UNC Botanical Gardens Capstone

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Flourishing Futures:

Reintroducing Native Plant Species into the Uwharrie National Forest

Through Suitability and Distribution Analyses

Abstract

This research aims to integrate GIS analysis and public outreach strategies to support the restoration and conservation of rare plants in North Carolina's Uwharrie National Forest. By using the MaxEnt modeling software, we developed a habitat suitability model to identify optimal reintroduction sites based on various environmental factors. Our study emphasizes the importance of using local ecotypes and seed material for effective conservation. Through the use of the North Carolina Botanical Garden's seed banking efforts, we accessed a repository of occurrence data for the targeted species. This data, which includes the precise information on where these species have been found, was crucial for developing habitat suitability models. By integrating this occurrence data into our GIS-based modeling, we were able to create accurate predictions for optimal reintroduction sites. Ground Truthing efforts validated our species distribution models and ensured that the selected habitats meet ecological needs of the species and aligned with our observed field conditions. The results of the study demonstrated that the models accurately identified suitable habitats, facilitating successful reintroduction efforts. The comprehensive approach of our study prioritizes conservation resources, providing focus on areas with the highest potential for sustaining the targeted species. The generated maps will guide future reintroduction efforts by the North Carolina Botanical Gardens and the U.S. Forest Service. Additionally, the use of various public outreach strategies and efforts will increase awareness and knowledge about plant conservation. Through targeting both policymakers and the general public through engaging, data-driven content, we will be able to accomplish this goal.

Keywords: Species Distribution Model, Habitat Suitability, Plant Conservation, Uwharrie National Forest, Plant Reintroductions, Ground Truthing

Introduction

Phenotypic plasticity within plants consists of the ability for a plant to change traits in response to environmental conditions which is vital for their adaptation to the various changes of the climate (Stotz et al., 2021). This plasticity allows plants to survive and thrive in a range of different environments to become more resilient to climate change. Each population of a plant species can show local adaptation, where specific traits are more fine-tuned to the local environmental conditions rather than on a larger scale(Stotz et al., 2021). This localized adaptive through phenotypic plasticity is crucial for conservation efforts because it shows the importance in preserving diverse habitats (Stotz et al., 2021). When reintroducing plant species to altered habitats, it is completely necessary to match genetic and phenotypic characteristics of the plants to the specific conditions of the restoration site (Stotz et al., 2021). Without doing this, the plant populations can experience poor survival and establishment rates.

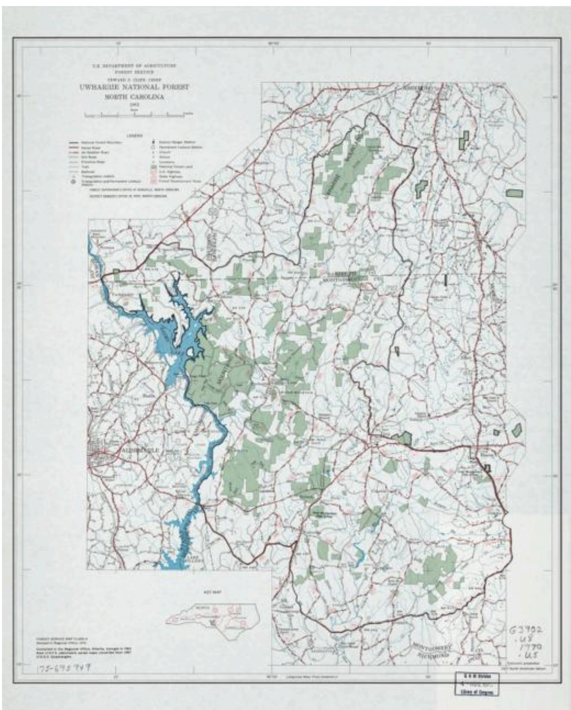


Figure 1 Original map of Uwharrie National Forest from the Library of Congress. The black line indicates original boundaries of 1964. (LOC)

There are many gardens and conservation programs that aim to address these challenges. Recognizing the importance of this challenge, the North Carolina Botanical Garden (NCBG) aims to inspire appreciation and conservation of plants while encouraging a more sustainable relationship between nature and people. With this, the NCBG developed the Conservation Garden that represents the many conservation activities that are conducted by the gardens. This conservation includes propagating native plants, seed banking for reintroduction and protection, and conserving habitats. Seed banking is a location at suitable conditions to preserve seeds of different plant species. The NCBG's rare plant seed banks protect over 500 individual accessions that represent 72 different species (North Carolina Botanical Garden, n.d.). The United States Forest Service (USFS) manages public lands in national forests and works to sustain the health, diversity, and productivity of these ecosystems for the present and for future generations. They support conservation through practices like habitat restoration, species protection, and sustainable land management. The forest service's restoration plan focuses on rehabilitating ecosystems and improving forest health, while their logging practices are managed to ensure sustainable use of timber resources and balances economic needs with environmental conservation (USDA Forest Service, 2011).

Species Distribution Models (SDMs) are algorithms that create probability of occurrences across a landscape based on a set of ecological variables and presence/absence data. (Miller, 2010). Habitat Suitability models (HSMs) are used to identify areas that are suitable for a species based on the environmental variables without the need for point data (Miller, 2010). The SDMs and HSMs can be used to study the specific environmental variables such as climatic variables, topography, and NDVI (Normalized difference vegetative index) that affect species distribution. A study by Bowen and Stevens (2020) demonstrates how environmental variables such as temperature, topography, soil, and NDVI drive habitat preferences for shade-tolerant invasive grass. They used MaxEnt and GIS to properly predict and identify suitable habitats for a species of invasive grass on both a regional scale and a smaller scale (Bowen and Stevens, 2020).

This paper aims to integrate GIS analysis and public outreach strategies to support restoration and conservation of rare plants in North Carolina's UNF. The primary goal is to develop a habitat suitability model to identify optimal reintroduction sites based on environmental factors and ensure effective conservation efforts.

Marshallia obovata var. *obovata* is a species that thrives in specific soils and habitat conditions. This species is commonly found in soils that are classified by their ability to retain moisture and their tendency to crack during dry periods, which creates a microhabitat that supports the species growth (Weakley, 2012). These specific ecological conditions suggest that the species will succeed in reintroduction efforts in the UNF where these soil characteristics are present. *Asclepias tuberosa* var. *tuberosa* can be found statewide across North Carolina in dry and sunny locations (Patterson, 2016).

Figure 2 Photos of four target species: Asclepias tuberosa var. tuberosa, Monarda fitsulosa var. fitsulosa, Marshallia obovata var. obovata, Eryngium yuccifolium var. yuccifolium (LOC)

The Uwharrie District of North Carolina has a diverse topography with different habitats, including dry sloped woodlands that can benefit the *Asclepias tuberosa* var. *tuberosa* can be successfully reintroduced. *Eryngium yuccifolium* var. *yuccifolium* is widely acknowledged to be a dominant species within the prairie environment. It primarily disperses its seeds through wind which is essential to reproduction and reintroduction within habitats (Albee, et al, 2023). *Monarda Fistulosa* var. *fistulosa* has been widely witnessed to grow in areas with high levels of available light such as roadways and pastures (Missouri Botanical Garden). Our hypothesis is appropriate because it is based on the specific ecological characteristics and habitat preferences of the targeted plant species. Each of the plant's preferred conditions are derived from existing ecological research and observations.

We hypothesize that *Marshallia obovata* var. *obovata* will have a higher probability of occurring in areas of Uwharrie National Forest (UNF) with high soil drainage, clay-rich soil deposits, and mixed light availability. Similarly, *Asclepias tuberosa* var. *tuberosa* is predicted to have a higher probability of occuring in areas that are dry and well-drained soil at woodland borders with low vegetation coverage in the Uwharrie National Forest. The habitat suitability of *Eryngium yuccifolium* var. *yuccifolium'* is predicted to be higher in the Uwharrie Forests in open areas with exposure to wind, and minimal anthropogenic disturbance (meadows and prairies, deep within the park). The habitat suitability of *Monarda fistulosa* var. *fistulosa* is predicted to have a higher probability of occurring within the Uwharrie Forest with areas of high light availability and lower vegetation coverage (areas such as meadows and roadsides).

Although our hypotheses are based on current ecological factors, some alternative outcomes are biologically possible. For example, *Marshallia obovata* var. *obovata* might be found in unexpected soil types or light conditions due to its potential for wider ecological plasticity; which allows it to adapt to a broader range of environmental conditions (Austin and Van Niel, 2011). While *Asclepias tuberosa* var. *tuberosa* could be present in highly dense vegetative areas or in soils that are not well-drained, which indicates its potential resilience and adaptability to different soil moisture availability (Guisan and Zimmerman, 2000). Additionally, *Eryngium yuccifolium* var. *yuccifolium* could potentially thrive in areas that experience a higher amount of anthropogenic disturbances or in more shaded areas, which would indicate its ability to tolerate moderate disturbance levels and more fluctuated light availability (Pearson and

Dawson, 2003). *Monarda Fistulosa* var. *fistulosa* might experience a higher vegetative coverage or lower light availability that could reflect its shade tolerance in more dense areas (Pearson and Dawson, 2003).. These alternative outcomes are based on the species' known ecological traits and their adaptability potential in various environmental conditions.

Methodology

Study Location

In order to create a maximum entropy model (MaxEnt) for the four selected species, our study area is the Uwharrie National Forest (UNF) in the North Carolina Piedmont. UNF's topography includes 50,814 acres of land, including sloped hills, long ridges, and a range of elevations from 400 to 1000 feet above sea level (U.S. Department of Agriculture, 2024). The Uwharrie National Forest is made up of pines such as loblolly pines, short-leaf pines, and hardwoods. As the Uwharrie National Forest's main focus includes the logging of pine trees, there is a diverse range of sunlight coverage depending on the density of the trees present which can affect the volume of undergrowth vegetation. The Uwharrie National Forest was chosen due to the garden's interest in reintroducing native North Carolina species within this forest. The figure below compares the broader regional distribution of the species and the localized occurrences within the forest.

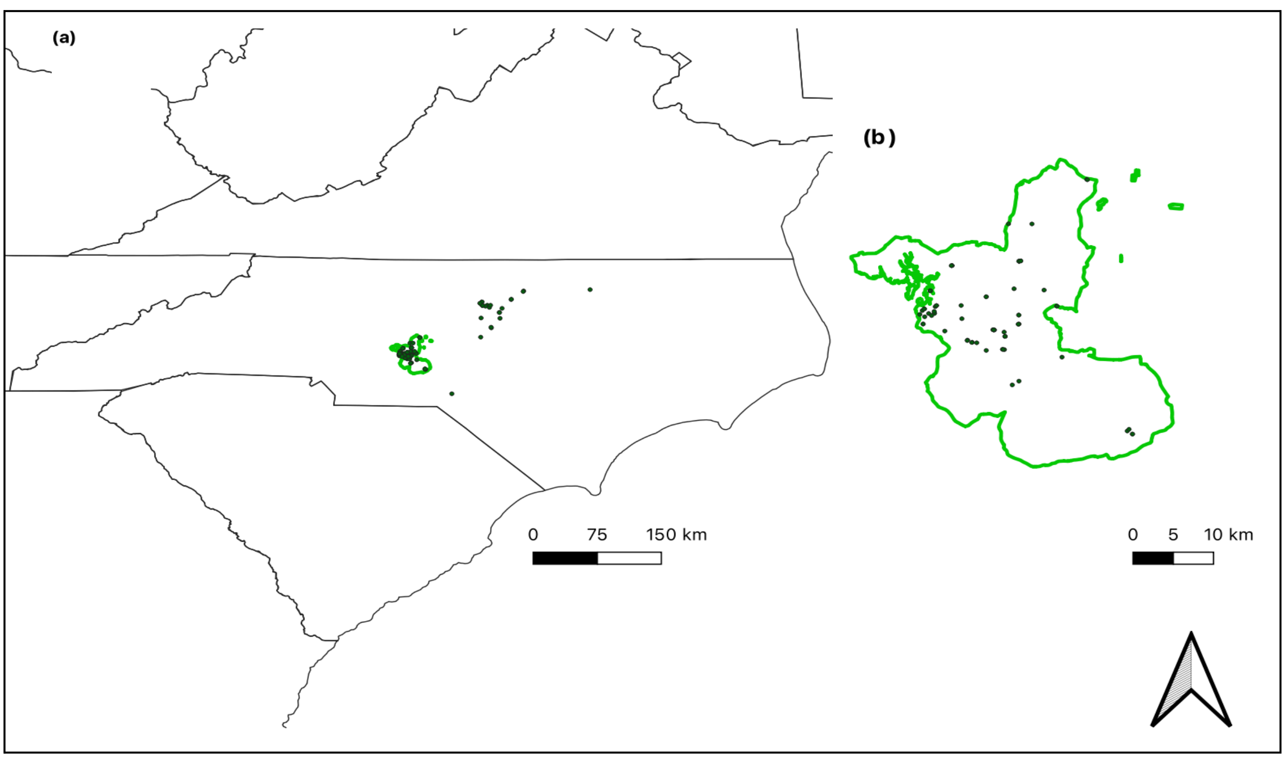


Figure 3. A depiction of the study area representing species points throughout North Carolina (a) and showing the distribution of the four targeted species within the Uwharrie National Forest (b). This visualization aids in pinpointing critical areas for species observations and data collection.

Species Distribution Modeling

We integrated multiple datasets that were used to optimize the MaxEnt process for assessing habitat suitability for each species. The USGS 19 bioclimatic layers (BIOCLIM) are a set of environmental variables that represent various aspects of climate, such as temperature and precipitation, which are necessary for modeling species distribution and habitat suitability.

For this model, we used a total of 25 environmental layers which included 19 BIOCLIM layers, three DEM layers, a Time Integrated NDVI (TIN) layer, and an Average Water Content (AWC) layer at 400m^2 resolution from USGS along with a Percentage of sand in top 30 cm of soil layer from USDA (Fig. 4). This allowed the model's output size to be reduced and gave more exact information on species habitability by clipping the variables to the state of North Carolina and the UNF boundary. Clipping the environmental variables to the state of North Carolina and the UNF provides more precise and relevant information for modeling habitat suitability within those corresponding geographic areas of interest.

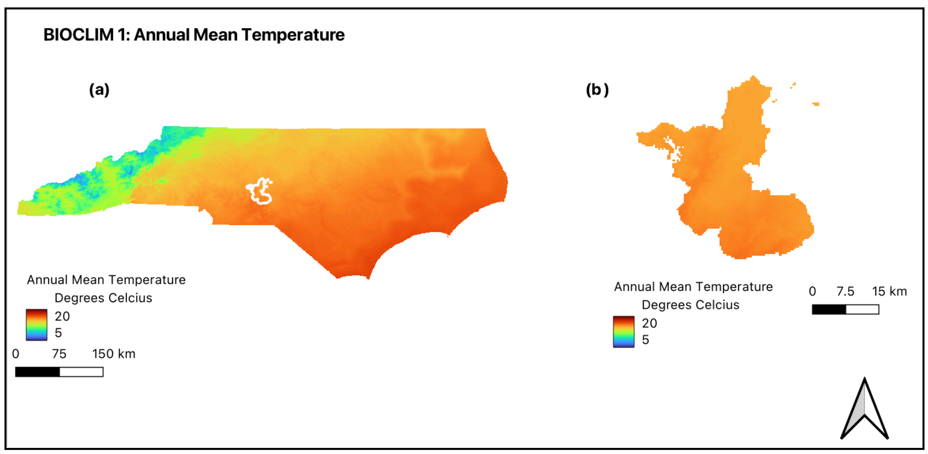


Figure 4. USGS BIOCLIM Variable 1: Annual Mean Temperature. Yearly average of climate inputs of the average temperature for each month in Degrees Celsius.

The cleaned species distribution data containing the latitude, longitude, and species name/code were then uploaded alongside the previously masked environmental layers over both the Uwharrie National Forest boundary and North Carolina State at a resolution of 400m^2 into the software program. There were a total of 97 species presence points for all four species, consisting of 39 *Asclepias tuberosa* var. *tuberosa* coordinates, 21 *Monarda fistulosa* var. *molis* coordinates, 16 *Eryngium yuccifolium* var. *yuccifolium* coordinates, and 21 *Marshallia obovata* var. *obovata* coordinates. The distribution data used was collected from the North Carolina Botanical Garden (NCBG) and supplemented with research-grade iNaturalist observations due to the small sample size (iNaturalist, 2024). The species presence points were not spatially filtered and the hinge feature was applied to the models due to the small sample size.

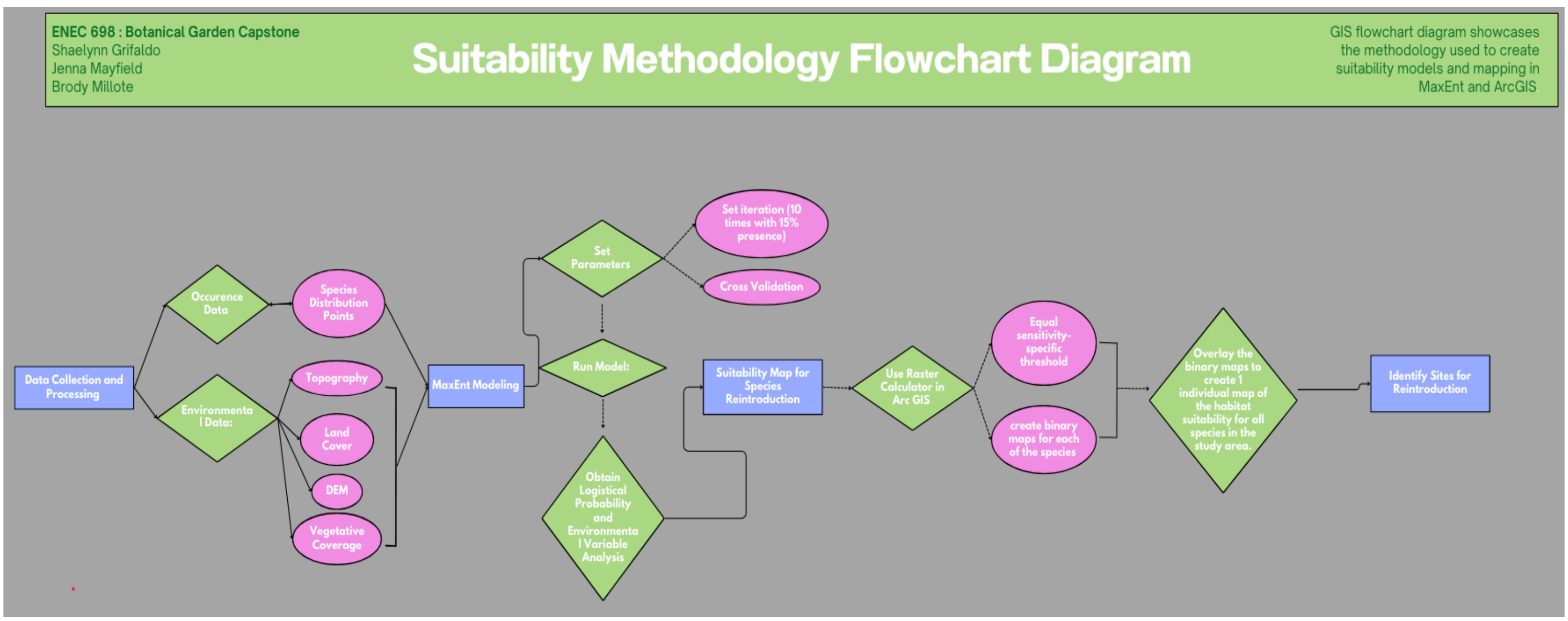


Figure 5. This figure shows the flowchart of the methodology for the creation of the habitat suitability models in MaxEnt and the ArcGIS mapping for the four rare plant species (Miller, 2010).

The parameters used in the model were the adjustment of the regularization multiplier to best fit each species, and we were able to analyze which environmental variables affected the habitat suitability of each species. The regularization multiplier is a parameter in MaxEnt that adds new constraints to the model so that the model is better fitted to the range of environmental variables (Morales, 2017). The regularization multiplier changed from species to species with values ranging from 0.8-1 (Table. 2). A feature class is the mathematical transformation of the different covariates which allows the model to access the complex relationship between environmental layers and data points (Morales, 2017). We used the hinge threshold feature class as this feature estimates changes in the model fit which finds change points within the distribution data (Terrill, 2021). We avoided using categorical data such as soil types and land cover so that MaxEnt would not overemphasize the categorical data causing overfitting for specific environmental layers (Bowen, 2020).

For each species, the non explanatory environmental variables were omitted from the model and then ran to accumulate the average suitability over the course of 1000 replicates using the hinge feature and cross-validation. Through the analysis of the generated jacknife tests and response curves, we were able to remove layers that showed signs of overfitting and/or autocorrelation (Table 3) so that we obtained the best AUC value for each model (Halvorsen, 2016). All species distribution models were done in Maxent version 3.4.4 (Phillips, Dudík, & Schapire, 2024).

This same overall process was repeated for the entire land area of North Carolina due to some observation points occurring outside of the UNF boundary. With adjustments to the regularization multiplier and the removal of layers to avoid overfitting and autocorrelation, the models were set at relatively the same parameters. However, changes were made regarding the feature class, the random test percentage, and replicate run type (Table 5). These changes were due to the larger area of the North Carolina boundary compared to the UNF boundary. The omitted variables for the state-scale model include Annual Mean Temperature, Min Temperature of Coldest Month, Annual Temperature Range, and Mean Temperature of Wettest Quarter (Table

3). A map of North Carolina was made to visualize the species observations as the seed banks were distributed outside of the Uwharrie National Forest Boundary.

Habitat Suitability Model

All final species distribution models that were generated in Maxent were imported and edited in QGIS. A raster average map was created in QGIS by using the Raster Calculator and then edited. The raster calculator allows you to perform calculations for raster layers and creates a new output layer (Dragos, 2017). The raster of the individual species models were aligned and averaged across cells, creating the final habitat suitability model. A final model with 1,000 replicates was also created within QGIS on a binary scale to visualize which areas would be considered suitable versus unsuitable. The suitability of these areas were shown within the specificity threshold, with a specificity of 0.5 and above meaning highly suitable. All final species habitat suitability maps were created in QGIS version 3.36.3 (QGIS Development Team, 2024).These methods were used to generate the habitat suitability model across North Carolina as well as just the UNF boundary.

Ground Truthing

The sites for ground truthing were split between the already-known sites from the North Carolina Botanical Garden and new sites for introduction picked out from the habitat suitability maps created. The final model was overlaid onto Google Earth Pro to find accessible sites for the research team to visit in person. These efforts ensured a comprehensive selection of sites for accurate data collection.

The visitation of these selected sites allowed us to record the GPS coordinates of the species that were seen on a GPS Coordinate tracker and gain information on the habitats' surroundings and soil types through written field notes. This process was essential for verifying the presence of the species in the predicted habitats and understanding their environmental conditions. The general soil characteristics of each site were briefly noted, providing critical information about the soil-related factors that may influence species distribution.

Results

Species Distribution Modeling

For each of the species, the species distribution models (SDM) of the Uwharrie National Forest boundary have shown to have a high suitability value (Above 0.5). Each of the species distribution models have a different configuration of environmental predictors based on the modeling from MaxEnt. Taking this into consideration, the Uwharrie National Forest has shown to be a location with the highest suitability based on the presence point modeling and is a good candidate for the reintroduction of these four species.

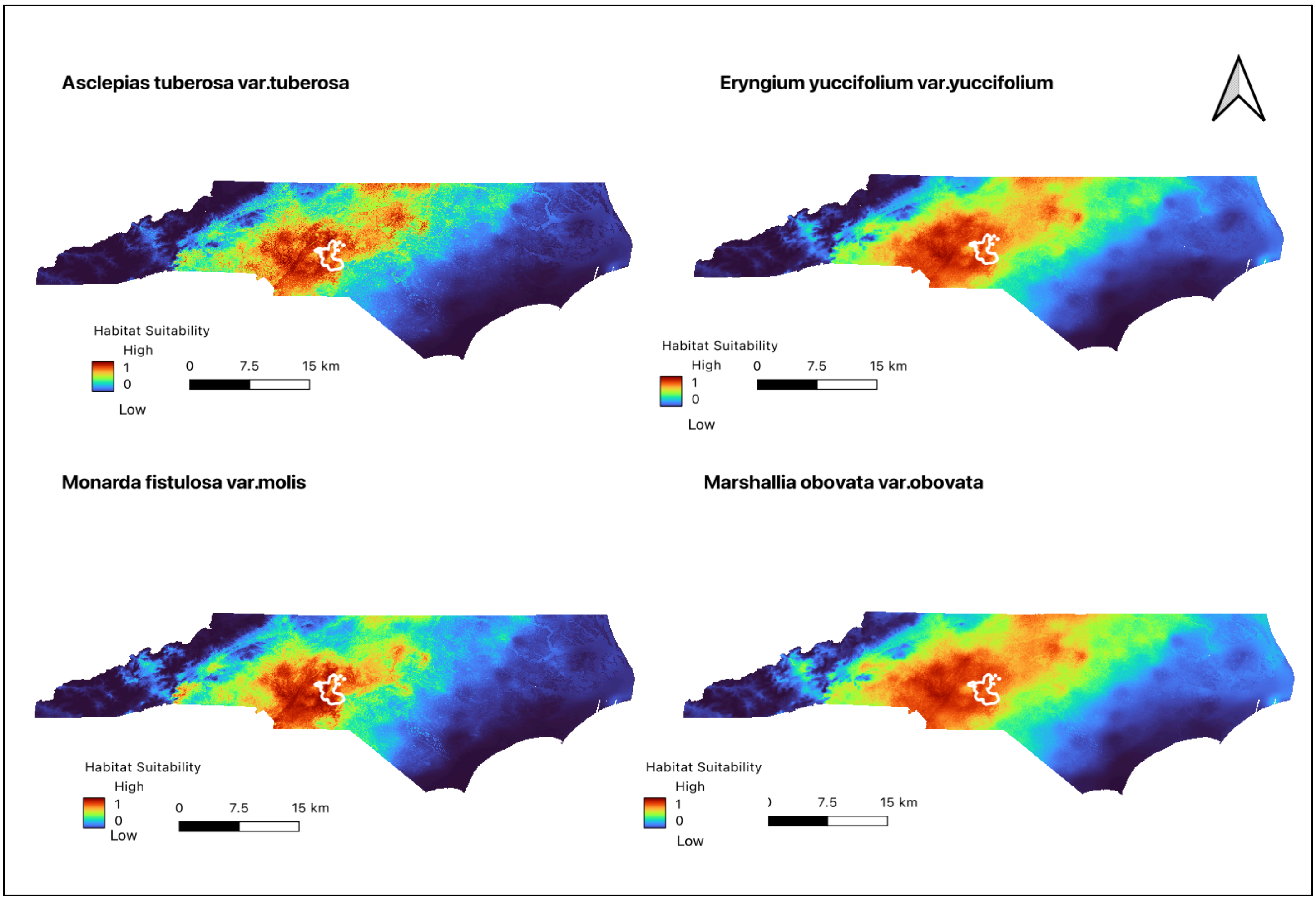


Figure 6. MaxEnt modeling maps showing the distribution of species occurrence for the four targeted species focused on the state-scale. Created across 1,000 cross-validated replicates on a scale from high to low occurrences.

The species distribution model for Asclepias tuberosa var. tuberosa has shown to have the largest range of species distribution across the Uwharries boundaries (Figure 7). *Eryngium yuccifolium* var. *yuccifolium* model has been shown to have the largest area of species distribution with most of that area being concentrated towards the edge of Badin Lake along with the mid-region of the Uwharrie National Forest (Figure 7). Overall the mid regions of the Uwharries should be prioritized for reintroduction as it is the region that shows the highest suitability for the final averaged models and for the models of each individual species.

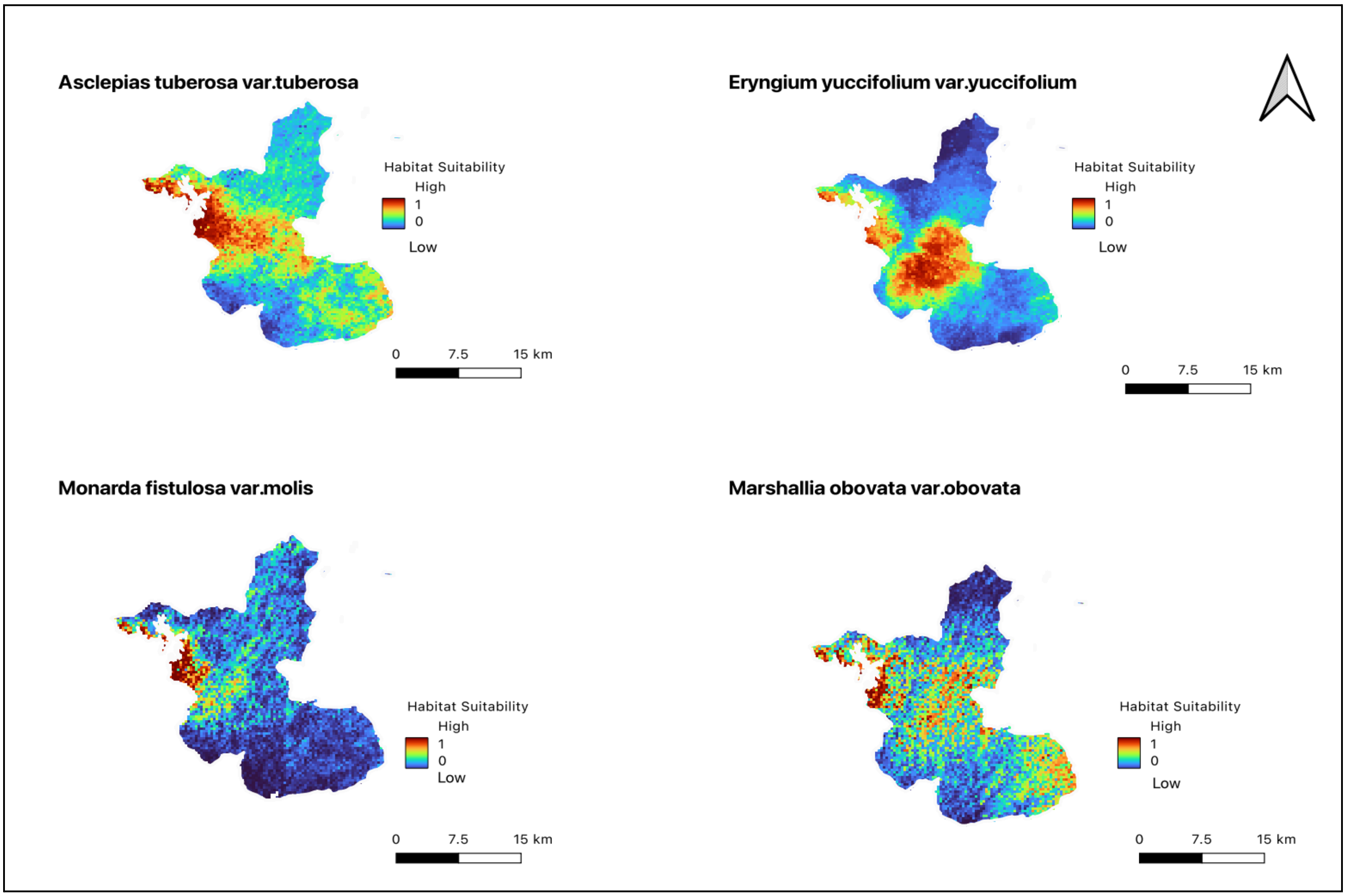


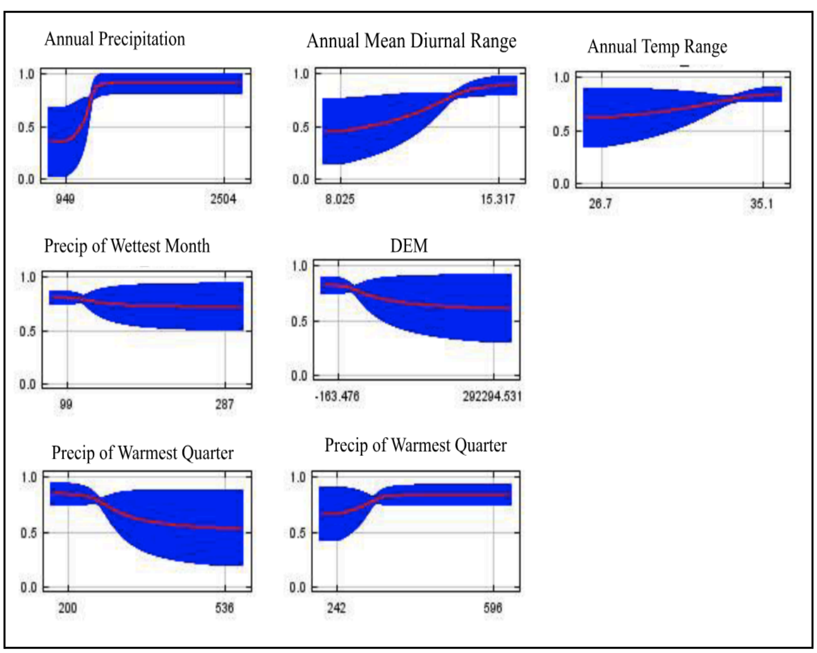
Figure 7. MaxEnt modeling maps showing the distribution of species occurrence for the four targeted species focused on the study area. Created across 1,000 cross-validated replicates on a scale from high to low occurrences.

Percent contribution represents the proportional contribution of each of the environmental variables in the model’s predictions of habitat suitability (Phillips, 2006). Permutation importance assesses the influence of each environmental variable on the model's prediction performance by measuring how much the model’s accuracy fluctuates when the values of the variables are shuffled at random (Phillips, 2006). For the state model, the top contributing variable for *Asclepias tuberosa* var. *tuberosa* (ACTUB) and *Monarda fistula* var. *fistula* (MONFIS) were the Bio 12: Annual Precipitation environmental variable with a percentage of 45.5% for ACTUB and 62.6% for *Eryngium yuccifolium* var. *yuccifolium* (Table 6.1-6.2). The top contributing variable for *Eryngium yuccifolium* var. *yuccifolium* was BIO 12: Annual Temperature with a percent contribution of 36.8% (Table 6.2). The top contributing variable for *Marshallia obovata* var *obovata* is Aspect which has a percent contribution of 33.4% (Table 6.3).

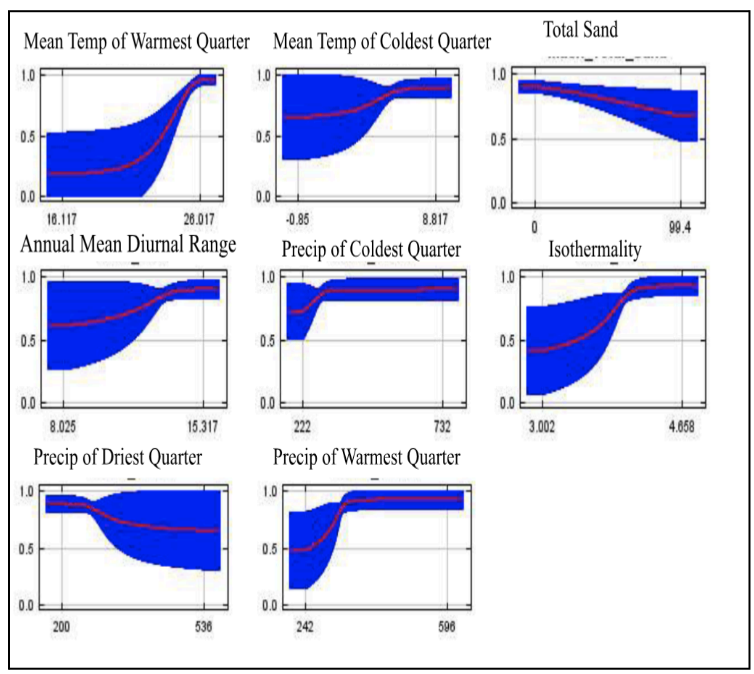
Response curves (ROC) are used to indicate a graphical illustration of the relationship between environmental variables and the predicted outcomes of the model. Within the state model, the species *Asclepias tuberosa* var. *tuberosa* precipitation of driest quarter, precipitation of coldest quarter, and precipitation of driest month had the strongest correlation with the highest suitability for the models. For the species *Monarda fistulosa* var. *fistulosa* annual mean diurnal range, precipitation of coldest quarter, and the percentage of sand in the soil layer had the strongest correlation with the highest suitability for the models. For the species *Eryngium yuccifolium* var. *yuccifolium* precipitation of coldest quarter, slope, and annual mean precipitation had the strongest correlation with the highest suitability for the models. For the species *Marshallia obovata* var. *obovata* annual mean precipitation, precipitation of coldest quarter, and precipitation of driest quarter had the strongest correlation with the highest suitability for the models.

For the study-area model, the response curves illustrate that for the species *Asclepias tuberosa* var. *tuberosa* factors such as AWC, aspect, and precipitation during the driest and warmest quarter significantly influence habitat suitability. For the species *Monarda fistulosa* var. *fistulosa*, there is a high sensitivity to precipitation in the coldest and driest quarters as well as temperature during the wettest quarter and the minimum temperature during the coldest month. *Eryngium yuccifolium* var. *yuccifolium* shows a positive response to higher annual precipitation, precipitation during the coldest quarter, and mean temperatures of both the warmest and the wettest quarters, but it is less suitable in areas with more extreme temperatures. As well as this, *Marshallia obovata* var. *obovata* prefers stable temperature seasonality and moderate precipitation during the driest quarter. This species is also sensitive to the minimum temperature of the coldest month and mean temperature of the wettest quarter.

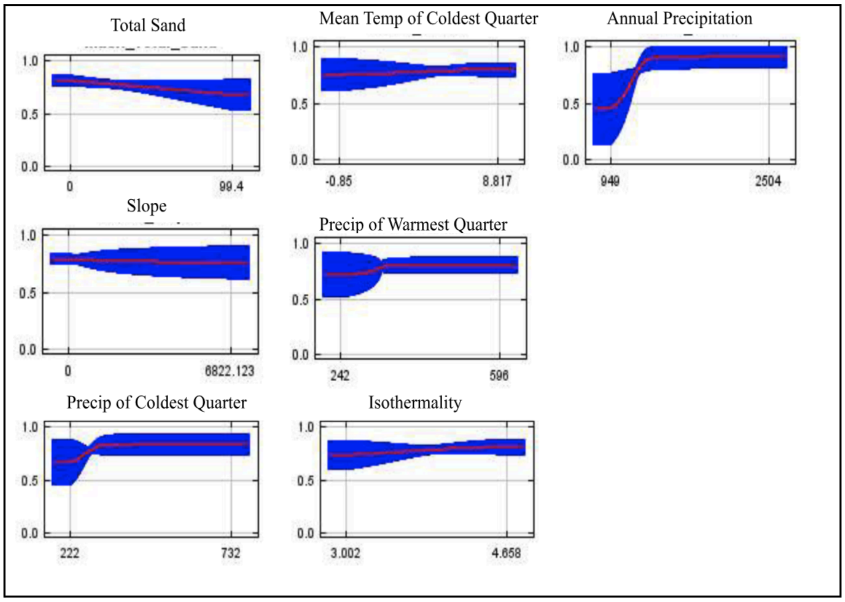
8a. *Asclepias tuberosa* var. *tuberosa*



8b.*Monarda fistulosa* var. *molis*



8c. ***Eryngium yuccifolium* var. *yuccifolium***



8d. ***Marshallia obovata* var. *obovata***

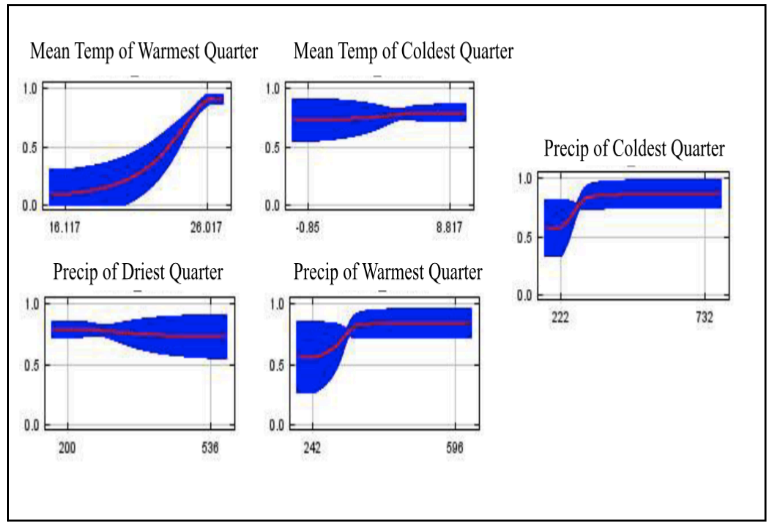
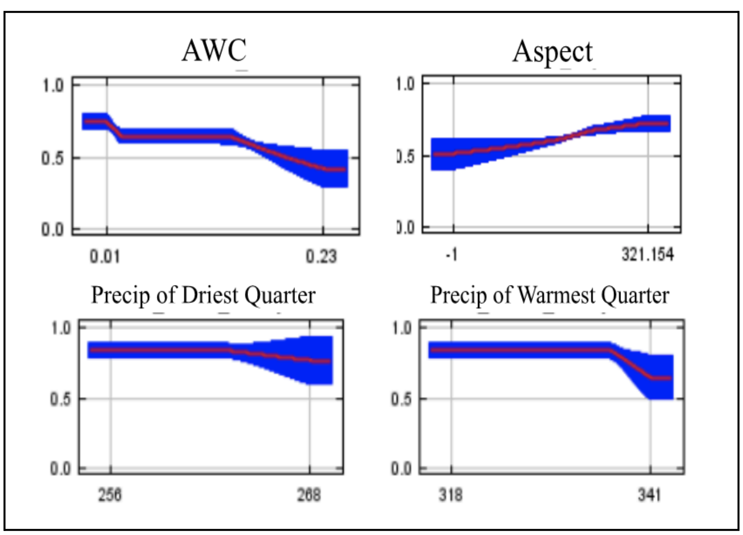
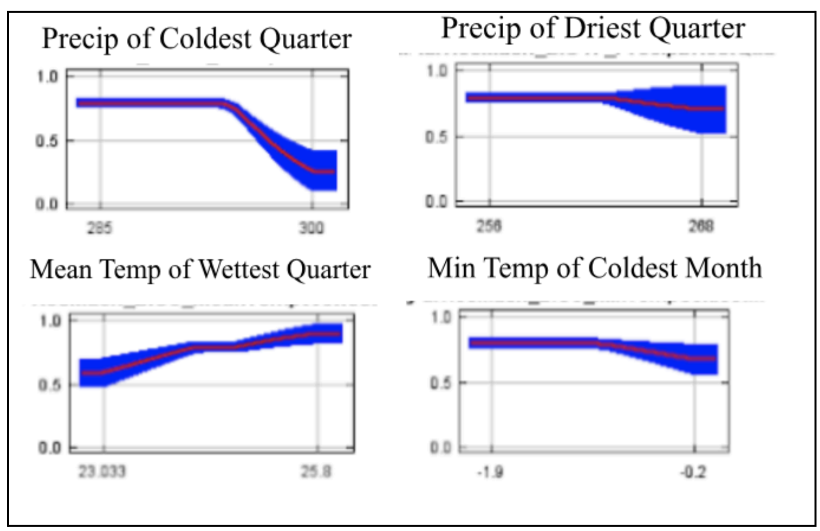


Figure 8. Visualizations of the response curves generated through 1,000 cross-validated replicates in MaxEnt. These MaxEnt suitability predictions (y-axes) and the predictor(x-axes) in the final models for the state-scale. The red lines within these ROCs indicate the means across the 1,000 cross-validated replicates, with the blue area of the curves indicating the standard deviation from that mean.

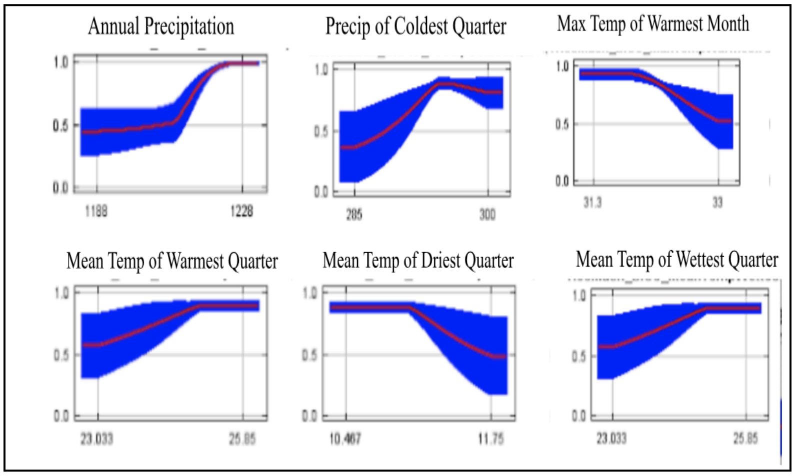
9a. ***Asclepias tuberosa* var. *tuberosa***



9b.***Monarda fistulosa* var. *molis***



9c. ***Eryngium yuccifolium* var. *yuccifolium***



9d. ***Marshallia obovata* var. *obovata***

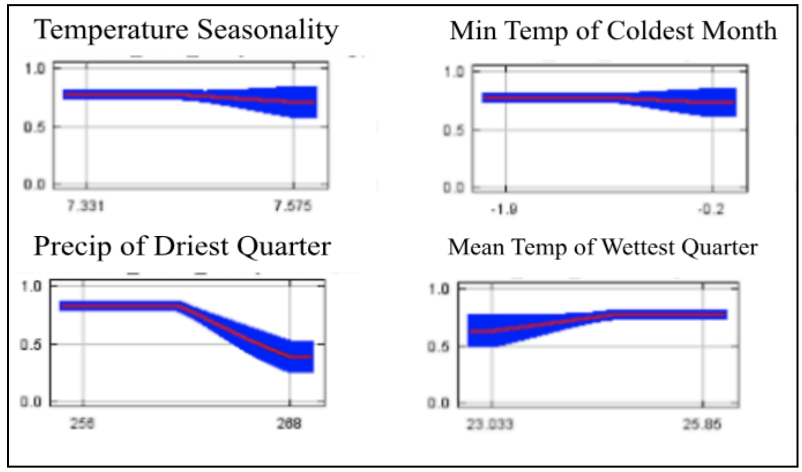


Figure 9. Visualizations of the response curves generated through 1,000 cross-validated replicates in MaxEnt.. These MaxEnt suitability predictions (y-axes) and the predictor(x-axes) in the final models for the study-area scale. The red lines within these ROCs indicate the means across the 1,000 replicates, with the blue area of the curves indicating the standard deviation from that mean.

Habitat Suitability Model

The predicted suitability model for *Asclepias tuberosa* var. *tuberosa*, *Monarda fistulosa* var. *molis*, *Eryngium yuccifolium* var. *yuccifolium,* and *Marshallia obovata* var. *obovata* presence points in the Uwharrie National Forest boundary in North Carolina. The highest predicted suitability in all of North Carolina is in the Uwharrie National Forest based on these parameters (Figure 10a). Inside of the Uwharrie National Forest boundary, the highest places of predicted suitability is located in the mid-region which is also near the most populated areas in the UNF (Figure 10b). Highly suitable habitat is an area that has a value of 0.5-1. This threshold was set to 0.5 because it represents a level of habitat suitability that is most likely to support the presence of the study species. Having the threshold at 0.5 indicates that the environmental conditions at these locations should meet the ecological needs of the study species (Figure 10c-10d). The calculated area of suitable land within the Uwharrie study area was 26.62%. Within all of North Carolina, 29.82% showed to be a highly suitable habitat.

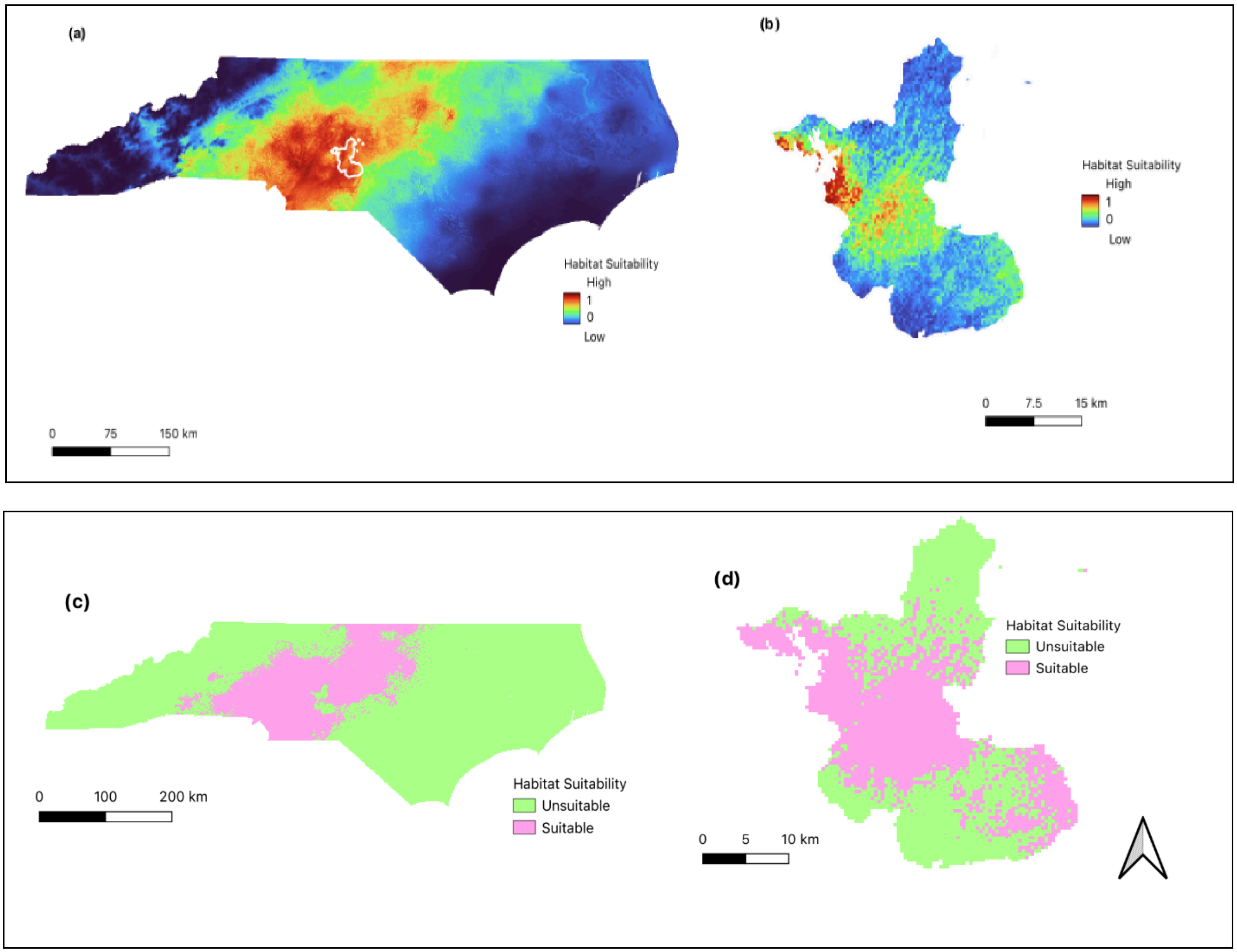


Figure 10. MaxEnt map results across 1,000 cross-validated replicates for both state-scale models
(a, c) and the study-area model (b, d). The average MaxEnt model maps show more suitable predicted habitats (a, b) and maps of suitable and unsuitable habitats on a binary scale (c, d).

Ground Truthing

The depiction of the coordinates and species that were seen through visiting the national forest can be seen below (Table 4). The accompanying figure (Figure 11) illustrates the exact location of these species within the Uwharrie National Forest. During our visitation of the UNF, our team collected GPS coordinates of eight occurrences of the targeted species. These included three occurrences of *Asclepias tuberosa* var. *tuberosa*, two of *Eryngium yuccifolium* var. *yuccifolium,* two of *Marshallia obovata* var. *obovata*, and one of *Monarda fistulosa* var. *molis.* Among these, four were previously known observances and four were new. All four species were found but only the species *Asclepias tuberosa* var. *tuberosa, Eryngium yuccifolium* var. *yuccifolium, Marshallia obovata* var. *obovata* had new occurrences.

The site for the observed *Monarda fistulosa* var. *molis* was located 100 meters south of the intersection on Baldin Lake Rd on the side of the road in a partially shaded forest area. This area was surrounded with canopy plants of Pine, White Oak, Poplar, and Sourwood with undergrowth of White Bush Clove, Carolina Rue, and Holly. The sites for *Asclepias tuberosa* var. *tuberosa* occurred in fire-mediated areas with canopy plants of Black Oak, Shortleaf Pine, and White Oak with undergrowth of Narrow Leaf Mountain Mint, Coral Honeysuckle, Goldenrod, Persimmon, White Ash, and sightings of *Marshallia obovata* var. *obovata.* This sighting of the two species within the same area would knowingly share the same canopy plants and undergrowth, but also share the same soil characteristics of a sandy-clay mixture. The sites of *Eryngium yuccifolium* var. *yuccifolium* were found in shaded ridges, with one occurring in a power line cut area. These sites share similar canopy plants of Longleaf Pine, Shortleaf Pine, Elm, and Walnut with undergrowth of Chinese Bush Clover, Pencil Flowers, Leafy Elephant Foot, and Helianthus. Knowing that these species are able to survive and withstand the habitat within this forest is crucial for mapping the suitability of the species being reintroduced in the same locations. This knowledge supports future conservation efforts and ensures that specific reintroduction strategies are based on ground truth models.

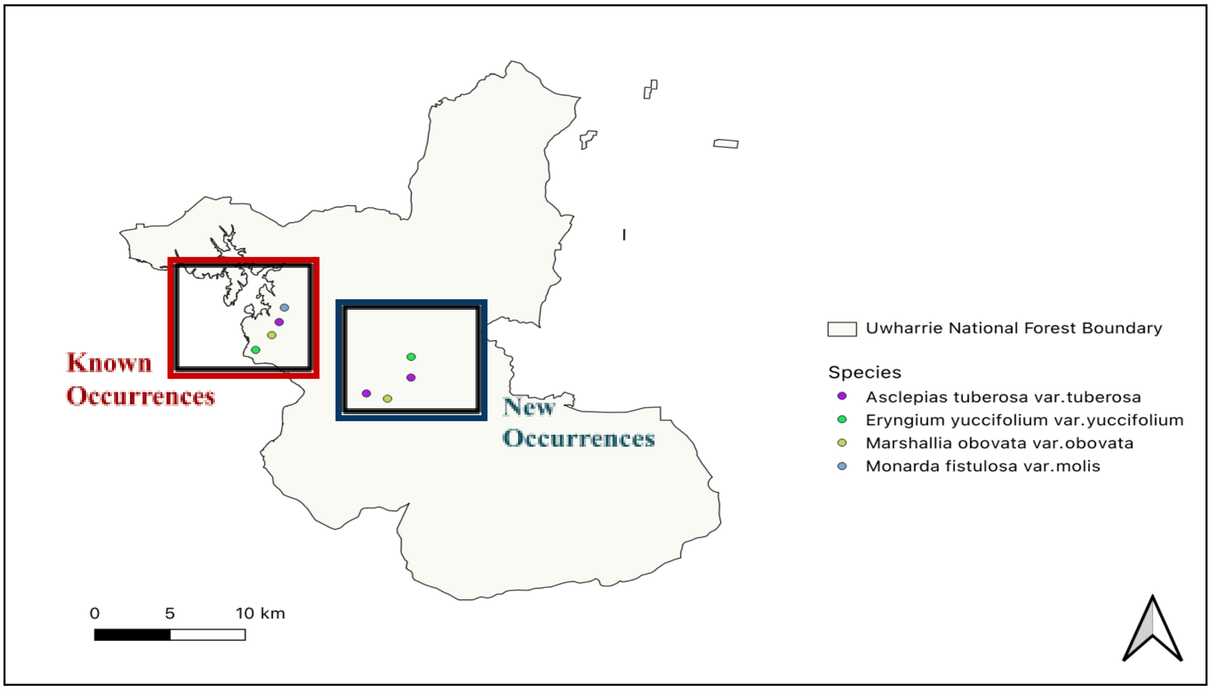


Figure 11. Depicts the species that were sited within the Uwharrie National Forest by type.

Discussion

Our results show that for each species, there are certain environmental variables that influence species distribution more than others. For *Asclepias tuberosa* var. *tuberosa* the highest suitability is linked to high levels of precipitation during the driest and coldest periods, which shows the species' sensitivity to seasonal moisture availability (Elith and Leathwick, 2009). This supports our hypothesis-that there would be a higher probability of the species occurring in areas that are drier and have well-drained soil, which are linked to precipitation and available water content in the soil (Franklin, 2010). *Monarda fistulosa* var. *fistulosa* shows suitability that is strongly predicted by both diurnal temperature range and soil-sand content. As well as this, within the state-scale model, *Monarda fistulosa* var. *fistulosa* was strongly influenced by TIN NDVI, an important measure of photosynthetic activity; a higher TIN NDVI means more photosynthetic activity which can correlate with increased canopy cover (Guisan and Zimmerman, 2000). This supports our hypothesis of this species occurring in areas that have a higher light availability and lower canopy cover. *Eryngium yuccifolium* var. *yuccifolium* has strong predictors with annual precipitation, cold quarter precipitation, and slope which highlights water availability and topographic features rather than open areas with minimal disturbance, as stated in our hypothesis. *Marshallia obovata* var. *obovata* shows high prediction with various precipitation variables, suggesting moisture availability is highly important to the habitat suitability of this species. This supports our hypothesis of this species occurring in areas of higher soil drainage because soil-sand percentage contributes highly to the habitat suitability of *Marshallia obovata* var. *obovata* (Elith et al., 2011) With the experience of ground truthing, we were able to note the general soil characteristics that surrounded the observation of this species; with doing this, we found clay-sand soils within the state.

The Uwharrie shows signs of meeting these ecological needs. The model revealed that 26.62% of the Uwharrie study-area is highly suitable for the seed bank material, compared to 29.82% of the entire state of North Carolina (Fig. 10c-10d). This indicates that while the UNF provides significant habitat for the seed bank material, there are also other suitable areas throughout the state.

The comparison between the state-scale model and the study-area model reveals key similarities and differences in the factors that influence habitat suitability for the specified species. For *Asclepias tuberosa* var. *tuberosa,* both models highlight the importance of precipitation but the state model emphasizes precipitation during the driest and coldest quarters (and months) while the UNF model points to AWC, aspect, and precipitation during the driest and warmest quarters. *Monarda fistulosa* var. *fistulosa* shows a high sensitivity to precipitation in both models, with the state model highlighting annual mean diurnal range and soil-to-sand percentage, and the UNF model emphasizes temperature during the wettest quarter and the minimum temperature of the coldest month. *Eryngium yuccifolium* var. *yuccifolium* responds positively to precipitation within both the state-scale and study-area models, however, the state-scale model also considers slope and annual mean precipitation while the study-area model focuses more on mean temperatures. For *Marshallia obovata* var. *obovata,* both models show high predictions towards precipitation. The differences observed between the state-scale and study-area can be attributed to various biological and ecological factors. A potential reason for these differences is the variability in microhabitats within the UNF compared to the broader state. The UNF's specific topography, soil types, and climatic conditions might influence the species habitat preferences and suitability differently than a more generalized condition of the state (Franklin, 2010). For *Asclepias tuberosa* var. *tuberosa,* the reliance on AWC and aspect in the UNF model might be due to the localized soil moisture and the orientation of slopes that affect sunlight exposure and various temperature changes. These climatic conditions are crucial in the growth of the plant population and survival that might not be as influential on the state-scale (Guisan and Zimmermann, 2000). For *Monarda fistulosa* var. *fistulosa* the sensitivity to the temperature variables in the UNF model could be linked to the temperature fluctuations during the wettest quarter and winter months. These temperature fluctuations could impact the seasonal growth patterns of the species that might not be noticeable at the state-scale (Austin and Van Niel, 2011). *Eryngium yuccifolium* var. *yuccifolium* response to slope and annual precipitation at the state level versus the influence by temperature at the local level suggests that the broader features of landscape (like slope) has more influence on the species distribution statewide rather than the UNF scale. Finally, *Marshallia obovata* var. *obovata's* emphasis on precipitation in both models shows the importance of moisture availability for this species; however, the varying influence of the other environmental factors between the two scales show the complex relationship between larger and smaller environmental conditions that determine habitat suitability (Perason and Dawson, 2003)

The models collectively highlight the mid region of the Uwharrie National Forest as the most suitable location for reintroduction. This area has consistently shown high levels of suitability across the SDMs and HSMs for both the state and UNF. These models show some differences due to their scale. The state-scale model incorporates broad climatic variables and analyzes the effect of plant distribution over a larger area while emphasizing factors like annual precipitation and slope. Contrastingly, the study-area model focuses more on localized conditions that may include more microhabitat preferences on a finer scale, such as soil moisture retention, aspect, and specific temperature variations.

Ground truthing increased knowledge of environmental variables that were not included within the modeling. Knowledge such as surrounding species composition and basic soil composition provided further indicators in which to analyze consistencies between different sites where target species were present. Clay-sand-decaying matter soil combination was present among all sites investigated through ground truthing. The similar makeup of soil was due to proximity of ground truthing sites (<15km) and provided information about soil type; it was not analyzed for drainage ability. Multiple sites had recently completed prescribed burns (within 1-2 seasons) which worked to increase light availability to the sites and worked to decrease the amount of decaying vegetation. Ground truthing provided ample information about exact species location that could not be determined through QGIS, all species were found along roadsides with full light - mixed light availability. This supported the hypothesis that *Asclepias tuberosa* var. *tuberosa* would be found along woodland borders. However, the results were inconclusive as to whether anthropogenic disturbance plays a role in the success of *Eryngium yuccifolium* var. *yuccifolium* due to species presence along roadways.

Some potential errors to take into account in this analysis include the possibility of overfitting or autocorrelation. Overfitting can cause conclusions about the species distribution to be misleading and have inconclusive results. Overfitting occurs when too many features or any overly complex relationships between features are included along with a smaller sample size to a number of environmental variables ratio (Elith, 2011). This issue has a major possibility of being present in our results due to our small sample size per species occurrences. Additionally, spatial autocorrelation can lead to biased results since nearby locations may be more similar than those that are further from one another.

To better improve our models and gain more insight on each species, it would be beneficial to enhance our analysis of soil characteristics. Specifically, focusing on soil drainage and clay content for certain species could help identify the moisture availability more precisely, which is crucial for plant survival and growth (Elith and Leathwick, 2009). Incorporating these soil characteristics can enhance habitat suitability predictions by being able to better match the species with their preferred moisture conditions (Franklin, 2010). Additionally, it would be beneficial to also incorporate land usage into the habitat suitability map. This layer would help identify areas that have high human activity, such as construction and urban development, which might negatively affect reintroduction efforts (Guisan and Zimmerman, 2000). By being able to pinpoint locations with a low amount of anthropogenic interference, we can select reintroduction sites that offer the best chances for species survival and growth while enhancing conservation strategies (Perason and Dawson, 2003).

Our analysis can help conservation efforts to increase biodiversity and ecosystem stability through the reintroduction of the specified species by the NCBG and USDA Forest Service by identifying optimal habitats and monitoring population dynamics. Our research can guide future restoration projects and ensure successful reintroduction of native species. The findings from our analysis can serve as a model for other conservation organizations that have a goal in preserving these species and many others. Our research will be beneficial in public outreach programs by educating the community about the specific needs of different plant species and the overall importance of habitat conservation. It also enhances seed banking efforts by identifying the optimal environmental locations for seed collection and storage. These insights contribute to more effective conservation strategies along with the reintroduction of native plants in suitable habitats. Effective and successful reintroductions provide marketing material for botanical gardens and improve the frequency of monetary donations from donors, enhancing the ability for future reintroductions to occur.

To gain a more comprehensive understanding of the environmental layers that contributed to the models and certain discrepancies between the North Carolina model and the Uwharries model regarding unsuitable areas, more testing needs to be done on the models. One way to possibly understand the discrepancies is to test the models on a mid-scale experiment in the Piedmont Region of North Carolina to gain an understanding on which areas are unsuitable and which areas are suitable. There are also limitations of pine plantations as habitats for certain plant species, which makes selecting appropriate sites for conservation efforts even more important. Pine plantations are often used for commercial timber production and the use of heavy machinery that is required can compact the soil, leading to poor root growth and reduced water infiltration for other plant species (Uribe, 2021). Further analysis on pine plantations as habitats would be more beneficial in conducting a more thorough investigation. Doing an experiment to a mid-scale size allows for detailed analysis and validation of the North Carolina State-scale model and the UNF model, making the conditions more relevant to the local conditions that come with the Piedmont environment instead of all of North Carolina or just the Uwharrie National Forest. Some other ways to refine and improve the SDMs and habitat suitability models would be to collect more samples and conduct species data collection, such as recording GPS coordinates of occurrences, assessing plant health and growth rates, and noting specific habitat conditions to validate how accurate the North Carolina models are compared to the UNF models.

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Figures

Table 1. Environmental Variables used in the MaxEnt model and their source location

Environmental Variables	**Source**
Aspect	United States Geological Survey
Average Water Content	United States Geological Survey
Original Demographic Elevation Model	United States Geological Survey
Slope	United States Geological Survey
TIN NDVI	United States Geological Survey
Total Sand	United States Department of Agriculture: Forest Service
Annual Mean Temperature	United States Geological Survey BIOCLIM
Annual Mean Diurnal Range	United States Geological Survey BIOCLIM
Isothermality	United States Geological Survey BIOCLIM
Temperature Seasonality	United States Geological Survey BIOCLIM
Maximum Temperature of Warmest Month	United States Geological Survey BIOCLIM
Minimum Temperature of Coldest Month	United States Geological Survey BIOCLIM
Annual Temperature Range	United States Geological Survey BIOCLIM
Mean Temperature of Wettest Quarter	United States Geological Survey BIOCLIM
Mean Temperature of Driest Quarter	United States Geological Survey BIOCLIM
Mean Temperature of Warmest Quarter	United States Geological Survey BIOCLIM
Mean Temperature of Coldest Quarter	United States Geological Survey BIOCLIM
Annual Precipitation	United States Geological Survey BIOCLIM
Precipitation of Wettest Month	United States Geological Survey BIOCLIM
Precipitation of Driest Month	United States Geological Survey BIOCLIM
Precipitation Seasonality	United States Geological Survey BIOCLIM
Precipitation of Wettest Quarter	United States Geological Survey BIOCLIM
Precipitation of Driest Quarter	United States Geological Survey BIOCLIM
Precipitation of Coldest Quarter	United States Geological Survey BIOCLIM
Precipitation of Warmest Quarter	United States Geological Survey BIOCLIM

Table 2. Showcases the species finalized regularization multiplier used for modeling with MaxEnt and the resulting AUC for each.

Species	**Regularization Multiplier**	**Model Area Under the Curve (AUC)**
Asclepias tuberosa var. *tuberosa*	1	0.649 AUC
Monarda fistulosa var. *molis*	1	0.855 AUC
Eryngium yuccifolium var. *yuccifolium*	0.8	0.704 AUC
Marshallia obovata var. *obovata*	1	0.657 AUC

Table 3. Indicates whether the environmental variables were 'Included' or 'Omitted' from the MaxEnt model for the respective species due to signs of overfitting or autocorrelation.

Environmental Variables	***Asclepias tuberosa* var. *tuberosa***	***Monarda fistulosa* var. *molis***	***Eryngium yuccifolium* var. *yuccifolium***	***Marshallia obovata* var. *obovata***	**State -Scale Species**
Aspect	Included	Included	**Omitted**	Included	Included
Average Water Content	Included	Included	Included	**Omitted**	Included
Original Demographic Elevation Model	**Omitted**	**Omitted**	Included	Included	Included
Slope	**Omitted**	Included	**Omitted**	**Omitted**	Included
TIN NDVI	Included	Included	Included	**Omitted**	Included
Total Sand	Included	**Omitted**	**Omitted**	Included	Included
Annual Mean Temperature	Included	Included	Included	Included	**Omitted**
Annual Mean Diurnal Range	**Omitted**	Included	**Omitted**	**Omitted**	Included
Isothermality	**Omitted**	Included	**Omitted**	**Omitted**	Included
Temperature Seasonality	Included	Included	Include	Included	Included
Maximum Temperature of Warmest Month	**Omitted**	**Omitted**	Included	Included	Included
Minimum Temperature of Coldest Month	Included	Included	Included	Included	**Omitted**
Annual Temperature Range	**Omitted**	**Omitted**	**Omitted**	Included	**Omitted**
Mean Temperature of Wettest Quarter	**Omitted**	Included	Include	Included	Included

Mean Temperature of Driest Quarter	Included	Included	Included	Included	**Omitted**
Mean Temperature of Warmest Quarter	**Omitted**	Included	Included	Included	Included
Mean Temperature of Coldest Quarter	**Omitted**	Included	Included	Included	Included
Annual Precipitation	Included	Included	Included	Included	Included
Precipitation of Wettest Month	**Omitted**	**Omitted**	**Omitted**	Included	Included
Precipitation of Driest Month	Included	Included	Included	Included	Included
Precipitation Seasonality	Included	Included	Included	Included	Included
Precipitation of Wettest Quarter	**Omitted**	**Omitted**	Included	Included	Included
Precipitation of Driest Quarter	Included	Included	Included	Included	Included
Precipitation of Coldest Quarter	Included	Included	Included	Included	Included
Precipitation of Warmest Quarter	**Omitted**	Included	Included	Included	Included

Table 4. Collected GPS coordinates of the sited species taken during ground truthing

Species	**Latitude**	**Longitude**	**Known Or New Occurrences**
Asclepias tuberosa var. *tuberosa*	35.43113	-80.06662	Known
Asclepias tuberosa var. *tuberosa*	35.37074	-80.00294	New
Asclepias tuberosa var. *tuberosa*	35.3842	-79.97041	New
Monarda fistulosa var. *molis*	35.44334	-80.06284	Known
Eryngium yuccifolium var. *yuccifolium*	35.40754	-80.08383	Known
Eryngium yuccifolium var. *yuccifolium*	35.4017	-79.97027	New
Marshallia obovata var. *obovata*	35.42006	-80.07204	Known
Marshallia obovata var. *obovata*	35.3663	-79.98755	New

Table 5. Adjusted MaxEnt Model Settings for the state of North Carolina, these parameters apply to all four species.

NC MaxEnt Model Settings	
Base Settings	
Feature Class	Linear
Random Test Percentage	20
Regularization Multiplier	4
Replicates	1000
Replicate Run Type	bootstrap
Advanced Settings	
Write Output grids	unchecked
Experimental Settings	
Use Samples with Some Missing Data	checked
Threads	24

Tables 6.1-6.4 Percentage contributions of the environmental variables that show most importance in the analysis of habitat suitability for species *Asclepias tuberosa* var. *tuberosa* (6.1), *Monarda fistulosa* var. *molis* (6.4), *Eryngium yuccifolium* var. *yuccifolium* (6.2), and *Marshallia obovata* var. *obovata* (6.3) across North Carolina.

(6.1)

Asclepias tuberosa var. tuberosa		
Variable	**Percent contribution**	**Permutation importance**
BIO 12: Annual Precipitation	45.5	36.6
BIO19: Precipitation of Coldest Quarter	29.1	39.2
TIN NDVI	13.8	3.7
AWC	5.6	6.4
BIO 18: Precipitation of Warmest Quarter	2.8	7.8
Aspect	2.4	3.9
BIO 14: Precipitation of Driest Month	0.4	0.4
BIO 17: Precipitation of Driest Quarter	0.2	1.1

BIO 9: Mean Temperature of Driest Quarter	0.1	0.6
BIO 1: Annual Mean Temperature	0	0.3

(6.2)

Eryngium yuccifolium var. yuccifolium		
Variable	**Percent contribution**	**Permutation importance**
BIO 12: Annual Precipitation	36.8	41.9
BIO 19: Precipitation of Coldest Quarter	21.8	21.8
BIO 9: Mean Temperature of Driest Quarter	21.5	12.4
BIO 8: Mean Temperature of Wettest Quarter	8.2	7.3
AWC	5	0.6
BIO 5: Max Temperature of Warmest Month	3.3	14.2
TIN NDVI	2	0.1
BIO 18: Precipitation of Warmest Quarter	1.3	1
BIO 17: Precipitation of Driest Quarter	0.1	0
DEM	0.1	0.3
BIO 15: Precipitation Seasonality	0	0.4

(6.3)

Marshallia obovata var. obovata		
Variable	**Percent contribution**	**Permutation importance**
Aspect	33.4	36.7
BIO 12: Annual Precipitation	20.8	1.2
DEM	18.2	8.7
Total Sand	13.6	9.4
BIO 19: Precipitation of Coldest Quarter	5.4	1.6
BIO 9: Mean Temperature of Driest Quarter	2	0
BIO 17: Precipitation of Driest Quarter	2	3.5
BIO 14: Precipitation of Driest Month	1.9	0.6

BIO 6: Min Temperature of Coldest Month	1.6	0.3
BIO 18: Precipitation of Warmest Quarter	0.4	0.7
BIO 17: Precipitation of Driest Quarter	0.4	1.8
BIO 8: Mean Temperature of Wettest Quarter	0.2	0.2
BIO 4: Temperature Seasonality	0	0.3
BIO 15: Precipitation Seasonality	0	34.9

(6.4)

Monarda fistulosa var. molis		
Variable	**Percent contribution**	**Permutation importance**
BIO 12: Annual Precipitation	62.6	45.4
TIN NDVI	14	22.9
Aspect	13.3	20.9
BIo 19: Precipitation of Coldest Quarter	3.2	0.9
BIO 18: Precipitation of Warmest Quarter	2.2	0.8
Slope	1.4	0.3
BIO 14: Precipitation of Driest Month	1.1	6.2
BIO 8: Mean Temperature of Wettest Quarter	1.1	0.2
BIO 6: Minimum Temperature of Coldest Month	0.4	0.7
BIO 17: Precipitation of Driest Quarter	0.3	1.6
BIO 3: Isothermality	0.2	0.1
BIO 9: Mean Temperature of Driest Quarter	0.2	0