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Direct and indirect effects of forest microclimate on pathogen spillover

Whalen W. Dillon [1,2,3,4] and Ross K. Meentemeyer [1,2]

[1]*Department of Forestry & Environmental Resources, North Carolina State University, Raleigh, North Carolina 27695 USA*
[2]*Center for Geospatial Analytics, North Carolina State University, Raleigh, North Carolina 27695 USA*

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Abstract. Disease dynamics are governed by variation of individuals, species, and environmental conditions across space and time. In some cases, an alternate reservoir host amplifies pathogen loads and drives disease transmission to less competent hosts in a process called pathogen spillover. Spillover is frequently associated with multi-host disease systems where a single species is more tolerant of infection and more competent in pathogen transmission compared to other hosts. Pathogen spillover must be driven by biotic factors, including host and community characteristics, yet biotic factors interact with the abiotic environment (e.g., temperature) to create disease. Despite its fundamental role in disease dynamics, the influence of the abiotic environment on pathogen spillover has seldom been examined. Improving our understanding of disease processes such as pathogen spillover hinges on disentangling the effects of interrelated biotic and abiotic factors over space and time. We applied 10 yr of fine-scale microclimate, disease, and tree community data in a path analysis to investigate the relative influence of biotic and abiotic factors on pathogen spillover for the emerging infectious forest disease sudden oak death (SOD). Disease transmission in SOD is primarily driven by the reservoir host California bay laurel, which supports high foliar pathogen loads that spillover onto neighboring oak trees and create lethal canker infections. The foliar pathogen load and susceptibility of oaks is expected to be sensitive to forest microclimate conditions. We found that biotic factors of pathogen load and tree diversity had relatively stronger effects on pathogen spillover compared to abiotic microclimate factors, with pathogen load increasing oak infection and tree diversity reducing oak infection. Abiotic factors still had significant effects, with greater heat exposure during summer months reducing pathogen loads and optimal pathogen conditions during the wet season increasing oak infection. Our results offer clues to possible disease dynamics under future climate change where hotter and drier or warmer and wetter conditions could have opposing effects on pathogen spillover in the SOD system. Disentangling direct and indirect effects of biotic and abiotic factors affecting disease processes can provide key insights into disease dynamics including potential avenues for reducing disease spread and predicting future epidemics.

Key words: *disease ecology; emerging infectious disease; microclimate; path analysis; pathogen spillover;* Phytophthora ramorum; *sudden oak death.*

Introduction

Pathogen spillover (Power and Mitchell 2004) is the driving process in a multitude of emerging infectious diseases that directly and indirectly impact human well-being, including Lyme disease (*Borrelia burgdorferi*; Brisson et al. 2008), bovine tuberculosis (*Mycobacterium bovis*; Nugent 2011), and Ebola (*Ebolavirus*; Chowell and Nishiura 2014). Spillover arises from asymmetries in the infectious competency of hosts, occurring when a reservoir host species amplifies and maintains a high level of a pathogen population relative to other less competent host species and causing higher infection rates in non-reservoir host species. Reservoir hosts support high levels of pathogen reproduction (amplification) and transmission, while tolerating infection and experiencing few ill effects. In contrast, non-reservoir hosts are much less tolerant of infection and experience more negative effects, including greater mortality. The asymmetries in host competency and tolerance of the host species are biotic factors that must naturally interact with abiotic environmental heterogeneity. Responding to and predicting emerging infectious disease outbreaks in this era of rapid global change requires disentangling the effects of biotic and abiotic factors in disease processes such as pathogen spillover.

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[3]Present address: Agronomy Department, University of Florida, Gainesville, Florida, 32611 USA.
[4]E-mail: whalendillon@gmail.com

Disease transmission and spillover fundamentally depend on the overlap of a pathogen, susceptible hosts, and abiotic environmental conditions that are favorable for disease. Abiotic factors such as temperature, rainfall, and physical landscape structure can all directly and/or indirectly affect the host-pathogen interactions that lead to disease. Abiotic conditions can directly affect pathogen survival especially when the pathogen is transmitted indirectly and must survive outside of the host. Landscape characteristics and changes in land use affect host density by influencing where hosts aggregate. Animal hosts may have preferred forage areas where they aggregate, while the aggregation of plant hosts is more strongly determined by landscape structure because individuals don't move. Plant host density only increases in areas on the landscape where conditions are favorable or where conditions are made favorable by human action. The hosts and the pathogen experience the same climatic conditions but respond at different temporal scales to exposure to hot/cold or wet/dry extremes. Extreme conditions for the pathogen can reduce pathogen survival thereby reducing transmission. Extreme conditions for the host may increase its susceptibility to infection at a later time by stressing the host and compromising its immune response. Pathogen reproduction is also dependent on the host community because of differences in species' competency for pathogen reproduction and tolerance of pathogen infection. For example, greater diversity may present more low-competency or non-hosts, which would reduce disease transmission through processes such as spillover. Although abiotic environmental conditions are a key component for many diseases, plant disease systems may be more regularly affected by abiotic environmental heterogeneity because the plants and their pathogens generally can't move to change the conditions they are experiencing.

Despite the fundamental role abiotic environmental heterogeneity plays in disease dynamics at multiple temporal and spatial scales (Altizer et al. 2006, Meentemeyer et al. 2012), most studies on pathogen spillover mainly focus on the biotic interactions affecting disease dynamics, occasionally including a broad scale measurement of the abiotic environment such as temperature or precipitation averages from nearby weather stations (e.g., Beckstead et al. 2010). The dearth of studies examining abiotic environmental heterogeneity in pathogen spillover is in part due to a lack of data available at spatial and temporal scales relevant to epidemiological processes. Still, the process of pathogen spillover is dominated by heterogeneity of the hosts and host community. Therefore, assessing the effects of the abiotic environment on pathogen spillover requires disentangling the host–pathogen–environment interactions across space and time. Parsing these relationships necessitates long-term monitoring of disease dynamics under heterogeneous environmental conditions and taking measurements at epidemiologically relevant spatial and temporal scales (Jules et al. 2002, Holdenrieder et al. 2004, Rohr et al. 2011, Meentemeyer et al. 2012).

We analyzed the relative effects of biotic and abiotic factors on pathogen spillover using sudden oak death (SOD) as a case study of an emerging infectious disease with asymmetric host competency and transmission patterns correlated with environmental heterogeneity (Rizzo and Garbelotto 2003). This multi-host plant disease has killed millions of trees in coastal forests of California and southwestern Oregon since its introduction in the mid-1990s (Meentemeyer et al. 2008, Lamsal et al. 2011). The pathogen, *Phytophthora ramorum*, causes two host-dependent diseases: (1) lethal canker infections on the stems of certain *Quercus* spp. and tanoak (*Notholithocarpus densiflorus*; Manos et al. 2008) and (2) typically non-lethal foliar infections on a wide range of other species (APHIS 2013). The pathogen is wind dispersed and thought to spread primarily between individuals of the primary foliar host, California bay laurel (*Umbellularia californica*, hereafter referred to as bay laurel), or from leaf and twig infections on tanoak (Rizzo et al. 2005, Cobb et al. 2010). The majority of disease spread occurs locally (<60 m; Swiecki and Bernhardt 2007, Dillon et al. 2014), though long-range dispersal events are an important contribution to regional spread (Meentemeyer et al. 2011). Infection of susceptible *Quercus* host species, such as coast live oak (*Q. agrifolia*), California black oak, (*Q. kellogii*), and canyon live oak (*Q. chrysolepsis*), which do not support pathogen sporulation (Rizzo et al. 2005, Swiecki et al. 2016), result in bole cankers that lead to tree mortality. Meanwhile, foliar infections on bay laurel do not negatively impact this host (DiLeo et al. 2009). These asymmetries in host competency and tolerance observed in this system are emblematic of pathogen spillover.

While asymmetric host competency and susceptibility clearly indicate the presence of a reservoir host (bay laurel) and multiple non-reservoir hosts (*Quercus spp.*), the temperature and moisture sensitivity of *P. ramorum* make this system particularly useful for studying less understood abiotic effects on pathogen spillover. Fluctuations in moisture and temperature moderate the pathogen population on timescales of weeks to months (Davidson et al. 2005), and host species over years to decades. Experimental and observational studies indicate that temperature extremes (hot or cold) and/or lack of moisture reduces pathogen sporulation, and therefore transmission (experimentally [Englander et al. 2006, Tooley et al. 2008], observationally [Davidson et al. 2005, 2008, Eyre et al. 2013]). Temperature and moisture are in turn influenced by landscape topography through orographic effects and cool air pooling (Dobrowski 2011). By observing fluctuations of environmental factors influencing pathogen reproduction and host susceptibility over time at multiple locations across a landscape, we aimed to disentangle the relative contributions of key biotic and abiotic factors on pathogen spillover in this disease system.

The inherent complexity of ecological systems presents analytical challenges because multiple factors have direct and indirect causal relationships within and across ecological hierarchies. We addressed this challenge using path analysis to examine the direct and indirect effects of multiple variables within a confirmatory causal framework. Specifically, we analyze the influence of topography, understory microclimate, and the tree community on pathogen spillover in the sudden oak death disease system.

Landscape characteristics, such as topography and geology, influence moisture persistence, the vegetation community, and, indirectly, disease dynamics. Forest microclimate conditions, i.e., temperature and moisture, directly influence pathogen reproduction and may also affect host susceptibility. While the hosts and the pathogen experience the same abiotic conditions, they respond differently to extremes (too hot or too cold, too wet or too dry) because what is "extreme" for a microscopic pathogen is not the same as an "extreme" for a tree. Pathogen reproduction is also dependent on the host community because of differences in competency for pathogen reproduction and tolerance of pathogen infection.

We hypothesize that biotic factors, in this case the density of bay laurel and diversity of the tree community, affect spillover more strongly than abiotic factors of microclimate and landscape topography, but that abiotic factors still significantly affect the spillover process (Fig. 1). Specifically, as a very competent host greater bay laurel density will generate greater pathogen loads and directly drive infection of susceptible oak species (i.e., pathogen spillover). At the same time, infection of oaks is expected to be reduced by greater tree diversity (i.e., a dilution effect) because all other tree species in the community have low competency compared to bay laurel. Greater exposure to warm and wet conditions is hypothesized to directly increase pathogen loads and disease prevalence of oaks by creating favorable conditions for the pathogen. We also hypothesize that greater exposure to "extreme" temperatures for the pathogen will directly reduce pathogen load in the bay laurel canopy due to poorer conditions for pathogen reproduction and increased abscission of leaves damaged by infection. Finally, we hypothesize that the landscape context (topography) will indirectly affect pathogen spillover by influencing bay laurel density, heat exposure, exposure to warm and wet conditions, and tree diversity. Higher values of the topographic index are expected to correlate with greater bay laurel density, less heat exposure, greater exposure to warm and wet conditions, and lower tree diversity. Our results provide insights into the relative effects of biotic and abiotic environmental heterogeneity on disease dynamics, particularly the process of pathogen spillover, which can improve predictions of disease risk and strategies for disease control.

Methods

Study area

During 2003–2004, we established 202 plots (each 225 m^2) in potential *P. ramorum* host habitat (i.e., forests or woodlands) across a 275-km^2 study area in southeastern Sonoma County, California, USA (Fig. 2). Plots were located on public and private lands with varying levels of forest cover and development across the surrounding landscapes. Elevation at plots ranged from 55 m to 800 m (mean 378 m) and minimum Euclidean distance between plots was 137 m. Vegetation is diverse across this landscape, including stands of mixed evergreen forest dominated by oak species and bay laurel, as well as stands dominated by coast redwood (*Sequoia sempervirens*) or Douglas-fir (*Pseudotsuga menziesii*). Chaparral

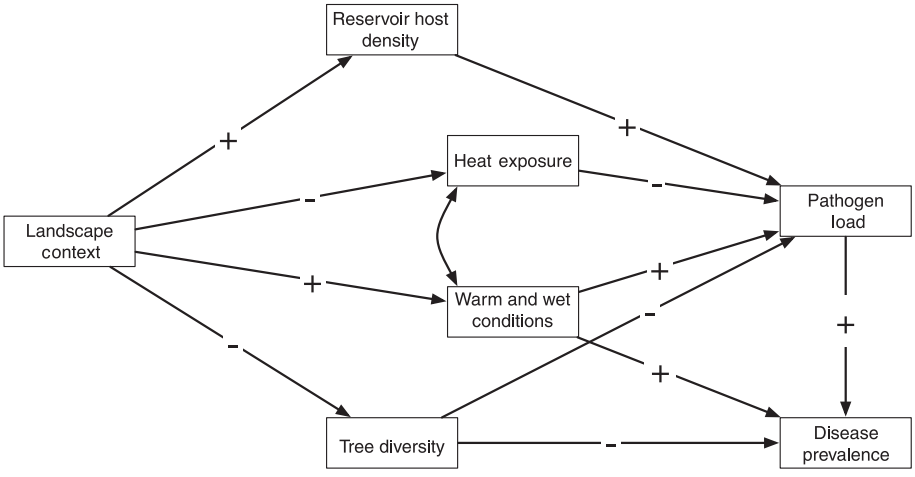

Fig. 1. Conceptual path model describing the hypothesized causal relationships between factors influencing pathogen spillover in the sudden oak death disease system. The curved, double-headed arrow indicates a correlative but not causal relationship between variables, and this relationship is excluded from the model fitting.

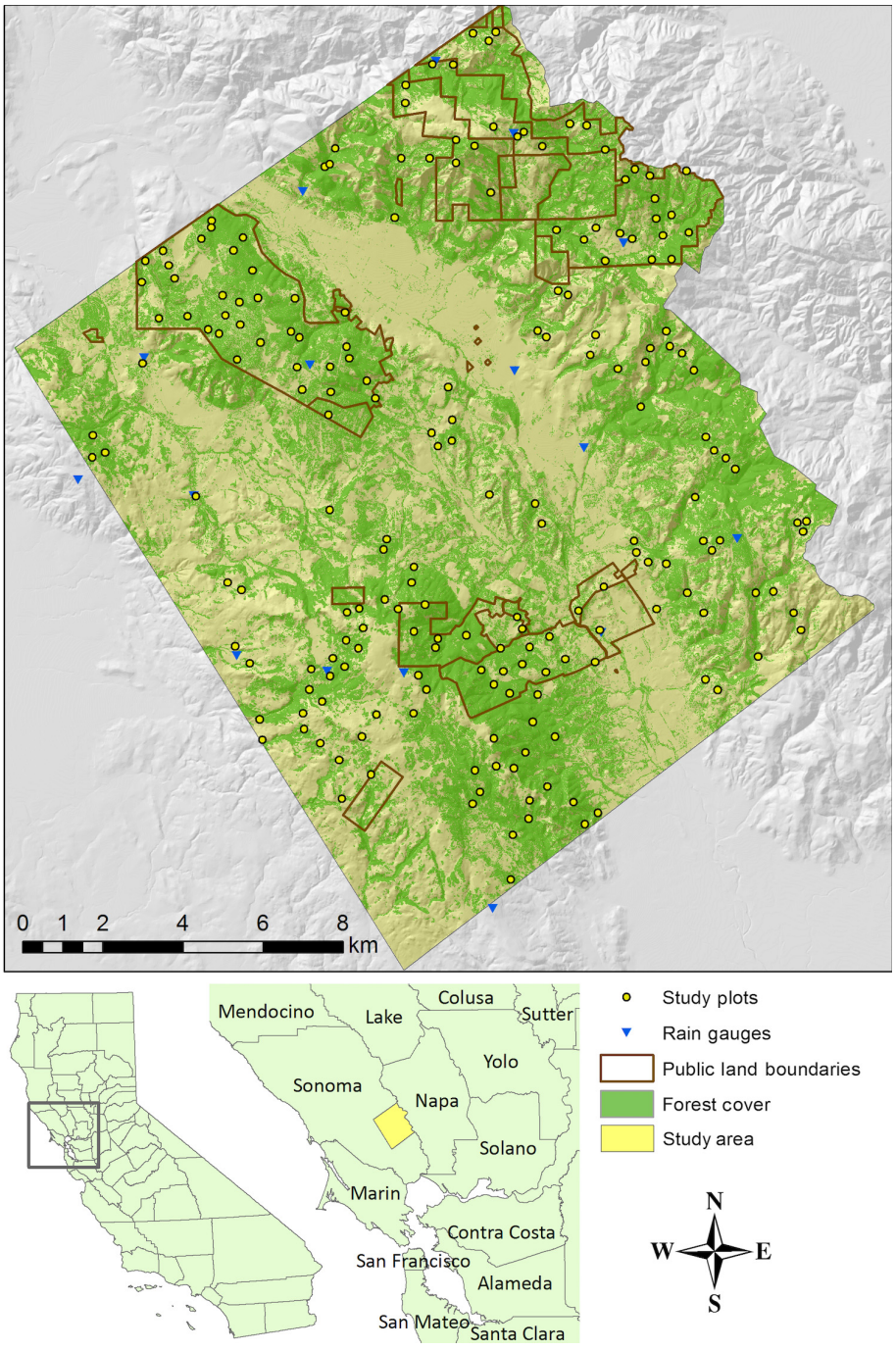

FIG. 2. Study area in Sonoma County, California, USA. Points are plot locations, inverted triangles (blue) are rain gauge locations, outlined areas are parks and public lands, and darker shading (green) indicates forest cover.

vegetation occurs at higher elevations of the Mayacama Mountain range along the eastern border of the study area, characterized by manzanita (*Arctostaphylos* sp.), chamise (*Adenostoma fasciculatum*), and *Ceanothus* shrub species interspersed with a few oak species. Tanoak is relatively rare across this study area, only found in eight plots, typically occurring with coast redwood or Douglas-fir. This region of California has a Mediterranean climate with distinct wet and dry seasons. Precipitation predominantly falls as rain from October through April, followed by a dry season with higher temperatures and lower humidity from May through September.

Data collection

We collected data in the field each spring during peak *P. ramorum* symptom expression from the onset of the study at plot establishment through 2012, and then began sampling every other year, with the most recent data used in this analysis collected during the spring of 2014. We recorded individual stem characteristics, disease intensity and prevalence, and understory microclimate temperature in each plot. We assessed stems measuring ≥2 cm diameter at breast height (DBH; breast height = 1.4 m) of five epidemiologically important host species: coast live oak, California black oak, canyon live oak, tanoak, and bay laurel. Some species commonly form multi-stemmed trees, so a stem was defined as a major branching that separated from the base or main trunk of the tree below breast height. We quantified disease intensity on bay laurel by counting the number of symptomatic leaves on each stem for 60 seconds (Condeso and Meentemeyer 2007, Haas et al. 2016). Infected oak species were identified by visual inspection for cankers characteristic of *P. ramorum* infection on main stems or major branches. At plot establishment, the stems of all other tree species rooted in the plot and ≥ 5 cm DBH were identified to species, deemed alive or dead, and their DBH was recorded.

We measured ambient understory temperature in each plot using HOBO temperature loggers (model H08-032-08 from 2003 to 2008 and model UA-001-64 from 2008 to 2014, Onset Computer, Bourne, Massachusetts, USA) housed inside a solar radiation shield (Model RS1, Onset Computer) secured 1 m above the ground in the center of each plot. The temporal resolution of these data sometimes changed between years and some data were missing each year due to user error, logger malfunction, vandalism, and/or battery failure. Using methods described in Tonini et al. (2016), we developed a complete set of temperature measurements at an hourly resolution for each plot for the entire study period. We recorded rainfall using tipping-bucket rain gauges (model RG3, Onset Computer) at 14 locations capturing the topographic variability across the study area. Using these data, we developed variables to quantify pathogen spillover and major abiotic and biotic factors relevant to this process in this disease system.

Pathogen spillover

During each sampling year at each plot, we quantified the pathogen load on bay laurel and the disease prevalence on oak trees as measures of the pathogen spillover process. We estimated pathogen load by summing the symptomatic leaf counts on bay laurel stems that were rooted within, or had foliage overhanging, the plot boundary. We calculated disease prevalence on living stems of susceptible oak species as the ratio of infected to uninfected stems based on observed canker symptoms.

Landscape context

To account for heterogeneity of the physical landscape in the sudden oak death disease system we calculated the topographic wetness index (TWI) for each plot from a 15-m resolution digital elevation model using the r.topidx function (Cho 2000) implemented in GRASS GIS (GRASS Development Team 2016). The index is calculated by dividing the upslope contributing area of a location on the landscape by the tangent of the local slope gradient (in radians) assuming soil transmissivity is constant (Moore et al. 1991):

$$w = \ln\left(\frac{A_s}{\tan\beta}\right) \quad (1)$$

where w is the wetness index, A_s is the catchment area, and β is the local slope gradient. Higher index values indicate the potential for more water to flow through or accumulate at a location, prolonging moisture persistence.

Plant community

To assess effects of the plant community on disease dynamics we calculated reservoir host density and tree diversity. We estimated reservoir host density as the number of bay laurel stems per hectare based on the number of stems ≥2 cm rooted in the plot area (225 m^2). We calculated Shannon's diversity index for tree species in each plot using the following equation (Krebs 1999:444–445):

$$H' = -\sum_{i=1}^{s}(p_i)(\log_2 p_i) \quad (2)$$

where H' is the Shannon's diversity index, s is the number of species, and p_i is the proportion of the total sample belonging to the ith species. Larger values of H' indicate greater diversity. We used the diversity function from the R package vegan (Oksanen et al. 2016) to calculate Shannon's index for each year that we collected data on all tree species (2005, 2012, and 2014). Index values were strongly correlated between these years (Pearson's $r > 0.9$), so we used values from the 2005 sample year in this analysis, because this resulted in the largest number of plots for analyses due to some being decommissioned later in the study.

Microclimate

To capture effects of microclimatic variability on bay laurel foliar pathogen load and oak infection we calculated heat exposure in excess of 25°C or warm and wet conditions at each plot using the rainfall and temperature data collected across the study area. We calculated heat exposure as the number of hours above 25°C during

the dry season (June–September) prior to sampling the following spring. We chose this threshold because inoculum is reduced with greater exposure to high temperatures due to increased leaf abscission and direct mortality of the pathogen (Davidson et al. 2005, Englander et al. 2006, Tooley et al. 2008). We defined warm and wet conditions as the average number of hours that the temperature was between 14°C and 22°C on wet days during November through May of the current and previous sample year for each plot. A plot was determined to have a wet day if its nearest-neighbor rain gauge recorded >0.25 mm of precipitation during a 24-h period.

Statistical analyses

We used structural equation modeling (SEM) to assess the hypothesized relationships between biotic and abiotic drivers of pathogen spillover in the sudden oak death disease system because it enables conceptualizing and examining direct and indirect effects of multiple processes in ecological systems (Grace et al. 2010). Using published knowledge of sudden oak death disease dynamics, we developed a structural model describing hypothesized direct and indirect relationships between the landscape, forest microclimate climate, tree community, and disease (Fig. 1) and assessed this model structure using path analysis (Wright 1921, Shipley 2004). Each box in Fig. 1 that has at least one arrow pointing to it is a response variable in the model and each of the boxes where that arrow is rooted is a predictor variable. In our evaluation of the path model, we excluded modeling the relationship between the microclimate variables because they were assumed to be correlated due to a shared driver (regional climate), indicated by the curved and double-headed arrow (Fig. 1). All analyses were conducted using R statistical software (R Core Team 2017).

We used the piecewiseSEM package (Lefcheck 2015) to evaluate our model because it allows linear mixed models with different distributional forms to be evaluated together in the same SEM. We modeled all response variables (except oak infection) as normally distributed. To better meet assumptions of normality, we applied a natural logarithm transformation to the pathogen load, host density, and warm and wet conditions variables. Since diversity for each plot was calculated from a single year (not a repeated measurement) and also had a static predictor variable in the topographic index we fit an ordinary least squares bivariate regression to model this relationship. For variables repeatedly measured at each plot during each year, we used mixed-effects models with scalar random effects for the sample year and plot, which account for this cross-replication by allowing the intercepts for these grouping factors to vary. The random effect for year accounts for correlation between measurements made during the same year, while the random effect for plot accounts for correlation between the measurements from the same plot. Accounting for this induced correlation provides more accurate estimates of confidence intervals and P values by preventing variation from being attributed to measured variables. We modeled the repeatedly measured symptomatic leaf count and the microclimate variables using linear mixed-effects regressions of the following form using the lmer function in the R package lme4 (Bates et al. 2015):

$$\mathbf{y} = \mathbf{X} \times \boldsymbol{\beta} + \mathbf{Z} \times \mathbf{b} + \mathbf{e} \tag{3}$$

$$\mathbf{b} \sim N(0, \mathbf{D}) \tag{4}$$

$$\mathbf{e} \sim N(0, \sigma^2) \tag{5}$$

where $\mathbf{y}$ is the n-length vector of observed values for the response variable; $\mathbf{X}$ is the $n \times p$ fixed-effects matrix of predictor variables, with p equal to the number of explanatory variables; $\boldsymbol{\beta}$ is the p-length vector of fixed-effects coefficients to be estimated that apply to all years and plots; $\mathbf{Z}$ is the $n \times q$ "random effects" matrix, where q is the number of random effects in $\mathbf{Z}$; and $\mathbf{b}$ is a q-length vector of random effects that is assumed to have a multivariate normal distribution. The scalar random effects for sample year and plot are assumed to be independent within and between groups, resulting in the covariance matrix $\mathbf{D}$ having diagonal structure with the estimated variance (diagonal matrix values) homogeneous within each group and the covariance (off-diagonal matrix values) equal to zero (Bates et al. 2015). The n-length vector $\mathbf{e}$ is the residual error in the model (unexplained variation in $\mathbf{y}$), and assumed to be normally distributed with a mean of zero and a variance σ^2.

We fit a binomial generalized linear mixed effects model to yes/no observations of oak infection with random effects for sample year and plot using the glmer function from the lme4 package:

$$(\mathbf{y}|\mathrm{B} = \mathbf{b}) \sim \mathrm{Binomial}(\mathbf{n}, \mathbf{p}|\mathbf{b}) \tag{6}$$

$$\mathrm{logit}(\mathbf{p}|\mathbf{b}) = \mathbf{X} \times \boldsymbol{\beta} + \mathbf{Z} \times \mathbf{b} \tag{7}$$

where $\mathbf{y}$ is binomially distributed with an occurrence probability $\mathbf{p}$, which is modeled as a function of linear predictors using the logit link function (Eq. 7). The details of the linear predictors in Eq. 7 are the same as those in Eqs. 3 and 4.

We assessed collinearity of predictor variables prior to inclusion in each model to ensure that Pearson's r was $<|0.5|$, and examined residual plots of each model for heteroscedasticity that would indicate violation of model assumptions. Models were checked for spatial autocorrelation by plotting and comparing correlograms of the response variable and the residuals from the fitted models. There was some minor spatial correlation in the modeled response variables, but the model residuals showed no significant spatial autocorrelation, indicating

that the models accounted for any significant spatial correlation in the response. We fit models using unstandardized variables, and then calculated standardized (mean = 0, variance = 1) coefficients for continuous variables.

Model interpretation

Path coefficients are interpreted as either direct or indirect effects on the response variables. Direct effects are from variables that have an arrow pointing directly to a response variable. Variables with indirect effects are those that are at least once removed from another response variable. For example, landscape context indirectly influences disease prevalence through direct effects on species diversity and heat exposure (Fig. 1). Indirect effects are calculated by multiplying the coefficients along the path to the response variable. The total effect of a variable is the sum of the direct and indirect effects. We report standardize coefficients in Fig. 3 showing the relative strength of direct effects in the pathogen spillover process. Unstandardized coefficient estimates and test statistics are reported in Appendix S1: Table S1.

Results

We tested our path model using data from 164 plots sampled between 2005 and 2014 (n = 1,433 observations) that met the criteria of having one or more susceptible oak trees and found an acceptable fit to the model structure (P = 0.409, Table 1). Biotic factors of species diversity, bay laurel density, and pathogen load had stronger effects on pathogen spillover relative to abiotic factors, but abiotic environmental factors of exposure to temperatures >25°C, optimal pathogen temperatures (14–22°C), and topographic index also significantly influenced the pathogen spillover (Fig. 3). The statistically insignificant hypothesized relationships included those between topographic index and bay laurel density and optimal pathogen temperatures, tree diversity and symptomatic leaf count, and optimal pathogen temperatures and symptomatic leaf count.

The terminus of pathogen spillover, oak infection, was most strongly affected by the biotic factors of tree diversity and symptomatic leaf count, but also was influenced by exposure to optimal pathogen temperatures on wet days. Diversity and pathogen load had opposite effects on oak infection with greater symptomatic leaf count resulting in greater oak infection (Fig. 4a) and greater tree diversity resulting in lower oak infection (Fig. 4b). Greater exposure to optimal pathogen temperatures resulted in greater oak infection (Fig. 4c). Bay laurel density had the strongest direct effect on symptomatic leaf count but symptomatic leaf count was also influenced directly by greater exposure to temperatures >25°C during the June through September dry season and indirectly by the landscape context indicated by the topographic index. Plots with greater bay laurel density had higher symptomatic leaf count (Fig. 4d), but greater exposure to temperatures >25°C reduced symptomatic

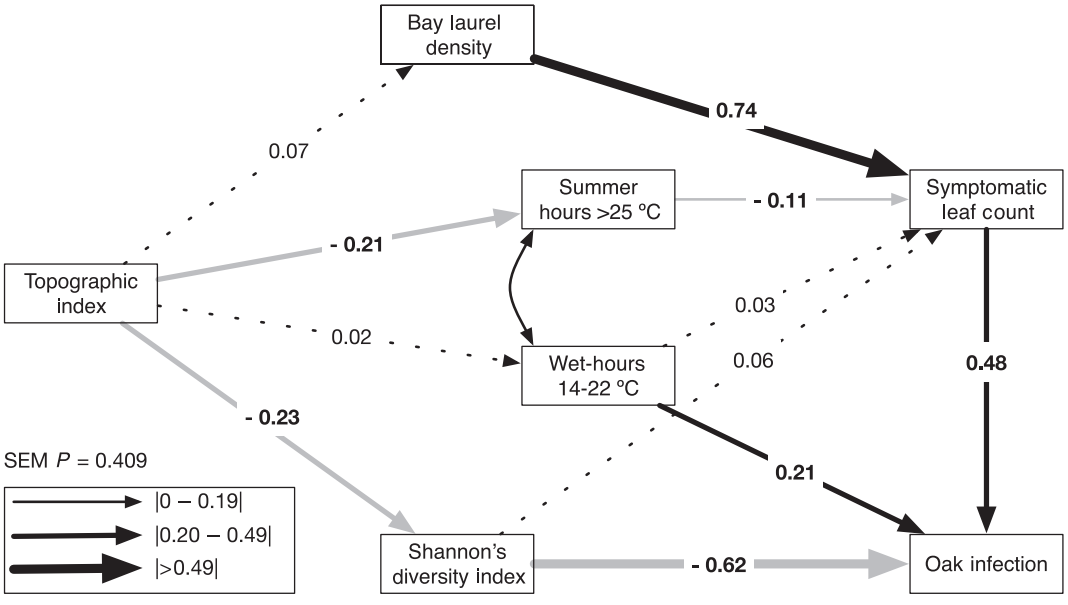

Fig. 3. Standardized coefficients for each relationship in the path model. Gray lines are negative relationships and dotted lines are statistically insignificant at P = 0.05 (full P-values are reported in Table S1). Direct effects are variables pointing unimpeded toward response variables; variables with indirect effects have one or more intermediate variables between them. The line weights are scaled to the absolute value of the standardized path coefficient. Oak infection represents disease prevalence; symptomatic leaf count represents pathogen load; summer hours >25°C represents heat exposure; wet-hours 14–22°C represents "optimal" warm and wet conditions; bay laurel density is reservoir host density; Shannon's diversity index is species diversity; and topographic index represents landscape context.

TABLE 1. Results for the fitted path model indicating relationships between pairs of variables with missing path(s) and the metrics to assess the fit of the entire path model.

Missing path	Estimate	SE	df	Crit-value	*P*
Pathogen load ~ Landscape context	0.047	0.049	165.8	0.966	0.336
Disease prevalence ~ Landscape context	0.083	0.084	NA	0.987	0.324
Reservoir host density ~ Diversity	0.126	0.241	161.0	0.523	0.602
Heat exposure ~ Diversity	3.170	22.78	160.8	0.139	0.890
Warm + Wet ~ Diversity	0.202	0.181	160.4	1.117	0.266
Heat exposure ~ Reservoir host density	−5.363	6.322	300.9	−0.848	0.398
Warm + Wet ~ UMCA density	−0.082	0.056	198.1	−1.473	0.143
Disease prevalence ~ UMCA density	0.120	0.176	NA	0.678	0.497
Disease prevalence ~ Heat exposure	−0.001	0.001	NA	−1.289	0.197

Notes: All missing paths are statistically insignificant and the overall model fit is acceptable ($P = 0.409$). Fisher's $C = 18.72$; SEM df = 18; SEM $P = 0.409$. Estimate, estimated coefficient; SE, standard error; df, model degrees of freedom (not estimated for the binomial response disease prevalence); Crit-value, critical value of the test statistic; Fisher's *C* as described by Shipley (2004); *P*, path model *P* value.

leaf count (Fig. 4e). Topographic index (landscape context) had a net positive indirect effect on oak infection that was mediated by tree diversity, and the path through exposure to temperatures >25°C and symptomatic leaf count (Fig. 3). Plots with greater topographic index values had less exposure to temperatures >25°C (Fig. 4f) and lower tree diversity (Fig. 4g). Multiplying the coefficients along the significant paths from the topographic index to symptomatic leaf count (Fig. 3) shows a positive indirect effect of landscape context on symptomatic leaf count through the direct effect on exposure to temperatures >25°C (Fig. 4f).

Discussion

Pathogen spillover in the sudden oak death disease system was most strongly influenced by biotic factors, but these biotic factors were still significantly influenced by abiotic environmental heterogeneity. Higher densities of the reservoir host increased pathogen load, which resulted in greater disease prevalence (i.e., pathogen spillover) in susceptible oaks, but this was countered by a relatively strong negative effect of tree species diversity. Microclimate conditions in the plot simultaneously influenced the reservoir host pathogen load and disease prevalence in non-reservoir hosts, with heat exposure reducing pathogen load, but warm and wet conditions increasing disease prevalence. The tree community and microclimate were influenced by landscape topography, where locations with greater potential for moisture accumulation and persistence had higher pathogen load and disease prevalence.

We confirmed that bay laurel density was the strongest driver of pathogen load (total symptomatic leaf count) in the plot, but also that microclimate conditions have significant influence. Previous studies indicated individual variation in bay laurel susceptibility and competency (Anacker et al. 2008, Hüberli et al. 2011) that would be obscured by aggregation to the plot level in our study. However, disease on individual trees can be overridden at the landscape scale by climatic conditions (Anacker et al. 2008) reinforcing our findings of abiotic effects on pathogen load at the plot level. Still, individual variation in susceptibility indicates the possibility of superspreader (Heesterbeek et al. 2015) bay laurels existing in the population, or in particular locations that would not be detectable in this analysis. By aggregating disease prevalence across all stems of all susceptible oak species we also obscured the variation among species' and individual's disease resistance, susceptibility, and tolerance that may be conferred by genetic differences (Dodd et al. 2005). Since this individual variation exists, efforts that preserve the genetic diversity of susceptible species increase resilience to this and other diseases.

The negative relationship between disease prevalence on susceptible oaks and species diversity is evidence for the dilution effect, whereby species diversity reduces disease risk (Keesing et al. 2006, 2010), which was previously shown in the sudden oak death disease system (Haas et al. 2011). Our analysis using a longer data set from a geographically distinct plot network with different forest types reinforces the previous finding in this system. The magnitude of the dilution effect may be influenced by individual variation, which we did not account for in our models, and by cryptic infections of bay laurel leaves or oak trees that are impossible to detect visually (Swiecki et al. 2016). In the case of cryptic infection, it is possible that we underestimated pathogen load or disease prevalence, however, any visual underestimation is likely to be equally biased across the range of tree diversity. An interesting contrast in our results is that the dilution effect did not extend to pathogen load (symptomatic leaf count) on bay laurel. One possible reason for observing a dilution effect of diversity on oak disease prevalence but no effect on pathogen load is because the *P. ramorum* infection on bay laurel occurs at the scale of the leaf, while on oaks it occurs at the scale of the tree (i.e., bole cankers). At the leaf scale, a single bay laurel tree with thousands of leaves could support a very large pathogen load even as the only bay

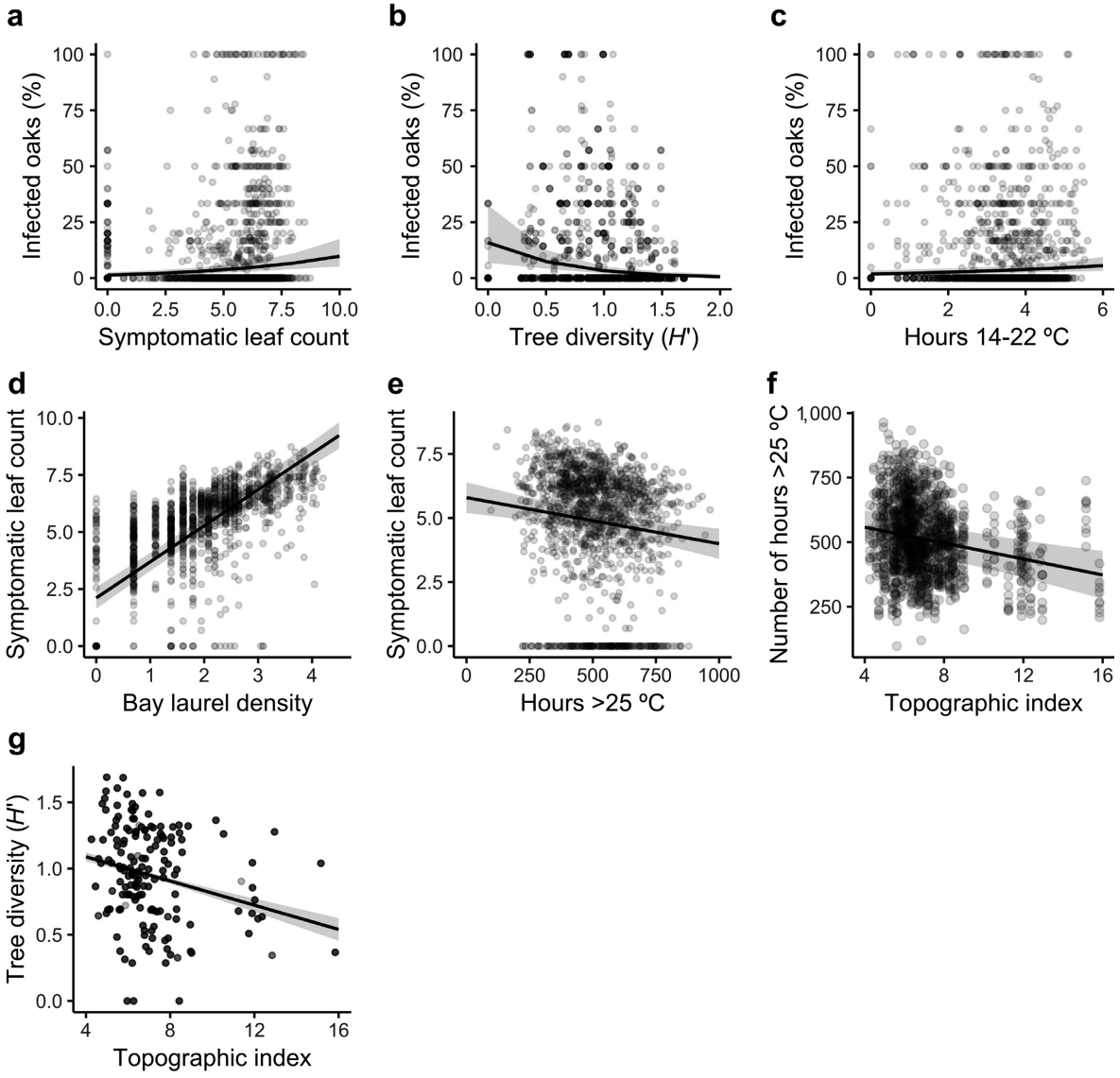

FIG. 4. Plots of the predicted relationships (smooth line) for significant paths between response and explanatory variables from fitted models of the path analysis in Fig. 3; (a) oak infection vs. symptomatic leaf count, (b) oak infection vs. tree diversity (Shannon's H'), (c) oak infection vs. hours at optimal pathogen conditions during the wet season (November–May), (d) symptomatic leaf count vs. bay laurel density, (e) symptomatic leaf count vs. time above 25°C during summer months (June–September), (f) time above 25°C during summer months vs. topographic index, (g) tree diversity vs. topographic index. Plots were generated using the R packages sjplot (Lüdecke 2018), ggplot2 (Wickham 2016), and cowplot (Wilke 2018).

laurel among a group of susceptible oaks. In this low-diversity scenario, all of the oaks near the bay laurel tree would be at high risk of becoming infected. Therefore, increasing tree diversity to include more species with low or no competency (essentially any species that is not bay laurel) for *P. ramorum* infection would likely reduce the encounter rate between dispersed spores and susceptible oak species.

Alongside the host(s) and pathogen(s), abiotic environmental conditions is the third key component of disease dynamics. Our finding that heat exposure during the summer reduced pathogen inoculum load the following spring corroborates findings showing that *P. ramorum* survival on bay laurel leaves decreased during the summer months (Davidson et al. 2005) and with the number of days exceeding 30°C (DiLeo et al. 2014). Several experimental studies also found decreased survival of *P. ramorum* exposed to high constant temperatures for several hours (Tooley et al. 2008, 2014, Tooley and Browning 2015). Although infection on bay laurel leaves can rebound quickly following drought conditions (Eyre et al. 2014), our results suggest that this may not necessarily increase pathogen spillover, because we also found that disease prevalence was greater when temperatures are

consistently warm and wet. A generally warming climate would likely mean warmer temperatures year-round, potentially increasing the success of pathogen spillover during the wet season, but also increasing heat exposure and reducing the pathogen load during the dry season. While these conditions could offset each other, the relative strength of the effects of heat exposure on bay laurel pathogen load and of optimal warm and wet temperatures on oak infection in our results suggests that greater exposure to warm and wet conditions favorable to pathogen growth and reproduction could override the limiting influence of summer heat exposure on pathogen load. Additionally, changes in rainfall patterns, such as shifts in the timing and length of the wet and dry seasons and the amount of rainfall, would dramatically affect sudden oak death disease dynamics. For example, extended drought would reduce pathogen loads and stress trees, potentially increasing oak tree susceptibility to infection during epidemic outbreaks by compromising their defense response.

In a review of studies examining the relationship between drought and plant diseases, Desprez-Loustau et al. (2006) found that 67% showed evidence for drought favoring disease, and in some cases the interaction was synergistic. However, only 50% of the studies on *Phytophthora*-related diseases in this review reported positive drought–disease interactions. The findings of positive interactions were frequently attributed to indirect effects of drought on the pathogen where host susceptibility to disease was increased by drought. A possible reason for this 50/50 finding for drought interactions with *Phytophthora* diseases is that in some situations the cost of drought to the host is greater than the cost of drought to the pathogen and in some cases the cost is greater for the pathogen than the host. The effect of drought also depends on the size and life-history of the organism: a drought for a pathogen would generally be at a smaller spatial and temporal scale than a drought for a tree. Our results suggest an antagonistic interaction between drought and disease for a *Phytophthora*-caused disease, where greater exposure to temperatures >25°C during the summer dry season (June–September) reduced pathogen load in bay laurel canopy foliage and therefore spillover that causes disease on susceptible oaks; i.e., the cost of "drought" was greater for the pathogen than for the host. Reduction of pathogen load on canopy foliage is partly due to bay laurel leaves damaged by the pathogen being abscised during hot summers, as well as the pathogen being killed directly or forced into dormancy due to the prolonged exposure to hot and dry conditions. The species of susceptible oaks in the sudden oak death system are generally resilient to drought and more commonly occur where rainfall is generally lower than in our study area (Lamsal et al. 2011). Since we modeled oak infection, and not mortality, it is possible that sudden oak death has a positive interaction with drought in terms of increasing mortality of trees that become infected when abiotic conditions are favorable for the pathogen.

Landscape context such as topography influences biotic and abiotic conditions through orographic effects, cool air pooling, and soil moisture. Locations with greater potential moisture indirectly increased pathogen spillover by having lower species diversity and heat exposure, but higher pathogen load. These conditions likely increase the susceptibility of oak trees. Persistent moisture also can enhance pathogen sporulation and increase the likelihood that *P. ramorum* spores will survive long enough to infect a susceptible oak tree without necessarily altering the physiological susceptibility of the host.

Biotic and abiotic conditions should both be considered when attempting to reduce pathogen spillover and the spread of sudden oak death. The epidemiologically important tree species in sudden oak death dynamics (bay laurel and oaks) appears to be more resilient to drought conditions compared to the pathogen. With this in mind, management actions attempting to slow disease spread may be especially effective during seasonal drought conditions when the pathogen load has already been reduced. Slowing the spread can primarily be achieved by removing bay laurel, the reservoir host in the spillover process, which would also worsen microclimate conditions for the pathogen on any remaining host species. These efforts could simultaneously reduce and restructure potential fuel loads, helping prevent large wildfires that may be more intense due to disease-induced mortality (Metz et al. 2011). Low-intensity prescribed fire may be an effective tool for thinning forest understory that is dominated by bay laurel. In these cases, the substrate for *P. ramorum* to survive at relatively high levels during the dry summer months would be removed, thus reducing the inoculum available to seed epidemic spread during the following wet season.

A growing number of emerging infectious diseases (EIDs) involve multiple hosts with transmission processes that involve pathogen spillover. While prevention and control of EIDs is a common goal, more research is needed to understand how environmental heterogeneity may affect these efforts by simultaneously influencing pathogen loads on reservoir host(s) and susceptibility to infection of non-reservoir host(s). Plant disease systems, such as sudden oak death, are useful for investigating the effects of abiotic environmental heterogeneity because the host species don't move, which enables close monitoring of variation in disease–environment relationships. Analysis of detailed monitoring data can in turn provide insights into disease management such as when or where to apply treatments that target reservoir hosts or may be most effective to prevent or slow disease spread.

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