

# Modeling the Environmental Suitability for Bacillus Anthracis Spores Survival in the Qinghai Lake Basin, China

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## Research

**Keywords:** Anthrax, Bacillus anthracis spores, Environmental suitability, spatial modelling, MaxEnt

**Posted Date:** July 21st, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-45014/v1>

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# Abstract

**Introduction:** *Bacillus anthracis* spores may remain viable for many years in soil. Previous studies have identified East Qinghai and neighbouring Gansu in northwest China as a potential source of anthrax infection. This study was carried out to identify conditions and areas in the Qinghai lake basin that are environmentally suitable for *Bacillus anthracis* spore survival.

**Materials and Methods:** Anthrax occurrence data from 2005 – 2016 and environmental variables were spatially modeled by a maximum entropy algorithm to evaluate the contribution of the variables to the distribution of the spore. A Principal Component and Variance Inflation Analysis were adopted to limit the number of environmental variables and minimize multicollinearity.

**Results:** The three variables that contributed most to the suitability model for *B. anthracis* spores are a relatively high annual mean temperature (53%), soil type (35%), and a high human population density (12%). The most significant soil types were cambisols and kastanozems. The resulting distribution map identifies the permanently inhabited rim of the Qinghai Lake as highly suitable for the survival and persistence of the spores.

**Conclusion:** The highly suitable areas for spores could be considered as a risk zone for Anthrax infection by spores of the livestock and the human population. Our environmental suitability map and the identified variables provides the nature reserve managers and animal health authorities readily available information to devise a surveillance strategy in *B. anthracis* suitable regions to abate future epidemics.

## Background

Anthrax is an infectious, often fatal disease of wild and domestic animals and humans that is caused by the endospore-forming, soil-borne bacterium *Bacillus anthracis*. It is primarily a disease in herbivores and sometimes sparks an outbreak in human beings with potentially serious consequences (1). Herbivorous mammals are infected when grazing on infected land, bitten by tabanid flies with contaminated mouthparts or ingesting contaminated feed (2). Herders, livestock farmers, workers in abattoirs, meat and fur processing plants and veterinarians are exposed to the disease as an occupational hazard. Recently, the disease has transposed from industry to agriculture affecting farmers and herdsman in 87.6% of the human cases (3). *Bacillus anthracis*, the etiological agent of anthrax, exhibits a bimodal lifestyle consisting of the vegetative and the spore stage (1). Bacteria in the vegetative stage are shed by infected animals and may die rapidly in most environmental conditions. After sporulation from the vegetative cells, the *B. anthracis* can survive in the soil for decades (4). The bacillus replicates rapidly in the bloodstream to high concentrations and releases toxins resulting in septicemia, which soon kills the host. In soil and vegetation, the spore stage can remain viable and infectious for years until it comes in contact with and enters a new host, where it germinates and begin a new life cycle (4, 5). Human anthrax infections are caused by contact with infected animals or animal products, ingestion of undercooked infected meat; or exposure to processing of contaminated hides, wool, and hair in enclosed spaces (6).

Clinically, there are three forms of anthrax namely; cutaneous, gastrointestinal tract and pulmonary (inhalation).

Globally, cutaneous anthrax accounts for over 95% of the human cases. In China, for 97.7% (3). All three types of anthrax are implicitly fatal in wildlife, livestock, and human beings, if not treated promptly (7). Due to *B. anthracis*' virulence, tenacious anthrax cases and repetitive outbreaks, concerns have been raised across continents in recent years, e.g., sub-Saharan Africa (8), Asia (9), Europe (10), Australia (11), and the United States of America (12). Also due to its potential use for bioterrorism, anthrax is considered as a global public health threat (13). Effective vaccines have reduced the economic significance of the disease in developed countries where it now occurs sporadically in unvaccinated domestic stock and wildlife populations (14). In the Qinghai Province of China, Anthrax occurs sporadically and all year round. The prevalence of anthrax in Qinghai rose from 0.35/100,000 in 2012 to 1.17/100,000 in 2016. The incidence is gradually increasing as well (15). Qinghai province has recently been identified as one of the potential sources of *B. anthracis* (16, 17).

Zoonosis of anthrax via livestock has been controlled, but not that via wildlife. The behavior of avian and mammalian scavengers (18), rending the carcass apart, frees a multitude of *B. anthracis* bacteria. Subsequently, scavengers may disperse the bacteria over a wide range through ingestion and bathing resulting in contamination of water bodies (8). Human beings, livestock, and wildlife will invariably encounter each other or share habitats which would cause a spill back or spillover of the infection. Disease control in livestock through vaccination and intensive surveillance of both livestock and wildlife are essential for prevention (19). However, widespread surveillance is costly, and the vaccination of all animals is unattainable. Therefore, there is a need to concentrate on high risk areas. To identify these, it is expedient to upsurge our understanding of the ecology of the *B. anthracis* spore.

The mainstay of the rural economy in Qinghai province, including the lake basin is livestock husbandry (20). Livestock mainly includes sheep, goat and yak, but also some horse, cattle and donkey. Livestock numbers per household varied from dozens to more than 1000 (21). In spring or early summer most livestock is transferred to high-altitude pastures (4850 to 4950m a.s.l.) where milk is processed and flocks gain weight. After their return to the homestead (3190 to 3300m a.s.l.) in late summer, fodder (oats) and crop residues are provided as principal feed in addition to stubble grazing and grassy patches near the winter residence (22).

Previous studies found that soil type (cambisol), high pH, high calcium cation concentrations and high soil organic matter were associated with spore germination and maintaining spore viability (8, 23-25) as well as spore adhesion to soil particles (26). Many authors have described the soil types as the key factor in *B. anthracis* spore germination and maintenance of dormancy (11, 24, 27, 28). Similar studies elsewhere and China have identified climatic variables (11) and human population density as good predictors of *B. anthracis* spore suitability (16). Therefore, we tested the hypothesis that soil type, climatic variables and human population density are significant predictors of *B. anthracis* spore suitability in the Qinghai Lake basin. Presence-only modeling algorithms to predict the environmental suitability of *B.*

*anthracis* spores have been widely used, including maximum entropy (24) and GARP (genetic algorithm for the rule-set prediction) (11). During comparative model studies, MaxEnt outperformed other algorithms (29); Padilla et al., 2017). Therefore, in this study, MaxEnt was used to model *B. anthracis* spore's persistence and its spatial distribution (30, 31).

## Materials And Methods

### Study Area

Our area of interest (AOI) is the basin of the Qinghai Lake (98°37' - 101° 45' E and 36° 33' - 39° 14' N) in Qinghai province, Northwest China (Figure 1). The basin is approximately 29,600km<sup>2</sup>, and the lake about 4,300km<sup>2</sup>. The water surface is roughly situated at 3,193m a.s.l., with an average depth of 21m (32). Qinghai Lake is situated in a closed-basin (29,661 km<sup>2</sup>) with no surface water outflow. The entire watershed is in a high-altitude, cold and semiarid climate zone (33). More than 40 rivers flow into the Qinghai Lake, but most are intermittent. Qinghai Lake is the largest salt lake in China, an international wetland (34) and a breeding ground for migratory water fowl. Further, the mountains around the Qinghai Lake are perhaps the last refuge of the endangered Przewalski's gazelle (*Procapra przewalskii*) (35). The Lake basin is a center of ecotourism. Grassland is the major land cover, accounting for about 63% of the AOI (36). The Qinghai Lake basin has been identified as a modern, highly efficient animal husbandry production area where human beings and nature live in harmony (36). The human population in the entire watershed is about 110 thousand, mainly living around the Qinghai Lake (37).

### Anthrax occurrence data and preprocessing

We collected 37 cases of Anthrax in human beings, livestock and wildlife from spatial records (n=07) provided by the World Organization for Animal Health (38) and publications (n=30) (15-17). We removed records with geo-coordinate errors. The geo-coordinates were then saved in a comma separated value (CSV) format and imported into ArcGIS (version 10.3 ESRI) for editing. We filtered the presence points using the SDM Toolbox v1.1c (39) integrated into ArcGIS 10.3 to minimize potential spatial autocorrelation (30).

### Environmental variables and preprocessing

A total of 68 climatic, twelve incoming solar radiation (ISR) and eight (8) soil variables were used (Table 1 Suppinfo). We extracted the climatic variables from the WorldClim version 1.4 from 1950 – 2000 at 30 arc – second resolution (40). The categorical variable soil type and continuous soil variables in 1km grids were extracted from (41) including soil organic carbon, clay, silt and sand content as well as cation exchange capacity, soil pH, and land cover/use. Livestock (cattle, sheep and goat) population density/km<sup>2</sup> was obtained from <https://livestock.geo-wiki.org/home-2/> (42); the human population density from the Asia Continental Population Datasets (2000-2020) are publicly and freely available both through the WorldPop Dataverse Repository and the WorldPop project website (<http://www.worldpop.org.uk/data/>).

Principal component analysis (PCA) was used to reduce the number of continuous environmental variables (43, 44). During PCA, we used eigenvalues larger than 0.97 and the scree plot criterion for PCA in item level factoring (45). Suppression of unnecessary loading and rotation of factor pattern of climatic variables (46) were used to retain climatic variables. After variable reduction in PCA, we used VIF (linear regression statistics) in SPSS 22.0 to assess multicollinearity among both the remaining continuous and the categorical variables. A VIF >10 has been considered to indicate highly correlated variables to be removed from the input data set. Subsequently, only uncorrelated variables have been used. The Jackknife test, backward stepwise variable elimination, and the variable response curves were selected to identify the relative contribution of predictor variables to the model (31, 47-49).

## Model development and evaluation

A MaxEnt model was fitted using 100 bootstrap runs, a 70/30 partition percentage for the training/testing datasets and the default for all other settings and outputs including AUC. The stepwise elimination approach was used to remove variables that contributed less than ten percent (10%). Further, a smooth response curve was used as quality standard (30). We reclassified the MaxEnt spatial model output into two environmental suitability classes, namely high and low.

## Results

The filtering selected 25 out of 37 presence records. The PCA delivered five PCs, together accounting for 98.7% of the total variance (Table 1). After exclusion of unnecessary factor loading, thirteen predictor variables were retained. The model had a high AUC value (0.93) indicating that it had excellent ability to predict the suitability areas for *B. anthracis* spores (Suppinfo 5). Three variables contributed >10% (Table 2) namely annual mean temperature (53%), soil type (35%), and human population density (12%). The Jackknife test of variables shows that omitting any of these three variables affects the regularization gain, test gain and AUC in the model. The annual mean temperature has the highest training gain when each variable was tested as the only environmental variable (1.2), and the lowest values were observed in the human population density (0.4). The lowest training gain appeared when the annual mean temperature was excluded from the model, while the model has the highest gain when human population density (1.2) and soil type (1.3) were excluded (Table 3).

Annual mean temperature has the highest test gain values when used as the only environmental variable and soil type has the least test gain among the variables. Our model has a high training gain value when human population density and soil type were simultaneously excluded from our modeling process. The exclusion of annual mean temperature variable from the model results in a decline of the test gain (Table 3).

Observing our Jackknife test for AUC, the three important variables (annual mean temperature, soil type, and human population density), when used in isolation were not significantly different from each other. The AUC value of our model was excellent when soil type was excluded. There were no significant differences in AUC values of the other six variables in the model (Table 3).

The suitability for the anthrax spore peaked when the annual mean temperature increased from -2 to 0 °C, but declined briefly thereafter and maintained a constant probability across higher temperatures (Fig. 2). The soil types with the highest suitability are cambisol and kastanozem, both in their Haplic subtype (Fig. 2); Leptosols was unsuitable either in the presence of other variables or in isolation. The human population density response curve shows a gradual upward movement reaching a plateau at 40 individuals per km<sup>2</sup> (Fig. 2).

The highly suitable conditions are primarily found around Qinghai Lake. The northern and western part of the basin was predicted to be unsuitable for *B. anthracis* spore survival (Fig. 3).

## Discussion

Our study presents the first assessment of ecologically suitable areas for *B. anthracis* spore persistence in the Qinghai lake basin. Although, epidemiological analysis had been done in Qinghai province (15) and anthrax distribution mapping in mainland China (16).

We analyzed over ten years mixed anthrax outbreaks using MaxEnt algorithms to investigate the environment and the geographic distribution of *B. anthracis* in the Qinghai Lake basin. The identified sets of environmental predictors of the *B. anthracis* spore survival may represent factors directly or proxies thereof. This study supports the results of other studies, which have shown that anthrax outbreaks are associated by specific soils types (8), relatively high temperatures (50), and high human population density (16). We found that relatively high mean annual temperatures within our high alpine environment were predictive for the anthrax occurrence. Consequently, continued climate warming may increase suitability for anthrax spore (9) also in our AOI.

Soil types and certain soil characteristics, such as high levels of organic matter, pH or calcium, were thought to facilitate the survival of spores (Kracalik et al., 2013, Hugh-Jones and Blackburn, 2009). In our model, anthrax suitability was largely driven by two soil types; cambisols and kastanozems while leptosols show the lowest spore suitability. The cambisols, occurs on young alluvial deposits in our AOI as well as worldwide (WRB, 2006). The emergence of humus-rich kastanozems as the second most suitable soil type with the lowest standard deviations may be due to the presence of calcite (carbonate mineral) in its subsurface (26, 51). However, soil organic carbon pH, cation exchange capacity, silt content and calcium were not predictive for spores in our AOI.

We found that the human population density was associated with anthrax spores. However, sheep, goat, and cattle population density both contributed during the initial modeling but failed to meet the backward stepwise variable elimination criterion in our variable selection mode. The increase in human population density, settlement expansion, and seasonal migration would enhance human, livestock, and wildlife contact which would provide opportunity for *B. anthracis* transmission. The pastoralist nature of the population in Qinghai lake basin shows that human is frequent with the animal both in pasture or in corrals. They also practice mixed farming; rearing animals and crop cultivation which are often used as

fodder, tillage would transpose dormant spore to the surface which increases anthrax emergence rate. Sheep herding practices include high-altitude summer pasturing (Wiley et al., 2003) which may reduce exposure to *B. anthracis* at the high Anthrax risk zone around the Lake during summer months.

The areas with the highest suitability ranking are the low-lying area around the lake. The suitability could be dependent on the alluvial deposits, the various drainage channels from the higher elevation, characterized by the relocation of *B. anthracis* spores with soil through water, flooding or rain (26). The result of our model with low altitude (around 3200m a.s.l) as essential condition for survival of anthrax is in agreement with most similar study on anthrax (23). The moderate risk areas are the mid-elevated areas in our AOI. Our study reveals that bioclimatic and edaphic factors are fundamental conditions for *B. anthracis* spore survival and persistence. Also, human population density and other related activities are specific factors reshaping the spatial distribution of *B. anthracis*.

## Conclusion

We categorized the Qinghai Lake basin into two suitability classes for *B. anthracis* spore as high and low, and established that increase annual mean temperature, two soil types (cambisols and kastanozems), and a high human population density, were the contributing variables for predicting *Bacillus anthracis* spore suitability. Soil type as the only categorical and second most influential variable; this would strengthen the edaphic paradigm for *B. anthracis* in global *B. anthracis* suitability and anthrax epidemiology studies. Additionally, vaccination of both human and livestock would be essential for disease prevention and can be prioritized for high-risk regions identified in our work.

## Abbreviations

Maxent – Maximum entropy; ESRI – Environmental systems research institute; GIS – Geographic information system; AUC – Area under the curve; ROC – Receiver operating characteristics; ISRIC - International soil reference and information; USDA – United state Department of Agriculture; FAO – Food and Agriculture organization; SPSS - Statistical Package for Social sciences; PCA – Principal component analysis; AOI – Area of Interest.

## Declarations

### Acknowledgments

This study was supported by State Key Laboratory of Veterinary Biotechnology Foundation (Grant no. SKLVBF201904).

### Author contributions

XLW conceived and supervised the study. ATE, HNW, SK, LH and LJN contributed to the data filtering, analysis, interpretation and map design. HvG contributed to the methodology, cartography, discussion

and manuscript writing. All authors significantly contributed to the final manuscript and gave final approval for publication.

## **Funding**

Not Applicable

## **Data sources**

The datasets generated and/or analyzed during the current study are available in:

1. [WORLDCLIM] repository, [[www.worldclim.org](http://www.worldclim.org)]
2. [SOIL GRIDS] repository [[www.soilgrids.org](http://www.soilgrids.org)]
3. [WAHID] repository [[www.oie.int](http://www.oie.int)]
4. [Chen et al., 2016] repository [<http://doi.org/10.1371/journal.pntd.0004637>]
5. [Yu et al., 2018] repository [<https://doi.org/10.1371/journal.pone.0203267>]
6. [Zhang, Hua-Yi et al., 2018 in Chinese] repository [[http://en.cnki.com.cn/Article\\_en/CJFDTotal-XDYF201810003.htm](http://en.cnki.com.cn/Article_en/CJFDTotal-XDYF201810003.htm)]

## **Ethical approval and consent to participate**

Not applicable

## **Consent for publication**

All the co-authors consent the publication of this work.

## **Conflict of interest statement**

The authors declare no competing interests with respect to the research, authorship, and/or publication of this article.

## **Consent for publication**

Not applicable

## **Ethics approval and consent to participate**

Not applicable

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# Tables

**Table 1:** Ranking of Principal Components by Eigenvalues.

PC Rank	Eigenvalues		
	Total	Variance %	Cumulative %
1	42	63	63
2	16	24	87
3	4	6	93
4	2	3	96
5	1	2	98

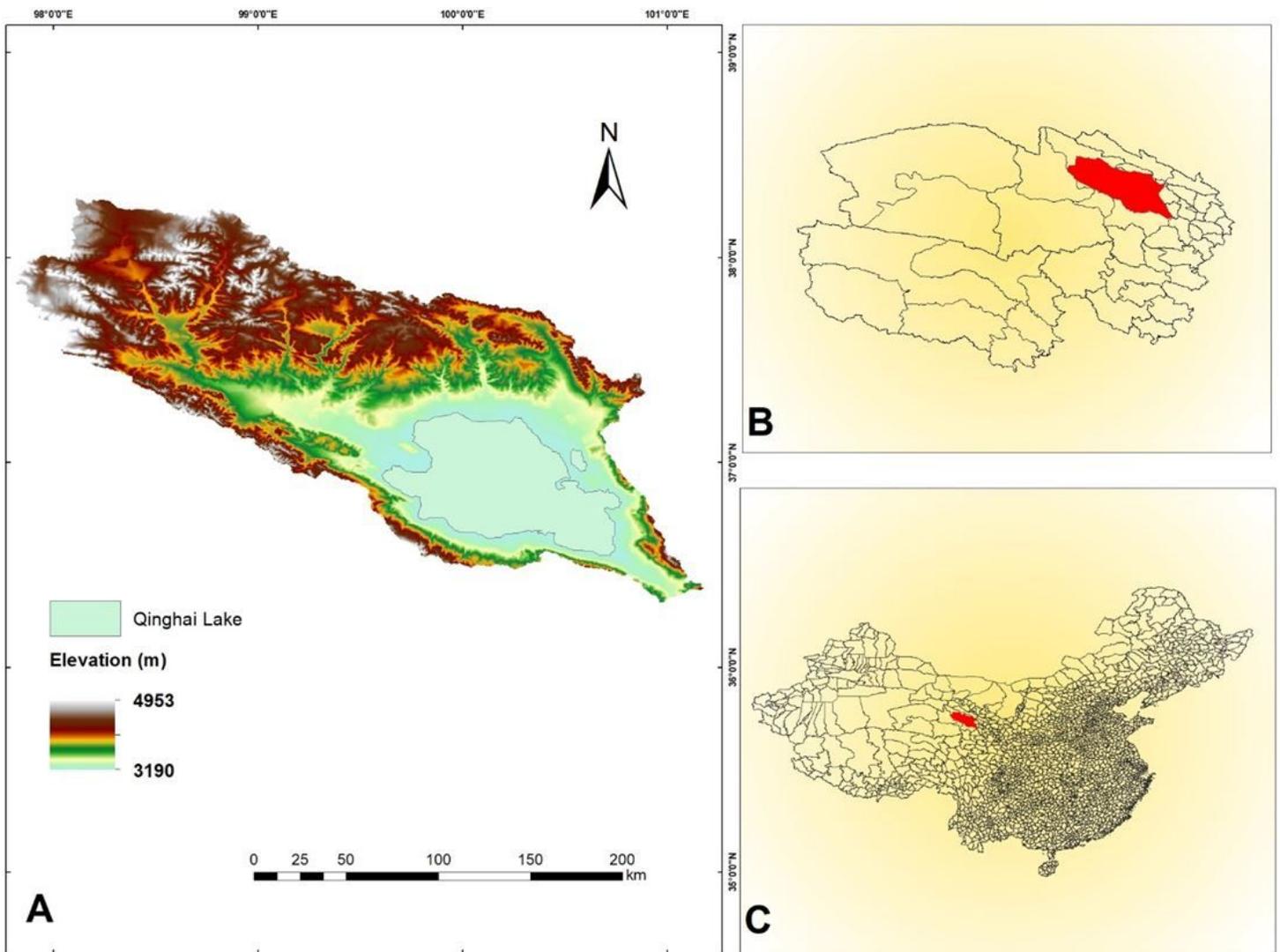
**Table 2:** Contribution of the three environmental predictors to the final suitability model.

Variable	Contribution (%)	Permutation (%)
Annual mean temperature	53	83
Soil type	35	4
Human population density	12	13

**Table 3:** Summary of the Jackknife analysis performed to determine importance per environmental variable.

Variable	Regularized		Test gain		Test AUC	
	Alone	Excluded	Alone	Excluded	Alone	Excluded
Annual mean temperature	1.2	0.8	1.2	0.8	0.86	0.87
Soil type	0.6	1.3	0.4	1.5	0.76	0.91
Human population density	0.4	1.2	0.8	1.0	0.89	0.83

## Figures



**Figure 1**

Area of Interest (AOI): A – Qinghai Lake basin; B – Qinghai province and C – People’s Republic of China.

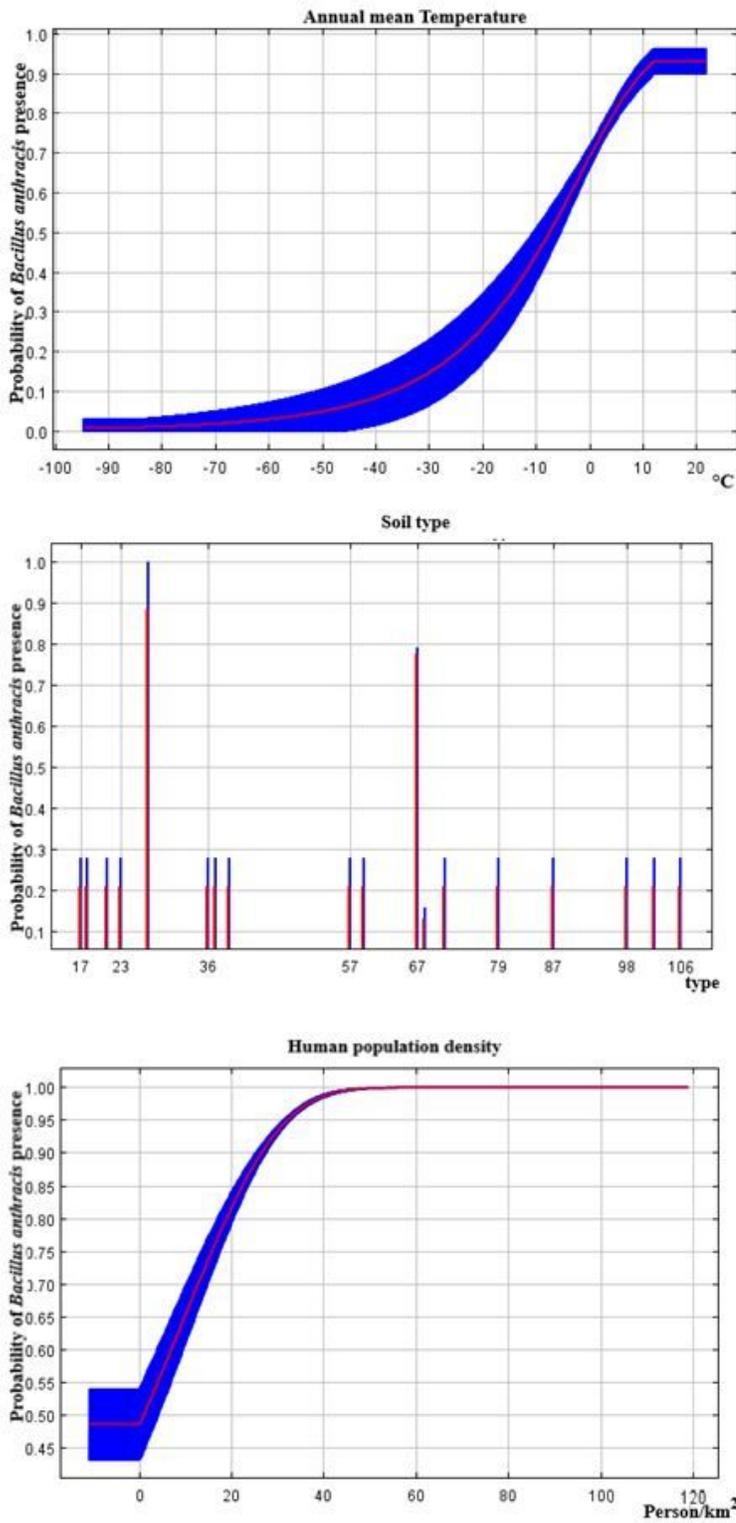


Figure 2

Response curves of predictive continuous variables (climate and human population density) and bar graph of predictive categorical variable (cover) for *B. anthracis* spore suitability in the Qinghai Lake basin. The red lines indicate the mean values while the blue denote the standard deviation. See Suppinfo 6 for Soil type legend.

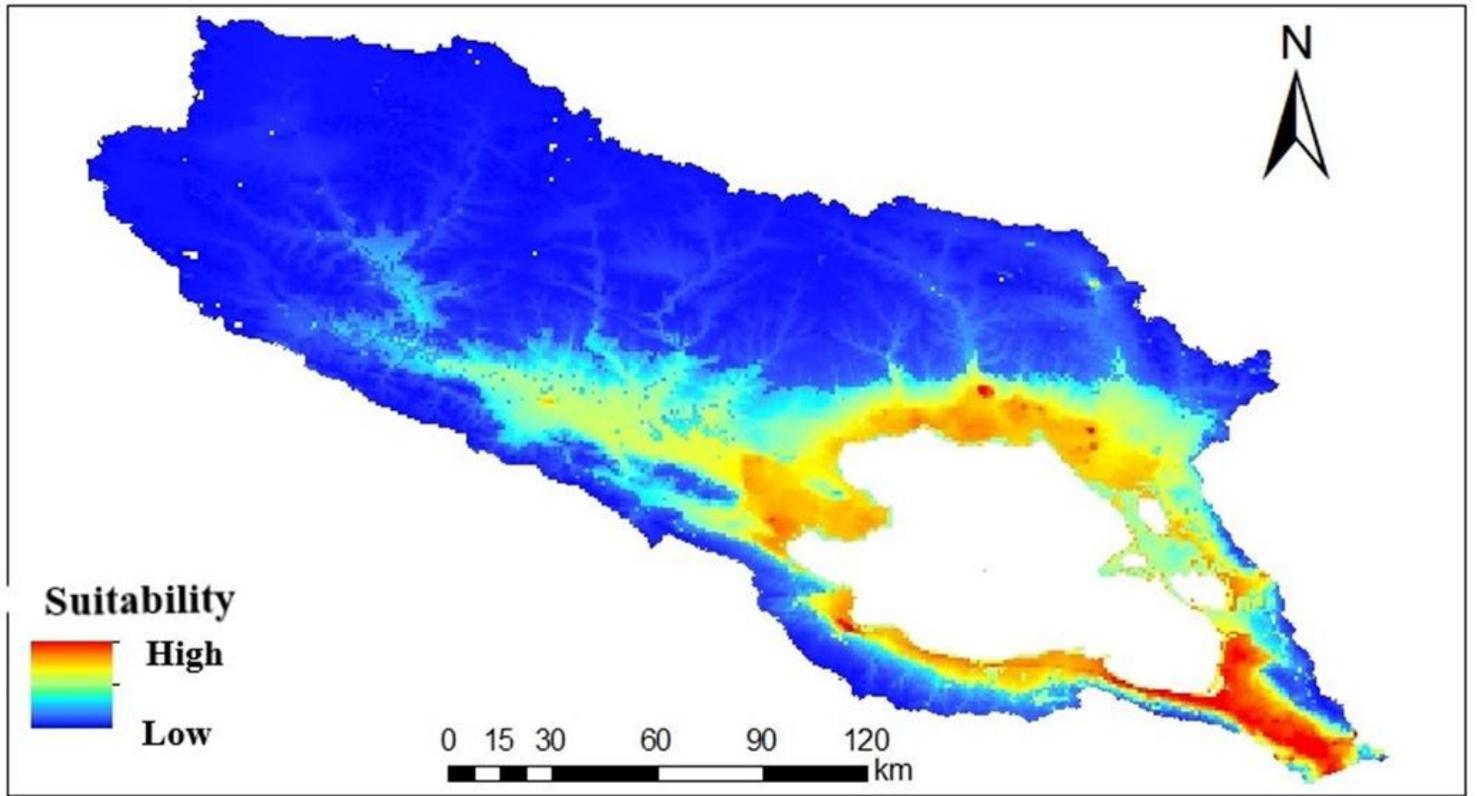


Figure 3

The environmental suitability map of *B. anthracis* spore survival in Qinghai Lake basin.