 2009a). Thus, the Dilution Effect hypothesis predicts that biodiversity alters intraspecific contact rates among potential hosts, ultimately influencing disease prevalence in a population. Our preliminary data show distinct differences in biodiversity across the SAD gradient, with low levels of SAD associated with increased plant and small mammal biodiversity and high levels of SAD associated with reduced plant and small mammal biodiversity. Likewise, SNV prevalence was greatest on sites with the high levels of SAD, and we found a strong negative relationship between SNV prevalence and small mammal species diversity. Collectively, these results indicate that the SNV–SAD system follows predictions of the Dilution Effect hypothesis, as it appears that SAD-induced changes in vegetation structure result in shifts in small mammal community composition, likely affecting both inter and intraspecific interactions of deer mice. According to predictions of the Dilution Effect hypothesis, this may have ultimately translated into differences in SNV prevalence across the SAD gradient.

A shift in small mammal community composition towards dominance by generalist species is a common outcome of habitat disturbance and fragmentation (Suzan et al. 2008). This outcome has potentially significant implications for human health, as generalist small mammals are often reservoirs for viruses that cause human disease (Mills 2006). Our results indicate that these patterns are valid for the SAD–SNV system, as both deer mouse densities and SNV prevalence were greatest on sites with the highest levels of SAD. Deer mice are often described as ''quintessential habitat generalists''; they are the most abundant and widely distributed mammal in North America and are known to exist in nearly every ecosystem type in the U.S. (Calisher et al. 1999). Because of this innate behavioral and ecological plasticity, deer mice can persist in even the most highly disturbed areas. Furthermore, deer mice are often displaced from stable ecological communities by more specialized small mammal species, opportunistically taking up residence in communities where interspecific competitive pressures are reduced (Mills 2006). Because these specialist small mammals are not hosts for SNV, such patterns of community assemblage may have important implications for the distribution of deer mice in forest ecosystems affected by SAD, ultimately increasing SNV prevalence.

It is noteworthy that we found greater standing biomass on sites with high levels of SAD, despite these sites having lower vegetation cover and more bare ground compared to low and moderate sites. These patterns suggest that the spatial arrangement of vegetation on sites with high levels of SAD was highly clustered, with small patches of dense understory vegetation interspersed with large areas of bare ground. Because small mammals nest and forage in areas of cover, this spatial orientation of vegetation may

result in animals living at locally higher densities on sites with the highest levels of SAD. According to principles of mass action, higher population densities may result in greater contact rates among deer mice, ultimately promoting transmission of SNV (Dobson and Hudson 1995; Suzan et al. 2008; Clay et al. 2009a.). Although we did not observe a relationship between SNV prevalence and deer mouse density, it is possible that there is a time lag effect in which seasonal increases in density are followed by later increases in SNV prevalence (Aldler et al. 2008; Madhav et al. 2007). This pattern would not be detected over the course of our short sampling season. Increased understory biomass on sites with high levels of SAD is likely the result of widespread reductions in canopy cover, as canopy loss results in increased light availability and higher air and soil temperatures in the understory. Our ambient temperature data appear to support this possibility, as mean daily temperatures increased with higher intensities of SAD. These patterns may have positive impacts on survival and reproductive success of small mammals who reside in the forest understory, as increased biomass translates into increased availability of forage. Life histories of herbivorous small mammals that occur in temperate climates are inherently linked to seasonal increases in plant biomass, as the timing of reproduction in these species typically coincides with increased availability of forage (Bronson and Perrigo 1987). Changes in seasonal availability of forage biomass are thus also linked to seasonal fluctuations in population density, as densities of temperate small mammals typically peak during late summer and fall and decline during winter and early spring (Levin 1976; Van Horne 1982). Although not well studied, extrapolation of patterns resulting from short-term increases in plant biomass suggests that changes in ecosystem structure associated with SAD that result in more permanent shifts in plant productivity may have substantial and long-term impacts on life history dynamics of small mammals and prevalence of zoonotic disease.

In addition to altering understory biomass for a given time period, changes in sun exposure and microclimate associated with SAD may also act to increase the overall length of the growing season for the understory plant community, ultimately increasing the time period over which forage is available to small mammals. Because deer mice are highly opportunistic breeders, shifts in the timing of plant phenology may have important implications for reproduction. Deer mice can be reproductively active for up to 11 months per year if food resources are abundant (Gashwiler 1979) and, in forest ecosystems, increased availability of herbaceous vegetation and woody debris are associated with higher deer mouse densities (Wilson and Carey 2000; Carey and Wilson 2001; Converse et al. 2006). Likewise, in wild deer mice, warm temperatures promote both earlier initiation and later cessation of reproductive activity (Miller and Gyug 1981). Thus, increased sun exposure resulting from SAD could facilitate an earlier and longer reproductive season for deer mice. Longer reproductive seasons could increase SNV prevalence via both density-dependent (Dobson and Hudson 1995) and density-independent mechanisms because, in addition to increasing population size, reproductive activity is associated with increased contact and aggression among deer mice (Glass et al. 1998; Klein et al. 2004). As such, reproduction is a critical time for transmission of SNV; the incidence of new hantaviral infections among hosts is generally highest during periods coinciding with peak reproductive activity (Klein et al. 2002). It is important to note that in our study, biomass was sampled once during the growing season. To obtain a more comprehensive understanding of how SAD influences the timing of understory phenology, future studies should sample biomass repeatedly across the growing season.

Conclusion

Whereas the direct effects of climate change can be difficult to observe across diminutive temporal and spatial scales, the synergetic interactions that occur across trophic levels in an ecological community can result in intensification of the effects of climate change, a process referred to as trophic amplification (Kirby and Beaugrand 2009). For the SAD–SNV system, we suggest a model of trophic amplification in which subtle changes in temperature and precipitation promote successively more severe changes in canopy cover in SAD affected stands, leading to shifts in microclimate and understory vegetation structure. In turn, these changes prompt even more substantial alterations in small mammal community composition, ultimately leading to increased SNV prevalence. Future research should focus on disentangling potential mechanisms through which increases in SAD are related to increased prevalence of SNV, focusing primarily on how changes in microclimate and vegetation community structure alter small mammal density and biodiversity, as these are two key regulators in many zoonotic disease systems.

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