SOIL PH AND CLAY CONTENT ASSOCIATED WITH CHRONIC WASTING DISEASE IN WHITE-TAILED DEER IN NORTHERN ILLINOIS

BY

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THESIS

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ABSTRACT

Soil is an important reservoir for chronic wasting disease (CWD) which is a prion disease that infects cervids through both direct contact with infected animals and contact with contaminated environments. I built a boosted regression tree model that accurately predicted (AUC = 0.954) the probability of CWD presence in northern Illinois based on soil characteristics (soil texture, pH, cation exchange capacity, organic matter, and water content), then used the outcome to assess possible pathways by which soil characteristics increase the probability of transmission via environmental contamination. The model indicates CWD is likely to be present where: soil pH is greater than 6.6, percent clay is lower than 20%, cation exchange capacity (CEC) is lower than 15 meq/100g, and soil organic matter is less than 4.5%. Soil pH and the abundance of clays and associated soil organic matter and CEC appear to alter the availability of prions immobilized in soil. The results suggest that exposure to prions through probable routes of infection such as inhalation or ingestion is greatest where pH is greater than 6.6 and the percent clay is less than 20%.

ACKNOWLEDGEMENTS

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INTRODUCTION

Soil contributes to the spread of several diseases that infect animals by serving as a long-term reservoir for diseases such as histoplasmosis, anthrax, and scrapie [1–3]. One disease for which soil has been more recently implicated as an important reservoir is chronic wasting disease (CWD)[4]. Chronic wasting disease, like scrapie, is a transmissible spongiform encephalopathy (TSE) caused by infectious prions that causes neurodegenerative effects in the infected host inevitably leading to death [5]. It predominately infects cervids including mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), elk (*Cervus elaphus*), and moose (*Alces alces*). Since its discovery in Colorado in 1967, CWD has been detected in 24 U.S. states, 2 Canadian provinces, the Republic of Korea, and Norway [5–8]. In Illinois, CWD was first detected in the northern part of the state in 2002 and has since been detected in 16 counties. In response, the Illinois Department of Natural Resources (IDNR) designed and implemented management strategies that included surveillance of harvested deer throughout the state and reduction of deer densities in CWD affected counties through increased harvest opportunities and localized culling in locations where CWD is known to occur [9]. These management strategies are aimed primarily at lowering deer densities in and around CWD affected areas to reduce the risk of direct transmission[10]. Direct transmission occurs through deer to deer contact of bodily fluids and also through vertical transmission from doe to fawn [11–15]. CWD is also transmitted indirectly through contact (ingestion or inhalation) with the contaminated environment (soil and plants) caused by prions shed from infected deer through excreta, bodily fluids, antler velvet, and also from decaying carcasses [4,11,16–22].

Because of its complex nature, the study of the indirect transmission of CWD through the environment continues to prompt questions. Empirical evidence suggest soils contribute to the environmental transmission of CWD by influencing prion availability and persistence [23]. Adsorption (defined here as the physical or chemical binding of a prion to a soil solid phase) has been repeatedly examined for its role in prion persistence and availability in soil [24]. Many studies have shown the capacity of whole soils and mineral components to adsorb prions and explored the mechanisms underlying prion-soil interactions [19,25–27]. Soil characteristics found to influence adsorption include texture (e.g. clay, sand, silt), organic matter, soil moisture, pH, ionic strength, and cation exchange capacity [26,28,29]. Because adsorption mechanisms and a soil's capacity to adsorb vary depending on these characteristics, the amount of prion stabilized in soil also varies [23]. Our understanding of factors that determine whether prions immobilized in soils remain bioavailable to organisms that could degrade and/or ingest them are hindered by the absence of reliable experimental models and the complexity of these systems [26].

Identification of areas that are highly susceptible to infection due to soil characteristics that enhance prion availability and persistence could improve our understanding of CWD spread across the landscape and inform CWD management. To date, predictive landscape models of CWD have been based primarily on landscape features related to deer habitat with limited input directly related to soil conditions[30–33]. This study is designed to isolate and understand the contribution of soil characteristics to the epidemiology of CWD in deer. Therefore, I aimed to identify chemical and physical soil characteristics that are associated with areas where deer are infected with CWD. To do this, I developed a predictive model of CWD on the Illinois landscape based on the association of CWD presence and soil characteristics. Study objectives were to 1) build a boosted regression tree (BRT) model using CWD presence/absence data and soil data to identify soil characteristics that have the greatest influence on CWD presence, 2) assess the effect of soil characteristics on CWD presence, and 3) predict the probability of CWD presence in northern Illinois based on soil characteristics.

METHODS

Study area

Five counties in northern Illinois (Jo Daviess, Stephenson, Winnebago, Ogle, and Boone) were chosen as the study area based on their long history of CWD testing. This five-county region is bordered by the Mississippi River to the west and contains the Rock, Pecatonica, and Kishwaukee Rivers as well as many smaller waterways and, according to the 2011 National Land Cover Database, has a mixture of cropland, pastureland, developed lands, deciduous forest, grasslands, and wetlands (Figure 1) [34]. The Boone-Winnebago County line is the location of first detection of CWD in Illinois, and CWD-positive deer have been consistently identified in these two counties since 2002 (n = 316). Jo Daviess and Stephenson Counties are situated to the west of Winnebago County and have had a smaller CWD outbreak (n = 50) with the first detected case in 2008. Ogle County, directly south of Winnebago and Stephenson Counties, has had relatively few cases of CWD (n = 21) with the first case detected in 2006.

CWD data

IDNR conducts CWD surveillance of harvested deer and performs localized culling of deer in areas where CWD is known to occur to reduce deer density and disease transmission. Tissue samples (retropharyngeal lymph nodes and obex) collected from deer were tested by the Department of Agriculture Illinois Animal Disease laboratories for the presence of CWD using the gold standard immunohistochemical examination. Based on the immunohistochemistry test results for each sample, a disease status of positive or not detected was specified. The location of each sample was recorded as the township, range, section number in which it was collected as defined by the Public Land Survey System (PLSS) referred to in this study as TRS. The CWD data were spatially joined with a PLSS map of the study area in order to classify CWD presence at the TRS level. TRSs (n = 2238) included in the dataset used to build and test the model had at least one deer tested between 2003 and 2015 (Figure 2). I defined TRS-level CWD presence as a TRS that contained three or more CWD positive deer detected between 2003 and 2015 (n = 33) so as to ensure that infected deer had repeatedly been detected in a TRS (indicating disease establishment) thus minimizing the chance of false positives for modeling purposes. I defined absence as a TRS that contained zero cases of CWD over the 13-year period (n = 2205) (Figure 3). Because TRSs with one or two CWD positive deer may represent either transient occupation by infected deer or initial emergence of CWD in the TRS, these were not included in the analysis, thereby reducing the likelihood of false negatives.

Soil data

I used the United States Department of Agriculture Natural Resource Conservation Service (NRCS) Soil Data Viewer in conjunction with ESRI ArcMap 10.3 to access and map soil type characteristics from the Soil Survey Geographic database [35]. From the soils database, nine soil characteristics (mean pH, maximum pH, minimum pH, percent clay, percent silt, percent sand, percent organic matter (OM), cation exchange capacity (CEC), and the percent water content at field capacity) were selected as predictors based on their association with interactions between soils and proteins/prions (Table 1). Soil type polygons delineate each soil type on the landscape where each polygon represents a continuous area designated to have relatively homogenous soil characteristics and topography. Most TRSs contained several soil type polygons; therefore, an area-weighted average was used to estimate the mean of each soil characteristic in each TRS. In the case of minimum and maximum pH, these values represent only the most extreme pH of all soil types in the TRS. In all cases, I used the surface layer soil properties only (top five centimeters of soil).

Boosted regression tree model to predict CWD presence

In order to assess the relationship between soil characteristics and CWD presence in a TRS, I created a boosted regression tree (BRT) model which uses a combination of boosting (a technique used to improve model accuracy by combining many simple models iteratively to reduce predictive error) and regression trees (models in which repeated binary splits in predictors are used to classify a response) [36,37]. Three main meta-parameters are used to maximize predictive performance and reduce overfitting (bag fraction, tree complexity, and learning rate) [37]. The bag fraction represents the proportion of the data used to fit the model that is used in each step. Tree complexity defines the number of nodes in each tree and represents the maximum order of interactions that can be fitted. Learning rate (or shrinkage rate) determines the contribution of each tree to the model as it grows. After testing several different settings, the predictive performance of this model was maximized when I used the default bag fraction of 0.75, a tree complexity of 7, and a learning rate of 0.001. I fit the BRT model using 80% of the data (n = 1790), and validated the model predictions with the remaining 20% (n = 448). The predictive ability of the model was determined based on the area under the receiver operator characteristic curve (AUC) where the area under a plot of the true positive rate versus the false positive rate assessed at various classification thresholds (in this case, values of the predicted probability of CWD presence) represented the model's ability to distinguish between presence and absence [38]. I used kappa scores to assess the level of agreement between the model predictions and the disease on the landscape and used the predicted probability of CWD presence at which kappa was maximized as the classification threshold for determining the CWD status of a TRS [39]. In addition to the TRSs used for model fitting and validation, I applied the model to all of the remaining TRSs in the study area regardless of CWD testing history to obtain model predictions for the entire region. I used the statistical program R and packages 'dismo' and ‘gbm’ to perform all analyses [40–42].

Determining the influence of soils

The relative influence of the soil characteristics on CWD presence was estimated as a part of the package ‘gbm’ in R [42]. The values for relative influence represent the number of times a soil characteristic was selected for splitting and the improvements made on the model as a result of that split [36]. These values have been transformed so that each assigned value reflects a percentage of contribution to the response. To determine the direction of the effects of each soil characteristic, I generated partial dependence plots for each soil characteristic on the probability of CWD presence. The partial dependence plots serve as visual guides for interpreting how each soil characteristic influences the model predictions after the effects of all other soil characteristics have been averaged out [43]. Finally, I assessed the relative strength of interactions present between soil characteristics (or the relative contribution of the interaction between two soil characteristics to the predictive performance of the model) and generated a three-dimensional partial dependence plot of the strongest interaction as described previously [36,37].

RESULTS

Predicted probability of presence of CWD in deer

The BRT model predicted the probability of CWD presence in each TRS within the test dataset with an AUC score of 0.954. The maximum kappa score, 0.74, was achieved with a threshold probability of 0.223 that delineated CWD presence from absence. Predicted probabilities ranged from 0.005 to 0.678 across all TRSs (Figure 4). The highest predicted probabilities were located in Boone and Winnebago Counties. Higher probabilities were predicted along the western and southern edges of Jo Daviess County relative to the rest of the county. Stephenson County, in general, had the least predicted probability of CWD presence with all TRSs predicted ≤ 0.03. Although the majority of Ogle County had a very low predicted probability of CWD presence (≤ 0.03), TRSs with an increased probability existed in the central and eastern portions of the county.

Effect of soils on the predicted probability of CWD presence

The four most important predictors (% relative influence) were mean pH (18.4%), percent clay (15.1%), CEC (14.6%), and percent OM (11.2%) (Figure 5). Partial dependence plots showed that below a mean pH of 6.6, the probability of CWD presence was low, whereas above a mean pH of 6.6, the predicted probability rose abruptly before plateauing around pH 6.7 (Figure 6a). When the percentage of clay exceeded approximately 19%, the predicted probability of CWD presence dropped from high to low (Figure 6b). The predicted probability also dropped from high to low when the CEC exceeded approximately 15 meq/100g (Figure 6c). A threshold was also observed for percent OM where the predicted probability of CWD presence was high in TRSs with soils containing less than 4.5% OM (Figure 6d). The strongest interaction effect was observed between clay and mean pH. A three-dimensional plot demonstrates that the probability of CWD presence due to clay is affected by pH when clay is below 20%. Once clay reaches and exceeds 20%, the probability of CWD presence is low regardless of pH (Figure 7).

DISCUSSION

This analysis suggests that soil characteristics affect the presence of CWD in the environment and that the soil can serve as a complex reservoir either by enhancing or inhibiting prion availability. According to the model, pH has the most influence on CWD presence. Soil pH effects are likely associated with changes in the adsorption behavior of prions that can be influenced by pH. Shifts in pH can alter prion size and degree of aggregation as well as the surface charge of soil minerals and organic matter and the size of the soil-water interface that controls adsorption-desorption behaviors[26,28,44,45]. The distinct threshold in the influence of pH on CWD presence is notable and could be associated with an isoelectric point (IEP; the pH at which the prion has a net zero charge) for the prion that is around pH 6.6. This falls within the range of IEP (pH 4.6 – 7.9) reported for prion proteins [26,46]. If the IEP for the pathogenic prion is at pH 6.6, then it would have a net positive charge associated with the N-terminal end at and below this pH and would therefore be attracted and bind to negatively charged soil and organic matter surfaces. Above pH 6.6, the prion would have a net negative charge and be repelled by negatively charged surfaces. This could leave the prion in solution where it would be more bioavailable and mobile within the environment. Desorption of prions affiliated through a variety of mechanisms can be induced at high pH [26,47].

The percent clay in the soil is an important factor in CWD presence as indicated by both this model as well as many other studies although its effect on infectivity is debated [19,31,48–50]. The clay fraction disproportionately influences soils' chemical interactions because small particles found in this size range (< 0.002 mm) have a relatively large surface area to interact with ions in soil solution and greater cation exchange capacity (3 – 150 meq/100g) than minerals found in the silt and sand sized fractions [51]. Clay is positively associated with soil organic matter and to the sorption of organic compounds through both ionic and partitioning interactions [28]. In this model, the probability of CWD presence is greatest at lower percentages of clay. The model actually indicates a threshold value for clay at around 20% where below this threshold percent clay has a positive influence on CWD presence. Above this threshold, percent clay has a negative influence on CWD presence. Soil organic matter levels would be expected to increase with clay content and with this, the potential for organic compounds to be retained by partitioning (organic sorption) [52]. This threshold may seem at odds with some previous studies that suggest prion disease incidence increases with clay abundance due to increasing prion immobilization and persistence; however, those works have been carried out in regions where average clay contents are lower than in those considered by this study [23]. Soils with clay contents above 20% may reflect reductions in prion bioavailability due to immobilization where clay and organic matter levels are higher or that the prion reservoir may actually decline. Reduced persistence could also be tied to prion decay rates which have been found to be greater when bound to soils with high organic matter content than those of prions bound to clay and sand minerals [53,54]. Interactions between prions and clay and organo-clay surfaces may be affected by pH, ionic strength, and the specific ions present [55,56]. Though it is in general agreement with a broader study in the same region, the effect of clay seen in this model is the opposite to that modeled in Colorado which showed that for every 1% increase in clay, there was an 8.9% increase in CWD [30,31]. According to the NRCS Web Soil Survey, many soils in northern Colorado have a higher electrical conductivity (a measure of the salinity of soil) than are found in northern Illinois [57] . In Colorado, the increased concentration of salts may compete for binding sites on the surface of clays preventing the adsorption of prions and increasing the concentration of unbound prions in the soil. This is consistent with an elevated pH that would promote desorption in the same way predicted by my model. Increased salinity also decreases microbial activity (demonstrated to aid in prion degradation) and is associated with less organic matter and consequently lower CEC [58,59].

The soils data strongly indicate that the physical mechanism of immobilization on the solid phase and remobilization are controlling prion availability. When immobilization is reduced (above pH 6.6, below 20% clay, or CEC below 15meq/100g), we see an increase in the probability of CWD. Reduced immobilization would result in increased prion movement and dispersal in aqueous forms and increases in abundance of wind-blown particles and eroded sediments. Lower abundances of clay and organic matter also result in the reduced physical stability of soils and increased susceptibility to wind erosion and dust emissions [28,60]. These less physically stable soils are more likely to be aerosolized during grazing or rutting and scent marking behaviors leading to enhanced exposure of deer to prions in the soil through inhalation and ingestion. Therefore, where immobilization is low, animal exposure goes up through probable routes of infection (inhalation and ingestion).

This study provided novel insights into field conditions that are conducive to the development of an effective CWD reservoir. The resulting model can help direct efforts toward the study of the mechanisms controlling prion availability in the soil. Additionally, this model has practical applications to directing disease management in free ranging white-tailed deer. Even though the predictions derived from this model were developed through correlative analysis, they can be used to initiate new surveillance areas and management tactics by identifying TRSs where predicted probabilities are elevated. Areas with higher deer densities that overlap TRSs with higher predicted probabilities of CWD presence could prompt management officials to intensify density reduction efforts to limit the load of prions shed into the environment. If there are travel corridors that connect TRSs where CWD is known to occur to TRSs where the predicted probability of presence is higher, this may encourage managers to implement strategies that reduce deer movement between those areas. To develop even more useful models to direct management efforts, combining soil characteristics, landscape features found to be associated with CWD, and deer contact rates into a comprehensive model may even better predict CWD spread and persistence.

Because TRSs with one or two cases were removed from the analysis, the model could be missing areas where disease emergence is occurring. The model predicted very low probability of CWD presence in an area in which a cluster of TRSs with one or two cases along the southern edge of the Jo Daviess – Stephenson county line occurs (Figure 8). It could be that the model is underpredicting this region potentially as a result of low sampling rates due to restricted access. However, it could also be interpreted that the soil conditions do not favor reservoir development, and instead disease spread is facilitated through direct contact as a result of higher deer densities and increased contact rates. For comparison, a model was also built in which TRSs with one and two cases were classified as absences, and the model results did not change (results not shown). Areas like this in which CWD cases have been detected but predicted probabilities are low may be especially important to disease management. If disease spread is mainly facilitated through direct contact between deer because soil conditions do not promote exposure or make prions less available through inhalation and/or ingestion, increased culling efforts may be extremely effective in halting further disease propagation.

CONCLUSION

This model indicates that soil characteristics can serve as a useful tool to predict the probability of CWD presence on the landscape. It has highlighted the importance of the presence and abundance of soil properties that affect the availability and persistence of infectious prions in the environment and can help identify mechanisms responsible for these differences particularly when considered along with studies of other areas where soils have been identified as a potential reservoir. While it is difficult to ascertain the exact nature of prion interactions with the soil without a complete understanding of prions and the forms in which they exist once shed into the environment one can assume that dominant chemical and biological mechanisms controlling prion fate can be identified. Mixed findings in the literature have cited the complexities of soil factors and associated mechanisms driving prion sorption to soils, leaving us with limited understanding of how the infectivity of prions might be affected across a broad soil range [19,25,27,48,61]. However, based on the interactions between proteins and soils, this model does show that real life conditions are acting according to our expectations of these systems. This model provides insight into the ranges of soil characteristic values and thresholds wherein controlling factors shift from enhanced disease presence where clay levels increase from low values up until abundances of about 20%. Once clay and associated OM exceed this value, CWD levels decline, likely due to immobilization, unless pH values are high enough to promote desorption or remobilization of the prions.

This study also demonstrates that the use of BRT models to predict disease presence is a practical tool for managers to use when delineating and prioritizing disease management areas. The method uses software that is available for free and supplies information that is relatively easy to interpret. By bringing attention to areas where soil conditions would promote infection through the environment, it may help to direct management activities that would slow spread or halt disease establishment in risky soil environments. The results from this study can only further enhance knowledge of prion-soil interactions and serve as a guide to perpetuate future studies.

TABLES AND FIGURES

Table 1. Soil-prion/protein interactions

Soil Variable	**Influence on CWD**
Clay	Binds strongly to prions, affects availability of prions [25,48,50]
Sand	Binds less with prions relative to silt and clay [26,48,55]
Silt	Binds less with prions relative to clay[62]
Organic matter	Binds with prions, affects availability [19,61]
Water content	Affects decomposition of proteins [45,63]
pH	Affects prion charge and adsorption/desorption to soil particles [26,28,44,45]
CEC	Affects binding to soil particles[64]

Figure 1. Majority land cover type in each TRS.

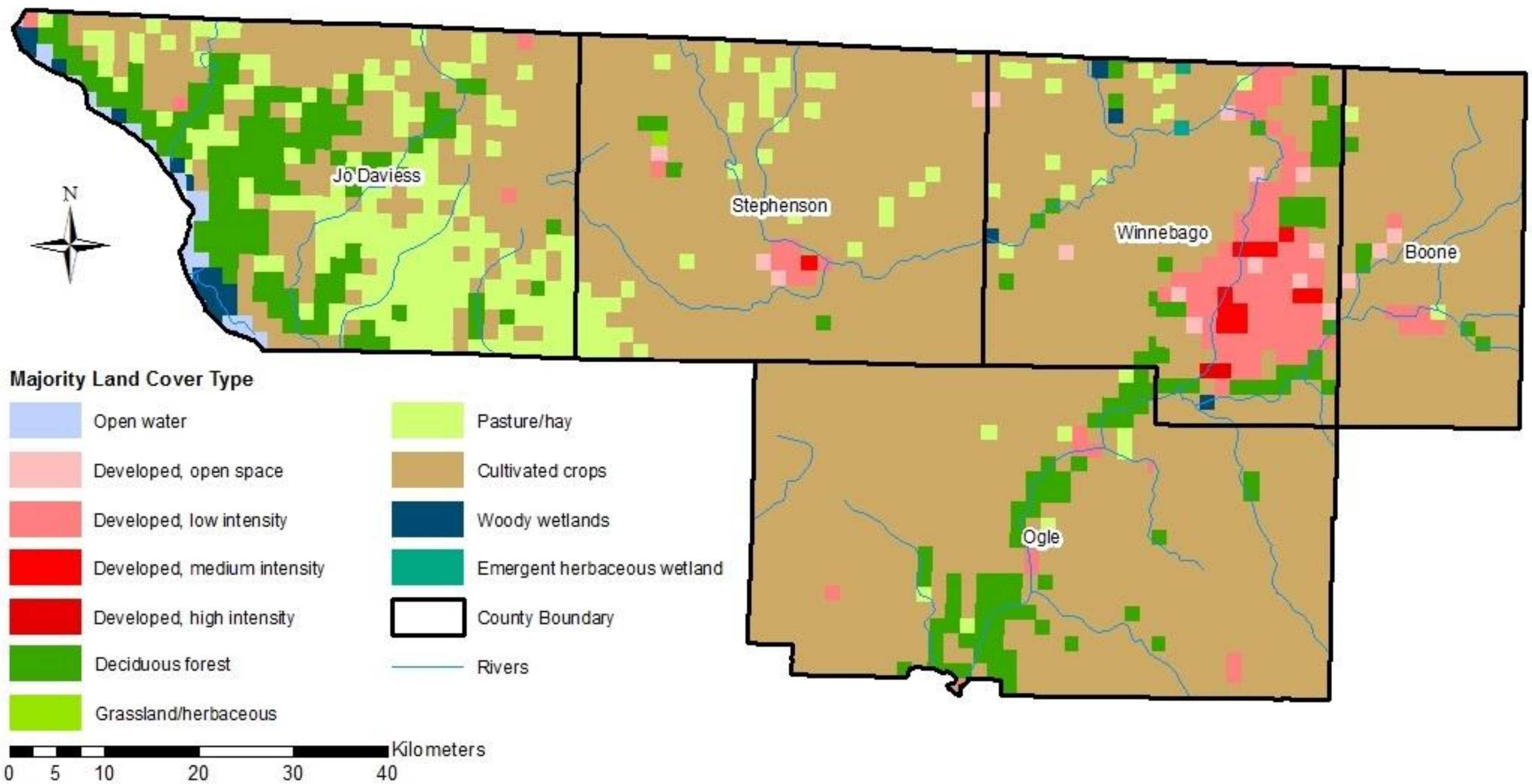


Figure 2. Tested locations in northern Illinois. Color coded TRS locations have had at least one deer tested for CWD from 2003-2015. Colors reflect the number of positive cases. Hashed TRSs have not been tested for CWD.

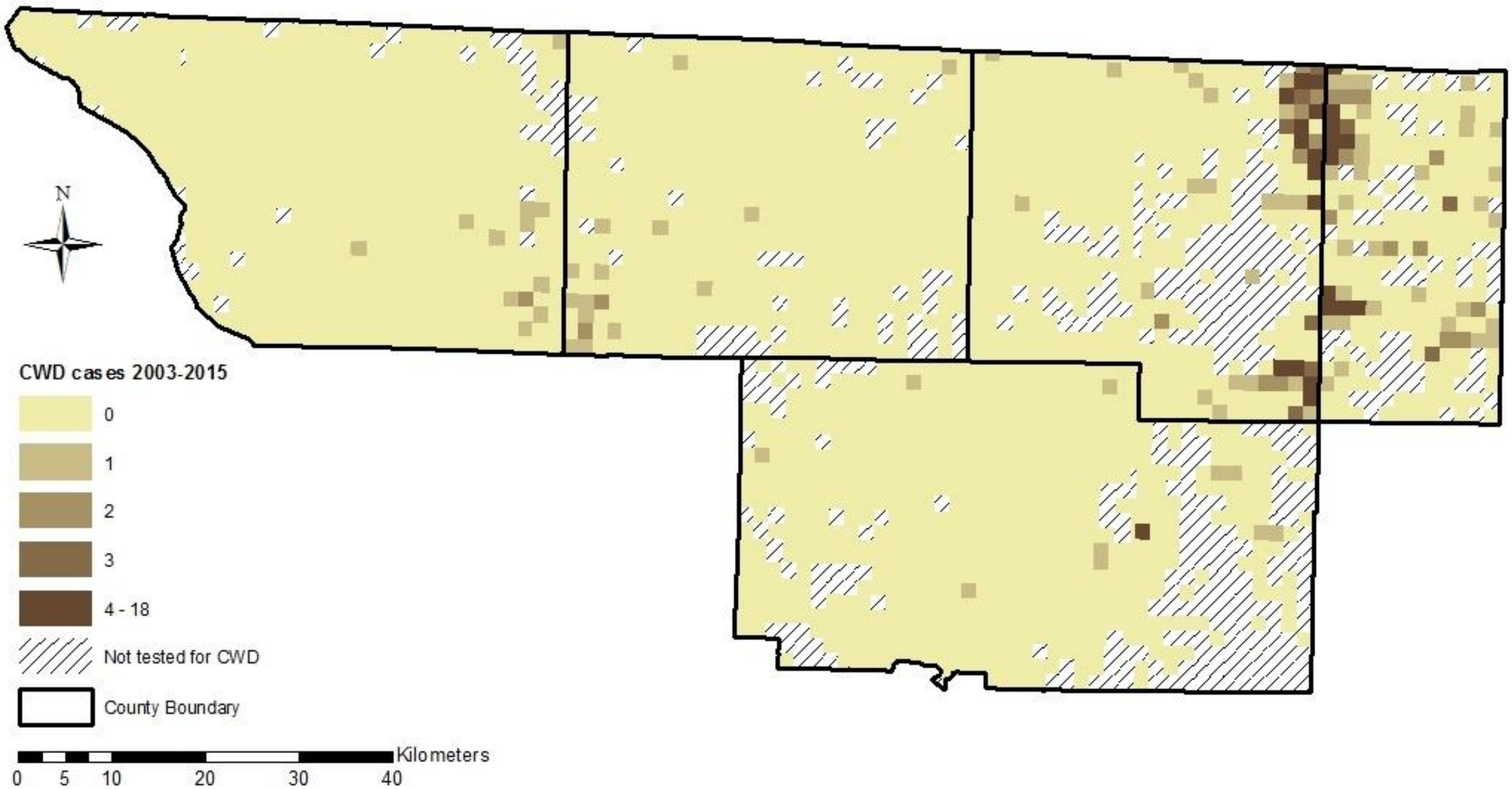


Figure 3. TRS locations of presence and absence of CWD used in BRT model. Presence was defined as TRSs with ≥ 3 CWD-positive deer detected (red). Absence was defined as TRSs with zero CWD-positive deer detected (gray). TRSs not included in the model consist of TRSs with one or two CWD cases detected and TRSs in which no deer were tested (hashed).

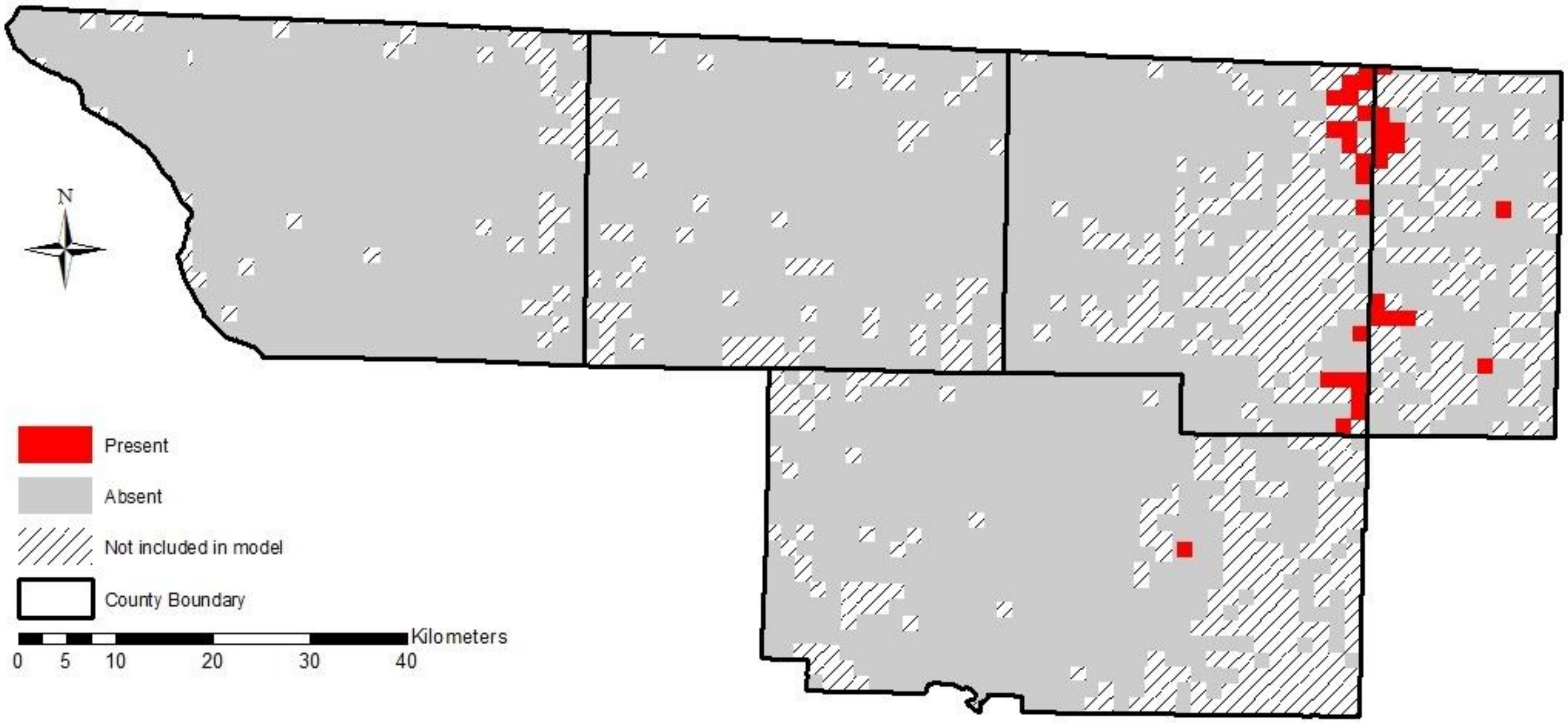


Figure 4. Predicted probability of CWD presence in the study region. Predicted probabilities increase as colors progress from light to dark.

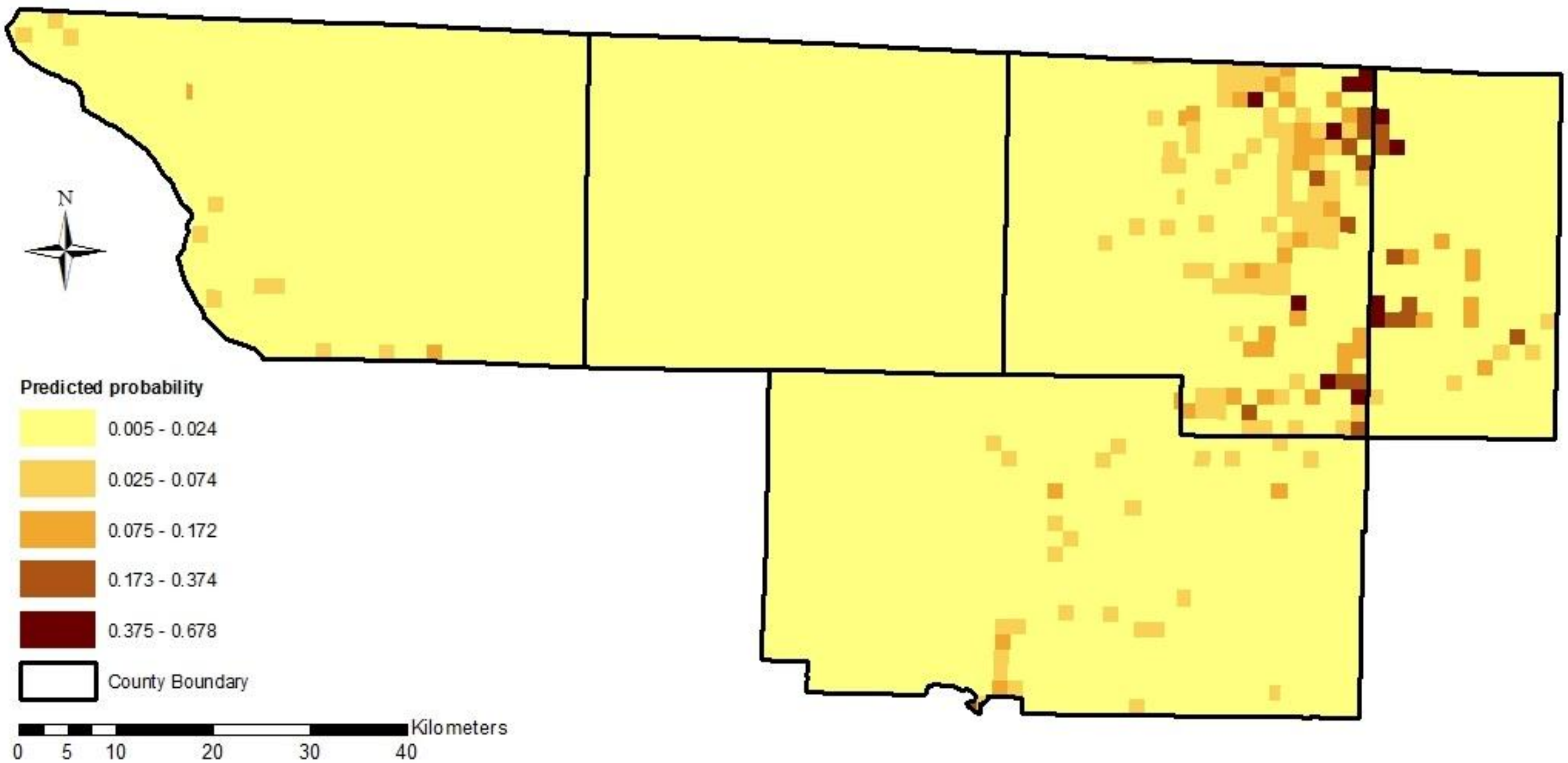


Figure 5. Relative influence of soil characteristics on CWD presence. CEC indicates cation exchange capacity, OM indicates organic matter, Max indicates maximum, Min indicates minimum.

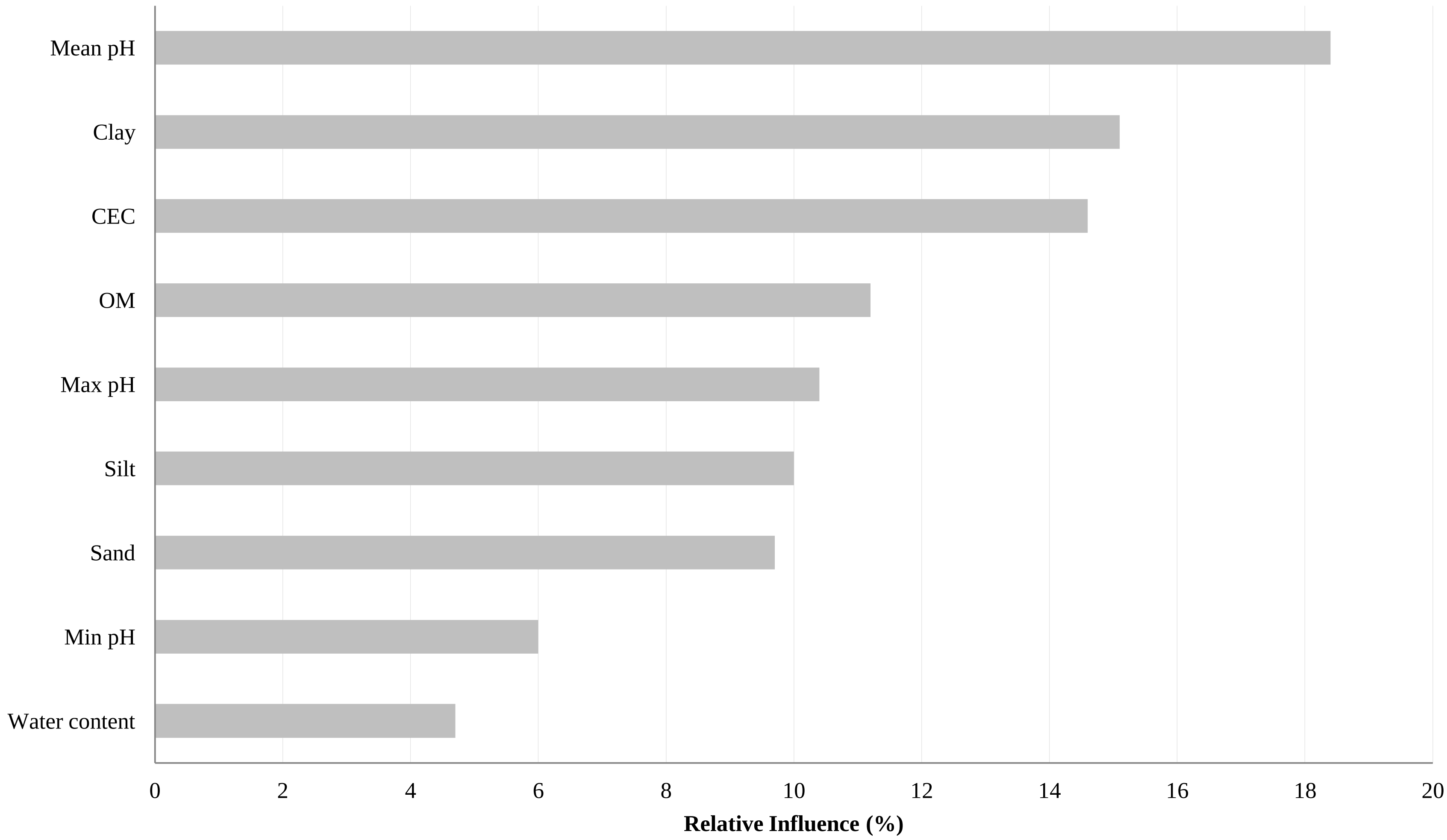


Figure 6. Partial dependence plots of soil characteristics with the relative influence (%). CEC indicates cation exchange capacity, OM indicates organic matter, Max indicates maximum, Min indicates minimum, h2ocont indicates water content.

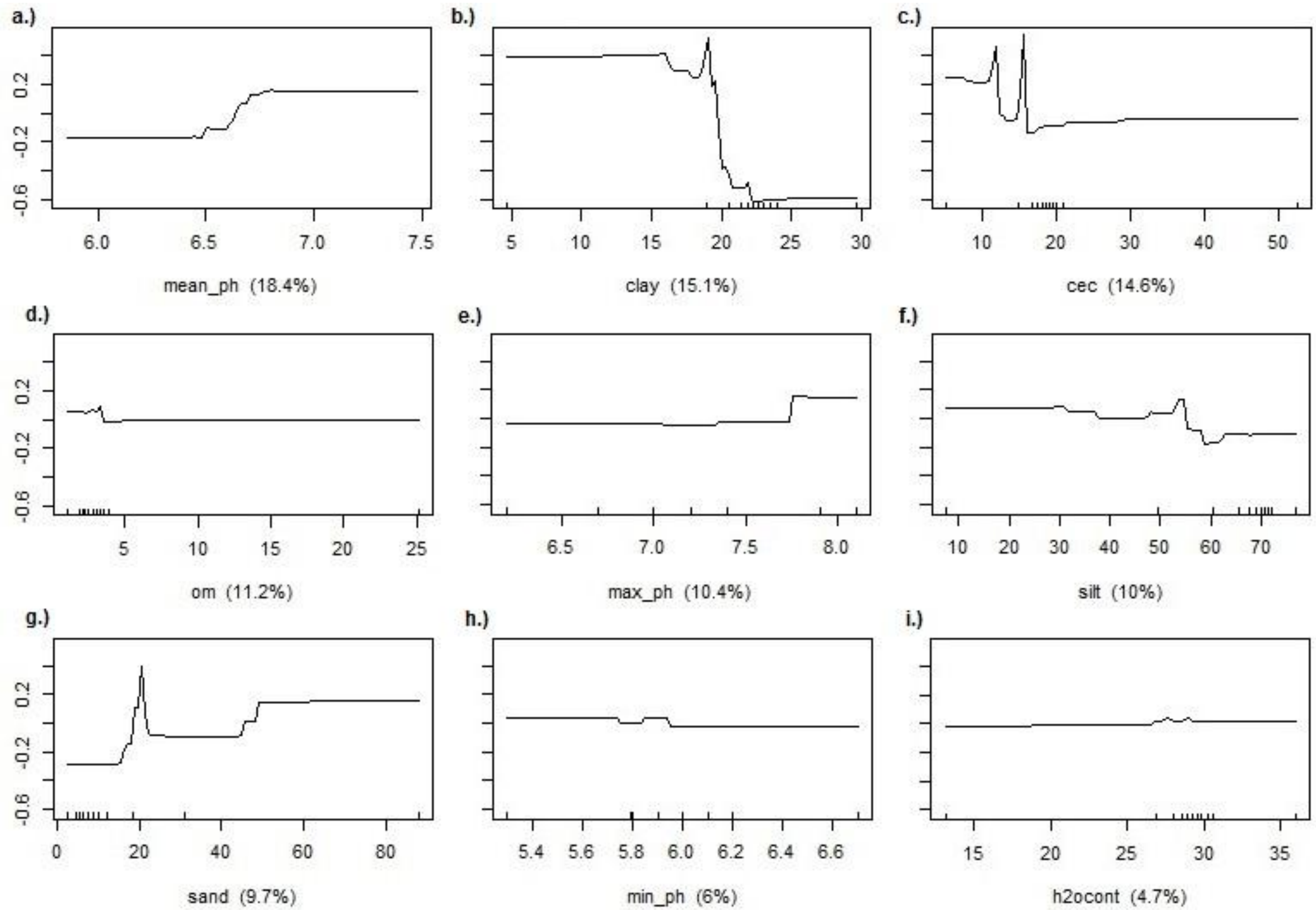


Figure 7. Three dimensional partial dependence plot of interaction between clay and pH

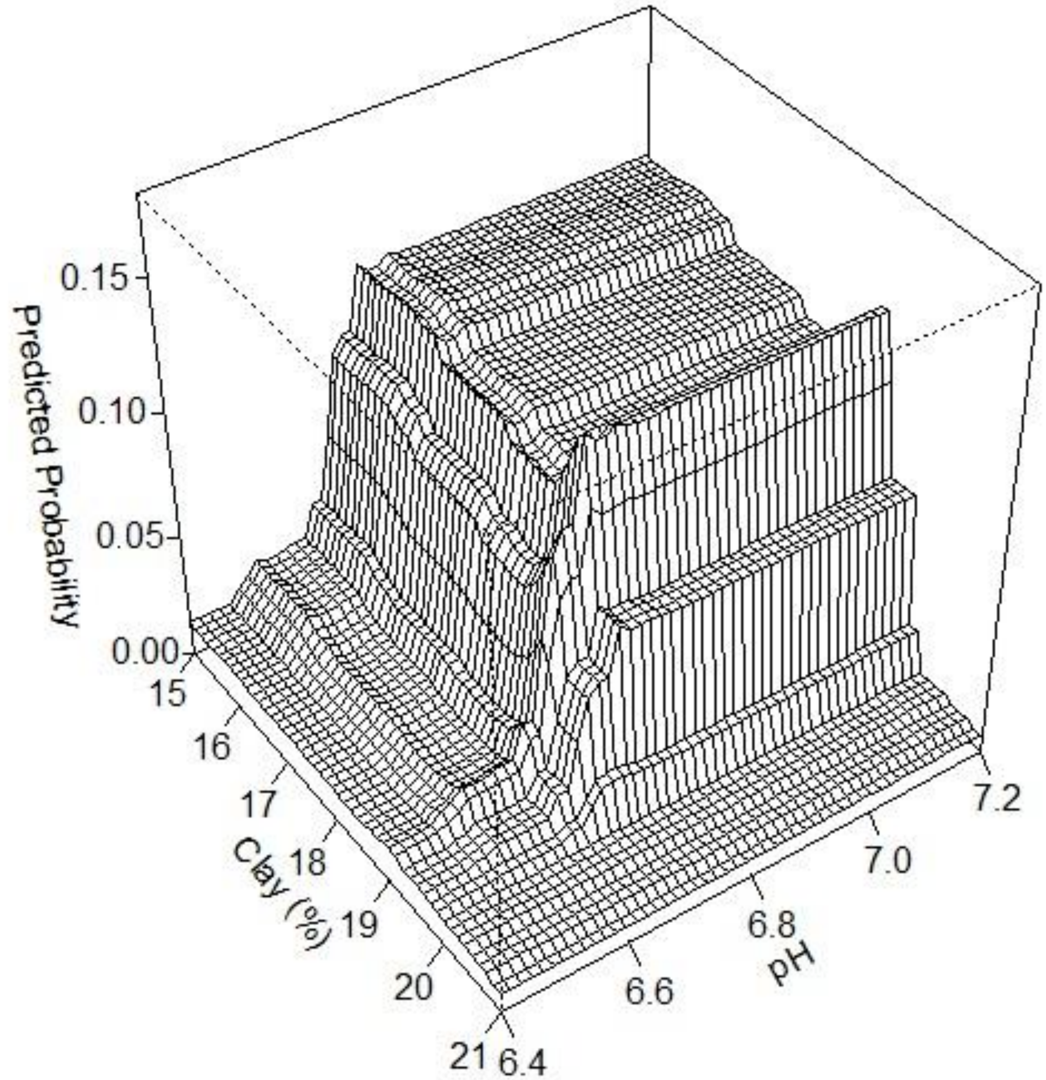
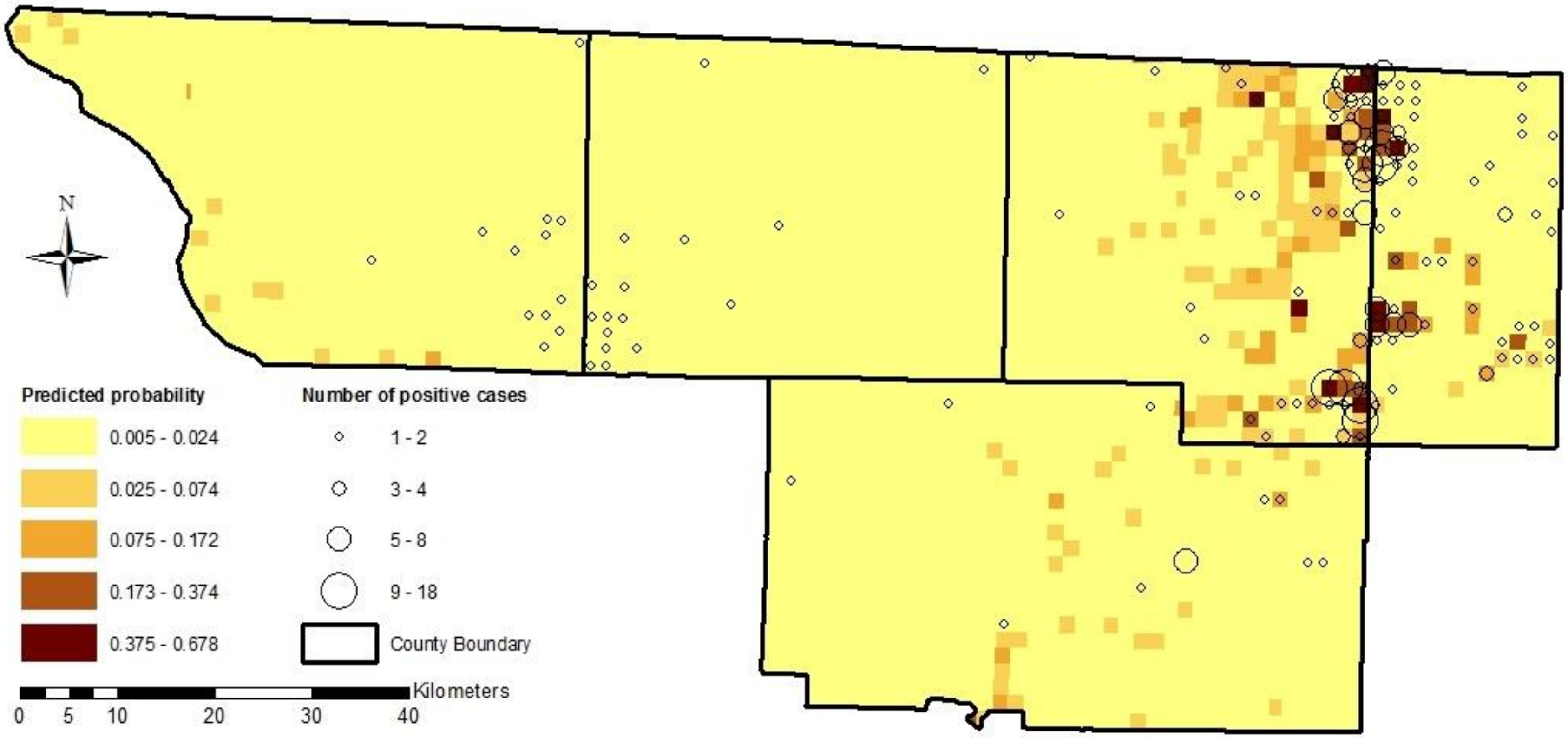


Figure 8. Predicted probability of CWD presence with the locations of CWD-positive cases. Predicted probabilities increase as colors progress from light to dark. Graduated circles indicate the number of CWD-positive deer in each TRS.



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