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Influence of Soil and Land Cover on Chronic Wasting Disease Prevalence in White-tailed Deer and Mule Deer across North America

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INFLUENCE OF SOIL AND LAND COVER ON CHRONIC WASTING DISEASE PREVALENCE IN WHITE-TAILED DEER AND MULE DEER ACROSS NORTH AMERICA

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Chronic wasting disease (CWD) is a fatal neurodegenerative disease that infects deer and is caused by a pathogenic prion. CWD is a concerning wildlife disease because it is incurable, potentially poses a risk to human health, and is spreading rapidly. CWD prions are transmitted both directly via bodily fluids and indirectly through environmental reservoirs such as soil. In this study, we investigated the influence of land cover and soil characteristics on CWD prevalence in white-tailed deer and mule deer. We acquired CWD prevalence data from seven North American regions and used ArcGIS to obtain land cover and soil characteristic data for each region. We input these environmental variables into a principal component analysis (PCA) to reduce multicollinearity and used the PC scores in generalized linear mixed models with CWD prevalence as the response variable. Our analysis indicated that land cover and soil characteristics explained variation in CWD prevalence in both deer species, and that the observed patterns were largely consistent across deer species and regions. Specifically, agricultural land cover, soil moisture and soil clay content were related to increased CWD prevalence while natural land cover was related to decreased CWD prevalence for both white-tailed deer and mule deer. These results imply that multiple pathways of disease transmission, both direct and indirect, are important in the spread of CWD. Uncovering the relationship between the environment and CWD provides wildlife managers valuable information in effectively controlling disease spread.

INFLUENCE OF SOIL AND LAND COVER ON CHRONIC WASTING DISEASE PREVALENCE IN WHITE-TAILED DEER AND MULE DEER ACROSS NORTH AMERICA

by

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Madison L. Miller

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INTRODUCTION

Chronic wasting disease (CWD) is a fatal neurodegenerative disease caused by a pathogenic prion that infects members of the family Cervidae (Mammalia). CWD was first discovered in 1967 in a Colorado captive mule deer and has since been found in 26 U.S. states, three Canadian provinces, three European countries, and in captive cervid herds in South Korea (Rivera *et al*. 2019). If an individual has been infected with CWD prions, an asymptomatic incubation phase begins which may last two to four years (Osterholm *et al*. 2019). During the incubation phase, prions can be detected in bodily fluids as early as six months after initial infection. In later stages of infection, pathologies include drastic weight loss, excessive thirst and urination, behavioral changes such as listlessness and aggressiveness, neurological degradation including lack of coordination and difficulty walking, and eventually death of the individual (Escobar *et al*. 2020; Rivera *et al*. 2019). CWD is an especially concerning disease because it is the only prion disease that affects free-ranging animals, there is no known treatment or cure, the possibility of zoonotic transmission cannot be ruled out, and it is spreading rapidly (Escobar *et al*. 2020; Rivera *et al*. 2019).

CWD prions are transmitted vertically (mother to offspring) or horizontally, either directly between individuals or indirectly by way of the environment (Escobar *et al*. 2020). The spread of horizontally transmitted diseases is influenced by the rate of contact between individuals which can be affected by population density, behavior, spatial distribution, and movement (Bradley & Altizer, 2007; Podgorski *et al*. 2018). In deer, behaviors such as migration, habitat selection, and home range size influences contact opportunities between individuals and these behaviors may vary with landscape composition (Habib *et al*. 2011). For example, deer aggregate seasonally in agricultural areas due to availability of growing crops as food (Kjær *et al*. 2008; Nixon *et al*. 1991; Urbanek *et al*. 2013; Vercauteren & Hygnstrom, 1998). Deer population densities are higher in areas with many patches of agricultural land (Urbanek *et al*. 2013). Developed land cover has also been associated with altered deer behavior and increased population density. For example, development is associated with reductions in hunting pressures and natural predation which increases survival and may prolong the time that animals are infected with CWD and are in contact with other individuals (Farnsworth *et al*. 2005; Polfus & Krausman, 2012; Urbanek *et al*. 2013). In addition, supplemental feeding in developed areas may concentrate deer and increase disease transmission (Kjær *et al*. 2008). Overall, the influence of landscape characteristics on deer behavior and movement suggests that opportunities for individual contact, and therefore direct CWD transmission, may differ as a result of variation in land cover and anthropogenic pressures among regions.

Exposure to prions contained in environmental reservoirs may also contribute to CWD transmission (Escobar *et al*. 2020). Once CWD prions are released into the environment by an infected individual, they can persist for many years and are resistant to heat, radiation, and most chemicals (Williams *et al*. 2019). Soil may be an important environmental reservoir for indirect CWD transmission as evidence suggests that prions bind readily to soil particles, prions may remain in soil for years, and soil is intentionally ingested by ruminants for mineral supplementation which may result in infection (Rivera *et al*. 2019; Saunders *et al*. 2012; Smith *et al*. 2011). Clay particles in soil may be particularly important for the persistence of CWD in the environment since prions have a high affinity for binding to clay particles in the lab (Saunders *et al*. 2012; Wyckoff *et al*. 2016) and some clay minerals may actually increase the retention, bioavailability, and infectivity of prions (Johnson *et al*. 2007; Wyckoff *et al*. 2016). Yet, evidence on the role of clay on CWD in the field is mixed, with some studies reporting higher

CWD occurrence in high clay content soils (Walter *et al.* 2011), other studies reporting lower occurrence (Dorak *et al*. 2017; O'Hara Ruiz *et al*. 2013), and even others reporting no effect of clay soil on CWD (Evans et al. 2016; Robinson *et al*. 2013; Storm *et al*. 2013). Other soil characteristics such as organic matter, pH, moisture, metal content, and texture may impact CWD prion persistence in soil, but their effects remain largely untested (Kuznetsova *et al*. 2014; Saunders *et al*. 2012; Smith *et al.* 2011). High concentrations of humic acid, a major component of soil organic matter, decreased infectivity and detection of CWD prions (Kuznetsova *et al*. 2018), while pH levels were positively correlated with higher CWD risk (Dorak *et al*. 2017).

Despite the interaction of CWD prions with soil and the influence of land cover on deer behavior and movement, few studies have simultaneously investigated the relationship between CWD, land cover and soil. Since CWD is widespread and endemic across a spectrum of land covers and soil types, it is vital to identify common environmental conditions that favor CWD transmission at a wide spatial scale. However, the studies that have investigated the link between CWD and environmental characteristics took place in different regions, utilized different approaches, and reached inconsistent conclusions. For example, Evans *et al*. (2016) investigated white-tailed deer in the Appalachian area of the United States, and Farnsworth *et al*. (2005) studied mule deer in Colorado. Both studies concluded that prevalence of CWD was higher in developed areas compared to undeveloped areas. By contrast, CWD risk in Midwestern white-tailed deer has been shown to increase in undeveloped, forested areas (Mateus-Pinilla *et al*. 2013; O'Hara Ruiz *et al*. 2013; Storm *et al*. 2013). The discrepancy on the role that land cover and soil plays in CWD among previous small-scale studies, and the lack of studies addressing CWD at a regional scale, emphasizes the need for a broader analysis.

In this study, we investigated the influence of land cover and soil properties on CWD prevalence in white-tailed deer and mule deer in multiple North American regions. We collected CWD prevalence data from six United States and one Canadian province and used ArcGIS to obtain data on land cover and soil properties in these areas. We expected that both land cover and soil characteristics would have a significant effect on CWD prevalence. Specifically, we predicted that that higher clay content soil would be associated with an increased risk of CWD infection (Saunders *et al*. 2012; Walter *et al*. 2011; Wyckoff *et al*. 2016). We also predicted that CWD prevalence would be positively correlated with altered landscapes such as developed land cover and agricultural land cover due to seasonal concentration of individuals, higher population densities, and changes in movement patterns associated with these areas which may elevate contact opportunities among individuals, and therefore increase disease transmission. The results of this study will help clarify the role that land cover and soil properties play in CWD infection risk in deer. Understanding the influence of environmental characteristics on CWD transmission at a large spatial scale is vital for identifying common areas across the range of CWD where risk of transmission is high. Identifying these areas can help wildlife managers and biologists target disease surveillance and inform management actions that reduce prevalence and spread of CWD.

METHODS

Data acquisition

We requested data from multiple jurisdictions in North America that are known to currently have deer infected with CWD. We retained CWD prevalence data for mule deer and white-tailed deer from seven North American regions: Illinois (USA), Michigan (USA), Montana (USA), Pennsylvania (USA), Wisconsin (USA), Wyoming (USA), and Saskatchewan

(CAN). Additional details of the data we obtained are listed in Table 1. We were unable to include CWD prevalence data from additional jurisdictions due to data sharing regulations (Kansas, USA; Alberta, CAN; South Dakota, USA; Utah, USA; Nebraska, USA), and differences in data resolution (Colorado, USA). Prevalence was recorded as the number of infected deer out of total number of samples tested for CWD in each county or wildlife management zone, referred to here as a spatial unit. A wildlife management zone is an area created to manage game species populations and hunting activities within its boundaries. In this dataset, CWD prevalence in spatial units for Montana, Wyoming, and Saskatchewan were collected in wildlife management zones and in Illinois, Michigan, Pennsylvania and Wisconsin, counties were used as the spatial units. CWD prevalence data was obtained for a total of 382 white-tailed deer and 184 mule deer spatial units.

Environmental Data

Land cover

We used ArcGIS to obtain environmental data and calculate the area (km^2) of each spatial unit for the six U.S. states and one Canadian province (Environmental Systems Research Institute, Redlands, California, USA). Raster land cover data for the United States was obtained from the National Land Cover Database 2016 (NLCD 2016). To simplify our analysis and account for consistent differences in dominant land cover types across regions, we used the reclassify tool in ArcGIS pro to create three land cover categories: natural, agricultural, and developed. The reclassify tool changes the values of a raster cell to a customized value which allows for regrouping a raster layer based on criteria, such as land cover type. In the United States, the natural land cover group consisted of deciduous forest, evergreen forest, and mixed forest, dwarf scrub, shrub/scrub, grassland/herbaceous and sedge/herbaceous (classes 41, 42, 43, 51, 52, 71, 72). Developed land cover consisted of developed open space, low intensity, medium intensity and high intensity (classes 21, 22, 23, 24). Agriculture land cover included pasture/hay and cultivated crops (classes 81 and 82). Land cover data for Canada, 2010 CAN-LC, was produced by the Canada Centre for Remote Sensing (CCRS) and downloaded from the Canada Open Government webpage. In Canada, natural land cover consisted of temperate forest, subpolar forest, needleleaf forest, tropical forest, broadleaf forest, evergreen forest, deciduous forest, mixed forest, tropical shrub, temperate shrub, sub-polar shrub, and lichen-moss shrub, tropical grassland, sub-tropical grassland, polar grassland, sub-polar grassland and lichen-moss grassland (classes 1,2,3,4,5, 6, 7, 8, 9, 10, 11, 12). Developed land cover included urban and built-up (class 17). Agriculture land cover consisted of cropland (class 15). To quantify the proportion of each land cover type in spatial units for both Canada and the United States, the tabulate area tool was used in ArcGIS pro. The total area of land cover groups was converted to meters squared and the proportion of each land cover type was calculated by dividing its area (m^2) by the total land cover area in each spatial unit.

Soil

STATSGO soil data for the United States was obtained from the United States Department of Agriculture (USDA) and used in conjunction with the Soil Data Viewer in ArcMaps 10.8.1. Each soil characteristic of interest was queried in advance mode with the rating options set to use the weighted average of the surface layer. Each soil characteristic layer was then downloaded and opened in ArcGIS pro. Soil data for Saskatchewan, with the exception of organic matter, was obtained from The National Soil DataBase, Government of Canada. In ArcGIS pro, the “Summarize Within (GeoAnalytics)” tool was used with the weighted area option to quantify the average soil data for each spatial unit. Soil characteristics of interest and their relationship with prions are shown in Table 2.

Data analysis

We performed a comprehensive data exploration protocol following the steps outlined in Zuur *et al* (2010). All analyses were performed in R studio, version 4.1.1 (R Core Team, 2021). We completed our data exploration protocol by first plotting descriptive statistics using the *ggplot2* package (Wickham, 2016) and testing for pairwise correlations among our dependent and independent variables using a Spearman correlation test. We tested for correlations among our dependent variables to determine if we needed to exclude any variables from the analysis if correlation was strong (rho=0.5-1.0; Sulaiman *et al*. 2019). Since many of our environmental variables were moderately correlated, we decided to use a principal component analysis (PCA) which avoids multicollinearity effects in subsequent analyses (Sulaiman *et al*. 2019). We ran the PCA in R using the *FactoMineR* package (Lê *et al*. 2008) with environmental variables for white-tailed deer data and mule deer data separately. Soil clay, silt and sand were highly correlated and proportional to each other (added up to 1) which meant we could not include all three in the PCA. We retained only soil clay in our analysis since previous studies have suggested it to be an important predictor of chronic wasting disease (Saunders *et al* 2012; Walter *et al*. 2011). A scree plot was created to graphically examine principal components and we retained PCs totaling at least 75% of variance, above the point of inflexion, and with eigenvalues > 1.0 for future analysis (Field *et al*. 2012).

We used generalized linear mixed models (GLMMs) to investigate the relationship between CWD prevalence and environmental characteristics, using separate models for mule deer and white-tailed deer. Restricted maximum likelihood estimation (REML) was used while creating candidate models (Zuur *et al*.2009). Prevalence was included in the model as the response variable using the "cbind" function in R to combine number the number of CWD positive individuals relative to the number of negative samples for each spatial unit: cbind(positives, negatives). This function was utilized to retain sample size for each spatial unit and account for differences in sampling effort among spatial units. The scores from the retained principal components were used directly in the GLMM as predictor variables. The residuals of our initial models run with a binomial distribution were overdispersed, therefore, we used the *glmmTMB* package (Brooks *et al*. 2017) to create GLMMs with beta-binomial distribution and a log-link function, recommended for modelling proportional data derived from counts and its ability to correct for overdispersion (Douma & Weedon, 2019). State/province was included in the models as a random intercept and a random slope to account for regional differences and for differences in the relationship between PCs among regions, respectively. Model residuals were plotted with the *DHARMa* package (Hartig, 2021) to quantify goodness of fit using a QQ residual plot, a plot of standardized residuals against model predictions, a nonparametric dispersion test, and a zero inflation test. Moran's I was tested on the residuals to determine if spatial autocorrelation was detected in the model using the function "moran.test" in the *spdep* package (Bivand & Wong, 2018). Akaike's Information Criterion (AICc) adjusted for small sample size was used to compare models to each other using in R using the *AICcmodavg* package (Mazerolle, 2020). Specifically, AICc, change in AICc, and AIC weight were used as determinants of model fit and we considered models that were < 2 AICc units within the best model to be competing models. If two models were competing based on AIC, a model was chosen based on the principle of parsimony, that the simpler explanation is more likely to be correct.

RESULTS

In total, 382 white-tailed deer and 194 mule deer spatial units were included in the analysis. Mule deer data came from three states or provinces: Montana, Saskatchewan, and Wyoming, while white-tailed deer data came from five: Illinois, Michigan, Montana, Pennsylvania, Saskatchewan, and Wisconsin. In our dataset, a total of 161,544 white-tailed deer and a total of 14,455 mule deer were tested for CWD. White-tailed deer CWD prevalence ranged from 0% to 69.5% with a mean prevalence of 0.0240% and mule deer CWD prevalence ranged from 0% to 67.1% with a mean prevalence of 0.0929% within spatial units.

White-tailed deer analysis

The white-tailed deer principal component analysis (PCA) incorporated eight environmental variables. We retained three principal components which in total explained 81.8% of the data variance (Table 3). The strongest positive loadings on PC1 were proportion agriculture, soil moisture, soil clay, while the proportion of natural land loaded negatively. Strong loadings on PC2 included the negative effects of soil cation exchange capacity and soil organic matter, and the positive effects of soil clay and soil moisture. Strong loadings on PC3 included the positive effects of soil pH and soil cation exchange capacity while proportion of developed land loaded negatively.

The addition of random slopes to our GLMMs did not improve the fit of models but adding region as a random intercept did. Two models were identified with ΔAICc <2 (Table 4), but the first model was the most parsimonius and was 2.68 times more likely than the second based on relative AIC_c weights. In addition, the confidence limits for the additional variable (PC3) in the second model encompassed zero, and so including this predictor did not improve the explanatory power of the model. Based on the top model there was a positive relationship between CWD prevalence with PC1 and PC2 (Table 5). CWD prevalence was higher in areas that had higher proportions of agricultural land cover, soils with high moisture and high clay content, and a smaller proportion of natural land cover. CWD prevalence was also lower in areas high in soil cation exchange capacity, high in soil organic matter and low levels of soil clay. Moran's I test of the model residuals indicated no significant spatial autocorrelation of residual errors.

Mule deer analysis

For the mule deer data, we retained two principal components which explained 74.8% of the variance (Table 3). In order, the strongest positive loadings on PC1 were percent soil moisture, proportion agriculture, percent soil cation exchange capacity, percent soil clay, and developed land cover while proportion natural land cover loaded negatively. Strong loadings on PC2 included the positive effects of soil organic matter and the negative effects of soil pH and percent soil clay. Candidate mule deer models are listed in Table 6 and three competing models were within 2 ΔAICc. The best-fit model had a positive relationship between CWD prevalence and PC1 and included the random intercept for region (Table 7). Specifically, this model indicated that CWD prevalence was positively associated with areas that had higher proportions of agricultural land cover, high levels of soil moisture, soil cation exchange capacity, and clay, and a smaller proportion of natural land cover. However, the slope for PC1 was low and marginally above zero, and overall evidence for this model was relatively weak. Although two other models had relatively low AICc values, the slopes for the additional fixed effect (PC2) in the second- and third-ranked models had confidence limits that overlapped zero. The random-intercept only model was slightly over 2 AICc units from the top model (ΔAICc = 2.01), but was 2.75 times less likely than the top model based on relative AICc weights. Moran's I test indicated that there was no significant spatial autocorrelation of residual errors in the mule deer models.

DISCUSSION

In this study, we obtained CWD prevalence, land cover, and soil characteristic data from seven North American regions to determine the influence of land use and soil characteristics on CWD prevalence. The results from our analysis supported our prediction that both land cover and soil characteristics will have a significant effect on CWD prevalence in deer. Specifically, our model indicated that a higher proportion of agricultural land cover, percent soil clay, and soil moisture content are positively associated with CWD prevalence for white-tailed deer. Conversely, proportion of natural land cover, percent soil cation exchange capacity, and percent soil organic matter are negatively related to CWD prevalence for white-tailed deer. Similarly, higher agricultural land cover, percent soil clay, soil moisture content, and lower proportion of natural land cover were positively associated with CWD prevalence in mule deer, but unlike for white-tailed deer, percent soil cation exchange capacity was positively associated with CWD prevalence in this species and soil organic matter was not important. In our analyses, land cover represented features are likely to influence deer movement and grouping behavior (Kjær *et al*. 2008; Koen *et al*. 2017; Nixon *et al*. 1991; Urbanek *et al*. 2013), which is likely tied to direct transmission, while soil characteristics represented features are likely to influence prion persistence in the environment (Kuznetsova *et al*. 2018; Saunders *et al*. 2012; Wyckoff *et al*. 2016) resulting in indirect transmission. The consistent effect of both land use and soil characteristics in explaining CWD prevalence across the two deer species provides compelling evidence that both direct and indirect transmission may be important in driving and maintaining CWD infection, and that the soil environmental reservoir may play a larger role than previously recognized. Further, because we utilized data from multiple regions and models including random slopes performed poorly relative to random intercept models, our results suggest that there are consistent patterns in the influence of environmental characteristics on CWD prevalence across regions and over large spatial scales.

Environmental reservoirs are important in the persistence of a disease by maintaining disease transmission autonomously of a host (Hoyt *et al*. 2020). Our model suggests that certain soil characteristics influence the likelihood of CWD transmission. In areas where soil clay and moisture content are high, CWD prevalence for both white-tailed deer and mule deer increased. Clay content was positively associated with CWD infection risk in a field study and a lab study found evidence that clay binds strongly to prions (Saunders *et al*. 2012; Walter *et al*. 2011). Soil organic matter was found to have a negative relationship with CWD prevalence for just white-tailed deer. Humic acid, a common component of soil organic matter, has been shown to decrease infectivity and recovery of CWD prions (Kuznetsova *et al*. 2018). Soil cation exchange capacity had a negative relationship with CWD prevalence for white-tailed deer and a positive relationship for mule deer; prior studies have not addressed the relationship of soil cation exchange capacity with CWD prions. In other disease systems, both indirect and direct disease transmission have been found to contribute to disease persistence. For example, chytrid fungal pathogen infects amphibians and transmission via environmental reservoirs has been shown to induce equivalent disease progression and mortality when compared to direct transmission (Burns *et al*. 2020; Kolby *et al*. 2015). Additionally, white nose syndrome in bats is transmitted both indirectly via environmental reservoirs in hibernacula and through direct transmission by individuals (Hoyt *et al*. 2021). The fact that multiple transmission pathways can have significant impacts on overall disease persistence underlines the importance of investigating all modes of transmission. Neglecting to consider either indirect or direct disease transmission in a system could lead to an incomplete understanding of disease dynamics and ineffective disease management strategies.

We found an association between CWD prevalence and land cover which could be due to changes in deer behaviors that influence direct disease transmission. In our model, agricultural land cover was positively related to CWD prevalence and natural land cover was negatively related to CWD prevalence for both white-tailed deer and mule deer. Deer herds aggregate seasonally in agricultural areas due to availability of crops as food resources (Kjær *et al*. 2008; Nixon *et al*. 1991; Urbanek *et al*. 2013). Deer have been shown to shift the center of their home range and decrease its size during crop growing seasons (Vercauteren & Hygnstrom, 1998). Deer sociality has been shown to increase in areas high in agriculture and low in forest (Koen *et al*. 2017). Also, deer population densities may be higher in areas with high patch density of agricultural land use (Urbanek *et al*. 2013). Previous studies have found conflicting results regarding the influence of land cover on CWD prevalence. However, these studies took place in different regions over small spatial scales and the differences in results among these studies may be related to more local scale factors influencing deer movement and grouping. Overall, the results from our study show that land cover features that influence deer grouping and movement may act to promote contact opportunities between individuals and therefore increase direct transmission of CWD.

The broad-scale patterns we observed with respect to the influence of land cover and soil characteristics on CWD prevalence are relevant at a large spatial scale, but local-scale dynamics may be influenced by additional factors not included in our study. The data included in our analysis were necessarily coarse in resolution (at the level of deer management unit or county), and local factors, such as availability of habitat corridors, linear barriers to deer movement such as rivers, and fine-scale habitat distribution may influence local patterns of prevalence (Kelly *et a*l. 2014 ; Robinson *et al*. 2013). Consideration of other factors that may influence direct transmission of CWD, such as sex, age, and deer population density, would give a more accurate measurement of contact rates among individuals and help characterize the influence of direct transmission. Further, our analyses did not include data from all areas in which CWD is present in North America, and it would be instructive to validate our conclusions by incorporating additional data from other affected regions, with different combinations of land covers and soil characteristics, in our models.

Our results demonstrated that agricultural land cover, high soil moisture, and high soil clay represent environmental conditions that increase CWD risk, while natural land cover was associated with decreased CWD risk for both mule deer and white-tailed deer. The finding that both land cover and soil characteristics influence disease prevalence suggests that both indirect and direct transmission pathways contribute to CWD persistence. Additionally, the relationship between CWD prevalence and environmental characteristics followed consistent patterns across a large spatial scale and was similar for both white-tailed deer and mule deer. Although CWD is widespread across North America, there are many regions where CWD has not been detected in wild cervid populations. The findings in our study suggest that certain environmental characteristics may act to mediate patterns of CWD infection when the disease is introduced to a new area, and that certain areas may be less likely to experience CWD outbreaks following introductions. Additionally, our results can be useful to wildlife managers who may concentrate management efforts in areas associated with an elevated CWD risk due to the presence of specific land cover and soil attributes.

REFERENCES

Bivand, R. S., & Wong, D. W. S. (2018). Comparing implementations of global and local indicators of spatial association. Test (Madrid, Spain), 27(3), 716–748.

Bradley, C. A., & Altizer, S. (2007). Urbanization and the ecology of wildlife diseases. *Trends in Ecology and Evolution*, *22*(2), 95–102.

Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Maechler M, Bolker BM (2017). “glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling.” *The R Journal*, 9(2), 378–400.

Burns, T. J., Scheele, B. C., Brannelly, L. A., Clemann, N., Gilbert, D., & Driscoll, D. A. (2021). Indirect terrestrial transmission of amphibian chytrid fungus from reservoir to susceptible host species leads to fatal chytridiomycosis. *Animal Conservation*, *24*(4), 602–612.

Dorak, S. J., Green, M. L., Wander, M. M., Ruiz, M. O., Buhnerkempe, M. G., Tian, T., Novakofski, J.E., Mateus-Pinilla, N. E. (2017). Clay content and pH: Soil characteristic associations with the persistent presence of chronic wasting disease in northern Illinois. *Scientific Reports*, *7*(1), 1–10.

Escobar, L. E., Pritzkow, S., Winter, S. N., Grear, D. A., Kirchgessner, M. S., Dominguez-villegas, *et al*. (2020). The ecology of chronic wasting disease in wildlife. *Biological Reviews*. *95*(2), 393–408.

ESRI, Environmental Systems Research Institute, Redlands, C. ArcGIS Pro, ArcMaps 10.8.1

Evans, T. S., Kirchgessner, M. S., Eyler, B., Ryan, C. W., & Walter, W. D. (2016). Habitat influences distribution of chronic wasting disease in white-tailed deer. *Journal of Wildlife Management*, *80*(2), 284–291.

Farnsworth, M. L., Wolfe, L. L., Hobbs, N. T., Burnham, K. P., Williams, E. S., Theobald, D. M. *et al*. (2005). Human Land Use Influences Chronic Wasting Disease Prevalence in Mule Deer. *Ecologica*, *15*(1), 119–126.

Field, A., Miles, J., & Field, Z. (2012). Discovering statistics using R. SAGE Publications.

Habib, T. J., Merrill, E. H., Pybus, M. J., & Coltman, D. W. (2011). Modelling landscape effects on density-contact rate relationships of deer in eastern Alberta: Implications for chronic wasting disease. *Ecological Modelling*, *222*(15), 2722–2732.

Hartig, F. (2021). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.4.

Hoyt, J. R., Kilpatrick, A. M., & Langwig, K. E. (2021). Ecology and impacts of white-nose syndrome on bats. *Nature Reviews Microbiology*, *19*(3), 196–210.

Hoyt, J. R., Langwig, K. E., Sun, K., Parise, K. L., Li, A., Wang, Y. *et al*. (2020). Environmental reservoir dynamics predict global infection patterns and population impacts for the fungal disease white-nose syndrome. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(13), 7255–7262.

Johnson, C. J., Pedersen, J. A., Chappell, R. J., McKenzie, D., & Aiken, J. M. (2007). Oral transmissibility of prion disease is enhanced by binding to soil particles. *PLoS Pathogens*, *3*(7), 0874–0881.

Kelly, A. C., Mateus-Pinilla, N. E., Brown, W., Ruiz, M. O., Douglas, M. R., Douglas, M. E. et al. (2014). Genetic assessment of environmental features that influence deer dispersal-implications for prion-infected populations. *Population Ecology*, *56*, 327–340.

Kjær, L. J., Schauber, E. M., & Nielsen, C. K. (2008). Spatial and Temporal Analysis of Contact Rates in Female White-Tailed Deer. *Journal of Wildlife Management*, *72*(8), 1819–1825.

Koen, E. L., Tosa, M. I., Nielsen, C. K., & Schauber, E. M. (2017). Does landscape connectivity shape local and global social network structure in white-tailed deer ? *PLoS ONE*, *12*(3), 1–21.

Kolby, J. E., Ramirez, S. D., Berger, L., Richards-Hrdlicka, K. L., Jocque, M., & Skerratt, L. F. (2015). Terrestrial dispersal and potential environmental transmission of the amphibian chytrid fungus (Batrachochytrium dendrobatidis). *PLoS ONE*, *10*(4), 1–13.

Kuznetsova, A., Cullingham, C., McKenzie, D., & Aiken, J. M. (2018). Soil humic acids degrade CWD prions and reduce infectivity. *PLoS Pathogens*, *14*(11), 1–11.

Kuznetsova, A., McKenzie, D., Banser, P., Siddique, T., & Aiken, J. M. (2014). Potential role of soil properties in the spread of CWD in western Canada. *Prion*, *8*(1).

Lê S, Josse J, Husson F (2008). “FactoMineR: A Package for Multivariate Analysis.” *Journal of Statistical Software*, 25(1), 1–18.

Mateus-Pinilla, N., Weng, H. Y., Ruiz, M. O., Shelton, P., & Novakofski, J. (2013). Evaluation of a wild white-tailed deer population management program for controlling chronic wasting disease in Illinois, 2003-2008. *Preventive Veterinary Medicine*, *110*(3–4), 541–548.

Mazerolle MJ (2020). AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.3-1

Nixon, C. M., Hansen, L. P., Brewer, P. A., & Chelsvig, J. E. (1991). Ecology of White-Tailed Deer in an Intensively Farmed Region of Illinois. *Wildlife Monographs*, (118), 3–77.

O’Hara Ruiz, M., Kelly, A. C., Brown, W. M., Novakofski, J. E., & Mateus-Pinilla, N. E. (2013). Influence of landscape factors and management decisions on spatial and temporal patterns of the transmission of chronic wasting disease in white-tailed deer. *Geospatial Health*, *8*(1), 215–227.

Osterholm, M. T., Anderson, C. J., Zabel, M. D., Scheftel, J. M., Moore, K. A., & Appleby, B. S. (2019). Chronic wasting disease in cervids: Implications for prion transmission to humans and other animal species. *MBio*, *10*(4), 1–8.

Podgórski, T., Apollonio, M., Keuling, O. (2018). Contact rates in wild boar populations: Implications for disease transmission. *Journal of Wildlife Management*, *82*(6), 1210-1218

Polfus, J. L., & Krausman, P. R. (2012). Impacts of residential development on ungulates in the Rocky Mountain West. *Wildlife Society Bulletin*, *36*(4), 647–657.

R Core Team (2021). R. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rivera, N. A., Brandt, A. L., Novakofski, J. E., & Mateus-Pinilla, N. E. (2019). Chronic wasting disease in cervids: prevalence, impact and management strategies. *Veterinary Medicine: Research and Reports*. *10*, 123–139.

Robinson, S. J., Samuel, M. D., Rolley, R. E., & Shelton, P. (2013). Using landscape epidemiological models to understand the distribution of chronic wasting disease in the Midwestern USA. *Landscape Ecology*, *28*(10), 1923–1935.

Saunders, S. E., Bartz J.C., Bartelt-Hunt,S.L.(2012) Soil-mediated prion transmission: Is local soil-type a key determinant of prion disease incidence? *Chemosphere*, 87, Issue 7,661-667

Smith, C. B., Booth, C. J., & Pedersen, J. A. (2011). Fate of Prions in Soil: A Review. *Journal of Environmental Quality*, *40*(2), 449–461.

Storm, D. J., Samuel, M. D., Rolley, R. E., Shelton, P., Keuler, N. S., Richards, B. J., & Van Deelen, T. R. (2013). Deer density and disease prevalence influence transmission of chronic wasting disease in white-tailed deer. *Ecosphere*, *4*(1).

Sulaiman, M. S., Abood, M. M., Sinnakaudan, S. K., Shukor, M. R., You, G. Q., & Chung, X. Z. (2019). Assessing and solving multicollinearity in sediment transport prediction models using principal component analysis. *ISH Journal of Hydraulic Engineering*, *00*(00), 1–11.

Urbanek, R. E., & Nielsen, C. K. (2013). Influence of landscape factors on density of suburban white-tailed deer. *Landscape and Urban Planning*, *114*, 28–36.

Vercauteren, K. C., & Hygnstrom, S. E. (1998). Effects of Agricultural Activities and Hunting on Home Ranges of Female White-Tailed Deer. *The Journal of Wildlife Management*, *62*(1), 280–285.

Walter, W. D., Walsh, D. P., Farnsworth, M. L., Winkelman, D. L., & Miller, M. W. (2011). Soil clay content underlies prion infection odds. *Nature Communications*, 6–11.

Wickham H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag. New York. ISBN 978-3-319-24277-4

Williams K, Hughson, A.G., Chesebro, B., Race, B. (2019) Inactivation of chronic wasting disease prions using sodium hypochlorite. *PLoS ONE*, *14*(10): e0223659.

Wyckoff, A.C., Kane, S., Lockwood, K., Seligman, J., Michel, B., Hill, D., Ortega, A., Mangalea, M.R., Telling, G.C., Miller, M.W., Vercauteren, K., and Zabel, M.D. (2016) Clay components in soil dictate environmental stability and bioavailability of cervid prions in mice. *Frontiers in Microbiology*. 7:1885

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, *1*(1), 3–14.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). Mixed Effects Modelling for Nested Data BT- Mixed effects models and extensions in ecology with R. (pp. 101–142). New York, NY: Springer New York.

Table 1: CWD data source detail in seven regions across North America.

Region	Species	Age/sex of deer	Years included	Data Source	Spatial unit type, mean area (km^2)
Illinois, USA	White-tailed deer	Male/Female	2017-2020	Hunter-harvested deer, publicly available	County 1,615,159
Michigan, USA	White-tailed deer	Male/Female	2018-2020	Hunter-harvested deer, publicly available	County 1,825,867
Montana, USA	White-tailed deer and mule deer	Male/Female	2017-2021	Hunter-harvested or agency removed deer, publicly available	Hunting district 2,585,830
Pennsylvania, USA	White-tailed deer	Male/Female	2018-2021	Hunter-harvested or roadkill deer, publicly available	County 1,749,894
Saskatchewan, CAN	White-tailed deer and mule deer	Male	2018-2020	Hunter-harvested deer, Saskatchewan Ministry of Environment	Wildlife management zone 4,953,219
Wisconsin, USA	White-tailed deer	Male/Female	2018-2020	Hunter-harvested or agency removed deer, publicly available	County 2,018,402
Wyoming, USA	Mule deer	Male	2018-2020	Hunter-harvested deer, publicly available	Herd unit 6,423,385

Table 2: List of soil characteristics obtained using ArcGIS and their relationship with prions/proteins adapted from Dorak *et al*. (2017).

Soil characteristic	**Description**	**Relationship with prions/proteins**	**Units**
Clay	Mineral soil particles that are less than 0.002 millimeter in diameter. The estimated clay content of each soil layer is given as a percentage, by weight, of the soil material that is less than 2 millimeters in diameter.	Binds strongly to prions, affects availability of prions	Expressed as proportion out of 1
Sand	Mineral soil particles that are 0.05 millimeter to 2 millimeters in diameter. In the database, the estimated sand content of each soil layer is given as a percentage, by weight, of the soil material that is less than 2 millimeters in diameter.	Binds less with prions relative to silt and clay	Expressed as proportion out of 1
Silt	Mineral soil particles that are 0.002 to 0.05 millimeter in diameter. In the database, the estimated silt content of each soil layer is given as a percentage, by weight, of the soil material that is less than 2 millimeters in diameter.	Binds less with prions relative to clay	Expressed as proportion out of 1
Organic matter	Plant and animal residue in the soil at various stages of decomposition. The estimated content of organic matter is expressed as a percentage, by weight, of the soil material that is less than 2 millimeters in diameter.	Binds with prions, affects availability	Expressed as average weight percentage
Cation exchange capacity (cation exchange capacity)	(cation exchange capacity-7) is the total amount of extractable cations that can be held by the soil, expressed in terms of milliequivalents per 100 grams of soil at neutrality (pH 7.0) or at some other stated pH value.	Affects binding to soil particles	Expressed as average mq/100 g soil
pH	The measurement of pH is of acidity or alkalinity using the 1:1 water method. A crushed soil sample is mixed with an equal amount of water, and a measurement is made of the suspension.	Affects prion charge and adsorption/desorption to soil particles	Expressed as average pH
Water content	The amount of soil water retained at a tension of 1/3 bar, expressed as a volumetric percentage of the whole soil.	Affects decomposition of proteins	Expressed as average volumetric percentage

Table 3: Principal component eigenvalues, percent variance and factor loadings for principal component analyses in white-tailed deer and mule deer data. Loadings >0.5 are shown in bold.

	White-tailed deer			**Mule deer**	
	PC1	**PC2**	**PC3**	**PC1**	**PC2**
Eigenvalue	2.76	2.39	1.41	4.21	1.77
Percent variance	34.4%	29.8%	17.6%	52.6%	22.2%
Factor loadings:					
Soil clay	**0.58**	**0.61**	0.17	**0.72**	**-0.52**
Soil pH	0.44	0.40	**0.60**	0.32	**-0.75**
Soil cation exchange capacity	0.36	**-0.71**	**0.53**	**0.86**	-0.072
Soil organic matter	0.26	**-0.88**	0.34	-0.31	**0.66**
Soil moisture	**0.77**	**0.53**	-0.12	**0.94**	-0.04
Natural	**-0.81**	0.41	0.35	**-0.87**	-0.40
Developed	0.13	-0.34	**-0.67**	**0.56**	0.43
Agriculture	**0.88**	-0.086	-0.20	**0.88**	0.38

Table 4: Set of generalized linear models used to test the effect of environmental variables on CWD prevalence in white-tailed deer ordered by AICc values. The response variable in every model is CWD prevalence, PCs are principal components, region is a random intercept, and slopes are random slope terms. Models shown in bold are competitive models (within 2 AICc units of the top model).

Model rank	Model structure	K	AICc	Δ AICc	AICcWeight
1	**PC1+PC2+(1|region)**	**5**	**1240.48**	**0.00**	**0.59**
2	**PC1+PC2+PC3+(1|region)**	**6**	**1242.42**	**1.94**	**0.22**
3	PC1+PC2+(1|region)+(PC1|region)+(PC2|region)	7	1243.56	3.09	0.13
4	PC1+PC2+PC3+(1|region)+(PC1|region)+(PC2|region)+(P3|region)	9	1247.73	7.25	0.02
5	PC2+ (1|region)	4	1248.22	7.75	0.01
6	PC1+ (1|region)	4	1248.54	8.06	0.01
7	PC2+PC3+(1|region)	5	1249.89	9.41	0.01
8	PC1+PC3+(1|region)	5	1250.11	9.63	0.00
9	PC2+(1|region)+(PC2|region)	5	1250.28	9.80	0.00
10	PC1+PC2	4	1250.52	10.04	0.00
11	PC1+(1|region)+(PC1|region)	5	1250.59	10.11	0.00
12	PC1+PC2+PC3	5	1252.21	11.73	0.00
13	PC2+PC3+(1|region)+(PC2|region)+(PC3|region)	7	1254.01	13.54	0.00
14	PC1+PC3+(1|region)+(PC1|region)+(PC3|region)	7	1254.05	13.58	0.00
15	(1|region)	3	1258.24	17.77	0.00
16	PC3+ (1|region)	4	1260.24	19.76	0.00
17	PC3+(1|region)+(PC3|region)	5	1262.29	21.81	0.00
18	PC1	3	1271.24	30.77	0.00
19	PC1+PC3	4	1272.25	32.26	0.00
20	PC2	3	1324.43	83.96	0.00
21	PC2+PC3	4	1325.30	84.83	0.00
22	PC3	3	1348.06	107.58	0.00

Table 5: Parameter values for the best candidate models describing the influence of environmental variables on CWD prevalence in white-tailed deer

Model term	**Estimate**	**Std. error**	**2.5 LCI**	**97.5 UCI**	**Z value**	**p-value**
Model 1: PC1+PC2+ (1|region)						
Intercept	-4.01	0.291	-4.58	-3.44	-13.8	<2e-16***
PC1	0.237	0.0801	0.0803	0.394	2.96	0.00305**
PC2	-0.270	0.0911	-0.449	-0.0915	-2.96	0.00304**
Model 2: PC1+PC2+PC3+ (1|region)						
Intercept	-4.00	0.299	-4.58	-3.41	-13.4	<2e-16***
PC1	0.232	0.0803	0.0750	0.390	2.90	0.00379**
PC2	-0.276	0.0922	-0.457	-0.0955	-3.00	0.00273**
PC3	0.0405	0.123	-0.201	0.282	0.330	0.742

Table 6: Set of generalized linear models used to test the effect of environmental variables on CWD prevalence in mule deer ordered by AICc values. The response variable in every model is CWD prevalence, PCs are principal components, region is a random intercept, and slopes are random slope terms. Models shown in bold are competitive models (within 2 AICc units of the top model).

Model rank	**Model structure**	**K**	**AICc**	**Δ AICc**	**AICc Wt**
1	**PC1+ (1|region)**	**4**	**673.21**	**0.00**	**0.33**
2	**PC2+ (1|region)**	**4**	**674.63**	**1.42**	**0.16**
3	**PC1+PC2+(1|region)**	**5**	**674.72**	**1.51**	**0.16**
4	**(1|region)**	**3**	**675.22**	**2.01**	**0.12**
5	PC1+ (1|region)+(PC1|region)	5	675.32	2.11	0.12
6	PC2+ (1|region)+(PC2|region)	5	676.06	2.85	0.08
7	PC1+PC2+(1|region)+(PC1|region)+(PC2|region)	7	678.07	4.86	0.03
8	PC1+PC2	4	746.96	73.75	0.00
9	PC1	3	756.57	83.36	0.00
10	PC2	3	801.71	128.49	0.00

Table 7: Parameter values for the best candidate models describing the influence of environmental variables on CWD prevalence in mule deer

Model term	**Estimate**	**Std. error**	**2.5 LCI**	**97.5 UCI**	**Z value**	**p-value**
Model 1: PC1+ (1|region)						
Intercept	-2.30	0.956	-4.17	-0.423	-2.40	0.0163*
PC1	0.191	0.0962	0.00249	0.380	1.99	0.0471*
Model 2: PC2+ (1|region)						
Intercept	-2.36	1.10	-4.50	-0.210	-2.15	0.0315*
PC2	0.213	0.127	-0.0358	0.461	1.68	0.0935
Model 3: PC1+PC2+ (1|region)						
Intercept	-2.35	1.01	-4.34	-0.364	-2.32	0.0204*
PC1	0.148	0.110	-0.0674	0.364	1.35	0.178
PC2	0.119	0.143	-0.162	0.399	0.829	0.407

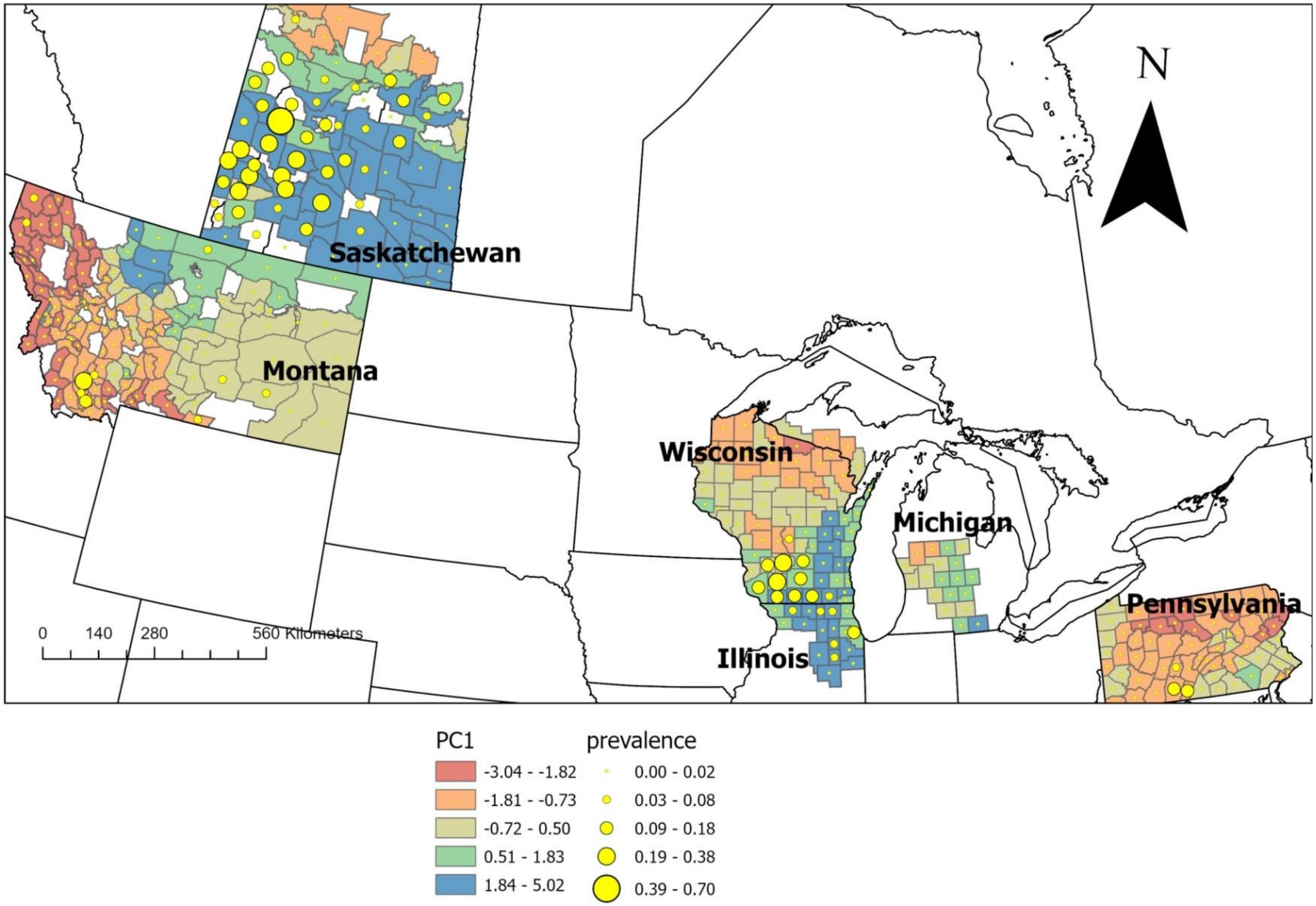

Figure 1: White-tailed deer prevalence (yellow circles) and PC1 scores in our study area. CWD prevalence had a significantly positive relationship with PC1 in our analysis. Spatial units with blue coloring have high principal component scores and are associated with areas high in agricultural land cover, soil clay, soil moisture content. Red colored spatial units have lower principal component scores and are associated with a higher proportion of natural land cover.

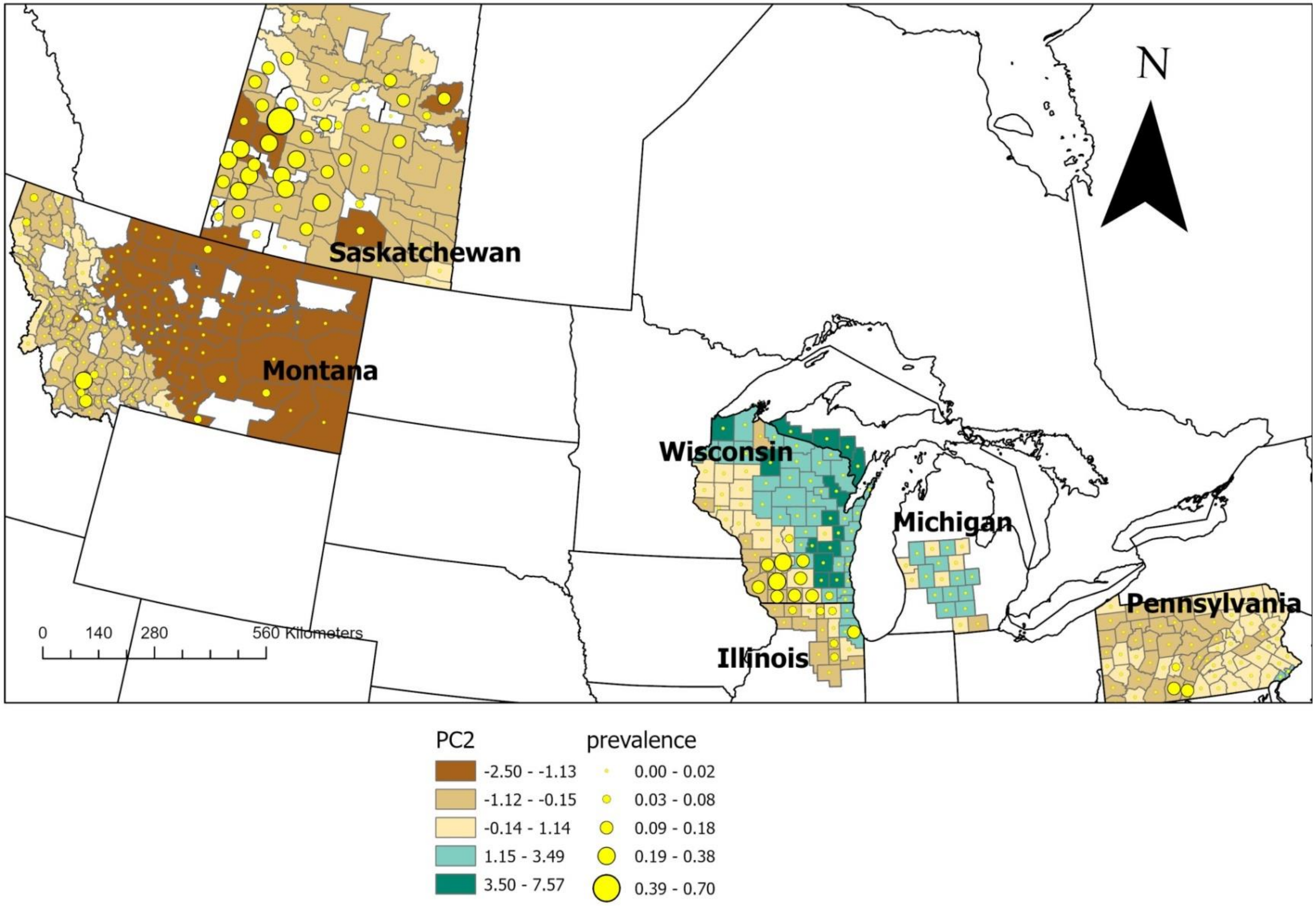

Figure 2: White-tailed deer prevalence (yellow circles) and PC2 scores in our study area. In the top model, CWD prevalence was negatively related to PC2. Spatial units with green coloring have high principal component scores and are associated with lands with higher soil cation exchange capacity and soil organic matter. Brown colored spatial units have low principal component scores and are associated with areas with higher clay content and soil moisture.

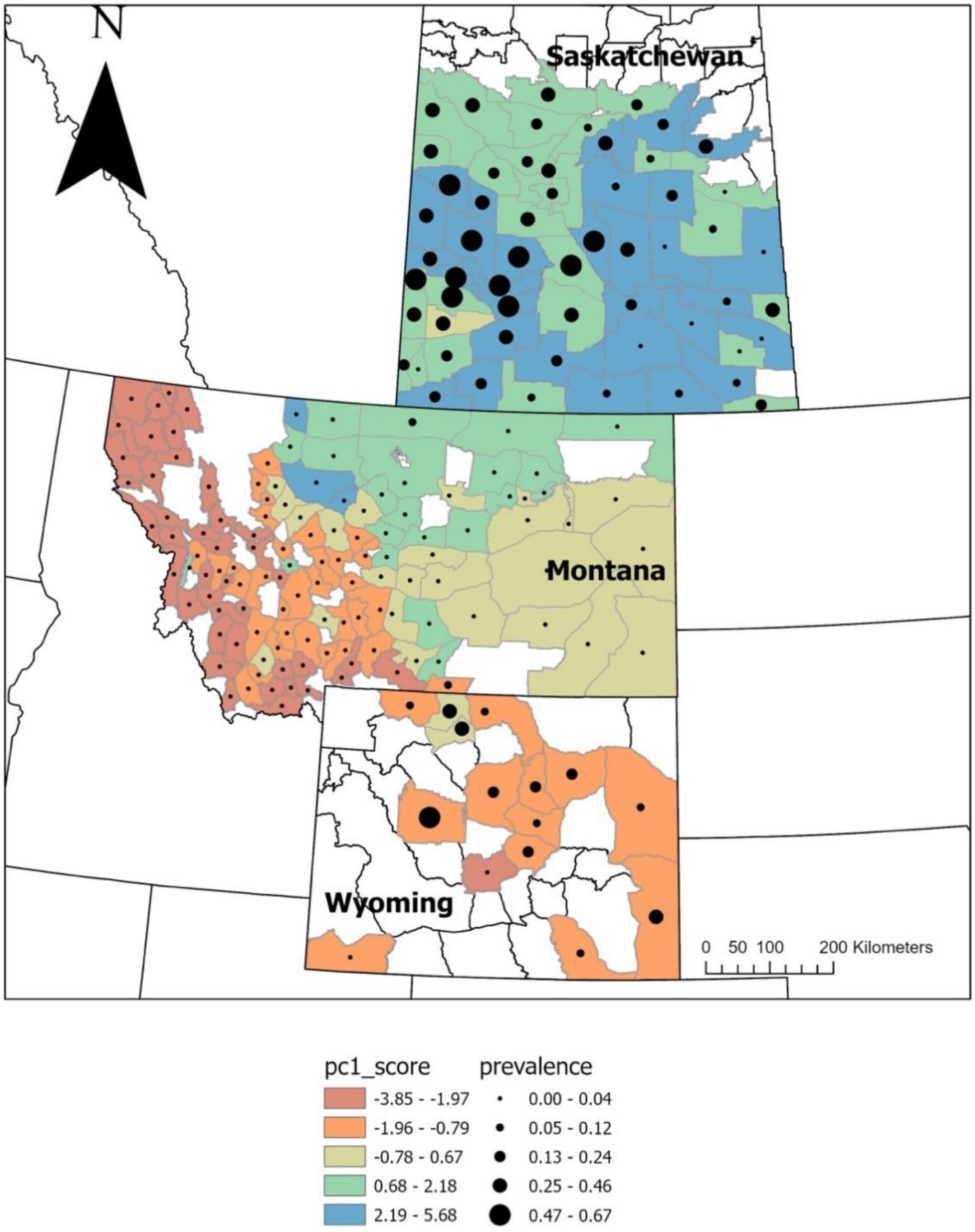

Figure 3: Mule deer prevalence (black circles) and PC1 scores in our study area. In the top model, CWD prevalence had a positive relationship with PC1. Spatial units with blue coloring have high principal component scores and are associated with higher proportion of agricultural land cover, higher soil clay, soil cation exchange capacity, and soil moisture content. Red colored spatial units have low principal component scores and are associated with a higher proportion of natural land cover.

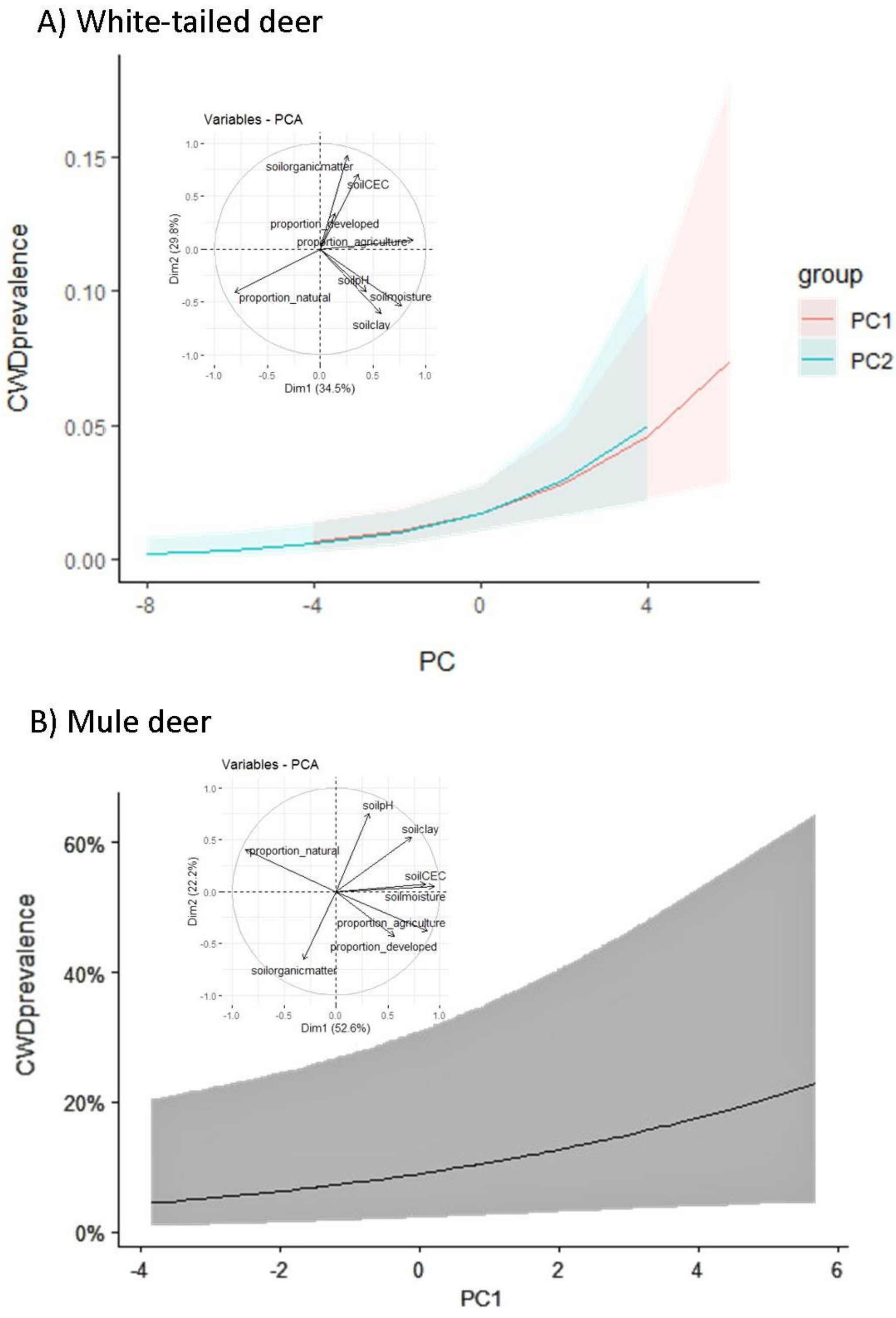

Figure 4: Relationship between environmental variables (shown in the principle component biplots) and CWD prevalence in a) white-tailed deer, and b) mule deer. In white tailed-deer CWD prevalence increases in relation to PC1 and PC2. In mule deer, CWD prevalence increases in relation to PC1.

SUPPLEMENTAL DATA

S1. CWD prevalence data sources for each region included in our analysis.

Region	Data Name	Source
Illinois	Illinois Department of Natural Resources: CWD Annual Reports	https://www2.illinois.gov/dnr/programs/CWD/Pages/default.aspx
Michigan	Michigan Department of Natural Resources: CWD Testing Results	https://www.michigan.gov/dnr/0,4570,7-350-79136_79608_90516_90536_90552_90560---,00.html
Montana	Montana Fish, Wildlife & Parks' 2020 Chronic Wasting Disease Surveillance and Monitoring Report	https://fwp.mt.gov/binaries/content/assets/fwp/conservation/wildlife-reports/cwd/2020-cwd-surveillance-report_final.pdf
Pennsylvania	Pennsylvania Game Commission: CWD Results and Surveillance	https://pgcdatacollection.pa.gov/CWDResultsLookup
Saskatchewan	CWD 3-YR Pooled Prevalence Estimates in White-Tailed Deer (2018-2020)	Obtained via personal correspondence with Iga Stasiak, DVM, DVSc, DACVPM, Provincial Wildlife Health Specialist, Saskatchewan Ministry of Environment
Wisconsin	Wisconsin Department of Natural Resources: CWD Deer Testing Results by County	https://dnr.wi.gov/wmcwd/Summary/County
Wyoming	Wyoming Game and Fish Department 2020: Chronic Wasting Disease Surveillance Report: May 2021	https://wgfd.wyo.gov/WGFD/media/content/PDF/Vet%20Services/2020-CWD-Surveillance-Report-final.pdf