ASSESSMENT OF CHANGE IN NORTH CAROLINA'S OUTER COASTAL PLAIN WETLANDS

Final Report to the US Environmental Protection Agency, Region 4
by North Carolina Department of Environmental Quality, Division of Water Resources

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1 Executive Summary

Wetlands everywhere hold tremendous value to humans and wildlife, economically, ecologically, culturally and recreationally. In North Carolina, the majority of the state's wetlands are located in the eastern third of the state, in the Coastal Plain ecoregion. Their location is part of their value, as they interface with coastal waters and provide refuge for commercially important fish and shellfish, as well as buffering from storms, water filtration, and recreational and aesthetic benefits. However, wetlands in the Coastal Plain, especially freshwater wetlands, also face challenges with sea levels rising and storm surges from hurricanes increasing. To assess vegetative changes and trends in these wetlands, North Carolina Department of Environmental Quality (NCDEQ) Division of Water Resources (DWR) analyzed historical and resampling data from 78 wetlands of various types across the Outer Coastal Plain of North Carolina. These sites included a combination of 36 wetlands resampled by the DWR wetland science team and 42 others resampled by project partners for other projects. Vegetation community data were collected from all sites, with soil and water chemistry data collected from a subset of sites.

Overall, wetland sites assessed in this study gained nonnative species (richness and cover), lost floristic quality, gained brackish tolerant species and coverage by those species, and lost shrub and tree cover while gaining herbaceous species. Forested wetlands showed the most changes in plant communities, particularly in terms of greater invasion by nonnative taxa and decreases in vegetation compositional quality (mean C). Cover of shrub, tree and vine taxa also decreased. Mapping trends in total tree taxa cover indicated a decrease all around the edges of the Albemarle-Pamlico Peninsula, as well as near the south end of the Pamlico Sound. Of the 66 forested and shrub wetlands with tree cover data available, just under 20% (13) lost more than 50% of their tree taxa cover between first and last sampling, with five (5) of these sites experiencing a greater than 90% reduction in their tree cover. Another 6% (4) gained more than 50% tree taxa cover between sampling events. Ten (10) sites, or 13%, completely changed community type - always from a more forested or woody type to less forested type. These losses in wetland tree cover are clearly seen around the North Carolina coast in the phenomenon of "ghost forests," with evidence of a past forest remaining for a time before full transition to brackish marsh.

The main nonnative species driving changes in freshwater forested wetland plant communities were Japanese honeysuckle (*Lonicera japonica*) and Japanese stiltgrass (*Microstegium vimineum*), which both had more frequent occurrences. Shrub and marsh wetlands appeared to change less than forested wetlands, although the nonnative common reed (*Phragmites australis*) increased in occurrence frequency between original sampling and resampling in marsh wetlands.

Freshwater and transitional salinity wetlands changed in a larger number of vegetative community aspects than brackish wetlands. Freshwater and transitional salinity wetlands, but not brackish wetlands, showed significantly greater invasion by nonnative species and reductions in floristic quality. Brackish wetlands did, however, show a significant reduction in herbaceous cover between sampling events, possibly due to increased flooding by seawater.

Freshwater forested wetlands showed recruitment of new individuals of woody species between original and resampling, but there was a drop in woody species recruitment between sampling events in transitional salinity forested wetlands. Additionally, larger size classes of trees were represented in the freshwater forested wetlands, but they were generally missing in the transitional forested wetlands during original sampling and more so during resampling.

Anderson et al. (2022) analyzed soil sodium in relation to plant species occurrence from 34 wetland sites in the Albemarle-Pamlico Peninsula, using occurrence of herbaceous plants along with shrub and tree species <4m in height. The "understory community" changepoint predicted from their analysis was 265 µg/g of soil sodium. With data on the entire wetland plant community, the same changepoint threshold analysis predicted a "whole community" change threshold of 383 µg/g (ppm) of soil sodium. Taken together, a top horizon soil sodium range of 265 to 380 µg/g could be considered a "red flag" zone, where a wetland could be vulnerable to whole plant community level shifts, especially under chronic exposure. The understory community changepoint of 265 µg/g soil sodium corresponds to approximately 0.54 parts per thousand (ppt) shallow groundwater salinity (1 m depth) or 1022 µS/cm specific conductivity based on the close relationship between the two. The whole community changepoint of 383 µg/g soil sodium corresponds to approximately 0.93 ppt shallow groundwater salinity or 1660 µS/cm specific conductivity. Groundwater salinity and specific conductivity are easily measured with water quality meters and could potentially be used as proxies for lab soil analysis of soil sodium. Surface water salinity was not well correlated with soil sodium, so should not be used as a proxy for soil analysis.

Nearly two-thirds of the 32 DWR sites with water chemistry data were categorized as "affected by seawater" based on water quality data chloride (Cl) ion concentrations greater than 100 mg/L, after Kelly (2005) and Konikow and Reilly (1999). Cl ion concentrations were significantly positively correlated with other elements of seawater including bromide (Br), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na) and sulfate (SO_4). Sites in the "affected by seawater" category had significantly higher top horizon soil sodium, higher salinity, greater changes in the composition of woody plant communities over time and higher proportions of brackish tolerant species, as well as cover by them. Therefore, surface or groundwater sample chloride ion concentration over 100 mg/L was determined to be a good indicator of the presence of seawater, when chloride is also correlated with other components of seawater.

Based on chloride ion concentrations, sites with surface or shallow groundwater salinities at 0.3 ppt or above could be considered impacted by seawater, if chloride ion concentrations are correlated with other elements of seawater. Vegetative communities are potentially able to tolerate chronic exposure to salinities between about 0.3 and 0.5 ppt, but the threshold analysis suggests that chronic exposure to salinities above 0.5 ppt could precipitate changes in the understory community in freshwater wetlands, where sensitive species clearly decrease in frequency of occurrence and more tolerant species increase.

Given the changes occurring in transitional salinity forested wetlands in particular, detection of freshwater coastal wetlands that are vulnerable to change, or affected by seawater, is imperative. Loss of forested freshwater wetlands comes with a loss of the tremendous value they provide to humans and wildlife - economically, ecologically, culturally and recreationally. Lack of recruitment in coastal forested wetlands is a concern that warrants attention through further research, management of existing areas, restoration of historical wetland areas and creation of new wetland areas. Indicators of seawater intrusion could be used to detect sites that are unimpacted or have been minimally impacted by seawater, presenting an opportunity to conserve, protect and restore these existing coastal forested wetlands. Priority could also be put on conserving land upslope of these important wetlands, as well as proactively managing them for future sea level rise.

2 Introduction

Detailed analysis of National Wetland Inventory data for the Coastal Plain of North Carolina indicates that this ecoregion contains at least 2.78 million acres (71%) of the state's wetlands, including marshes, swamps, forested riverine wetlands, pocosins and other wetland habitats (Street et al. 2005; Gale unpubl. data 2020). These Coastal Plain wetlands provide the state with an array of essential services. Coastal wetlands, especially forested wetlands, stabilize shorelines and serve as buffers against storms and erosion, a service valued at $25.6 billion per year in 2016 (NCDEQ 2016). They can store water and hence reduce flooding, creating value in protection from extreme rain or storm events (Narayan et al. 2017). Wetlands are needed as natural filters for water supplies, a service vitally important to the Coastal Plain aquifers which supply water for more than half of North Carolina's population as well as visitors (US EPA 2010).

North Carolina has a billion-dollar commercial and recreational fishing industry, ranking it among the nation's highest seafood producing states (NC DEQ 2016). The state's estuaries and associated wetlands are an essential economic resource, serving as nursery grounds for many commercially important fish and shellfish, such as crabs, shrimp and flounder species (DeWan et al. 2010). Tidal freshwater wetlands provide nursery habitat for aquatic species that live as adults in salt waters but rely on fresh and brackish waters for larval recruitment and development. Freshwater swamps and marshes occur along rivers or sounds in areas where flooding is influenced by wind or lunar tides.

Wetlands serve as refuges for beneficial pollinators and other insects and provide critical habitat for wildlife and plants. More than two-thirds of North Carolina's rare, threatened and endangered species of plants and animals live in wetlands (USGS 1996). Coastal wetlands serve as vital breeding and migration stopover points for many migratory birds, as well as key breeding areas for some bird species in decline (Defenders of Wildlife 2010). In particular, Coastal Plain freshwater wetlands are very important habitat for bitterns, rails, and a variety of other wading and shorebirds.

Additionally, North Carolina's wetlands provide value in aesthetics, tourism and recreation, especially in the Coastal Plain where large national wildlife refuges, game lands and preserves exist. Lake Mattamuskeet National Wildlife Refuge is just one example, where more than 58,000 visitors come annually for wildlife observation and other recreation to this major stopover for over 200,000 migratory and overwintering waterfowl (USFWS 2024).

For these reasons and more, wetlands are singled out as priority ecosystems by state agencies. The North Carolina Wildlife Resources Commission 2015 Wildlife Action Plan describes all wetlands statewide as priority habitat for conservation, including coastal wetland habitats such as tidal freshwater wetlands, bottomland and floodplain wetlands, pocosins, bays, isolated depressional wetlands, wet pine savannahs and brackish marshes (NCWRC 2015). The NC Division of Coastal Management's Coastal and Estuarine Land Conservation Program lists all coastal wetland habitats as priority for restoration and conservation (NCDCM 2007, 2011).

2.1 Wetland Loss in North Carolina

The National Oceanic and Atmospheric Administration Coastal Change Analysis Program (NOAA C-CAP) provides nationally standardized, raster-based inventories of land cover for the coastal areas of the United States using remotely sensed 30-meter data imagery. Based on 2016 aerial imagery, all wetland changes (natural and human induced) in North Carolina's Coastal Plain have resulted in the loss of 134,878 acres of non-tidal, freshwater (i.e., palustrine) wetlands between 1996 and 2016, with forested wetlands experiencing the greatest loss (NC DEQ 2021). These losses were initially due to conversions to agriculture (1996–2006), uplands (1996–2001 and 2006–2011) and development (2001–2016) but were more recently due to conversions to unconsolidated shorelines (2006–2016) and open water (2011–2016). These conversions were most likely caused by sea level rise, erosion from increasingly frequent and intense storms and water quality degradation (Baillie 2020). It is possible that there have been additional losses or gains in estuarine wetlands that the 30-meter NOAA C-CAP imagery data were unable to detect.

2.2 Vulnerability to Climate Variability and Sea Level Rise

Eastern North Carolina has 1,729 square miles of land equal to or less than one meter of elevation above current sea level (Figure 1), which make these low elevation coastal areas particularly vulnerable to sea level rise, temporary and permanent flooding, and coastal erosion. Estimates of future mean sea level under multiple scenarios using the 2022 NOAA Sea Level Rise Technical Report (NOAA et al. 2022) were obtained for two locations in North Carolina - Beaufort and Wilmington - from the US Army Corps of Engineers Sea Level Analysis Tool (https://climate.sec.usace.army.mil/slat/). Results are shown in Figure 2. Under the worst-case scenario (indicated as "NOAA et al. 2022 – High" on the graphs), these two areas are predicted to see sea levels rise to 0.5m above MSL as early as the 2050s and rise to 1.0m by the 2070's. Even under the "middle of the road" scenario ("NOAA et al. 2022 – Intermediate"), it is estimated that these same thresholds would be crossed in the 2060's (0.5m above MSL) and 2090's (1.0m above MSL).

Effects of higher water levels on forested wetlands and uplands are becoming obvious throughout the Coastal Plain of North Carolina, and also along the entire southern US Atlantic coast and the Gulf of Mexico. Coastal wetland forests in the Southeast have been experiencing tree die-off and decline from saltwater intrusion, increased tidal inundation, and altered freshwater flows (Conner et al. 2007; White et al. 2021). Changes in salinity can kill trees and make plant communities vulnerable to invasion by nonnative species such as the common reed (*Phragmites australis*), which is happening along the Albemarle Sound 40 miles from the ocean. Thousands of trees have died at the northern tip of Alligator River National Wildlife Refuge, 21.5 miles from the nearest ocean inlet (Beck 2021). In Hyde County, agricultural lands have changed into freshwater and brackish wetlands, timber tracts have changed from pine stands to brackish marshes and in some places residential land is transitioning to coastal marsh (Roberson 2012; Malijenovsky 2015). Some farmers in the Lake Mattamuskeet area have experienced more frequent flooding during minor precipitation events, as well as loss of available acreage to farm due to increased wetness (NC Coastal Federation 2018).

Rising sea levels threaten NC's outer Coastal Plain wetlands and increased intensity of storms, both in rainfall and wind, are also causing increased freshwater inundation and saline storm surges. Drainage ditches can facilitate increased flooding as well as saltwater intrusion into more inland areas (McPherson 2009). The presence of saltwater promotes rapid decomposition of peat soils by sulfate-reducing bacteria (Hackney and Yelverton 1990). Loss of peat causes soil subsidence which leads to increased inundation. Within wetlands, these changes are expected to result in plant community composition shifts and more open water habitat. Even if climate change causes an increase in estuarine wetlands, this may come at the expense of more diverse freshwater wetlands. With decreased biodiversity comes a loss in the variety of ecosystem services freshwater wetlands provide (Isbell et al. 2011). Of freshwater forested wetlands in coastal areas, Baldwin (2007) notes "these diverse, productive wetlands may be among the most susceptible ecosystems to small changes in water level and salinity resulting from global climate change."

Given the importance of coastal wetland systems to the environment and economy of North Carolina, their priority for wildlife and coastal management agencies and their vulnerability to water-related changes brought about by climate change, it is imperative to assess their current condition relative to historical condition. Most academics and state and federal agency staff rely on remote sensing or nationally available spatial datasets for change detection, but these only allow detection of substantial plant community shifts and erosion. Earlier, subtler indicators of change require on-the-ground data collection, which has been lacking particularly in freshwater wetlands vulnerable to impacts from saltwater. The goal of this project was to compare current wetland condition and function to historical on-the-ground data collected in freshwater wetlands, to understand if, and in what directions, North Carolina's Outer Coastal Plain wetlands have changed.

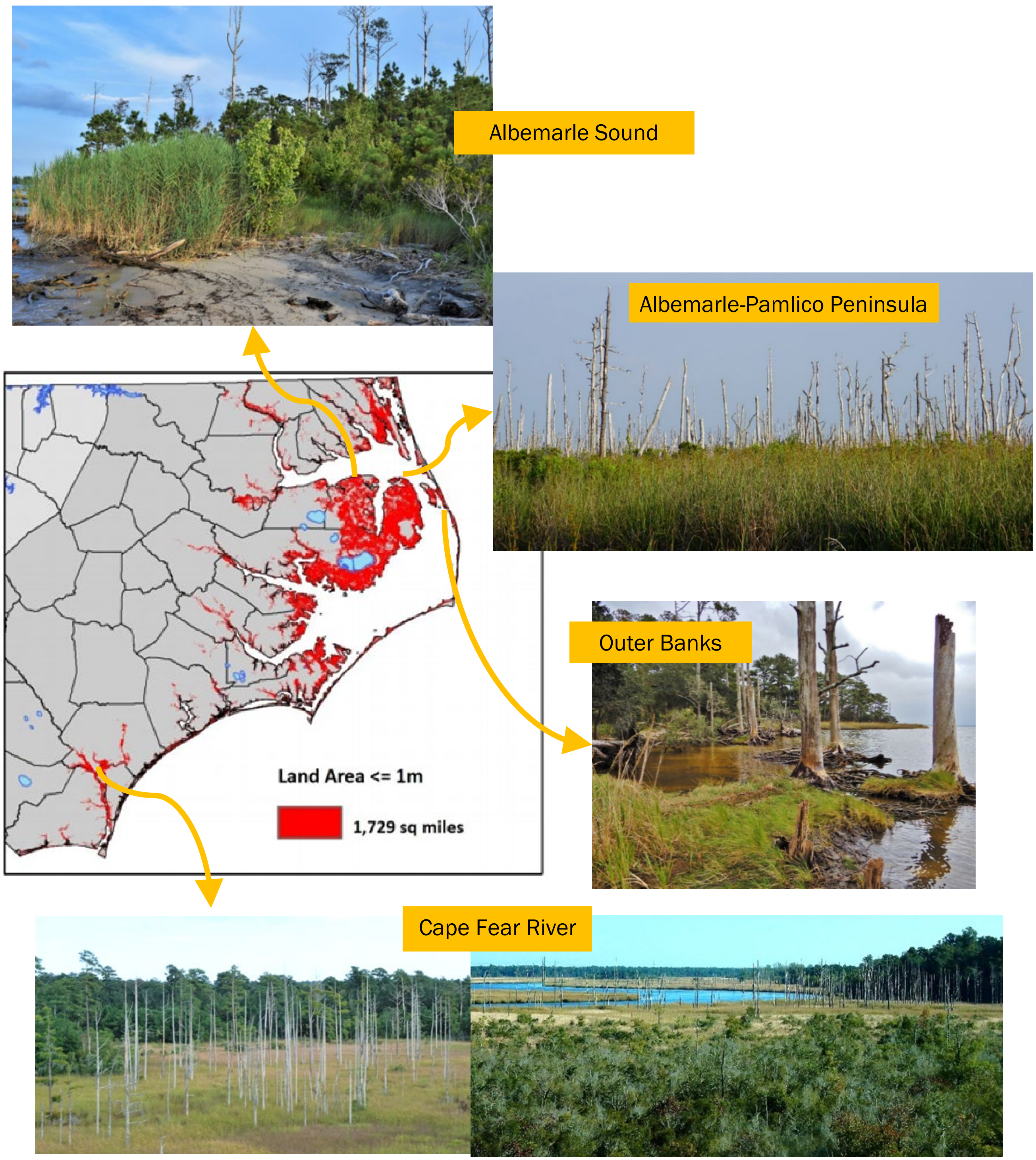
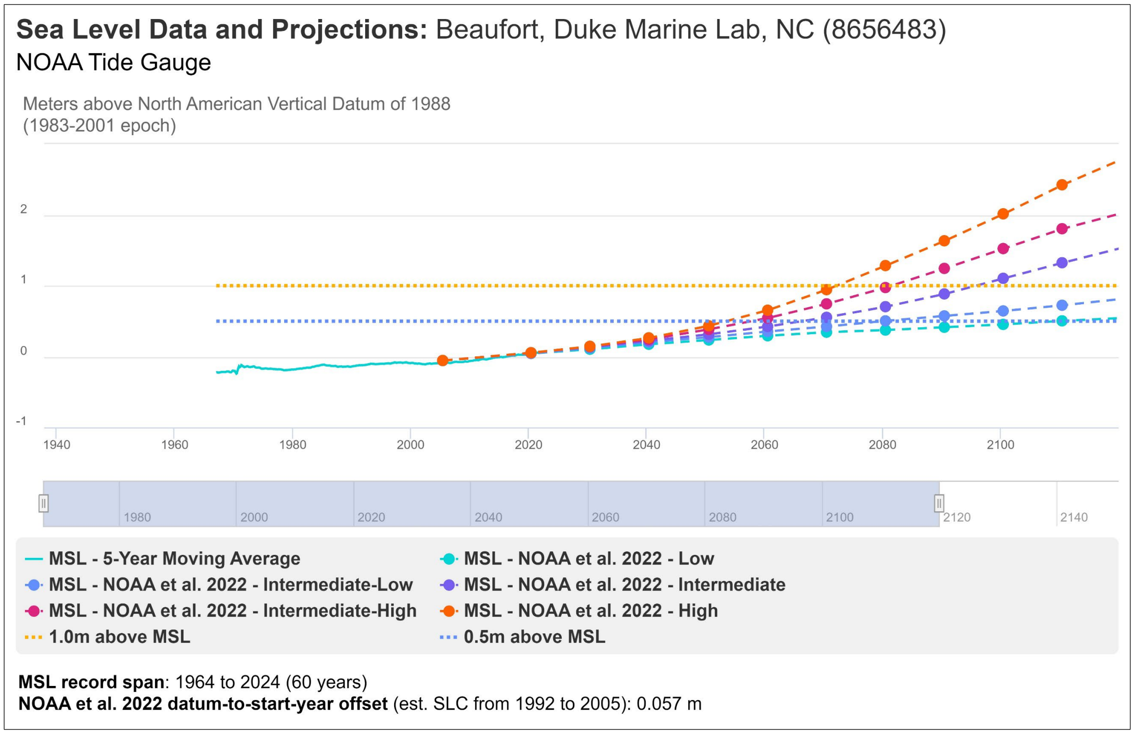


Figure 1. Wetland plant community shifts from forested wetlands or uplands to marsh are evident in many parts of the outer Coastal Plain of North Carolina (photos dated 2018). Map showing land area at an elevation within one meter of sea level. (source: NC Interagency Leadership Team. 2010. Climate maps)



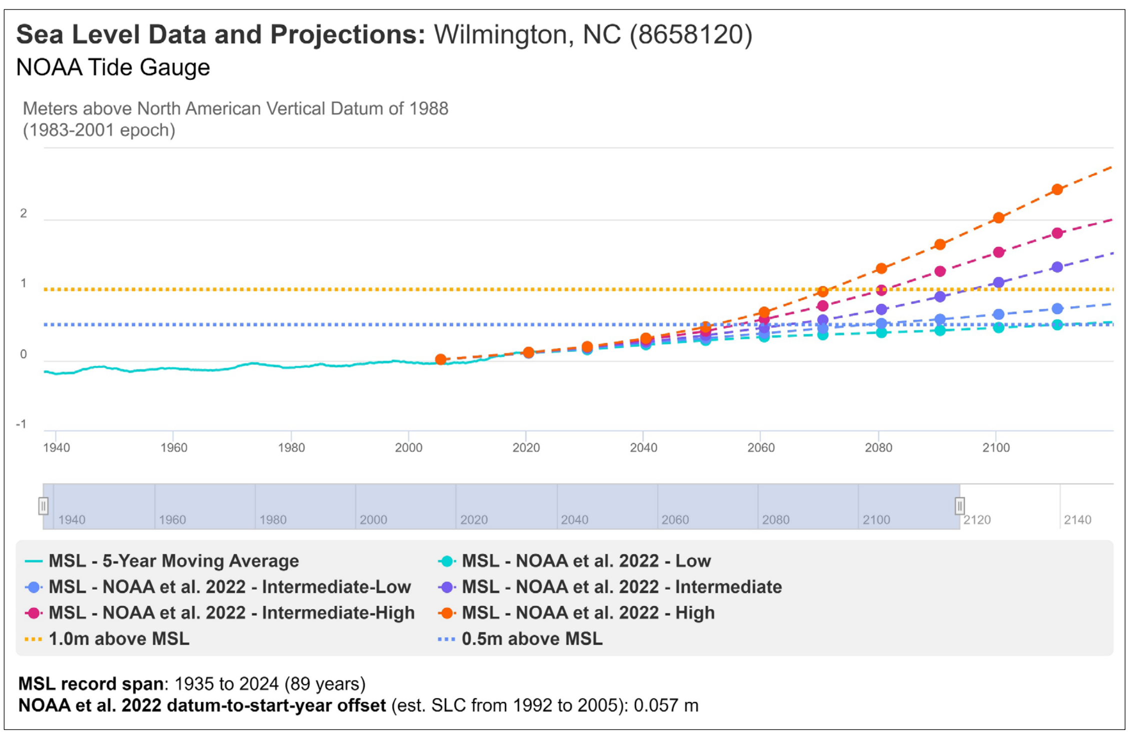


Figure 2. Sea level data and projected sea levels for Beaufort and Wilmington, NC from the US Army Corps of Engineers through 2120. Graphs created using the USACE Sea Level Analysis Tool. https://climate.sec.usace.army.mil/slat/

3 Methods

3.1 Project Area

This project focused on freshwater and transitional wetlands in the Outer Coastal Plain ecoregion of North Carolina (Figure 3). The Outer Coastal Plain of North Carolina is low-lying, with Carolina bay pocosins and lakes, isolated freshwater marshes, freshwater and saltwater tidal marshes, swamps, forested riverine wetlands, pocosins and other wetland habitats. This land area is particularly vulnerable to sea level rise, temporary and permanent flooding, hurricanes, and coastal erosion. Because of its low-lying nature, the Outer Coastal Plain has also been extensively ditched for agriculture and roadways, increasing its potential for saltwater intrusion.

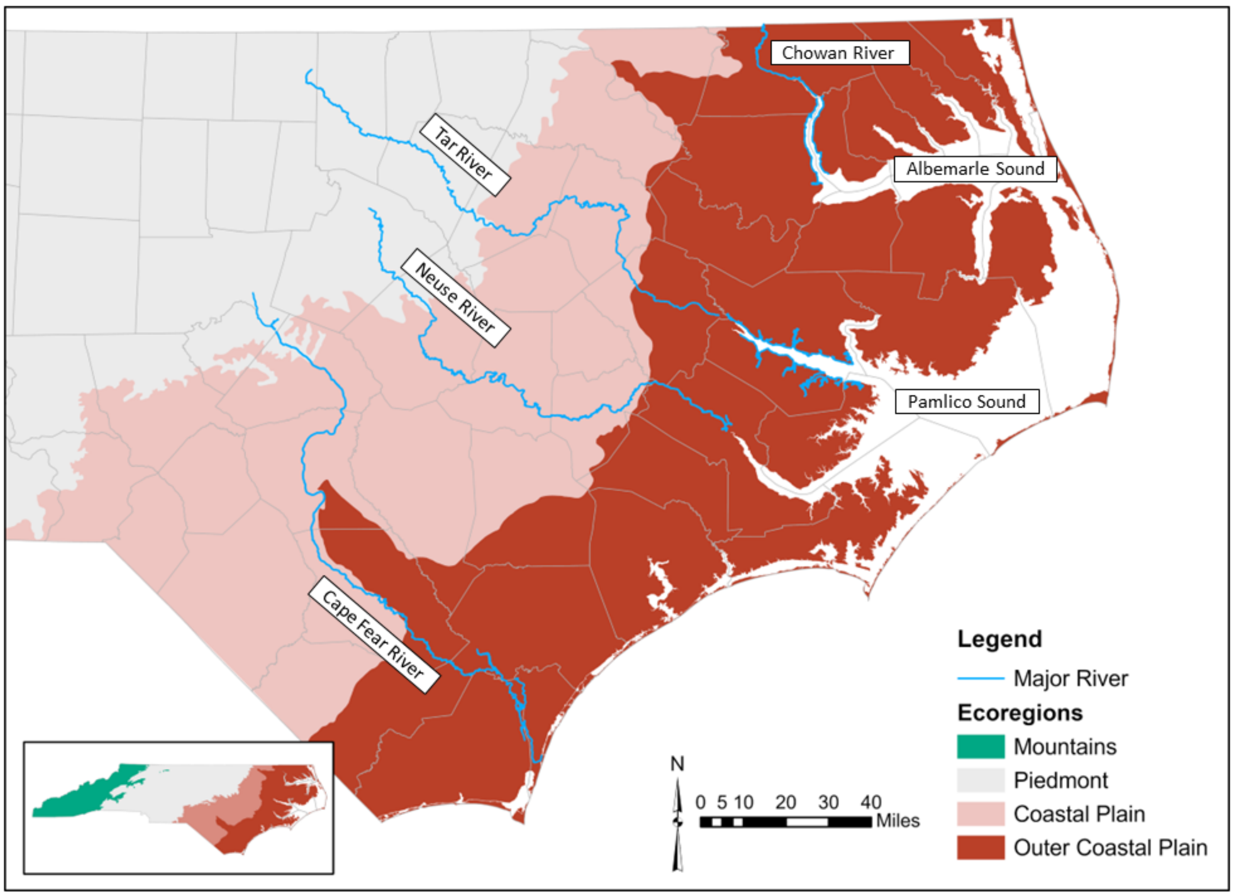


Figure 3. Map of Outer Coastal Plain of North Carolina (project area).

3.2 Site Selection

The target population for this study was freshwater and transitional salinity wetlands near a body of water that could potentially deliver saltwater. Wetlands had rooted vegetation during original sampling and —when present— open water less than one meter deep. A wetland's jurisdictional status under state or federal regulatory programs did not affect a site's status as a target wetland for this project.

All sites were in locations previously sampled for the EPA's National Wetland Condition Assessment (NWCA) or the Carolina Vegetation Survey (CVS; organized by the University of North Carolina Chapel Hill). All sites were first sampled between five and 34 years prior. NWCA site locations were randomly selected prior using a spatially-balanced Generalized Random Tessellation Stratified survey design applied to the National Wetland Inventory. This design ensured the sample was representative of wetland resources at the regional scale as well as the national scale. Six NWCA sites with historic data were selected by the EPA for resampling as part of the NWCA project, and two additional NWCA sites were selected for resampling for this project. All data collection on these NWCA sites was performed by the DWR wetland science team.

Twenty-eight (28) CVS sites were resampled by DWR for this project. Original CVS site locations were usually determined on a project-specific basis, designed to sample high-quality examples of plant communities present in the geographical area or community type of interest at the time of the survey. Sites resampled by DWR were selected to extend the geographic range of the resample data obtained for localized areas from project partners (Figure 4).

Data from 42 additional sites resampled by project partners for other research projects were also assimilated into the master database, for a total of 78 sites used in the data analysis. Project partners resampled previously sampled sites between 2016 and 2020 and recorded identical vegetation parameters and (often) soil chemistry using either the Carolina Vegetation Survey plot method or a similar plot method. Project partners included researchers from NC State University and Duke University and biological staff with the Nutrien Phosphate Company. Vegetation plot resample data were also obtained from the US Fish and Wildlife Service (USFWS). Site selection criteria were met for all sites included in the analysis. Criteria for selection of resample sites for this study were the following:

- freshwater or transitional/brackish wetland during initial sampling event (some partner sites were brackish wetlands in initial sampling)
- proximity to saltwater or ditches/canals such that current conditions could include effects from saltwater intrusion or hurricane storm surges (within 3 straight-line miles of a water body with a measured average salinity greater than 1.0 ppt) (Figure 5), or if greater than 3 straight-line miles from a saline water body, having an elevation of less than one meter above sea level
- latitude/longitude location accuracy to within 15m of original sampling location (for historical CVS sites)
- access permission granted to assess the site (for DWR sites)
- sampled/resampled >2 years apart

Sites were categorized as forest, shrub, or marsh based on the predominant vegetation present at the site at the time of sampling. Historical site and plant community descriptions were relied upon, as well as cover data for tree and shrub species and historical and current aerials. Sites were categorized as freshwater, transitional salinity, or brackish based on a wide variety of available factors including site metadata describing salinity during sampling, plant community composition, hydrologic regime and location in relation to salt water. A site was considered transitional salinity if it was a forested or shrub freshwater community adjacent to brackish marsh and at low elevation, with a strong potential for regular influence by salt water. Aerial photographs were consulted for the times of sampling and examined for brackish marsh vegetation (generally tan in color); areas that were forested but bordering brackish marshes were considered transitional salinity. Water salinity and soil sodium content data were taken into account when available to corroborate site identification of freshwater and brackish wetlands (low and high extremes of salinity and soil sodium), but not transitional salinity wetlands.

3.3 Site Locations

Wetland resample sites were distributed throughout the Outer Coastal Plain of North Carolina (Figure 4). Resample sites were located more often in the northern two thirds of the Outer Coastal Plain than the southern third, due in part to the low-lying terrain (ie. more wetlands) in the northern portion of the Coastal Plain. This distribution of resample sites was also due in part to a lack of historical sampling in wetlands in southern locations, especially those that met the selection criteria. Sites comprised a combination of freshwater, transitional salinity and brackish wetlands as well as a combination of forested, shrub and marsh wetlands (Table 1). All sites were assigned a 5 to 6 character alphanumeric code; details for each individual site are included in Appendix A and photographs of each site are included in Appendix B. Original (first) sampling occurred between 1988 and 2016; resampling occurred between 2016 and 2022 (Figure 6).

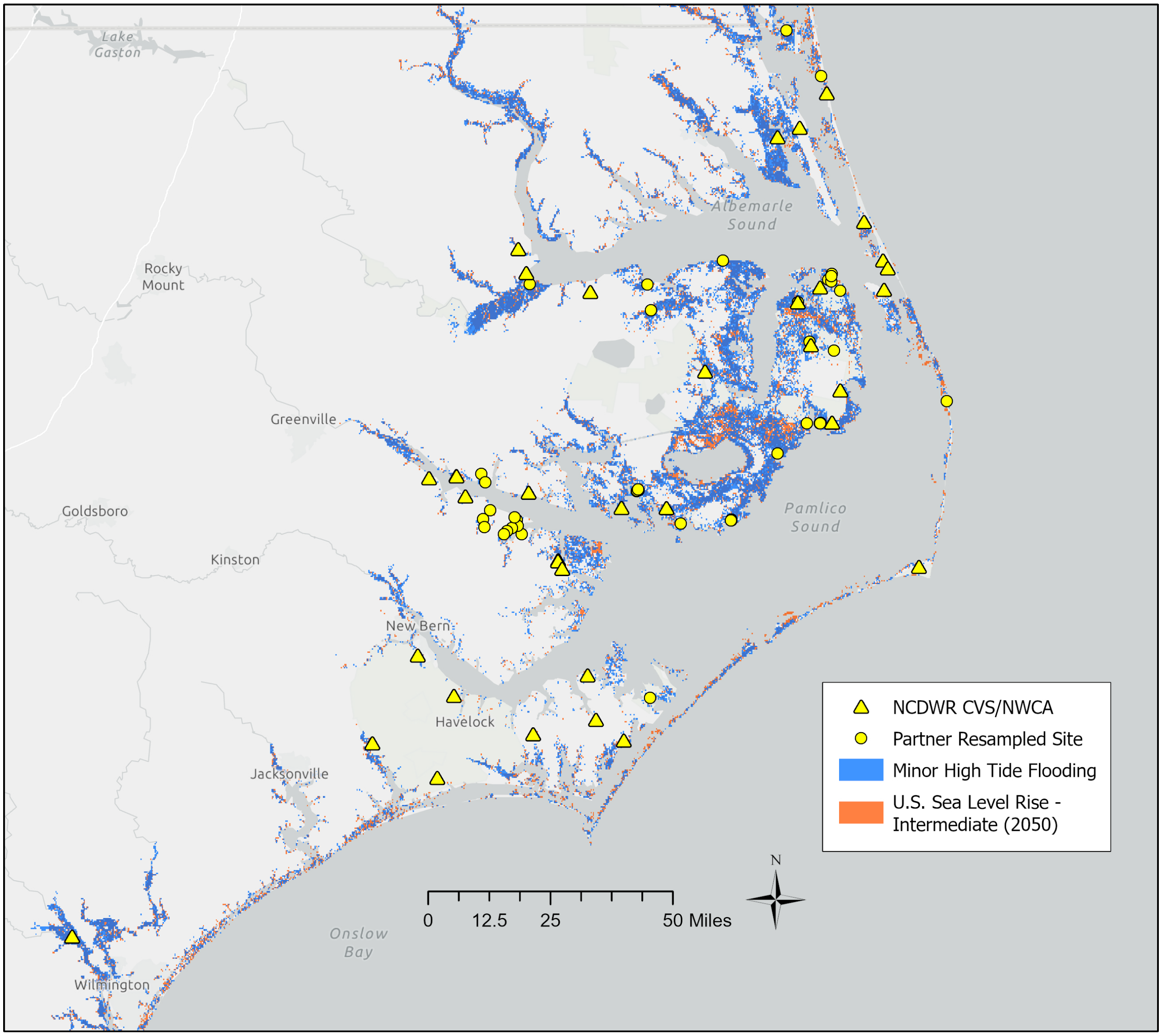


Figure 4. Locations of wetland resample sites in this study overlaid over minor high tide flooding and NOAA's 2050 intermediate sea level rise model.

The US Minor High Tide Flooding layer represents areas currently subject to tidal flooding, often called "recurrent or nuisance flooding" within the coastal areas of the United States. This is the official layer for the National Climate Organization, including the Climate Mapping for Resilience and Adaptation portal (https://resilience.climate.gov). Sea Level Rise Intermediate 2050 scenario from NOAA's Technical Report (Sweet et al. 2022).

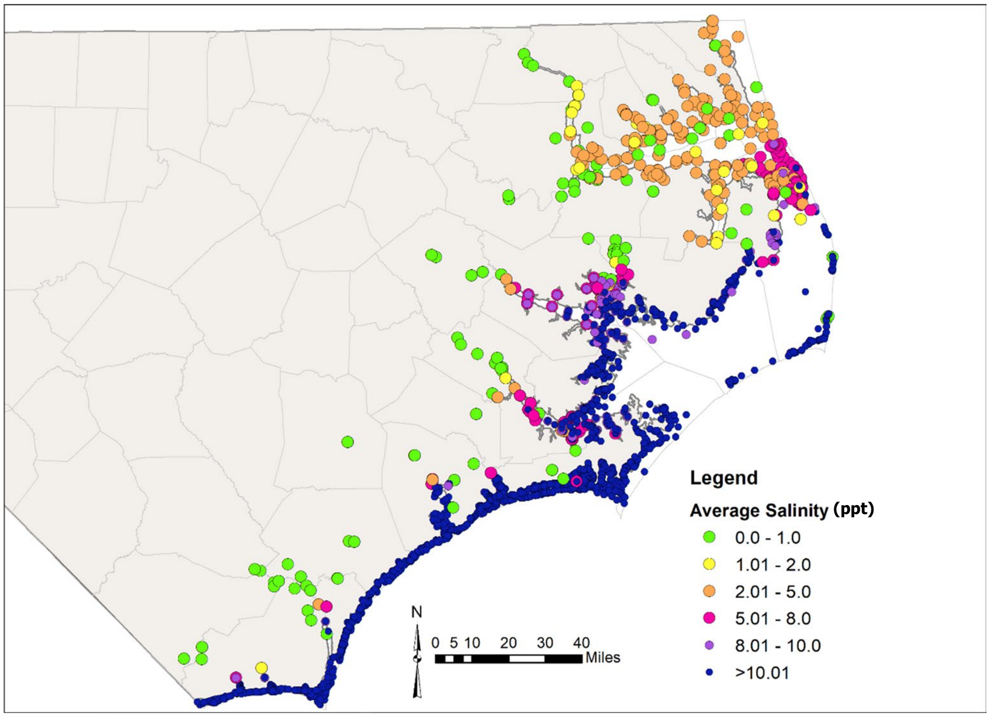


Figure 5. Average salinity around the Outer Coastal Plain of North Carolina; data from NC Division of Water Resources Ambient Monitoring Program (2000–2021) and NC Division of Marine Fisheries (1985–2021).

Table 1. Summary table describing the 78 resample sites and data obtained by DWR or by partners.

Wetland habitat types: FF = freshwater forest, FS = freshwater shrub, TSF = transitional forest, TSM = transitional marsh, BS = brackish shrub, BM = brackish marsh (transitional = intermediate between distinctively freshwater and distinctively brackish wetland).

Source	Number of Sites	Veg. Data Collection Method	Habitat Type(s)	Purpose of Resample Data Collection	Data collected
DWR resampling CVS sites	28	CVS method	FF, BS	Assessing change in wetland plant communities for this project	Vegetation composition and cover, woody basal area, soil chemistry, water chemistry, rapid assessments
DWR resampling NWCA sites	8	NWCA method	FF, BS, BM	As part of the EPA's National Wetland Condition Assessment every 5 years (beginning 2011)	Vegetation composition and cover, woody basal area, soil chemistry, water chemistry, rapid assessments
Partner Resample Sites					
Duke University resampling CVS sites	9	CVS method	FF, FS, TSF, BS, BM	Assessing change in wetland plant communities in the Albemarle-Pamlico Peninsula	Vegetation composition and cover, woody basal area, soil chemistry
USFWS resampling USFWS sites	8	CVS method	FF, BM	Monitoring vegetation communities in National Wildlife Refuges	Vegetation composition and cover, woody basal area, soil chemistry
NC State University graduate students resampling own plots	13	Plot-based method	FF, TSF, TSM, BM	Assess vegetation change in wetlands across gradient of saltwater exposure in the Albemarle-Pamlico Peninsula	Vegetation composition and cover, woody basal area, soil chemistry
Nutrien Phosphate Company resampling their own sites	12	Plot-based method	FF, TSF, TSM	Impact monitoring for permit requirements	Vegetation composition and cover

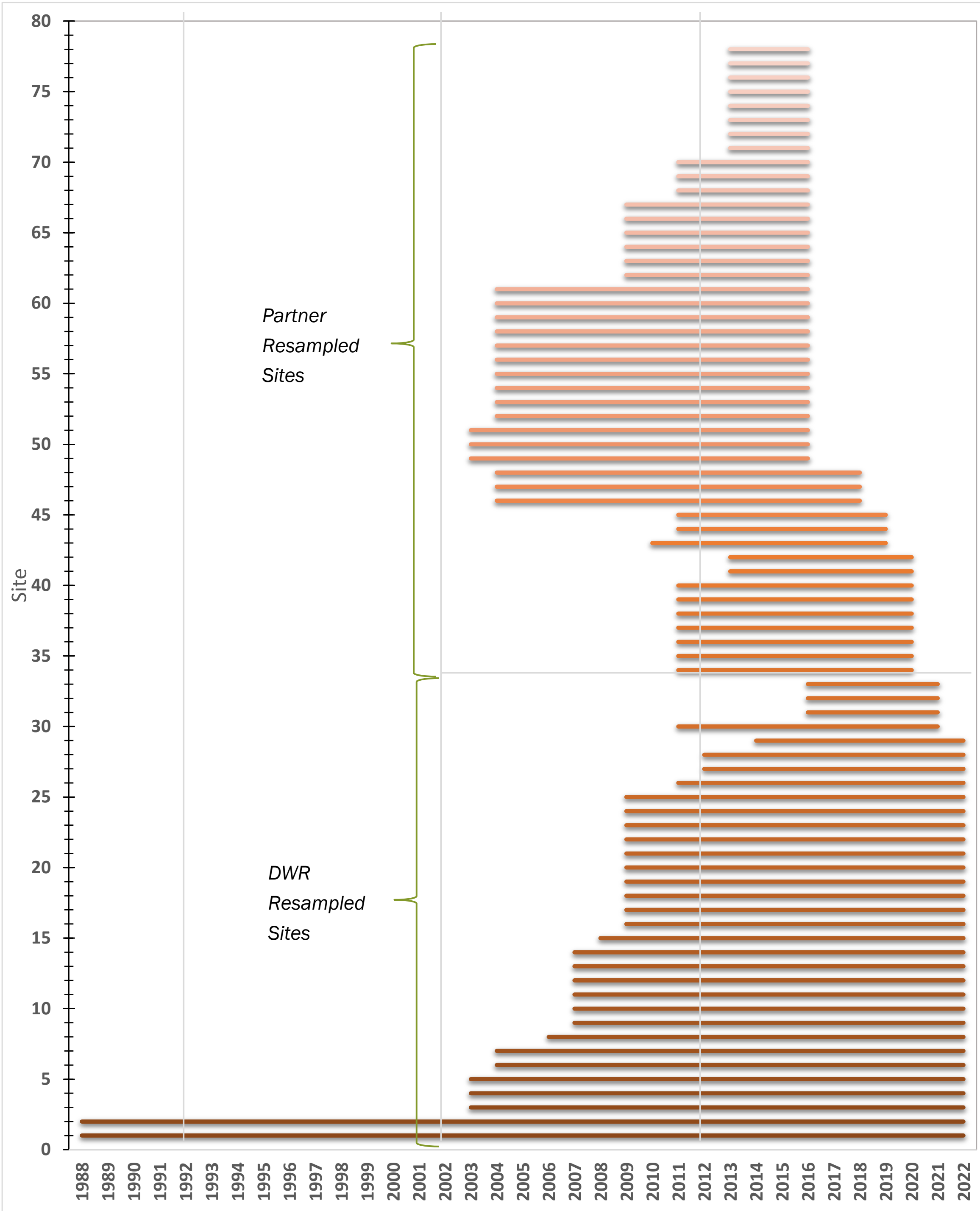


Figure 6. Schematic of lengths of time between first and last sampling at each site used in this study, as well as original and resampling dates. Vertical lines demarcate decades going back from 2022.

3.4 Vegetation Sampling Methods

Resampling protocols were matched to the original sampling protocol used during the original sampling event. For resampling of NWCA sites, the NWCA sampling protocol was used for vegetation data collection, with the exclusion of data collection from the buffer around the assessment area (USEPA 2011). For resampling of CVS plots, the CVS sampling method was followed (Peet et al. 1998).

Botanical identification keys by local authorities were used to identify species, including Weakley (2020) and Radford et al. (1968). Online resources such as USDA PLANTS and LeGrand et al. (2021) were also consulted for synonyms, updated nomenclature, and NC habitat and location occurrence information.

3.4.1 National Wetland Condition Assessment Method

Vegetation data collection on the eight NWCA sites took place during the growing season (April through September) in five 100 m² plots arranged systematically across an Assessment Area (Figure 7; Figure 8). NWCA vegetation sampling protocols are found in the 2011 and 2016 Field Operations Manuals (FOM) (US EPA 2011; US EPA 2016). The FOM Vegetation Chapter includes detailed instructions for establishing the vegetation plots in standard or alternate configurations. Vegetation composition, abundance and structure were assessed at the 100 m² scale. Each plot contained a series of nested quadrats established in two opposing corners to obtain estimates of species diversity, based on species presence at multiple spatial scales (1 m² and 10 m²). Forms for raw data collection data sheets are included in Appendix C.

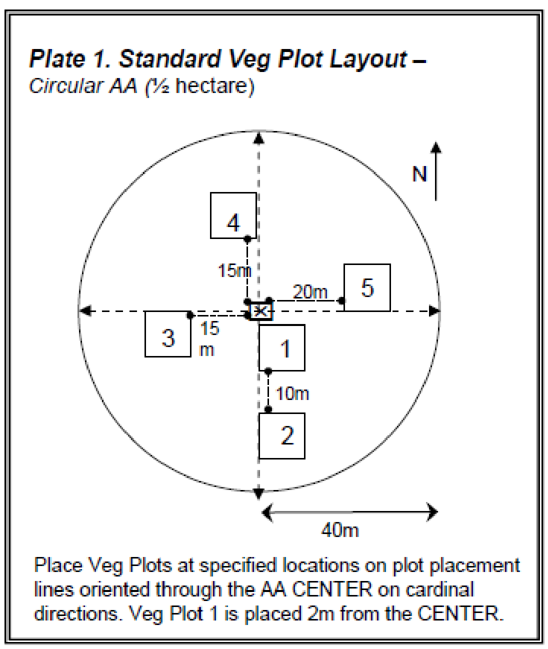


Figure 7. NWCA protocol vegetation sampling layout diagram, excerpted from the NWCA Field Operations Manual (US EPA 2011).

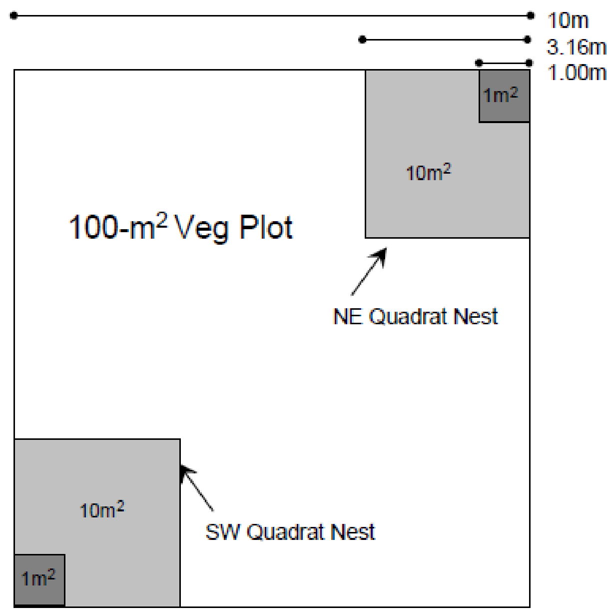


Figure 8. Locations of nested quadrats within plots for NWCA protocol. Excerpted from the NWCA Field Operations Manual (US EPA 2011).

3.4.2 Carolina Vegetation Survey Method

The CVS protocol is very similar to the NWCA protocol (which was largely based on the CVS protocol) and involves a set of 10 sampling modules, each 10 x 10 m (100 m^2), sampled during the growing season (Figure 9). Within these modules, nested quadrats are located in corners for more intensive recording of individual species and cover data. Raw data sheets are included in Appendix C.

It should be noted that flexibility on-site is incorporated into the CVS protocol, so it allows for fewer 10 x 10 m modules to be sampled depending on habitat conditions or size. At least one module was sampled at every site and number of modules ranged up to the standard full setup of four intensive modules with six residual modules added for extra species and tree-related data collection (Figure 9). A general site setup information datasheet was filled out during the original sampling, which indicated the number of modules sampled and overall setup. In resampling, very close attention was paid to original sampling setup and the sites were resampled in the exact manner as the original sampling. Efforts were always made to locate the original sampling location as closely as possible for resampling.

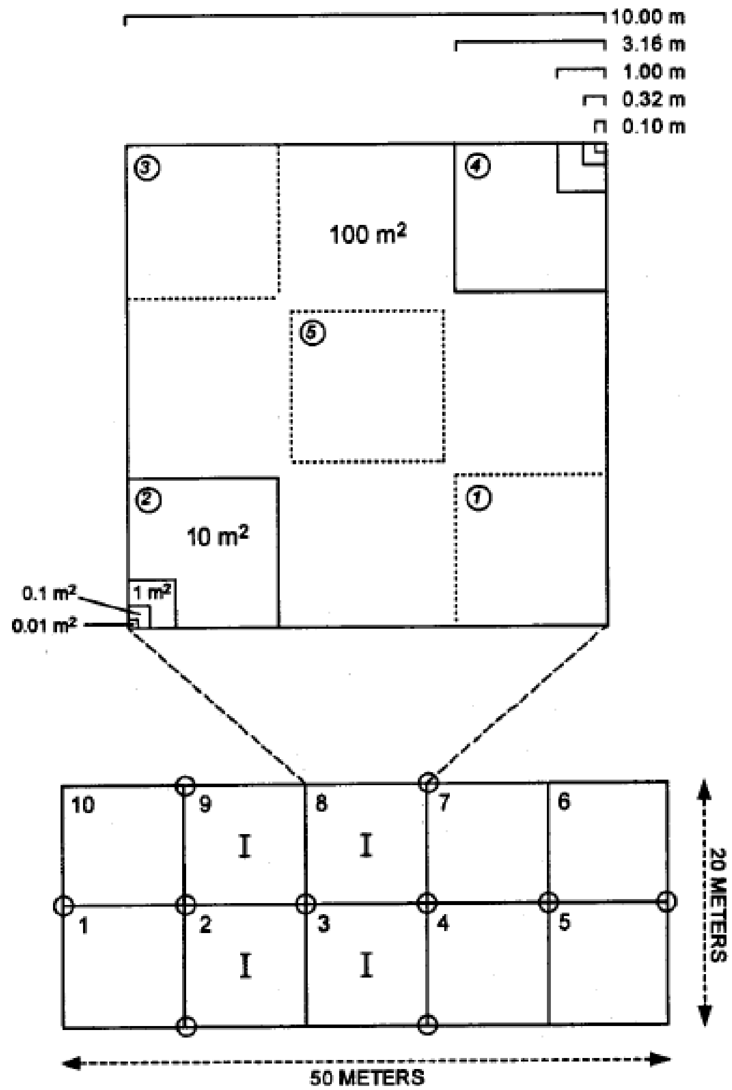


Figure 1. **Typical layout of an intensive module, and a set of 10 modules as a 0.1 ha plot. Modules are numbered counter-clockwise. The five standard locations in a module for nested quadrats are indicated, although in the standard 0.1 ha configuration only two nests are recorded (solid lines rather than dashed) in each of the four intensive modules. Typically, these intensive modules are 2, 3, 8 and 9 as intensive modules (marked I), with nested quadrats in the eight corners indicated. The remaining six modules are recorded as an aggregate. Corners within a module are numbered clockwise, starting along the centerline and moving initially along the centerline in the direction that the modules are numbered, as indicated for module eight. Typically a 50 m tape is placed along the centerline and two 20 m tapes cross the main tape along the outside edges of the four focal modules. Permanent metal stakes (circles in the 0.1 ha configuration) are located at the 10 locations where a tape touches the corner of a module.**

Figure 9. Figure and explanation excerpted from Peet et al. (1998), showing module layout and location of nested quadrats within modules, for vegetation data collection.

3.5 Water Quality Sampling

Water quality grab samples were obtained during vegetation resampling of sites resampled by the DWR field team. Grab samples were collected in accordance with DWR standard operating procedures (NCDWR 2013). When it was present, surface water was collected from a representative location within or adjacent to the vegetation plot.

Soil pit pore water (heretofore called shallow groundwater) was collected on these same sites whenever possible using the technique of Hackney and de la Cruz (1978), which constituted removing soil from a pit and sampling the water that seeps into the hole. Shallow groundwater was collected, when present, from the bottom of a 1 m soil pit dug in a representative location. One soil pit was sampled per site. Water samples were kept on ice until delivery to the DWR Chemistry Lab within the timeframes required by the lab. After analysis data were obtained, non-detects were included in the databases at the reporting limit of the lab (Table 2).

Table 2. DWR Chemistry Lab minimum reporting limits by parameter.

Parameter/Ion/Cation	Reporting Limit
Ammonia (NH_3-N)	0.02 mg/L
Bromide (Br^-)	0.4 mg/L
Chloride (Cl^-)	1.0 mg/L
Sulfate (SO_4^{2-})	2.0 mg/L
Calcium (Ca^+)	0.10 mg/L
Potassium (K^+)	0.10 mg/L
Magnesium (Mg^+)	0.10 mg/L

Concurrent with water sample collection (surface water and groundwater), an In-Situ water quality meter was used to measure certain parameters, specifically salinity, pH and specific conductivity when water was present. Calibration of the meters, weekly and/or on the day of the site visit followed manufacturer's instructions and DWR Standard Operating Procedures (NCDWR 2013).

3.6 Soil Chemistry Sampling

Soil sampling protocols matched or exceeded the original sampling protocol used during each original sampling event. On NWCA resample sites, one soil pit was sampled per site, the location specified by the 2021 NWCA protocol (the southeast corner of Veg Plot 1). On CVS resample sites, soil sampling protocol was outlined in Peet et al. (1998). Soil pits were located in a central, representative location within the overall sampling area, as the CVS protocol does not specify pit location. Although the CVS protocol specifies collection of the top 10 cm of the A horizon of mineral soil, to gather additional data discrete soil samples were collected from every horizon over 8 cm to a depth of 1 meter using a stainless steel 7.5-cm diameter tube. The number of horizons within the 1 meter varied by site up to 5 horizons. Excess set-aside soil from above the sampled interval was re-packed into the hole. Soil from each horizon was placed in its own sealable plastic bag for shipping to the Brookside Labs in Ohio for analysis. Complete soil profiles were recorded in field notebooks for all pits.

Analysis was obtained from Brookside Labs, which had performed the soil analysis for the original sampling by the Carolina Vegetation Survey. Selected parameters were analyzed including soil cations (sodium, magnesium, calcium), with the addition of chloride and sulfate ions, as they are components of seawater and have been found in North Carolina to be representative of monthly salinity measurements in shallow groundwater (Poulter 2008). Non-detects were included in the databases at the reporting limit of the lab (Table 3). Soil chemistry data from the original sampling were obtained from the Carolina Vegetation Survey databases (Peet pers. comm. 2022).

Table 3. Brookside Labs soils reporting limits by parameter.

Parameter/Ion/Cation	Reporting Limit
Soluble salts/Electrical Conductivity	0.01 mmhos/cm
Total Exchange Capacity	1 cmol(+)/kg
Ammonium (NH_4-N)	0.5 ppm = mg/kg
Sulfate (SO_4^{2-})	2 ppm = mg/kg
Chloride (Cl^-)	1.0 ppm = mg/kg
Calcium (Ca^+)	1.0 ppm = mg/kg
Potassium (K^+)	1.0 ppm = mg/kg
Magnesium (Mg^+)	1.0 ppm = mg/kg
Sodium (Na^+)	1.0 ppm = mg/kg

3.7 Rapid Assessments and Photographs

Wetland rapid assessment methods evaluate the condition and function of wetlands and are often used for regulatory purposes. Two rapid assessments (Ohio Rapid Assessment Method [ORAM] [Mack 2001]) and North Carolina Wetland Assessment Method ([NCWAM] [NC Wetland Functional Assessment Team 2010]) were completed at all DWR resample sites. Forms for these rapid assessment methods are included in Appendix C.

Site photographs were taken at cardinal directions from the assessment area point on NWCA sites, following the NWCA protocol. On CVS sites, photographs were taken at the outside midpoint of all sides of the plot layout, as well as any photograph locations indicated on the original sampling sheets from the first CVS data collection. Photographs were obtained for CVS sites whenever possible. Representative photographs of each DWR-sampled site are included in Appendix B, along with photographs from partner sites when available.

3.8 Partner Data Collection Methods

To supplement the project dataset of 444 vegetation plots, DWR partnered with several outside entities that had gathered resample data for vegetation plots in localized areas, such as the Albemarle-Pamlico Peninsula or tidal creek wetlands near a phosphate mine. All resample data included vegetation identity and percent cover in vegetation plots and resample methods were identical to the original sampling method. Upon assimilation into the master database, plot sizes were included and various size-dependent data fields (i.e., basal area, stem counts) were adjusted based on plot size, to standardize data across all data collection methods. Average percent cover was calculated by taxon at each site. Partners and their contributions are listed as follows:

Duke University researchers provided resample data, which was recorded using the CVS method upon resampling historical CVS sites in the Albemarle-Pamlico Peninsula. Historical CVS sites were selected based on location around the Albemarle-Pamlico Peninsula and vulnerability to saltwater intrusion. Original CVS data collection occurred in 2003 and 2009; sites were resampled by Duke University researchers in 2016. Vegetation identity and cover were recorded, and soil chemistry data were also obtained for the top 10 cm of soil.

NC State University researchers provided resample data which originally was collected in 2004 and resampled in 2016/2017. Cover of vascular species was recorded using 35 1-m^2 quadrats per site. Woody stem counts and diameter at breast height (dbh) were recorded using 12-m radius circular plots. Soil chemistry data were also obtained for the top 10 cm of soil.

Nutrien Phosphate Company provided wetland monitoring plot data, which they have been collecting for over 10 years as part of a permit condition for expansion of a phosphate mine in eastern North Carolina along the Pamlico River. Some of the Nutrien sites were reference sites across the Pamlico River from the mine, and some were tidal creek wetlands near the mining activities. First data collection occurred in 2010 to 2013; the last resample data collection added to the project database occurred in 2019 and 2020. Nutrien Phosphate Company wetland monitoring used permanent 40-meter transects with 10 quadrats each. Woody shrubs were evaluated with 4 x 4 m quadrats and herbaceous species evaluated with 1 x 1 m quadrats. Number of stems and percent cover by species were recorded on a yearly or near yearly basis. These data were collected by trained experts and reported as part of permit conditions, in a manner acceptable to the DWR permitting staff.

The USFWS utilized the CVS method for original (2013) and resample (2016) data collection on wildlife refuge lands across the Southeast, using three 10 x 10 m modules for each site. Vegetation data and top horizon soil chemistry data collected by USFWS were obtained for wetland sites in North Carolina by DWR through the USFWS publicly available data website (USFWS 2021).

3.9 Metric Calculations and Statistical Analysis

Sites were categorized by plant community structure as forest, shrub, or marsh and by salinity regime as freshwater, transitional and brackish. Sites were also categorized based on storm surge vulnerability in the case of a Category 1 hurricane, using NOAA's maps of vulnerable areas for a Category 1 hurricane, which is the most frequently occurring category of hurricane to historically hit North Carolina (Zachry et al. 2015).

A variety of descriptive and analytical metrics were calculated using the vegetation data (Table 4; Table 5) and supplementary soils and water data (Table 6). Floristic quality was assessed using the coefficient of conservatism (C) (Swink and Wilhelm 1994).

Percent of taxa that were considered brackish-tolerant was calculated using a "brackish tolerance database" created for this project and included in Appendix F. Brackish tolerance for each species was assigned on a yes/no basis using a variety of botanical sources, including many authoritative plant keys which included habitat information. If any brackish habitat was noted as a place where that species could be found, it was noted as brackish-tolerant. This information was then used to calculate metrics related to brackish tolerance.

The Wetness Affinity Index is a metric that was created for this project, using the National Wetland Plant List (NWPL) ratings by species to calculate an overall community affinity for wetlands (USACE 2020). "Wetness values" or weights were assigned to the different possible NWPL ratings (OBL=wetland obligate=5, FACW=facultative wetland=4, FAC=facultative=3, FACU=facultative upland=2, UPL=upland=1, not on list=0) and then averaged to generate the overall Wetness Affinity Index.

Soil chemistry data were obtained for the top soil horizon on all sites except Nutrien partner sites, so results are generally reported for top horizon only. Soil chemistry data for deeper horizons to one meter were obtained from DWR sites.

Metric medians were chosen for reporting because the data were not distributed symmetrically and consistently among groups or with all sites combined. Change per decade (CPD) for each metric for each site was calculated or extrapolated as a way to report differences between first and last sampling in a standardized manner (see formula below). The majority of the sites (54) had been sampled with a nine to 34 year gap between sampling events ("long interval" sites), so CPD was calculated for those sites. The remaining 30% of sites (24) were resampled at intervals of three to eight years ("short interval" sites), so CPD was extrapolated for those sites. Change per decade metric data were tested with Mann-Whitney U tests to determine if metric CPD significantly differed between short interval sites and long interval sites. If the groups did not significantly differ, the extrapolation for the shorter interval sites was considered reasonable.

CPD was calculated using the following formula:

Change in metric per Decade (CPD) (calculated or extrapolated)

$$= \left[\frac{\text{(metric value at last sampling-metric value at first sampling)}}{\text{number of years between last and first sampling}}\right] \times 10$$

Percent reduction/increase in cover was also calculated by site for the various plant types (tree, shrub, herbaceous and vine) using the following formula:

$$\textit{Percent reduction or increase in cover between sampling events} = \left[\frac{\text{(value at last sampling-value at first sampling)}}{\text{value at first sampling}} \right] \times 100$$

3.9.1 Multidimensional Analyses and Plant Community Change Analyses

Non-metric Multidimensional Scaling (NMDS) was used to assess the relative extent of change or shift in plant communities between original sampling and resampling. NMDS was performed on presence/absence data for all species, with very rare species excluded, using the Bray-Curtis dissimilarity index for plotting points in dimensionless space. The rarity cutoff was 0.5% of the total occurrences for a given species. Simple Euclidean distances were calculated between original sampling points and resampling points as a proxy for community composition change over time. Distances were normalized by number of years between resampling, to arrive at a rate of plant community composition shift over time. Results were then mapped to show locations of sites and the relative rate of overall plant community shift. NMDS was then repeated for all woody species to show extent of shifts in woody plant communities, excluding marsh sites.

For assessing detailed changes in vegetative communities, the similarity percentages breakdown procedure (SIMPER) was performed, which utilizes a Bray-Curtis similarity index matrix to assess percent dissimilarity between two datasets of species (Clarke 1993). The greater the deviation from the original sampled condition, the greater the percent dissimilarity. The advantage to using the SIMPER analysis is that it reveals which taxa are the more influential drivers of the dissimilarity between two datasets. SIMPER analyses were performed using PAST v. 4.13 biostatistical analysis software (Hammer et al. 2001) on presence/absence occurrence data on each site during original sampling and resampling. Data at the genus level were used to improve detectability of patterns. Then to avoid loss of more information than necessary, after detailed results were obtained, genera were expanded to species level wherever possible based on the particular species present in the dataset. In several instances, only one species was present for a given genus. Taxa driving the top 35% dissimilarity in community composition were considered "top taxa" for driving the changes between original sampling and resampling and reported in the results section below.

All analyses were performed using the statistical software packages JMP 17 (JMP Statistical Discovery 2022) and PAST v. 4.13 software.

Table 4. Vegetation metrics and explanation of calculations.

Richness and Diversity	
Richness All	Total number of individual taxa
Richness Herbaceous Taxa	Total number of herbaceous taxa
Richness Woody Taxa	Total number of woody taxa
Shannon Diversity All	Shannon's H Diversity Index for all taxa
Shannon Diversity Herbaceous Taxa	Shannon's H Diversity Index for only herbaceous taxa
Shannon Diversity Woody Taxa	Shannon's H Diversity Index for only woody taxa
Natives Only	
Native Taxa Richness	Total number of native taxa
Native Taxa Shannon Diversity	Shannon's H Diversity Index for only native taxa
Nonnatives	
Percent of Total Cover Occupied by Nonnative Taxa	Metric formula = (total cover occupied by nonnative taxa/total cover occupied by all taxa) x 100 Total cover occupied by all taxa = sum of mean percent cover for every individual taxon Mean percent cover for individual taxon = mean percent cover across all modules if more than one module or plot sampled per site
Percent Nonnative Taxa All	Metric formula = (total number of nonnative taxa/total number of all taxa) x 100
Percent Nonnative Herbaceous Taxa	Metric formula = (total number of nonnative herbaceous taxa/total number of all herbaceous taxa) x 100
Floristic Quality	
Mean C All	Average of Coefficient of Conservatism (C values) for all taxa
Mean C Herbaceous Taxa	Average of C values for all herbaceous taxa
Mean C Woody Taxa	Average of C values for all woody taxa
Floristic Quality All*	Metric formula = (mean C) x (square root of total number of taxa)
Floristic Quality Herbaceous Taxa	Metric formula = (mean C herbaceous taxa) x (square root of total number of herbaceous taxa)
Floristic Quality Woody Taxa	Metric formula = (mean C woody taxa) x (square root of total number of woody taxa)
Brackish Tolerance and Wetness Affinity Index	
Percent Brackish-Tolerant of All Taxa	Metric formula = (number of brackish-tolerant taxa/total number of taxa) x 100
Percent Brackish-Tolerant of Herbaceous Taxa	Metric formula = (number of brackish-tolerant herbaceous taxa/total number of herbaceous taxa) x 100
Percent of Total Cover Occupied by Brackish-Tolerant Taxa	Metric formula = (total cover occupied by brackish-tolerant taxa/total cover occupied by all taxa combined) x 100
Wetness Affinity Index All	Average of "wetness value" for all taxa [Wetness values assigned as follows: OBL=5, FACW=4, FAC=3, FACU=2, UPL=1, Not on NWPL=0]
Plant Structure and Biomass	
Woody Basal Area† (sq cm in 100 m^2)	Sum of basal area (sq cm) recorded for all taxa per 10 x 10 m area
Woody Stem Count† (in 100 m^2)	Sum of counts for all woody taxa per 10 x 10 m area
Total Herbaceous Taxa Cover	Sum of mean percent cover for each herbaceous taxon
Total Shrub Taxa Cover	Sum of mean percent cover for each shrub taxon
Total Tree Taxa Cover	Sum of mean percent cover for each tree taxon
Total Vine Taxa Cover	Sum of mean percent cover for each vine taxon

*Floristic Quality Index formula from Swink and Wilhelm 1994. †Some data sources did not record woody basal area and woody stem counts.

Table 5. Woody vegetation size classes. Size class 1 was not used with NWCA data collection method.

Size Class Designation	Diameter at Breast Height (dbh) (cm)	Analysis Value (cm)
1	0 – 4.9	2.45
2	5 – 9.9	7.45
3	10 – 24.9	17.45
4	25 – 49.9	37.45
5	50 – 74.9	62.45
6	75 – 99.9	87.45
7	100 – 200	150.00

Table 6. Supplementary data collected (water chemistry and soil chemistry).

Water Chemistry Data	N Sites with Data Available (Surface; Groundwater)	Soil Chemistry Data	N Sites with Data Available
pH (SU)	32;25	Soil pH	67
Specific Conductivity (µS/cm)	32;25	Soluble salts/Electrical Conductivity (EC) (mmhos/cm)	54
Salinity (ppt)	31;25	Total Exchange Capacity (CEC) (cmol(+)/kg)	54
Ammonia (NH_3-N) (mg/L=ppm)	32;23	Ammonium (NH_4-N) (mg/kg=ppm)	46
Bromide (Br^-) (mg/L)	21;24	Sulfate (SO_4^{2-}) (ppm)	46
Chloride (Cl^-) (mg/L)	28;24	Chloride (Cl^-) (ppm)	46
Sulfate (SO_4^{2-}) (mg/L)	28;24	Calcium (Ca^+) (ppm)	67
Calcium (Ca^+) (mg/L)	21;23	Magnesium (Mg^+) (ppm)	67
Magnesium (Mg^+) (mg/L)	21;23	Sodium (Na^+) (ppm)	67
Sodium (Na^+) (mg/L)	21;23	Potassium (K^+) (ppm)	67
Potassium (K^+) (mg/L)	21;23		

Water was not always present on a given site during sampling or resampling. Different sets of soil chemistry parameters were analyzed by each partner project. Water chemistry samples were collected from surface water and groundwater (1 m depth) when present. Soil chemistry samples were collected from the top soil horizon (or top 10 cm).

3.10 TITAN Community Changepoint and Species Salinity Sensitivity Analysis

The extensive database of plant community composition and soil sodium data accumulated for this project was used to estimate whole plant community changepoints using Threshold Indicator Taxa Analysis (TITAN) model (Baker and King 2015) in R software (R Core Team 2021; Version 4.2.1). The TITAN model was introduced as a way to detect changes in taxa distributions along an environmental gradient, as well as estimate community thresholds. Similar to a prior study on herbaceous and understory woody plant communities by Anderson et al. (2022), the TITAN model using the R package "TITAN2" was employed to model soil sodium thresholds from the species abundance (cover) and soil chemistry datasets (Baker et al. 2023). Soils were summarized using the "skimr" package in R after all data were arranged using "tidyverse" and "dplyr" packages (skimr: Waring et al. 2022; tidyverse: Wickham et al. 2019; dplyr: Wickham et al. 2023). Top horizon soil sodium and the sum of calcium and magnesium (Ca+Mg) were the two environmental gradients used for this analysis. All taxa observed in > 5 individual plots (194 species of 565 total recorded) and all sites where sodium, calcium and magnesium were measured were included in the analysis. The TITAN model predicts a change point, or threshold along the salinity gradient for each taxon, and change points for the decreasing (filtered sum z- score, fsumz-) and increasing (filtered sum z+ score, fsumz+) communities as a whole. Each taxon included in the model was assigned one of two categories: 1) sensitive/decreasing taxa (z-), or 2) tolerant/increasing taxa (z+) based on density probabilities calculated for each taxon and each community.

Parameterizing the TITAN model has flexibility in how to analyze and interpret results from the model. For this analysis, the model was kept as close to default and recommended values as possible to increase accuracy of the outputs even if the number of species that could be retained was decreased. However, since data along the soil sodium gradient were right skewed with many species occurring at lower sodium levels and several occurring at higher soil sodium, the default indicator values were not the models ultimately used for results. Model parameters are included in the code and listed, along with reasons for their selection or modification, in a separate report on the TITAN analysis (Appendix E).

Individual species information (as "increasers" or "decreasers") from the TITAN analysis was used to corroborate information in the brackish tolerance database. The resulting soil sodium threshold for whole plant community change was incorporated into mapping and other analyses.

4 Results

4.1 General Site Descriptions

Sites ranged in elevation from zero (0) meters above sea level to 3.7 meters, with the exception of one site (Croatan Bay in the Croatan National Forest) which was at 11.4 meters (Figure 10). Using NOAA's storm surge vulnerability maps based on the SLOSH model (Zachry et al. 2015), it was determined that 72 of the 78 study sites were vulnerable to a storm surge from a Category 1 hurricane (Figure 11; Figure 12), and nearly all sites (75) were vulnerable to a storm surge from a Category 2 hurricane.

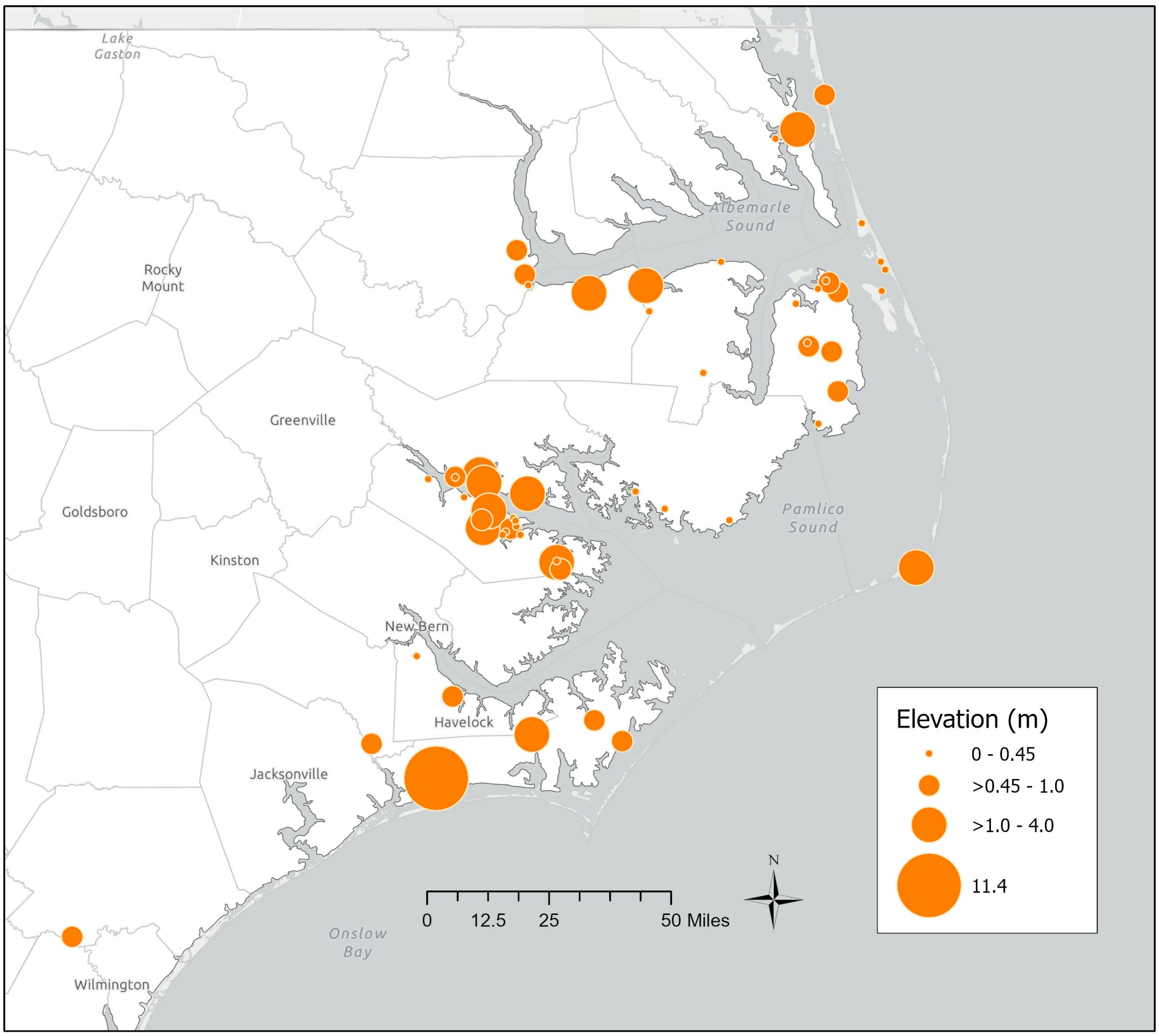


Figure 10. Map of elevation above mean sea level for resample study sites.

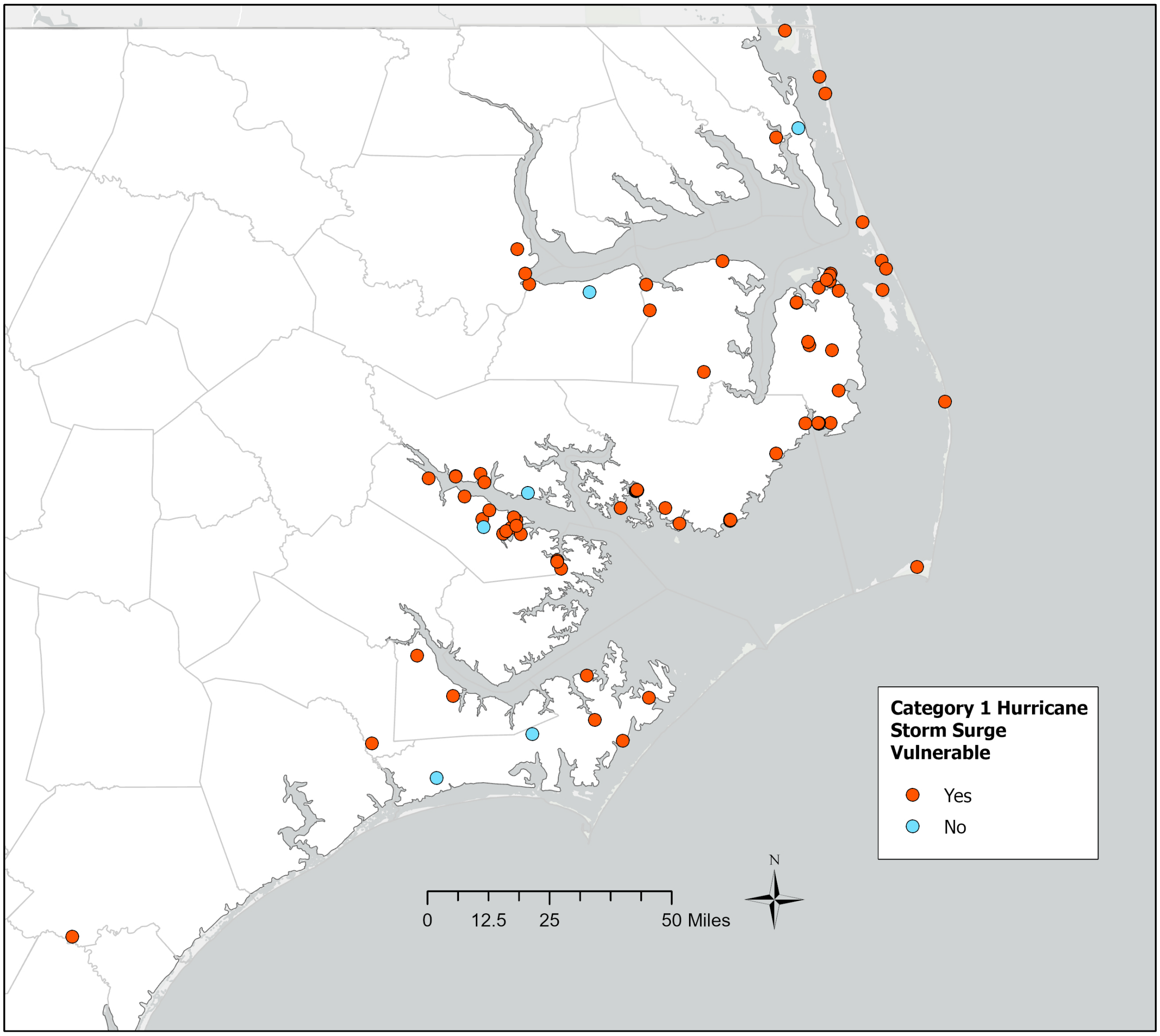


Figure 11. Map of vulnerability of resample study sites to a Category 1 hurricane storm surge (Vulnerability rated using Zachry et al. 2015).

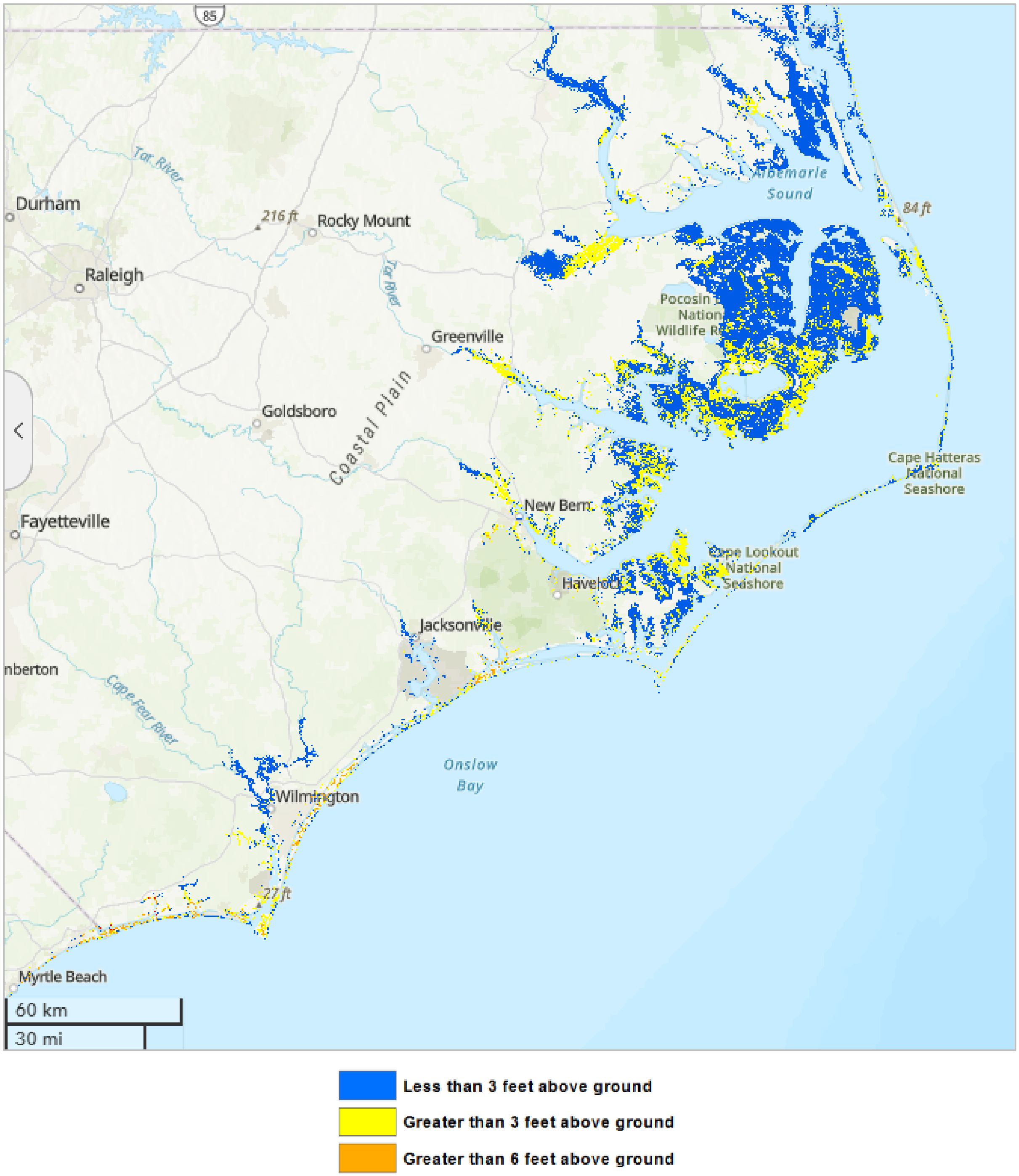


Figure 12. Map of storm surge vulnerability areas in North Carolina for a Category 1 hurricane. From National Hurricane Center Storm Surge Risk Maps for Category 1 hurricane (Zachry et al. 2015). Legend shows the inundation height of possible/predicted storm surge flooding by color.

Overall, median years between first and last sampling events was 12 years (range 3 to 34) for the 78 sites used in this study. Site types available were constrained by the requirement for historical data collection and other site selection criteria. At the time of the original sampling event, the majority of the sites were forested wetlands (53), while 12 were shrub wetlands or transitional wetlands (wetlands in transitional spaces between marshes and forested wetlands), and 13 were marshes (Table 7; Figure 13; Figure 14). Forty-one (41) sites were freshwater wetlands, 18 were transitional salinity wetlands and 19 were brackish wetlands. Ten (10) sites, or 13%, completely changed plant community type between original sampling and resampling: in four instances from forested to open water (ESTLK) or marsh (LSFOR, SEES4), in four instances from forested to shrub (BRTTBY, MAFOR, SEES18, STHRIV) and in three instances from shrub to marsh (MATRA, SWTRA, SWQTDK) (Figure 15). Unless otherwise noted, reported results are based on plant community structure at original sampling, which came from plant community information notes and historical aerial photointerpretation.

Rapid assessments were performed on all NWCA and DWR sites (36 sites) during the last resampling (in 2021 and 2022) and nearly all sites were rated "High" by NCWAM (Appendix D). Median ORAM score was 73 out of a total 90 points, with a range of 25 to 88 (Appendix D).

Table 7. Number of sites by category of original wetland plant community structure and salinity.

Number of Sites	Forest	Shrub	Marsh	All Plant Community Types Combined
Freshwater	40	1	No sites	41
Transitional Salinity	13	5	No sites	18
Brackish	No sites	6	13	19
All Salinity Regimes Combined	53	12	13	78

		No sites
Freshwater Forest (GTMNHS)	Freshwater Shrub (CRTNBY)	Freshwater Marsh
		No sites
Transitional Salinity Forest (STHRIV)	Transitional Salinity Shrub (GSCRKN)	Transitional Salinity Marsh
No sites		
Brackish Forest	Brackish Shrub (SWQTDK)	Brackish Marsh (PPNSBAY)

Figure 13. Photos of representative wetlands of the various types during last sampling. Site name in parentheses.

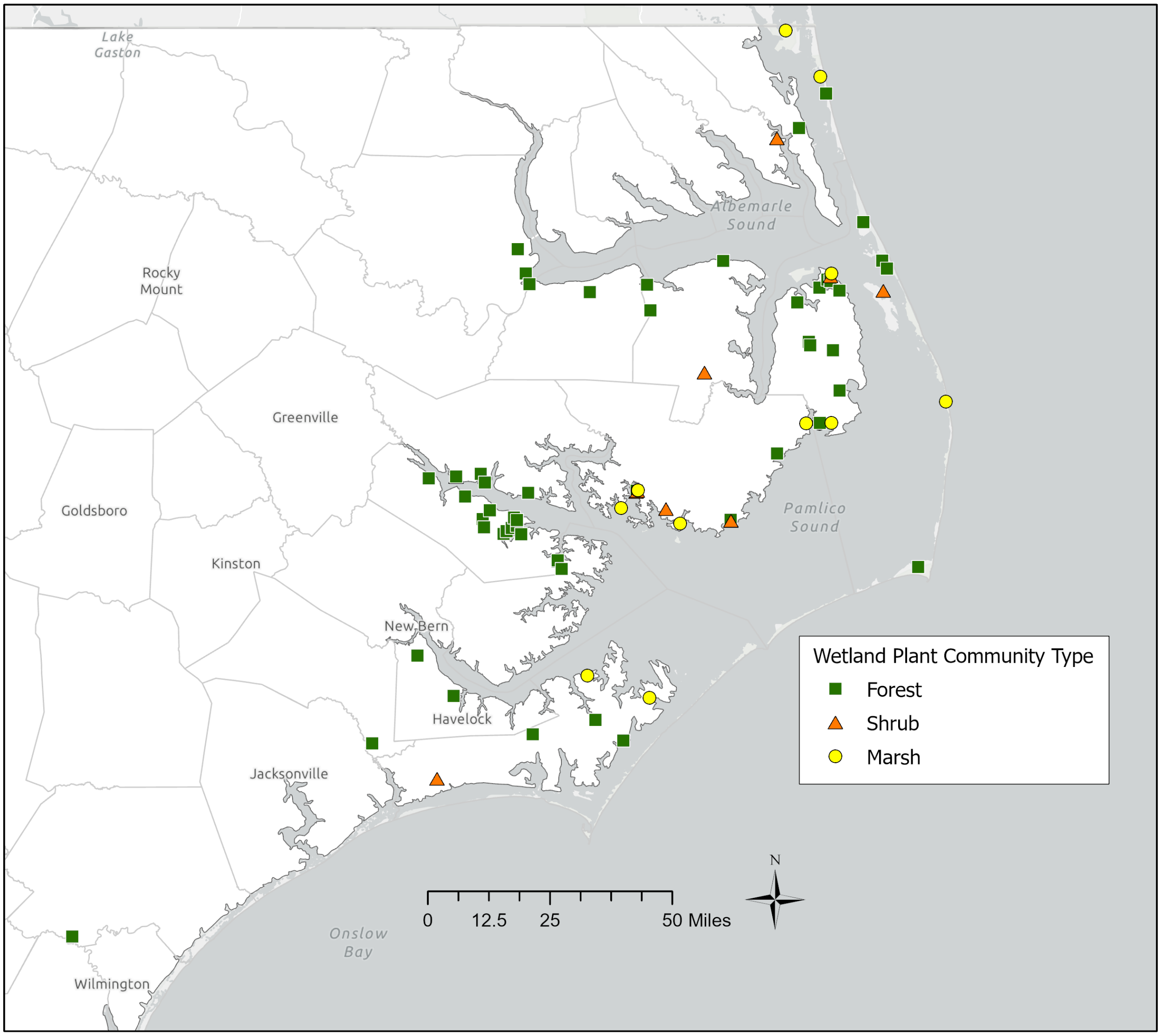


Figure 14. Spatial distribution of forest, shrub, and marsh resample study sites. Symbols are for plant community type at original sampling.

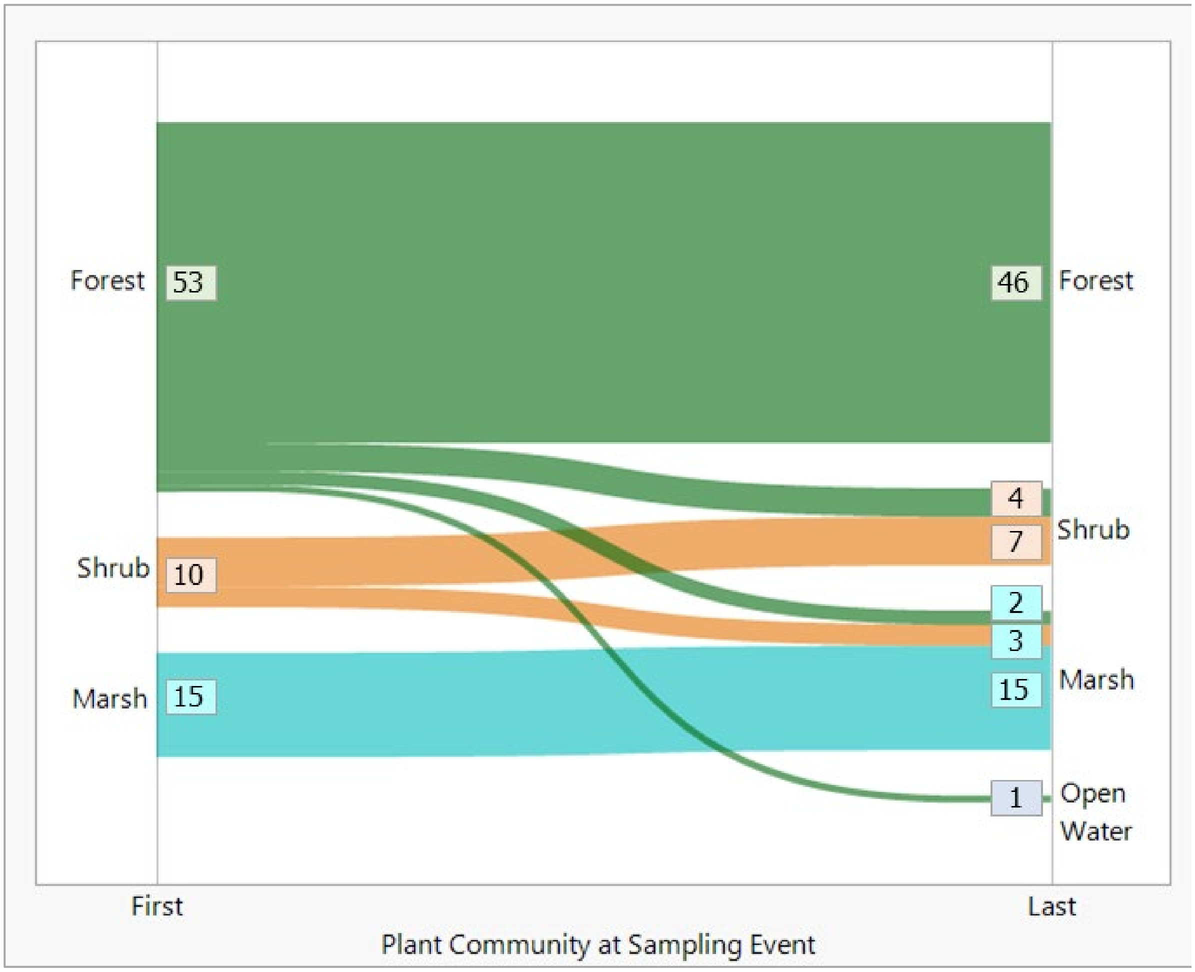


Figure 15. Plot of overall plant community change from first sampling to last sampling for all sites.

4.2 Vegetative Community Descriptions and Metrics

Included in this project analysis were data from a total of 2,035 vegetation plots and 14,430 individual plant species identifications made across all plots on the 78 sites. A total of 536 separate plant taxa were identified across all sites during original and resampling. A total of 32,438 woody stems or trees were counted and recorded. A total of 421 soil samples and 57 water samples were collected and analyzed.

Cumulative species lists were compiled for each original and resampling site visit, resulting in 4,431 total species occurrences (presence/absence) across all site visits. The most frequently encountered species across both original sampling and resampling was Eastern poison ivy (*Toxicodendron radicans*) (116 of 4,431 total occurrences of all species), followed by the tree and shrub species swamp redbay (*Persea palustris*), wax myrtle (*Morella cerifera*) and red maple (*Acer rubrum*). The next most frequently encountered species across all sites were common greenbrier (*Smilax rotundifolia*), Virginia creeper (*Parthenocissus quinquefolia*) and royal fern (*Osmunda regalis*). The frequency distribution of encounters by species was strongly right skewed; nearly a third (150) of the 536 identified species were only encountered one time across all visits to all sites, and another 39% (210 species) were encountered two to five times across all visits to all sites.

A variety of descriptive and analytical metrics were calculated using the vegetation data. Median values are presented from last data collection for forest, shrub and marsh sites (Table 8). Metric results are presented in Figure 16 through Figure 29. Descriptive boxplots are presented for last data collection only, because the time intervals between first and last sampling varied.

Differences between the "long interval" (nine to 34 year sampling interval) and "short interval" (three to eight year sampling interval) groups, in terms of metric CPD, were detected statistically in three of the 27 metrics, two of three being related to each other (Mann Whitney U test; $p<0.05$; metrics: percent nonnative taxa of all taxa, percent nonnative herbaceous taxa of all taxa, and woody stem count). Close examination of the data for these metrics indicated that the differences detected statistically between these two groups in these metrics were not meaningful ecologically (e.g. 1.8% mean decrease in percent nonnative taxa vs. 2.0% increase *per decade*; no change in woody stem count vs. decrease of two stems *per decade*), so it was determined that combining the long and short interval sites was reasonable for the data analysis.

Table 8. Median values for descriptive vegetation metrics in forest, shrub and marsh wetland sites and all sites combined, last data collection. Plant community type from original sampling. Descriptions of metrics are included in Table 4.

Vegetation Metric	Forest	Shrub	Marsh	All Combined
Number of Sites	53	12	13	78
Richness and Diversity				
Richness All	28	21.5	8	23
Richness Herbaceous Taxa	13	7.5	6	9
Richness Woody Taxa	18	10.5	1	15
Shannon Diversity All	3.6	3.2	2.2	3.3
Shannon Diversity Herbaceous Taxa	2.7	2.1	1.9	2.4
Shannon Diversity Woody Taxa	3.0	2.6	0.8	2.9
Natives Only				
Native Taxa Richness	27	18.5	7	20
Native Taxa Shannon Diversity	3.4	3.0	2.1	3.2
Nonnatives				
Percent of Total Cover Occupied by Nonnative Taxa	0.1	0.0	0	0.1
Percent Nonnative Taxa All	2.7	4.9	0	2.6
Percent Nonnative Herbaceous Taxa	1.8	3.1	0	1.8
Floristic Quality				
Mean Coefficient of Conservatism (C) All Taxa	5.0	4.9	5.67	5
Mean C Herbaceous Taxa	5.5	5.2	6.0	5.5
Mean C Woody Taxa	4.7	4.6	4.1	4.6
Floristic Quality All	25.1	21.1	17.3	22.9
Floristic Quality Herbaceous Taxa	16.7	15.8	17.0	16.5
Floristic Quality Woody Taxa	20.4	13.9	3.0	17.6
Brackish Tolerance and Wetness Affinity Index				
Percent Brackish-Tolerant of All Taxa	25.9	55.5	100	37.5
Percent Brackish-Tolerant of Herbaceous Taxa	24.7	61.9	100	38.5
Percent of Total Cover Occupied by Brackish-Tolerant Taxa	38.6	86.3	100	54.3
Wetness Affinity Index All	3.7	4.0	4.5	3.8
Plant Structure and Biomass				
Woody Basal Area (sq cm in 100 m^2)	198.8	30.6	0	167.5
Woody Stem Count (in 100 m^2)	23.4	3.6	0	9.0
Total Herbaceous Taxa Cover	272.5	309.0	462.0	278.9
Total Shrub Taxa Cover	138.6	140.1	0	94.6
Total Tree Taxa Cover	175	5.3	0	110.0
Total Vine Taxa Cover	14.1	0	0	3.5

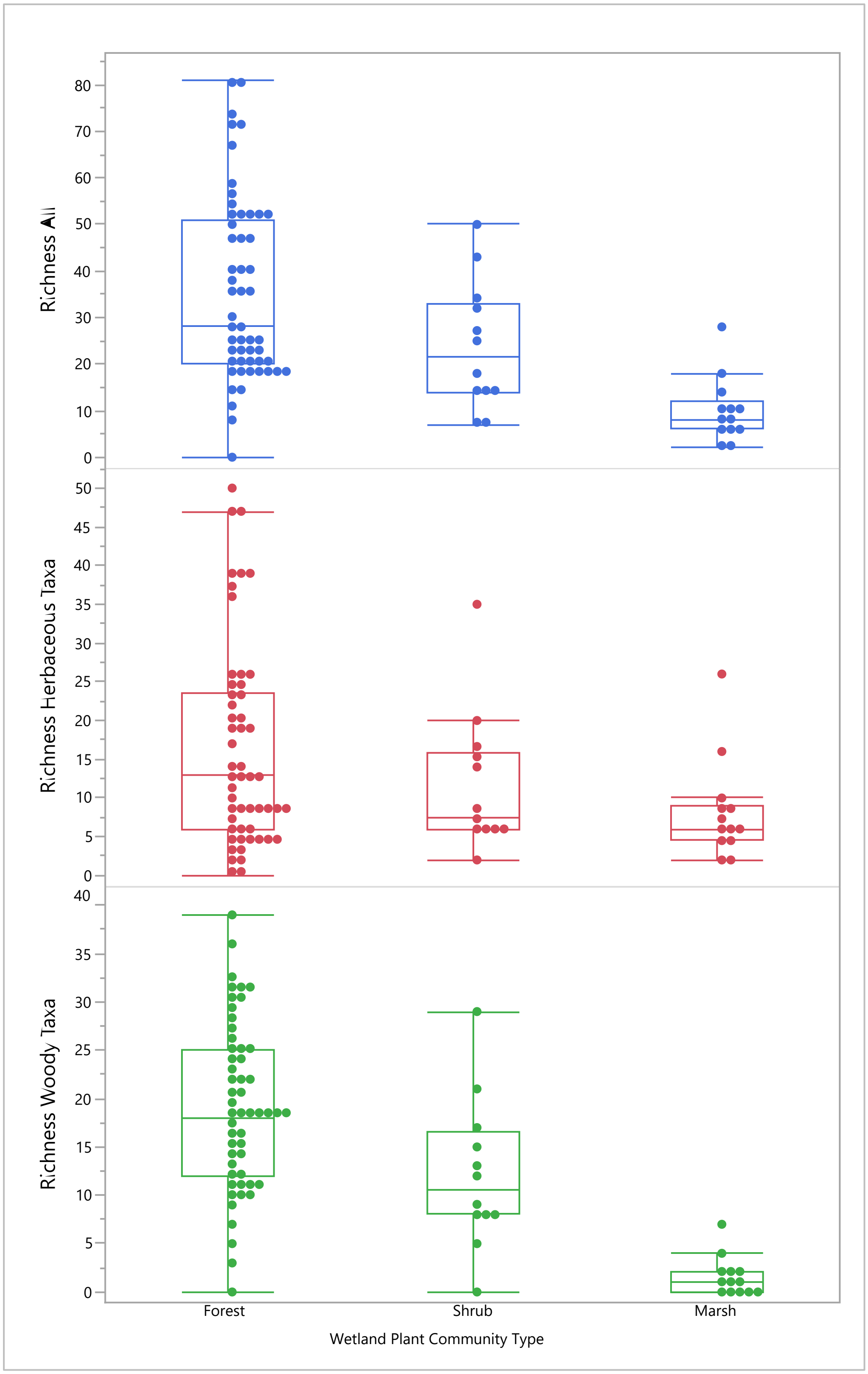


Figure 16. Descriptive boxplots for overall richness metrics by original plant community type. Overall richness metrics from last data collection.

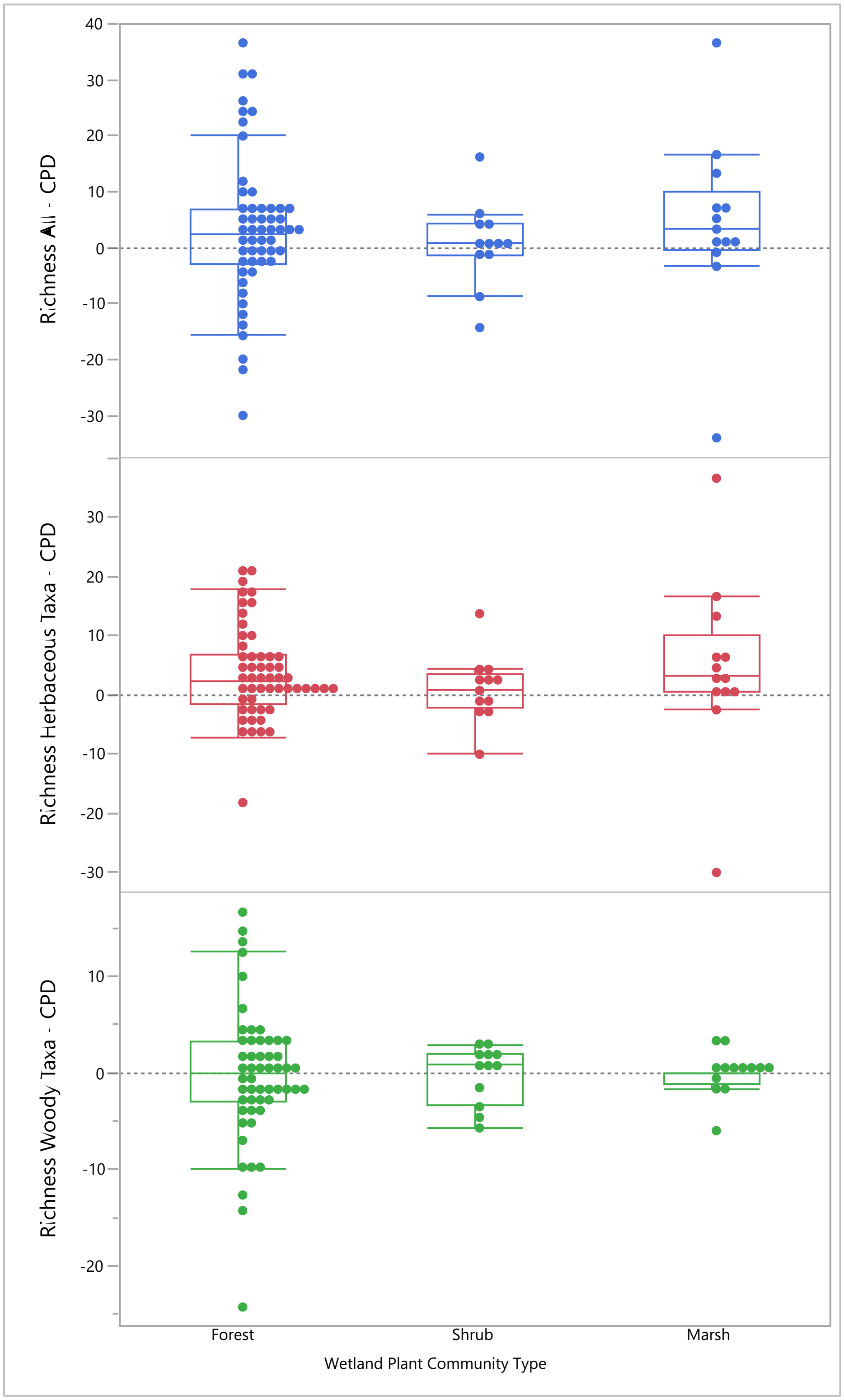


Figure 17. Change per decade (CPD) in richness metrics by original wetland plant community type. A value of zero means no change detected between first and last sampling events.

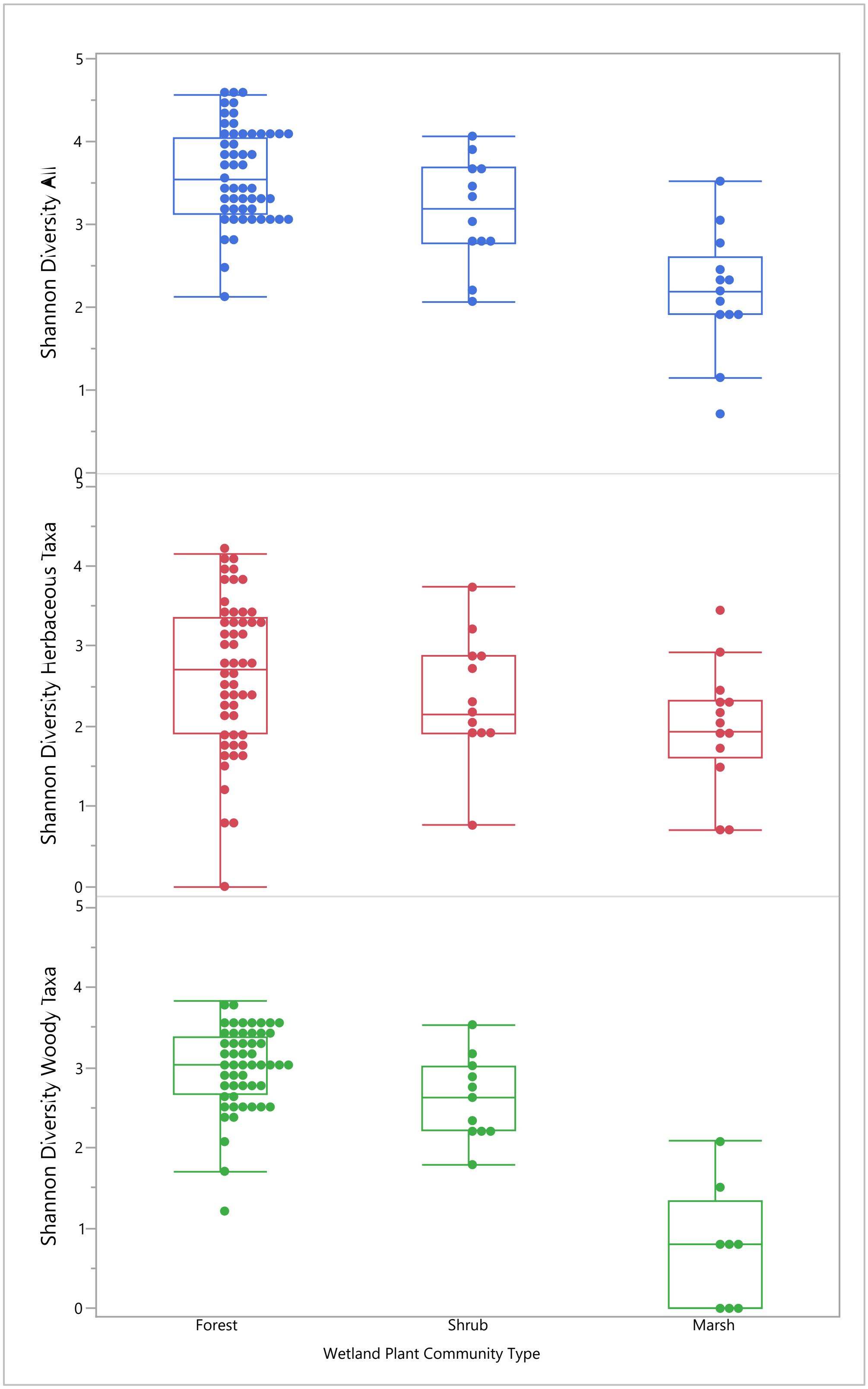


Figure 18. Descriptive boxplots for diversity metric by original plant community type. Diversity metrics calculated from last data collection. A value of zero means only one species present.

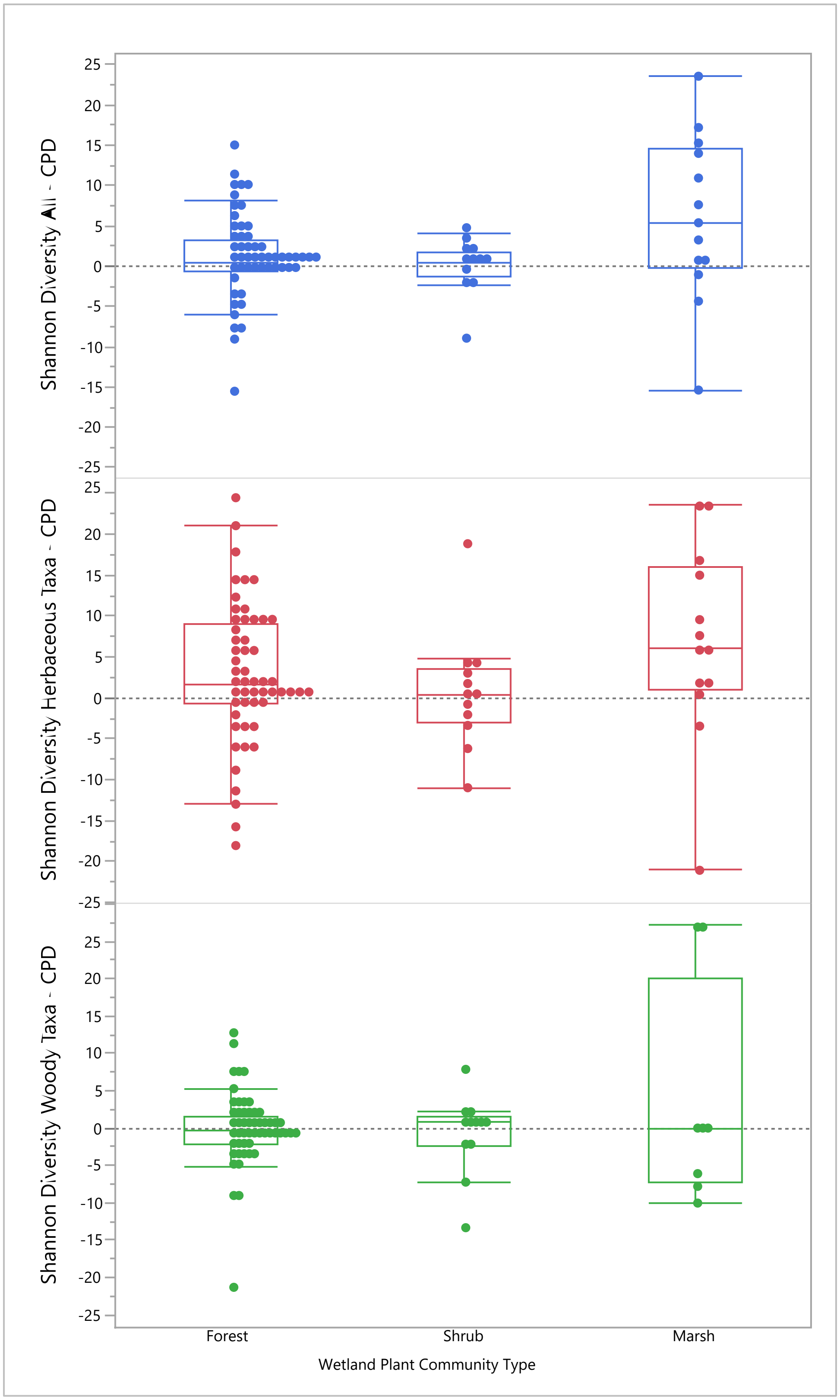


Figure 19. Change per decade (CPD) in diversity metrics by original wetland plant community type. A value of zero means no change detected between first and last sampling events.

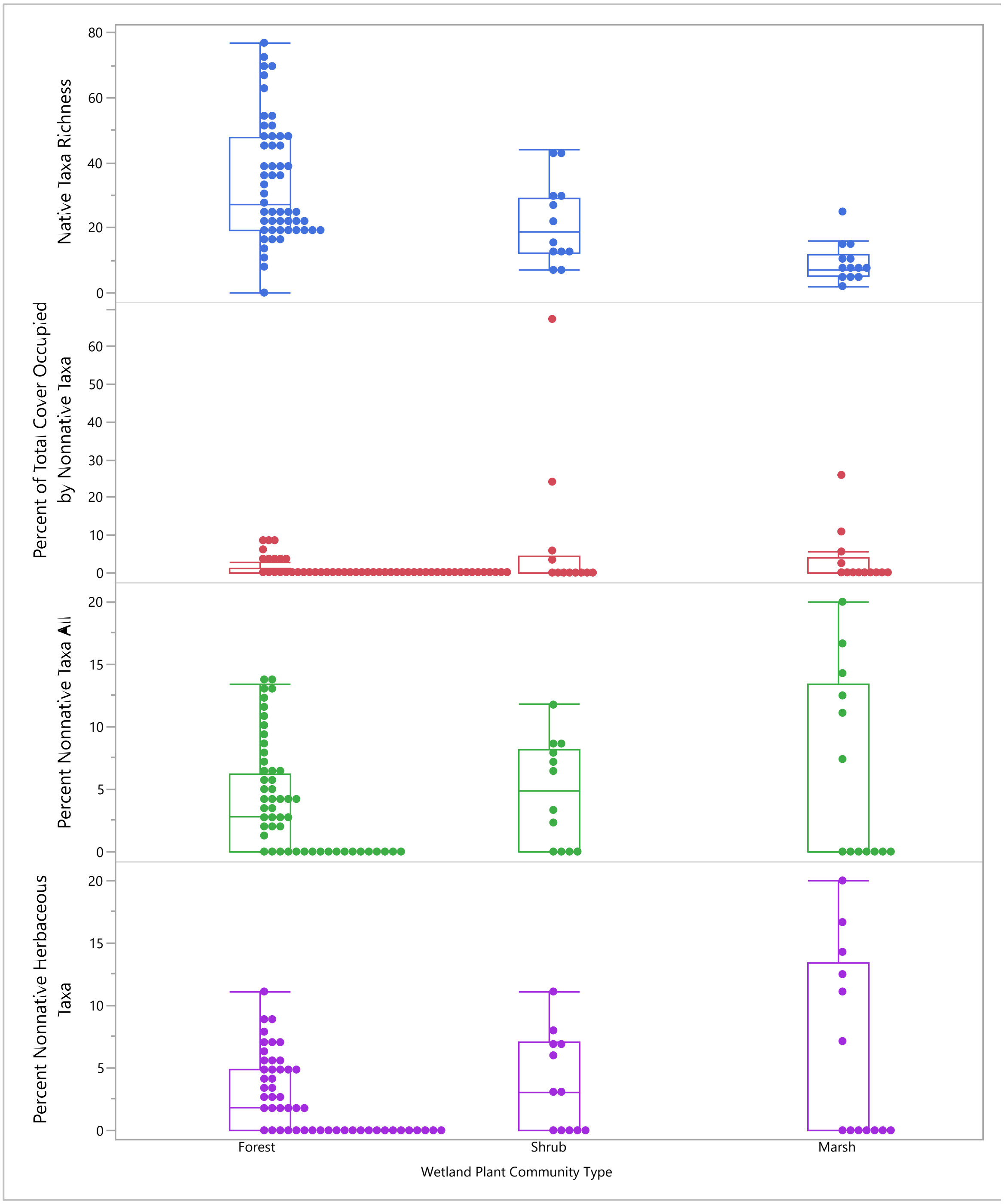


Figure 20. Descriptive boxplots for native/nonnative vegetation metrics by original plant community type. Metrics calculated from last data collection.

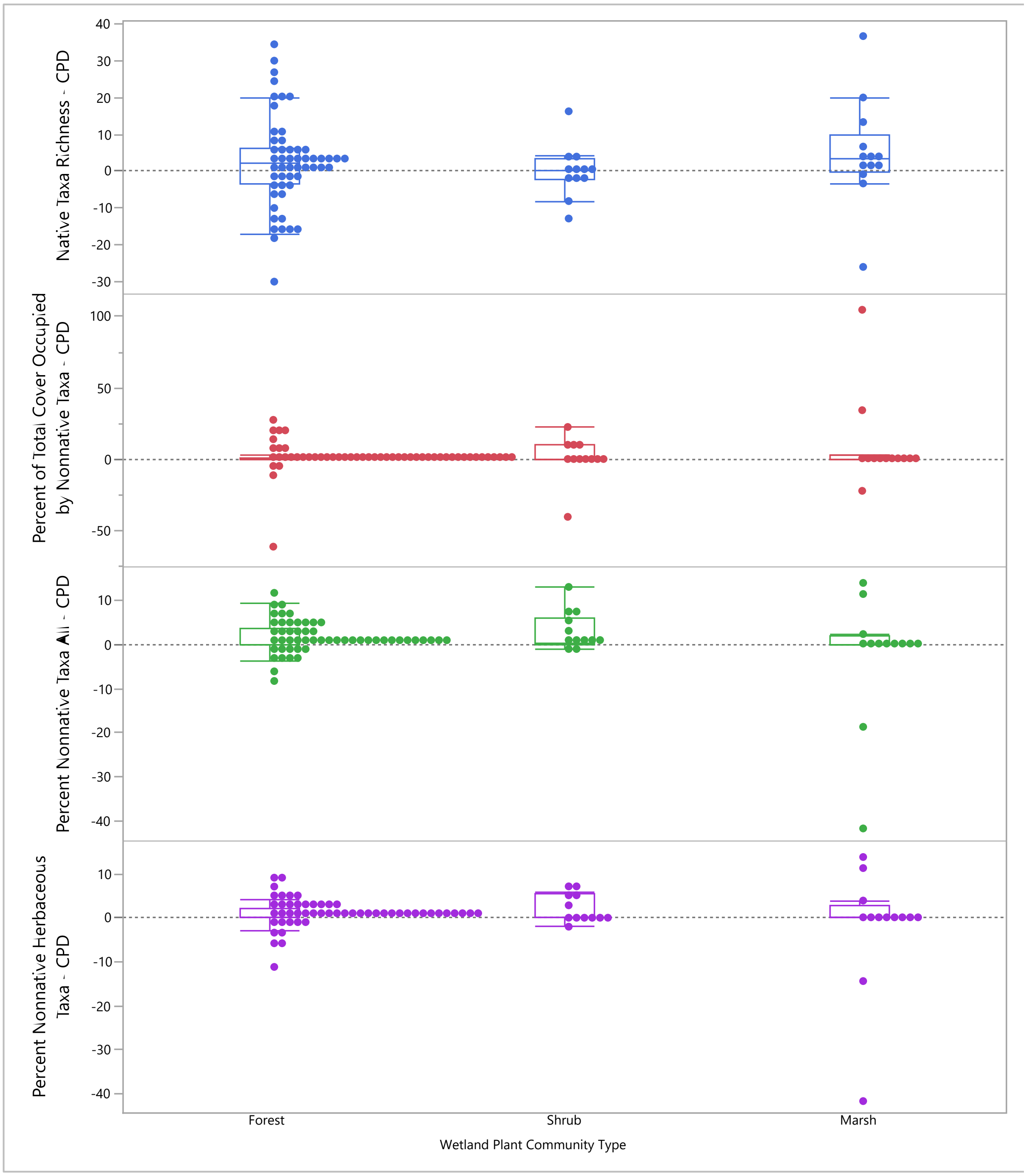


Figure 21. Change per decade (CPD) in native/nonnative vegetation metrics by original wetland plant community type. A value of zero means no change detected between first and last sampling events.

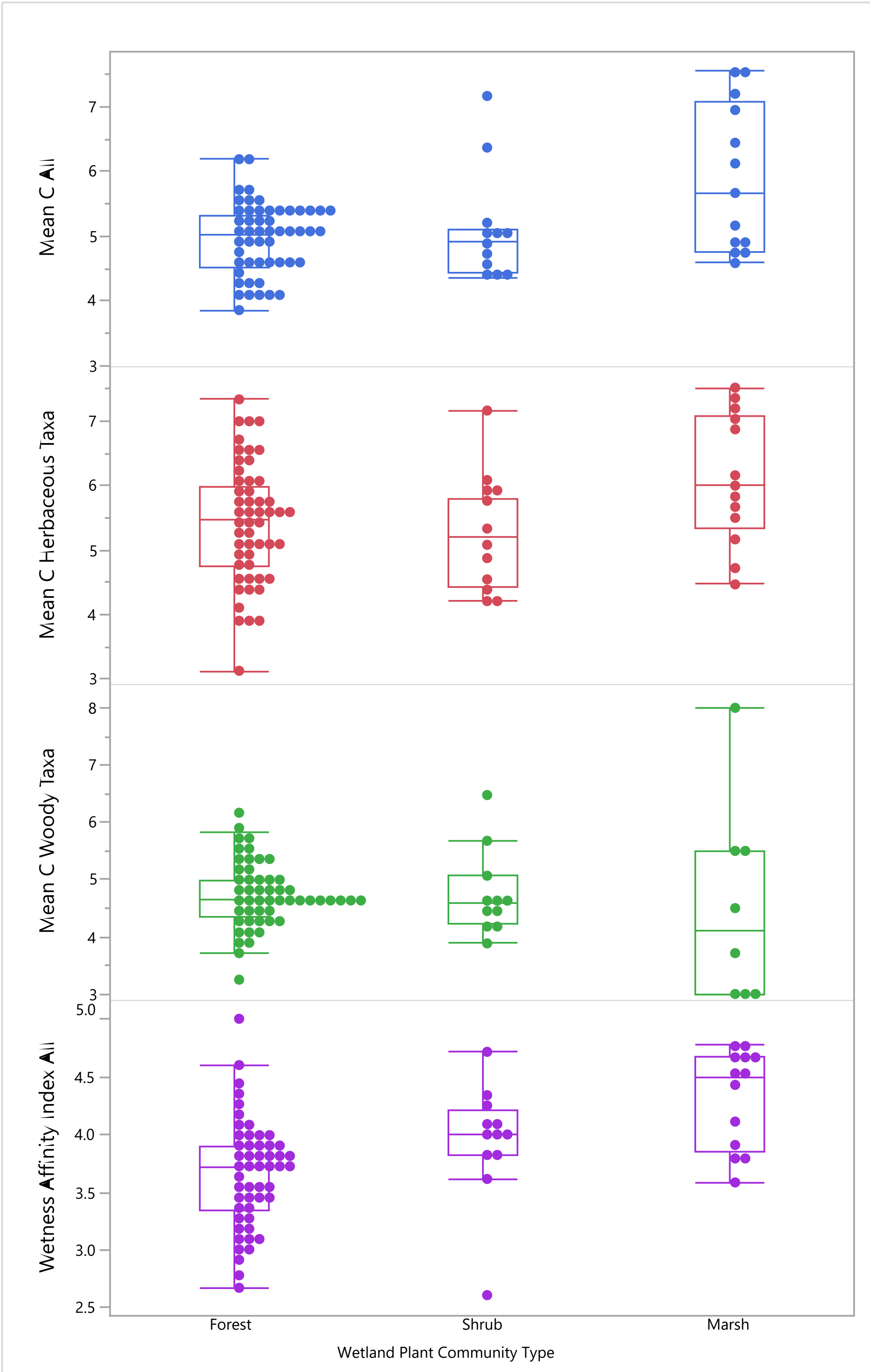


Figure 22. Descriptive boxplots for mean C and wetness affinity index metrics by original plant community type. Mean C and wetness index metrics calculated from last data collection.

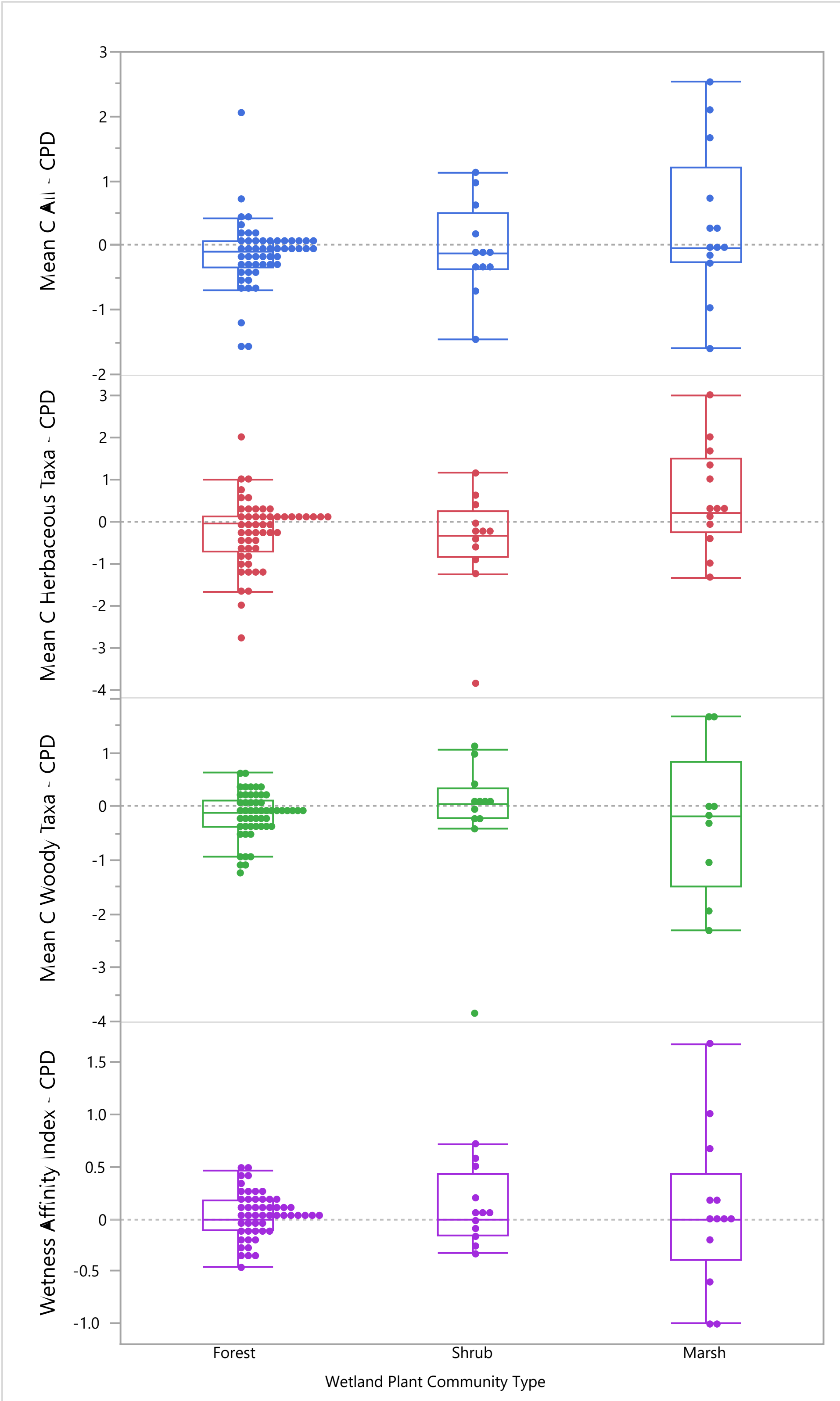


Figure 23. Change per decade (CPD) in mean C and wetness affinity index metrics by original wetland plant community type. A value of zero means no change detected between first and last sampling events.

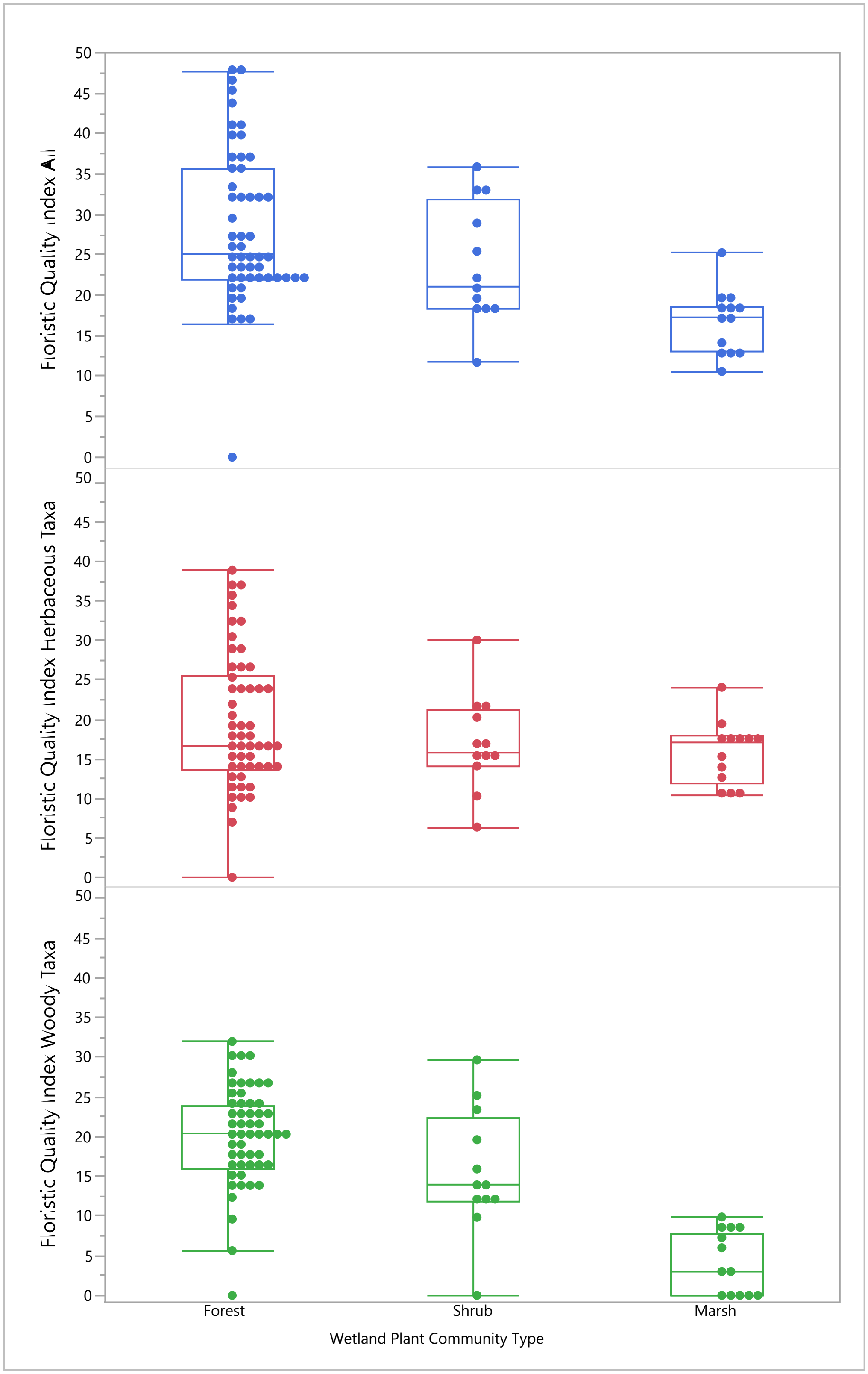


Figure 24. Descriptive boxplots for Floristic Quality Index (FQI) measures by original plant community type. FQI calculated from last data collection.

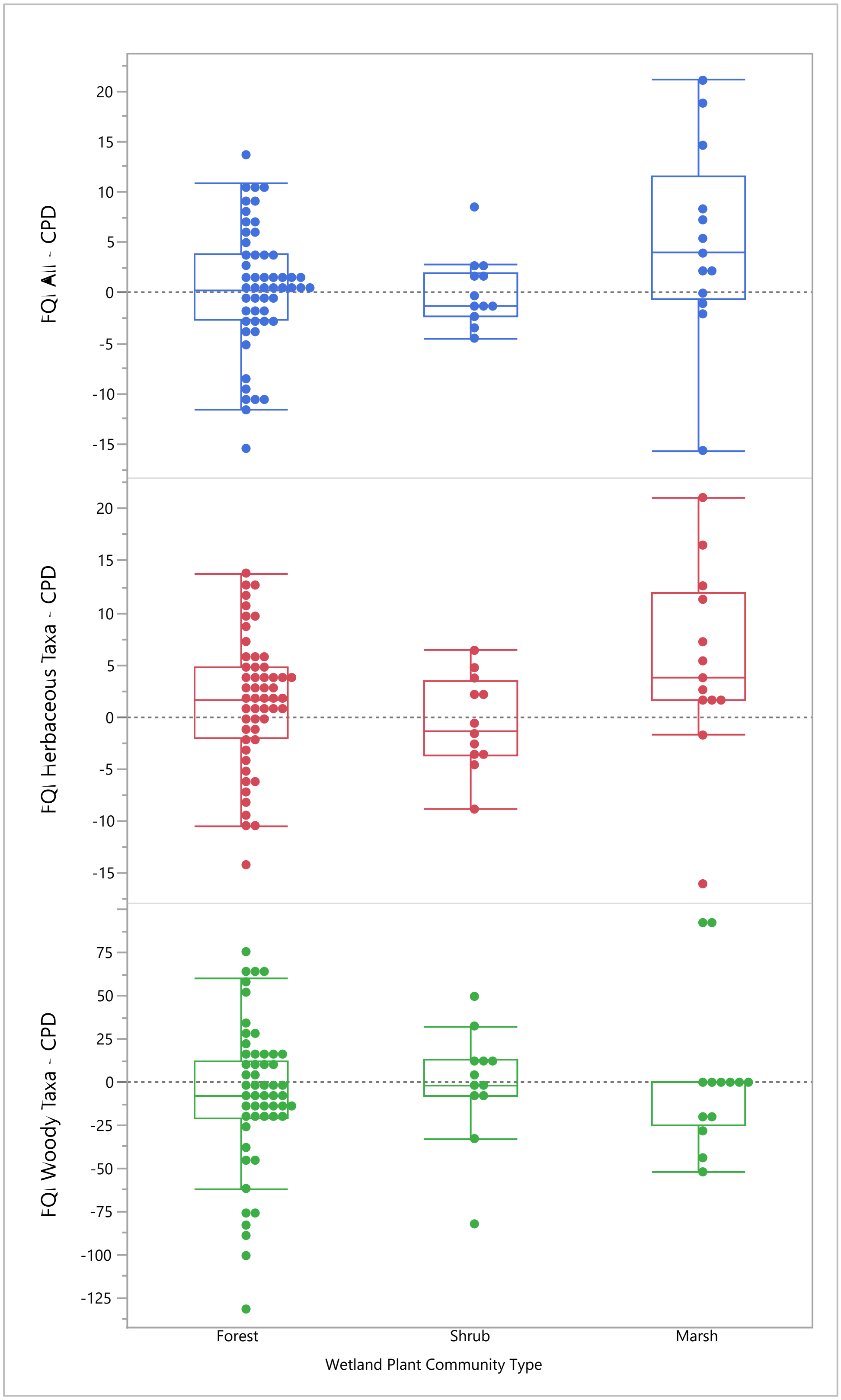


Figure 25. Change per decade (CPD) in Floristic Quality Index (FQI) metrics by original wetland plant community type. A value of zero means no change detected between first and last sampling events.

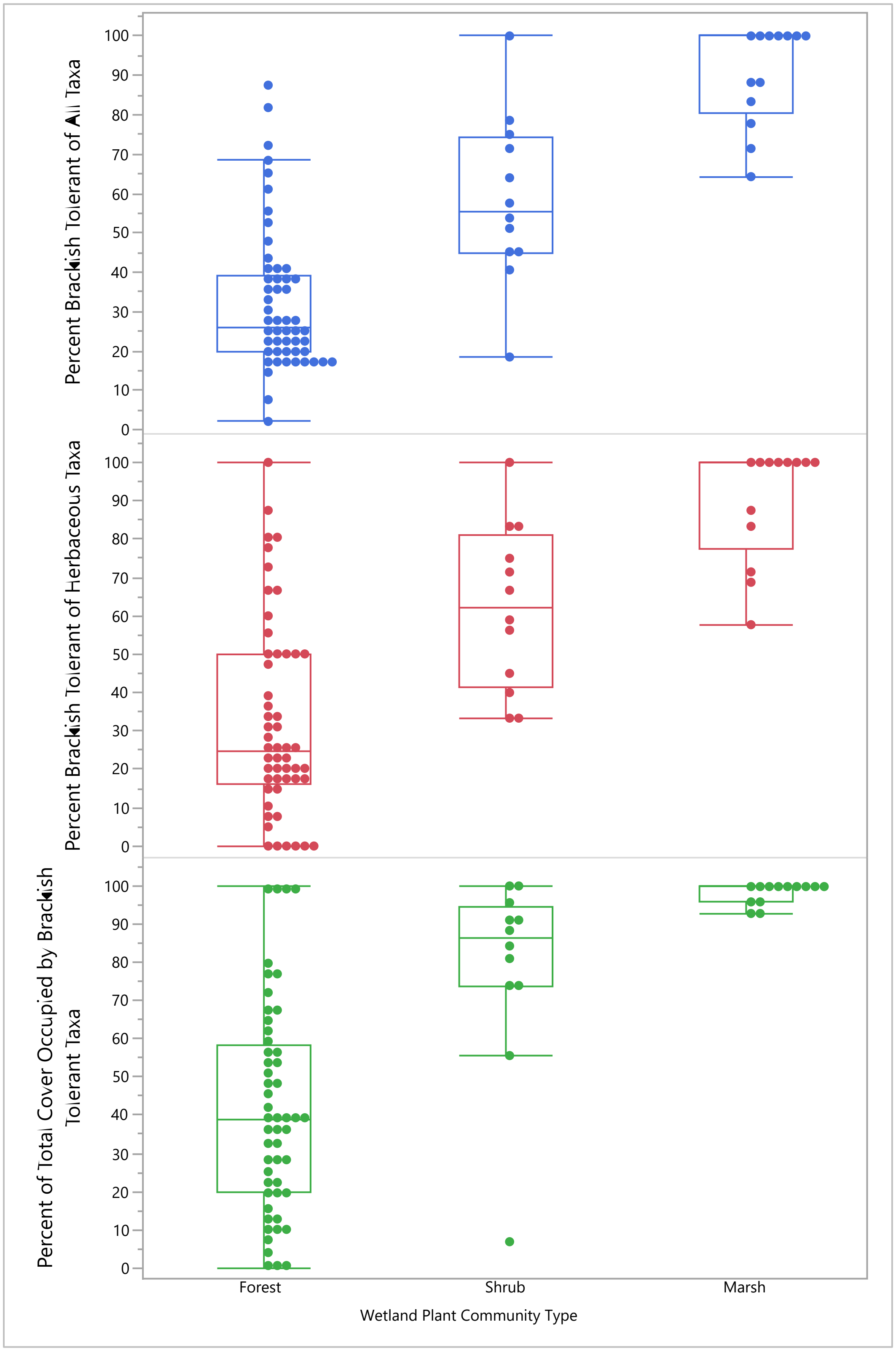


Figure 26. Descriptive boxplots for brackish tolerance metrics by original plant community type. Metrics calculated from last data collection.

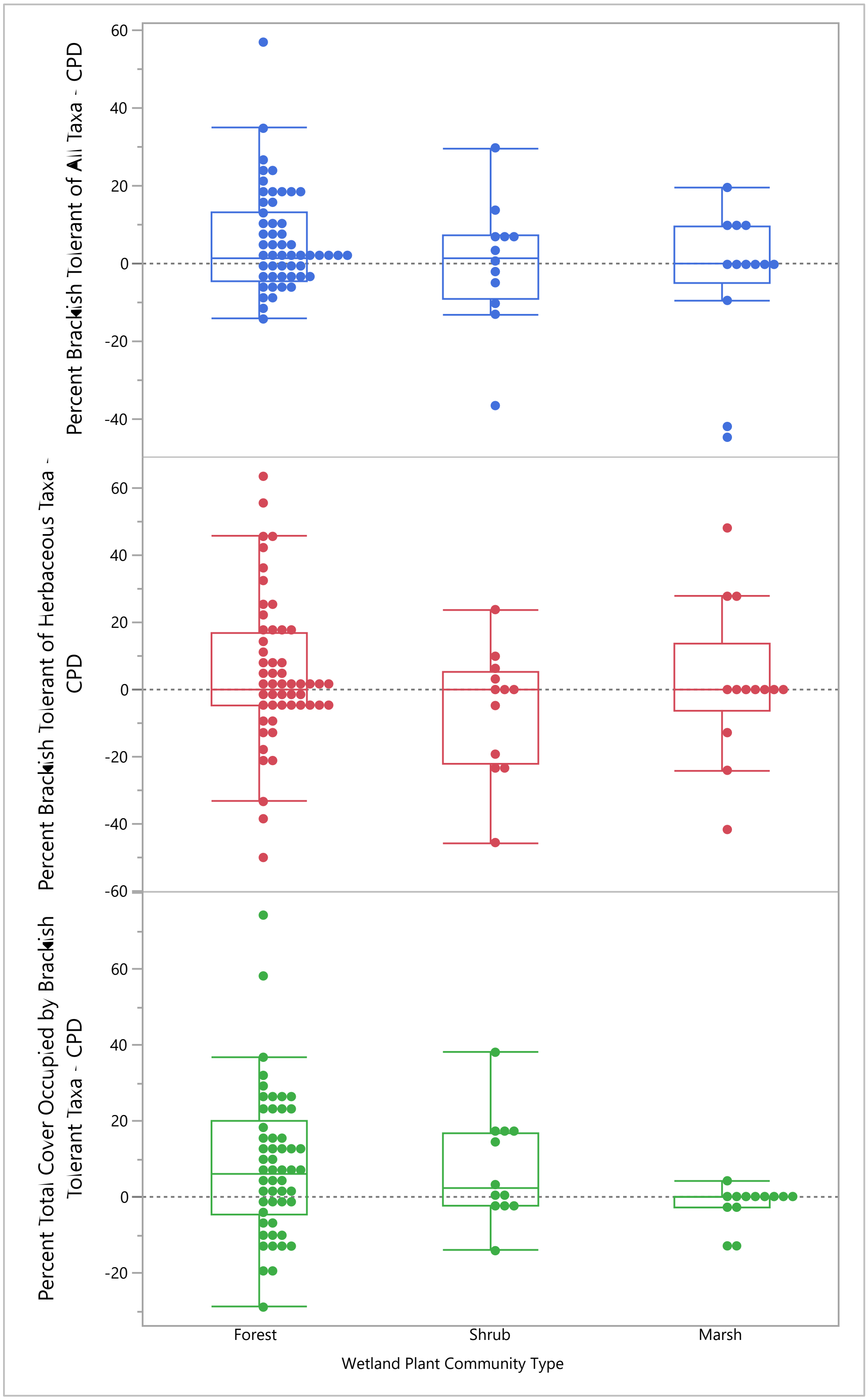


Figure 27. Change per decade (CPD) in brackish tolerance metrics by original wetland plant community type. A value of zero means no change detected between first and last sampling events.

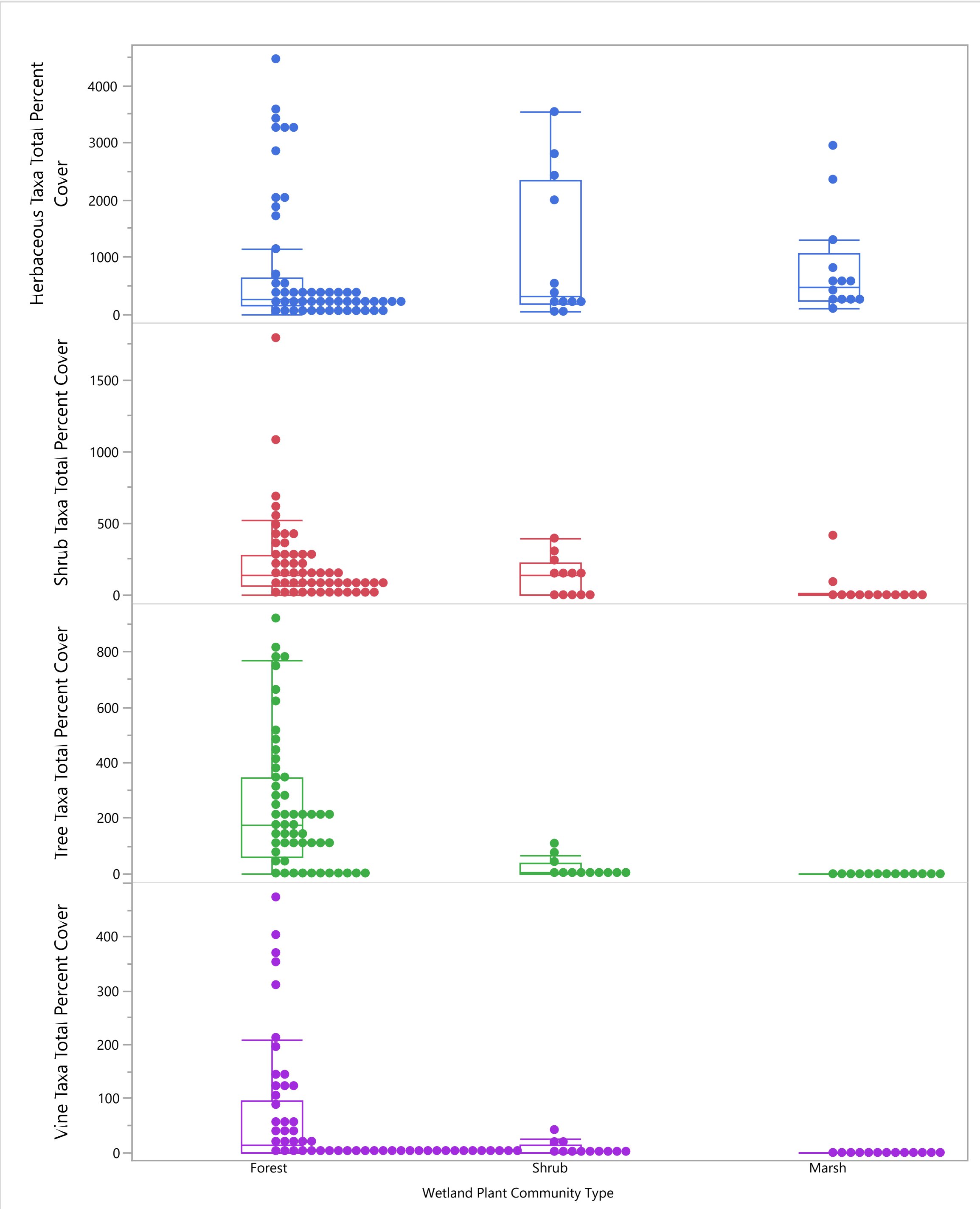


Figure 28. Descriptive boxplots for plant community structure metrics by original plant community type. Metrics calculated from last data collection.

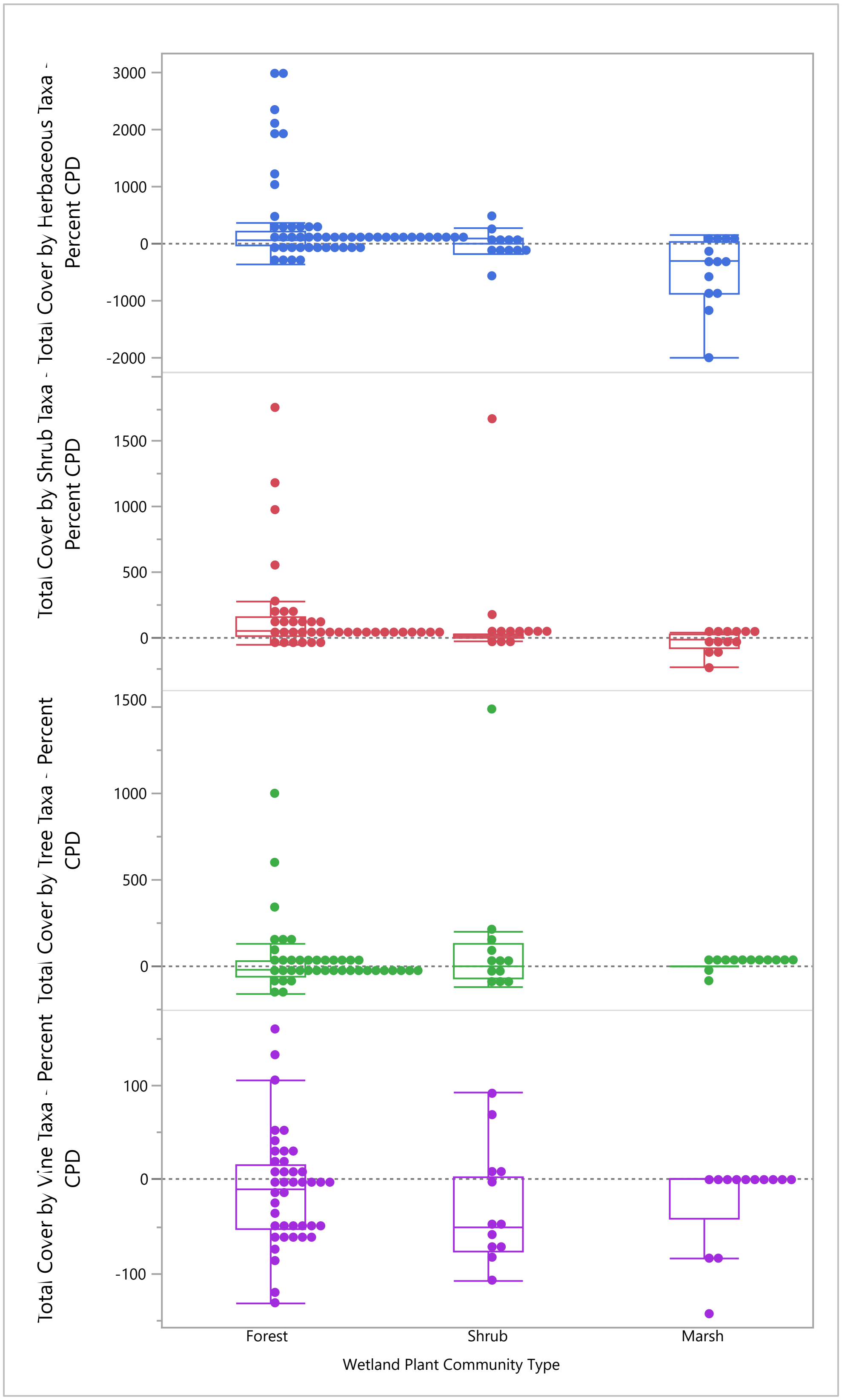


Figure 29. Percent change per decade (CPD) in total cover by plant taxa type. Cover percentages are additive for all taxa. A value of zero means no change between first and last sampling events.

Table 9. Results of Wilcoxon matched pairs tests to detect difference in medians between first and last sampling events in various vegetation metrics, all sites combined. Not significant (ns) means p>.05.

	p value	Direction of Change When Significant
Richness and Diversity		
Richness All	p=.022	Higher in resample
Richness Herbaceous Taxa	p<.001	Higher in resample
Richness Woody Taxa	ns	
Shannon Diversity All	p=.022	Higher in resample
Shannon Diversity Herbaceous Taxa	p=.001	Higher in resample
Shannon Diversity Woody Taxa	ns	
Natives Only		
Native Taxa Richness	p=.043	Higher in resample
Native Taxa Shannon Diversity	p=.029	Higher in resample
Nonnatives		
Percent of Total Cover Occupied by Nonnative Taxa	p=.002	Higher in resample
Percent Nonnative Taxa All	p=.001	Higher in resample
Percent Nonnative Herbaceous Taxa	p=.007	Higher in resample
Floristic Quality		
Mean C All	p=.007	Lower in resample
Mean C Herbaceous Taxa	p=.007	Lower in resample
Mean C Woody Taxa	p=.002	Lower in resample
Floristic Quality All	ns	
Floristic Quality Herbaceous Taxa	p=.010	Higher in resample
Floristic Quality Woody Taxa	p=.029	Lower in resample
Brackish Tolerance and Wetness Affinity Index		
Percent Brackish-Tolerant Taxa of All Taxa	p=.033	Higher in resample
Percent Brackish-Tolerant Taxa of Herbaceous Taxa	ns	
Percent of Total Cover Occupied by Brackish-Tolerant Taxa	p=.005	Higher in resample
Wetness Affinity Index all	ns	
Plant Structure and Biomass		
Woody Basal Area	ns	
Woody Stem Count	ns	
Herbaceous Taxa Cover	p=.019	Higher in resample
Shrub Taxa Cover	ns	
Tree Taxa Cover	p=.006	Lower in resample
Vine Taxa Cover	ns	

Table 10. Results of Wilcoxon matched pairs tests to detect difference in medians between first and last sampling events in various vegetation metrics, by original wetland plant community type. Not significant (ns) means p>0.05.

Vegetation Metric	Forest	Shrub	Marsh	Direction of change where significant
Number of sites*	53	12	13	
Richness and Diversity				
Richness All	ns	ns	ns	
Richness Herbaceous Taxa	p<.001	ns	ns	Higher in resample
Richness Woody Taxa	ns	ns	ns	
Shannon Diversity All	p=.030	ns	ns	Higher in resample
Shannon Diversity Herbaceous Taxa	p=.003	ns	p=.040	Higher in resample
Shannon Diversity Woody Taxa	ns	ns	ns	
Natives Only				
Native Taxa Richness	ns	ns	ns	
Native Taxa Shannon Diversity	ns	ns	ns	
Nonnatives				
Percent of Total Cover Occupied by Nonnative Taxa	p=.027	ns	ns	Higher in resample
Percent Nonnative Taxa All	p=.003	p=.031	ns	Higher in resample
Percent Nonnative Herbaceous Taxa	p=.038	p=.039	ns	Higher in resample
Floristic Quality				
Mean C All	p=.001	ns	ns	Lower in resample
Mean C Herbaceous Taxa	p=.005	ns	ns	Lower in resample
Mean C Woody Taxa	p=.001	ns	ns	Lower in resample
Floristic Quality All	ns	ns	ns	
Floristic Quality Herbaceous Taxa	p=.016	ns	p=.047	Higher in resample
Floristic Quality Woody Taxa	ns	ns	ns	
Brackish Tolerance and Wetness Affinity Index				
Percent Brackish-Tolerant of All Taxa	p=.006	ns	ns	Higher in resample
Percent Brackish-Tolerant of Herbaceous Taxa	ns	ns	ns	
Percent of Total Cover Occupied by Brackish-Tolerant Taxa	p=.004	ns	ns	Higher in resample
Wetness Affinity Index All	ns	ns	ns	
Plant Structure and Biomass				
Woody Basal Area	ns	ns	ns	
Woody Stem Count	ns	ns	ns	
Total Herbaceous Taxa Cover	p=.003	ns	p=.005	Higher in forest site resample; lower in marsh site resample
Total Shrub Taxa Cover	p=.039	ns	ns	Lower in resample
Total Tree Taxa Cover	p=.004	ns	ns	Lower in resample
Total Vine Taxa Cover	ns	p=.034	ns	Higher in resample

*Woody basal area and woody stem count metrics were from 41 forested, 12 shrub and 13 marsh sites.

Table 11. Results of Wilcoxon matched pairs tests to detect difference in medians between first and last sampling events in various vegetation metrics, by salinity. Not significant (ns) means p>0.05.

Vegetation Metric	Freshwater	Transitional	Brackish	Direction of change where significant
Number of sites*	40	19	19	
Richness and Diversity				
Richness All	p=.020	ns	ns	Higher in resample
Richness Herbaceous Taxa	p=.001	ns	ns	Higher in resample
Richness Woody Taxa	ns	p=.017	ns	Lower in resample
Shannon Diversity All	p=.009	ns	ns	Higher in resample
Shannon Diversity Herbaceous Taxa	p=.003	ns	ns	Higher in resample
Shannon Diversity Woody Taxa	ns	p=.048	ns	Lower in resample
Natives Only				
Native Taxa Richness	p=.023	ns	ns	Higher in resample
Native Taxa Shannon Diversity	p=.021	ns	ns	Higher in resample
Nonnatives				
Percent of Total Cover Occupied by Nonnative Taxa	p=.030	ns	ns	Higher in resample
Percent Nonnative Taxa All	p=.012	p=0.026	ns	Higher in resample
Percent Nonnative Herbaceous Taxa	ns	p=0.027	ns	Higher in resample
Floristic Quality				
Mean C All	p=.007	p=.008	ns	Lower in resample
Mean C Herbaceous Taxa	p=.035	p=.008	ns	Lower in resample
Mean C Woody Taxa	p=.020	p=.005	ns	Lower in resample
Floristic Quality All	p=.019	ns	ns	Higher in resample
Floristic Quality Herbaceous Taxa	p=.004	ns	ns	Higher in resample
Floristic Quality Woody Taxa	ns	p=.003	ns	Lower in resample
Brackish Tolerance and Wetness Affinity Index				
Percent Brackish-Tolerant of All Taxa	ns	ns	ns	
Percent Brackish-Tolerant of Herbaceous Taxa	ns	ns	ns	
Percent of Total Cover Occupied by Brackish-Tolerant Taxa	p=.040	ns	ns	Higher in resample
Wetness Affinity Index All	ns	ns	ns	
Plant Structure and Biomass				
Woody Basal Area	ns	ns	ns	
Woody Stem Count	ns	ns	ns	
Total Herbaceous Taxa Cover	p=.005	ns	p=.004	Higher in forest site resample; lower in marsh site resample
Total Shrub Taxa Cover	ns	ns	ns	
Total Tree Taxa Cover	ns	p=.025		Lower in resample
Total Vine Taxa Cover	ns	ns	ns	

*Woody basal area and woody stem count metrics were from 30 freshwater, 17 transitional and 19 brackish sites.

Overall, wetland sites in this study slightly gained nonnative species, lost floristic quality, gained brackish-tolerant species and coverage by those species (more often than not), and lost tree cover while gaining herbaceous species (Table 9). Freshwater forested wetlands showed the most changes in plant communities, particularly in terms of greater invasion by nonnative taxa and a decrease in plant community quality (mean C) (Figure 21; Table 10). Shrub and marsh wetlands appeared to have changed less than forested wetlands. However, shrub wetlands lost floristic community quality, with increases in nonnative taxa and cover (Figure 21; Figure 23; Figure 25).

Freshwater and transitional salinity wetlands changed in more vegetative community aspects than brackish wetlands. Brackish wetlands showed very little change between original sampling and resampling, except that total plant cover decreased significantly (Table 11). Freshwater and transitional salinity wetlands showed greater invasion by nonnative species and corresponding reductions in floristic quality (Table 11). Transitional salinity wetlands also showed a significant decrease in cover by tree taxa over time, in contrast to freshwater forested wetlands.

Most sites (n=55, or 71%) showed only slight increases or decreases in the Wetness Affinity Index (changes +/- 0.25 units of zero per decade) (Figure 23; Table 9; Table 10). Of the remaining 23 sites with larger index changes per decade, 12 sites showed increases greater than 0.25 units per decade (mean increase of 0.62 units), indicating plant community shifts toward species that can tolerate wetter conditions. Eleven (11) additional sites showed decreases of more than 0.25 units per decade (mean decrease of 0.48 units), indicating plant community shifts toward species that require drier conditions. Variation in the Wetness Affinity Index CPD in shrub and marsh wetlands was large, with some sites showing substantial increases in proportions of species with an affinity for wetness (Figure 23). Across all sites, median change in the Wetness Affinity Index between original sampling and resampling was zero.

Sites with the largest rates of increase in cover by nonnative species were concentrated in the Pamlico River area (Figure 30). Changes per decade in overall taxa richness were mapped for forested and shrub wetlands (brackish marsh sites by their nature had very low taxa richness to begin with) and most sites showed stable or increasing taxa richness over time (Figure 31). However, the main change was increasing herbaceous species in forests (Figure 17). Sites with the most extreme drops in taxa richness were on the Albemarle Pamlico Peninsula and in the Beaufort area, east of Havelock. Spatial patterns in floristic quality changes (mean C) were similar to those with taxa richness, with sites experiencing the most rapid decreases in mean C in the Albemarle Pamlico Peninsula, in the upper Neuse River area and east of Havelock (Figure 32).

Mapping trends in percent brackish-tolerant taxa showed increases in sites on the eastern and southern sides of the Albemarle-Pamlico Peninsula, as well as along the Pamlico River (Figure 33). Mapping trends in total tree taxa cover indicated a decrease all around the edges of the Albemarle-Pamlico Peninsula, as well as near the south end of the Pamlico Sound (Figure 34). Of the 66 forested or shrub wetlands with tree cover data, just under 20% (13) lost more than 50% of their tree taxa cover between first and last sampling. Five (5) of these experienced a greater than 90%

reduction in their tree cover (EASTLK, BRTTBY, LSFOR, STHRIV, SEES4). Four (4) sites, or 6%, gained more than 50% tree taxa cover between sampling events (GSCRKS, WSEES3, AYDLTT, BRCCRK). Sites with the greatest loss in tree taxa cover were located around the outer coastal edges of the study area, especially in the Albemarle-Pamlico Peninsula, with one in the Outer Banks and one west along the Pamlico River (Figure 35). Where historical photographs are available, the visible changes are dramatic (Figure 36).

Freshwater forested wetlands showed fairly consistent recruitment of new individuals of woody species between original and resampling, but the data showed a drop in recruitment between sampling events in transitional salinity forested wetlands (Figure 37). Additionally, larger size classes of trees were represented in the freshwater forested wetlands, but they were missing in the transitional forested wetlands during original sampling and more so during resampling. Total woody basal area per hectare showed a noticeable drop between sampling events in transitional forested wetlands (Figure 38).

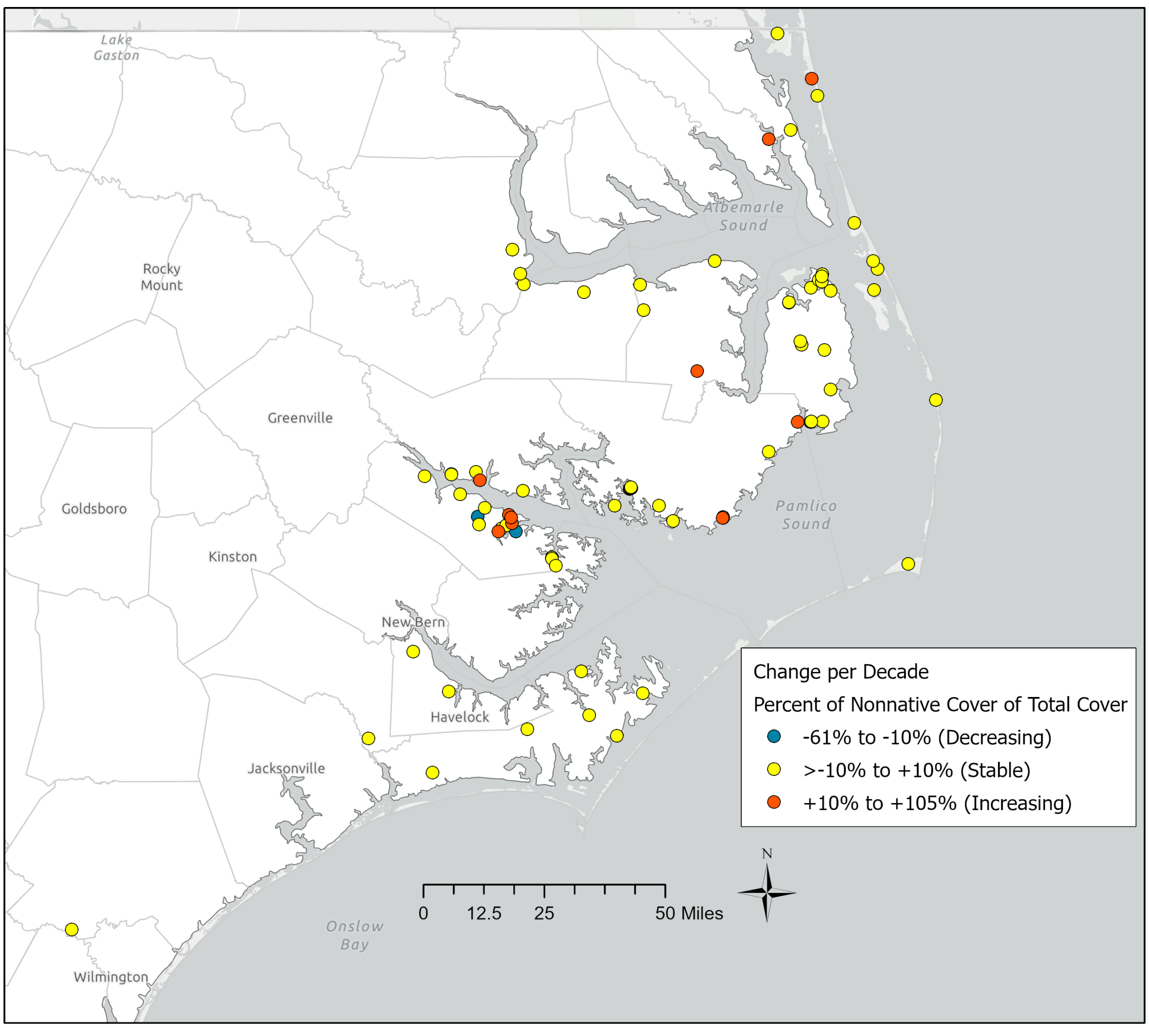


Figure 30. Map of change per decade in percent of total cover occupied by nonnative taxa.

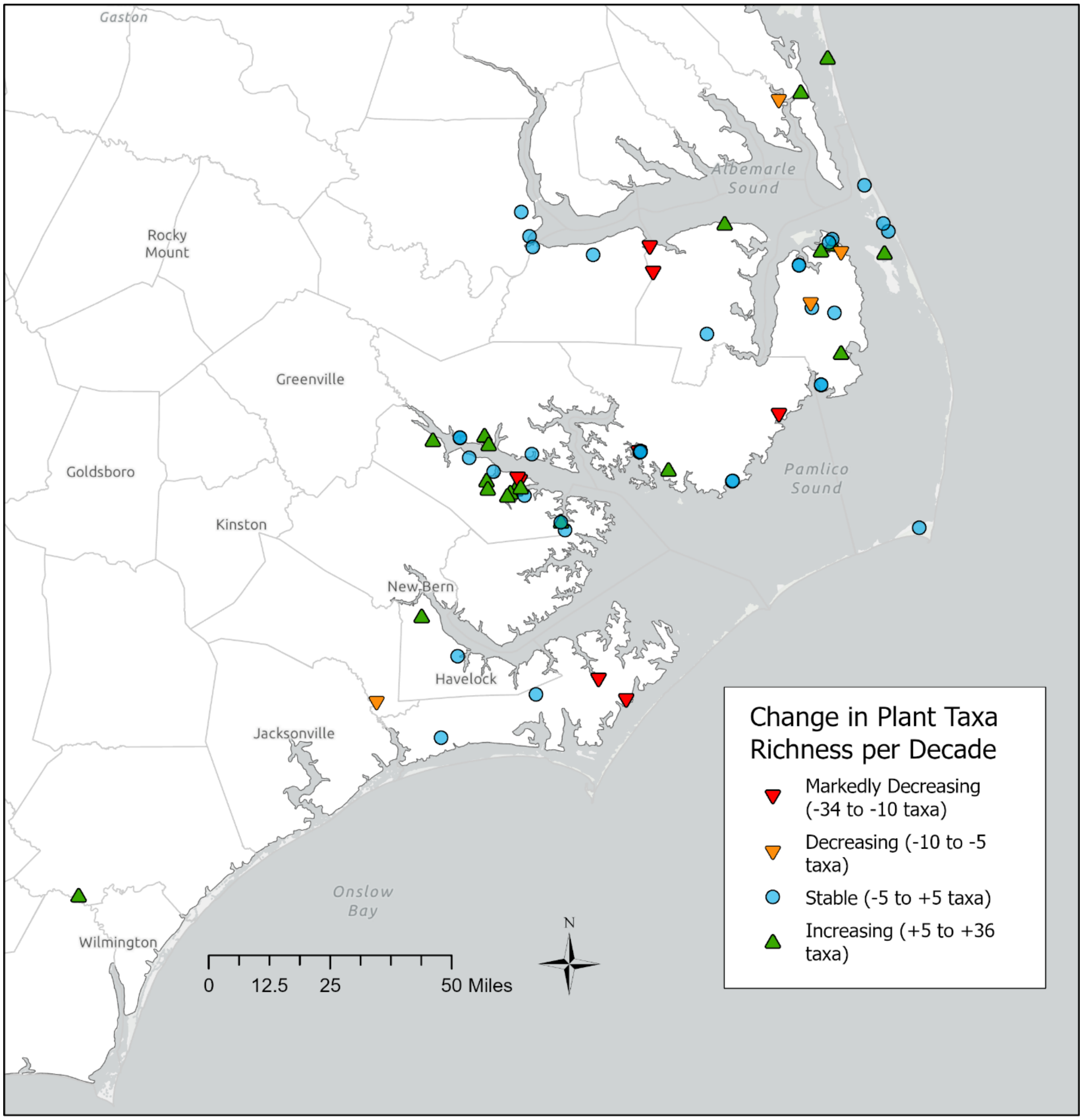


Figure 31. Map of change per decade in plant taxa richness in forested and shrub wetlands.

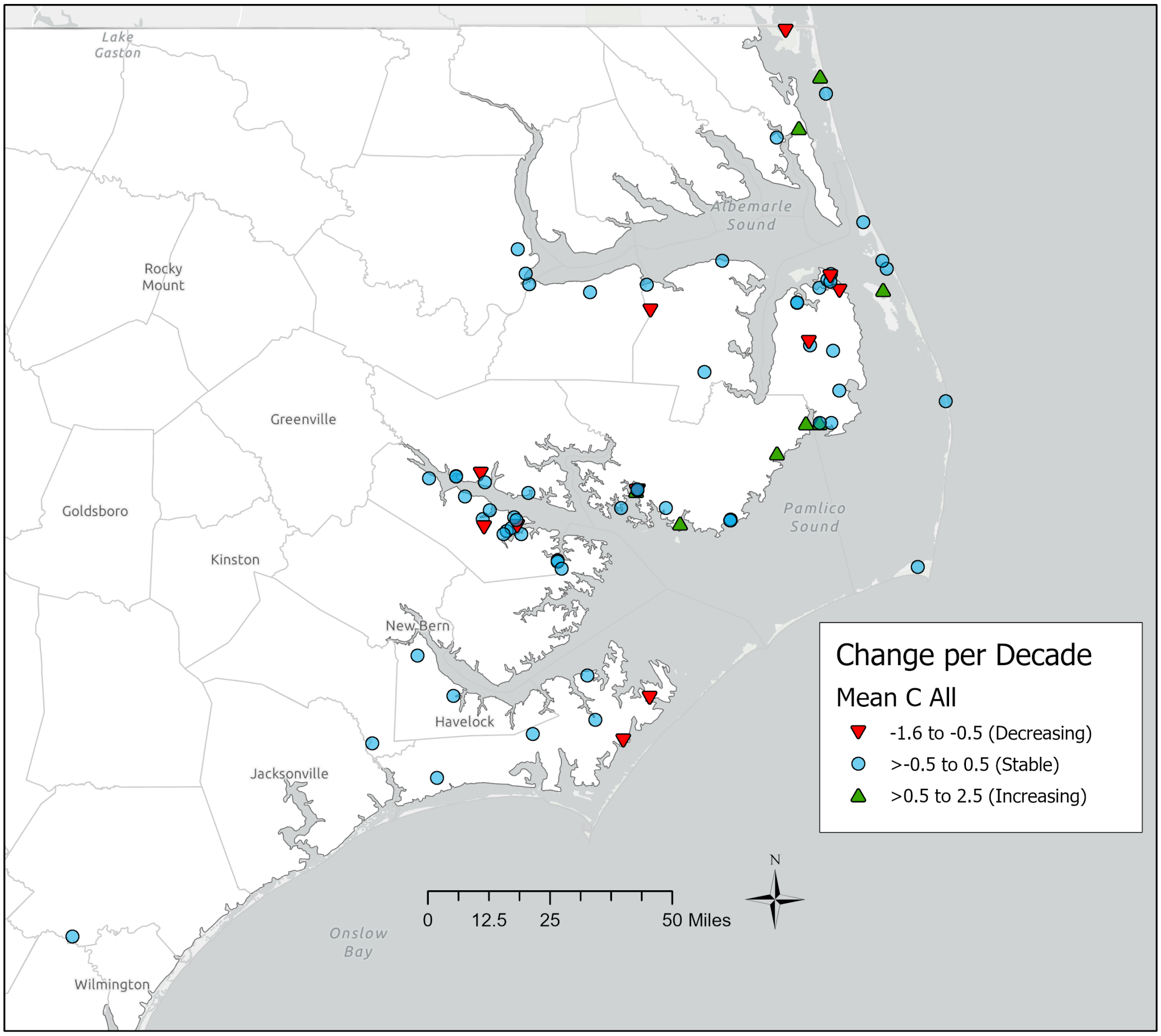


Figure 32. Map of change per decade in mean C value of plant communities.

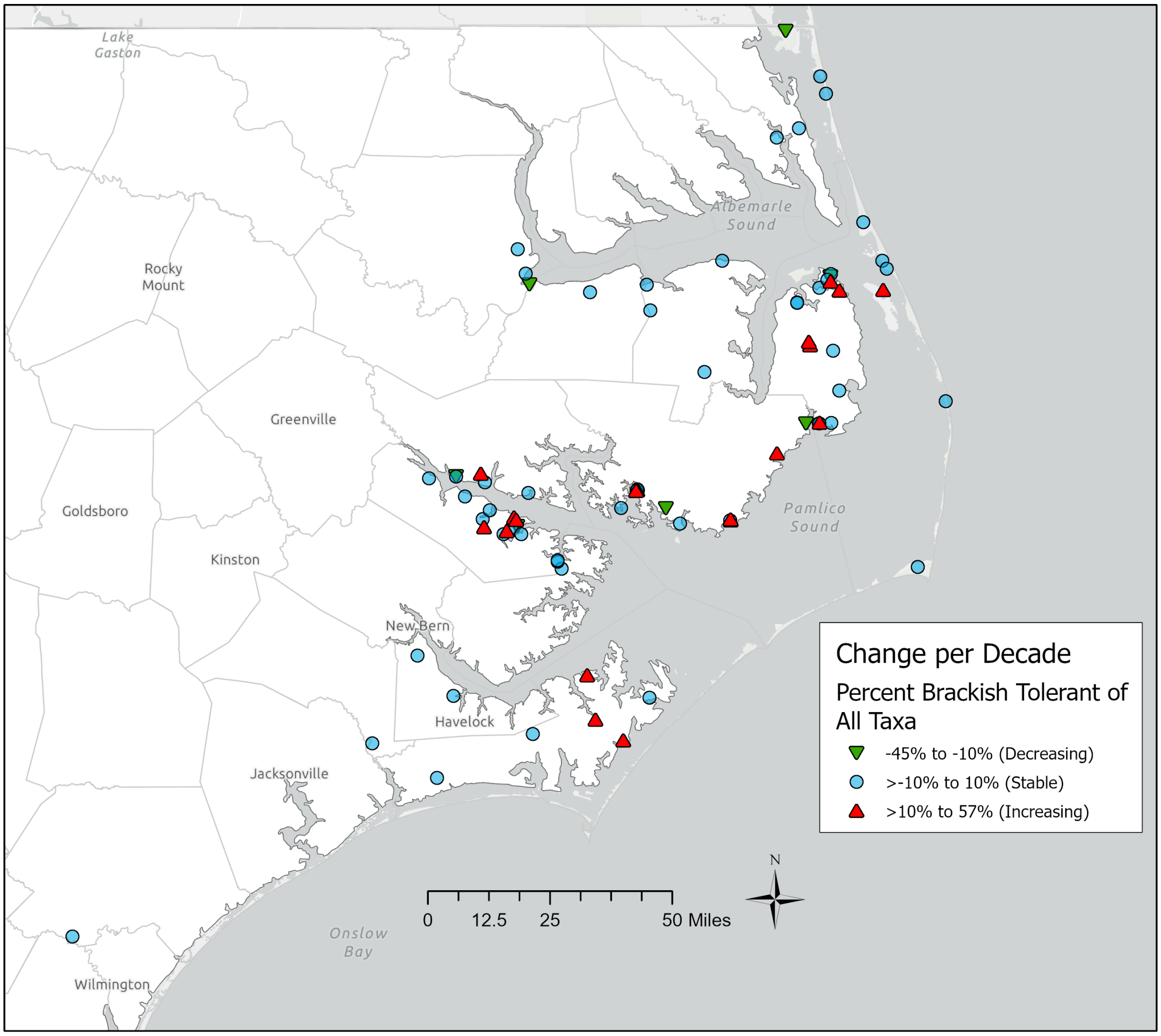


Figure 33. Map of change per decade in percent brackish-tolerant taxa out of all taxa.

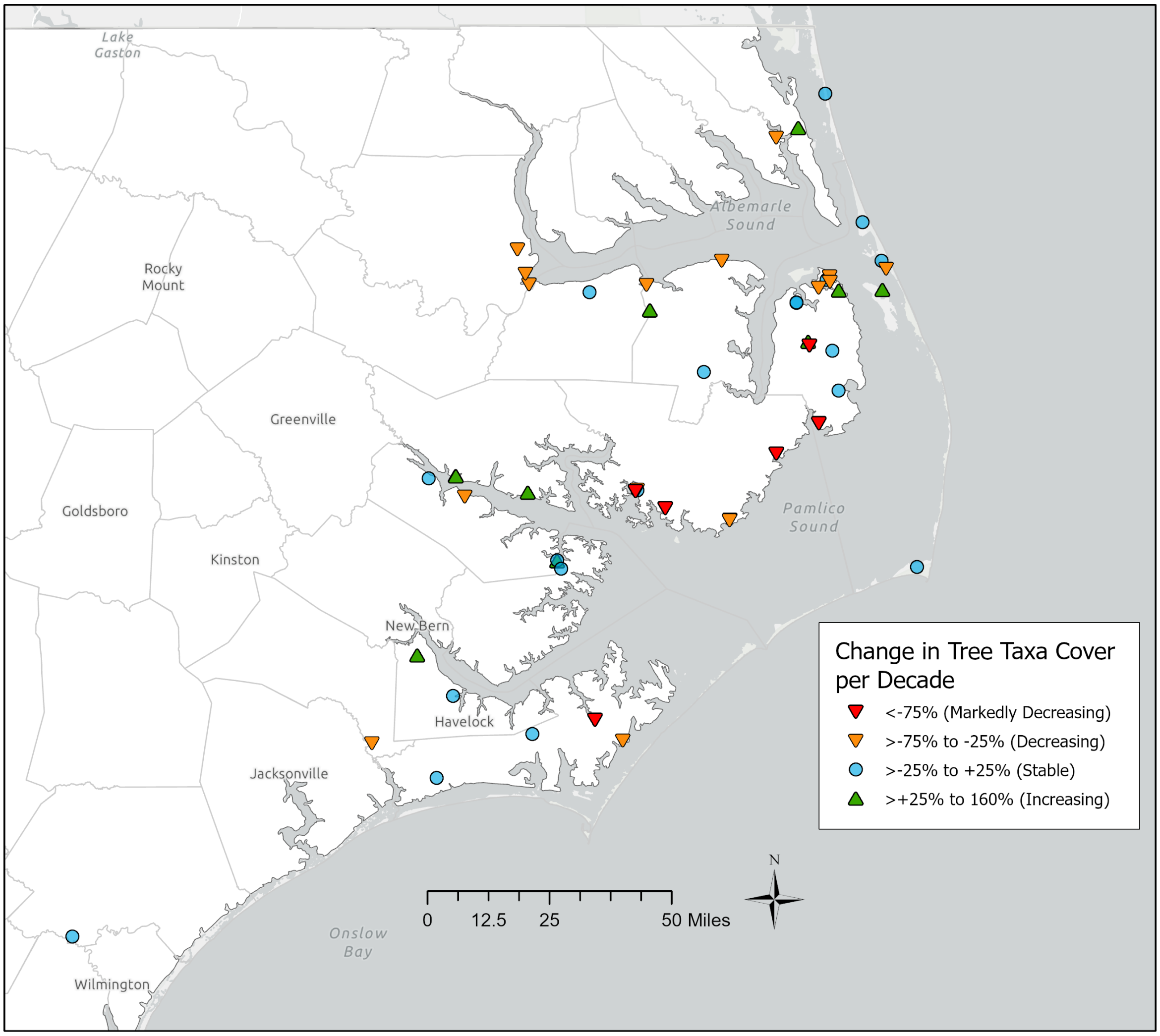


Figure 34. Map of change per decade in total tree taxa cover. Marsh sites excluded and Nutrien sites excluded (no canopy tree data collected).

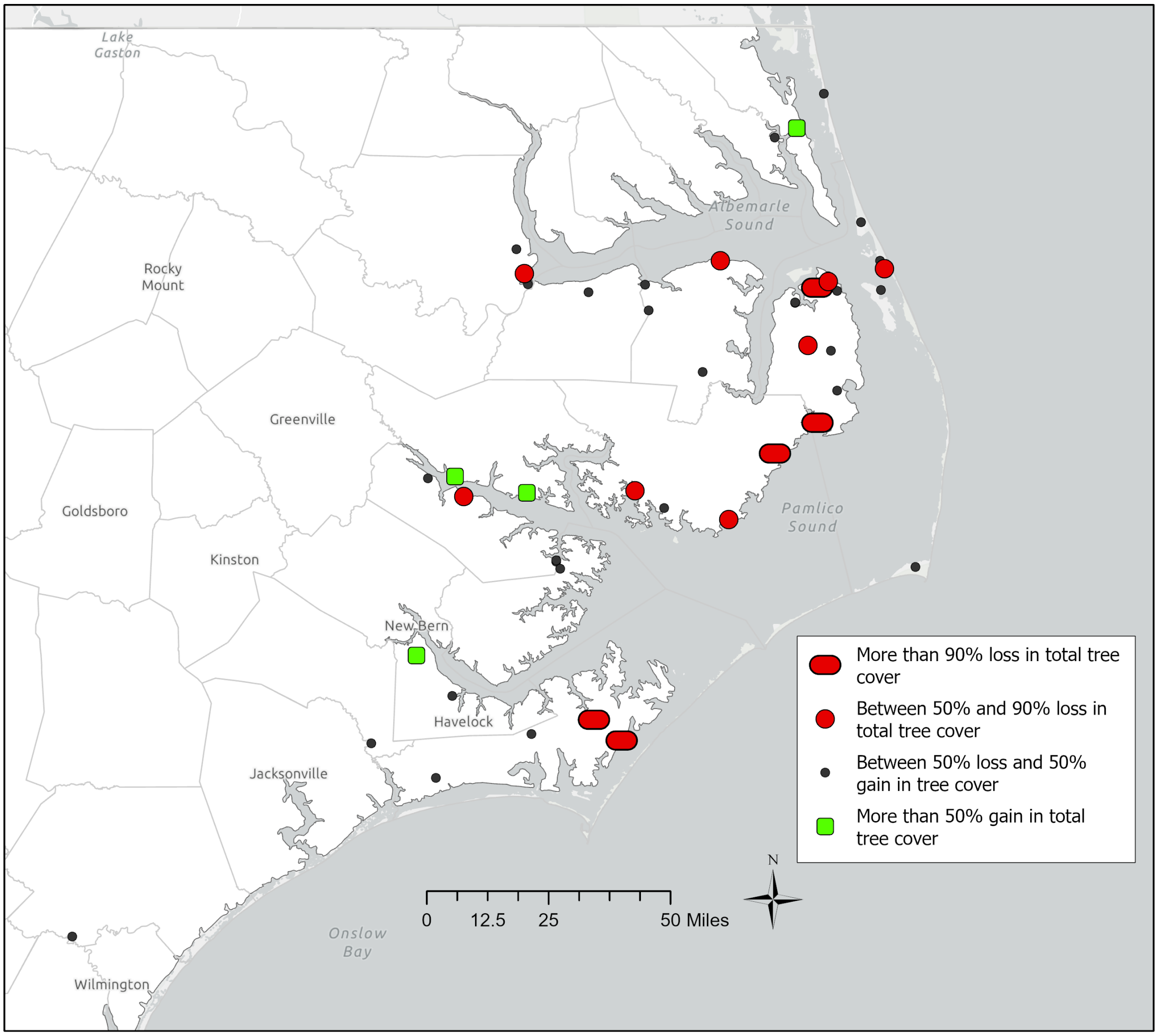
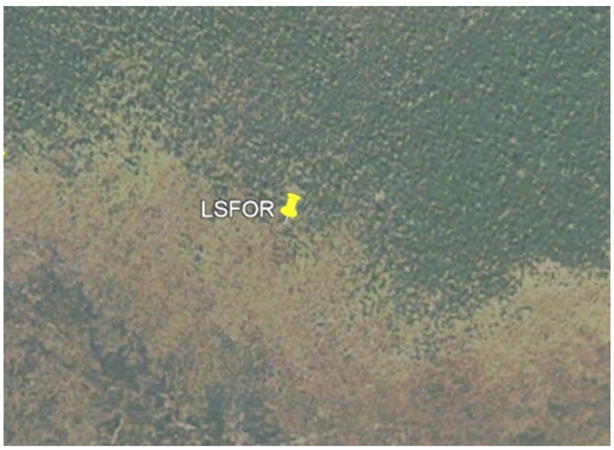


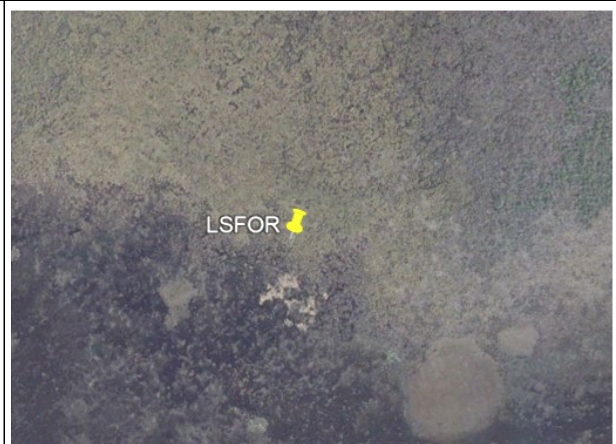
Figure 35. Map of sites with > 50% loss in total tree taxa cover between first and last sampling, sampling interval ranging from 5 to 19 years. Marsh sites excluded and Nutrien partner sites excluded (no canopy tree data collected).

BRTTBY – 2007 (first sampling)

BRTTBY – 2022 (last sampling)



LSFOR – 2006 (sampled 2004)

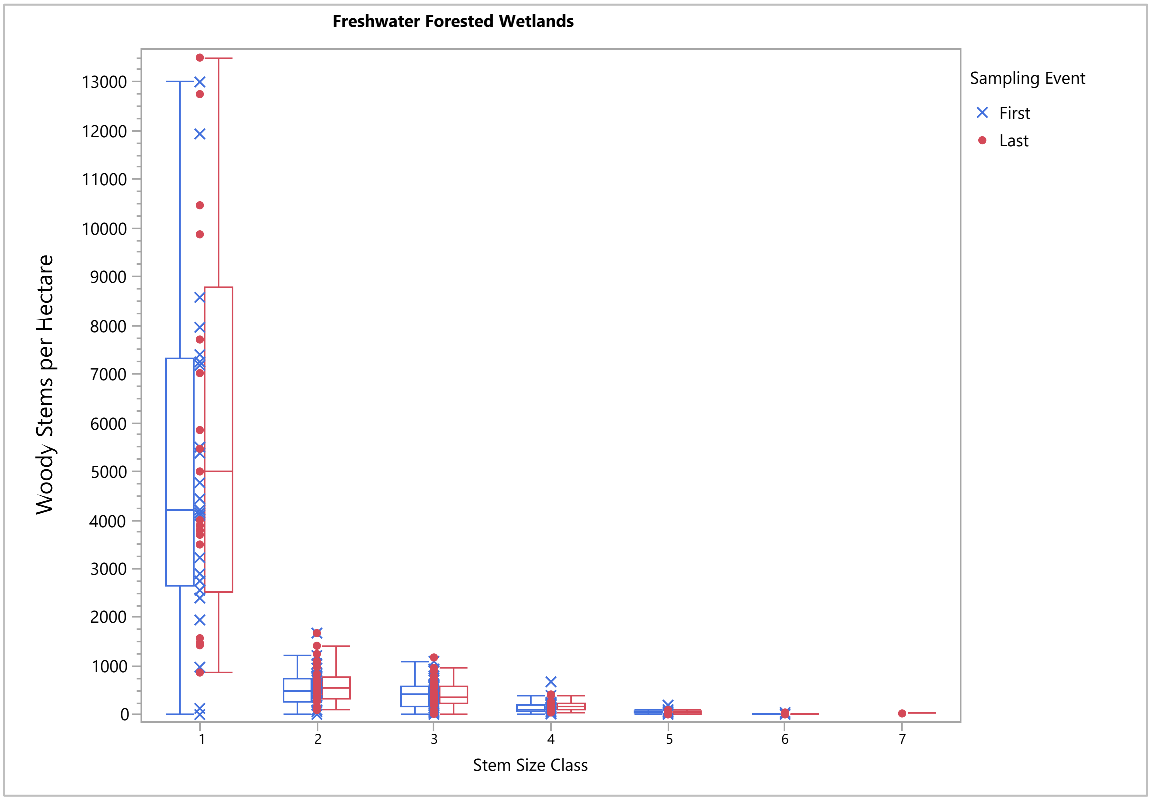


LSFOR – 2017 (sampled 2017)

SEES4 – 2009 (sampled 2009)

SEES4 – 2017 (sampled 2016)

Figure 36. Best available past/present photographs of three of the sites which experienced the greatest loss in tree taxa cover between first and last sampling. Photos taken in same location and orientation.



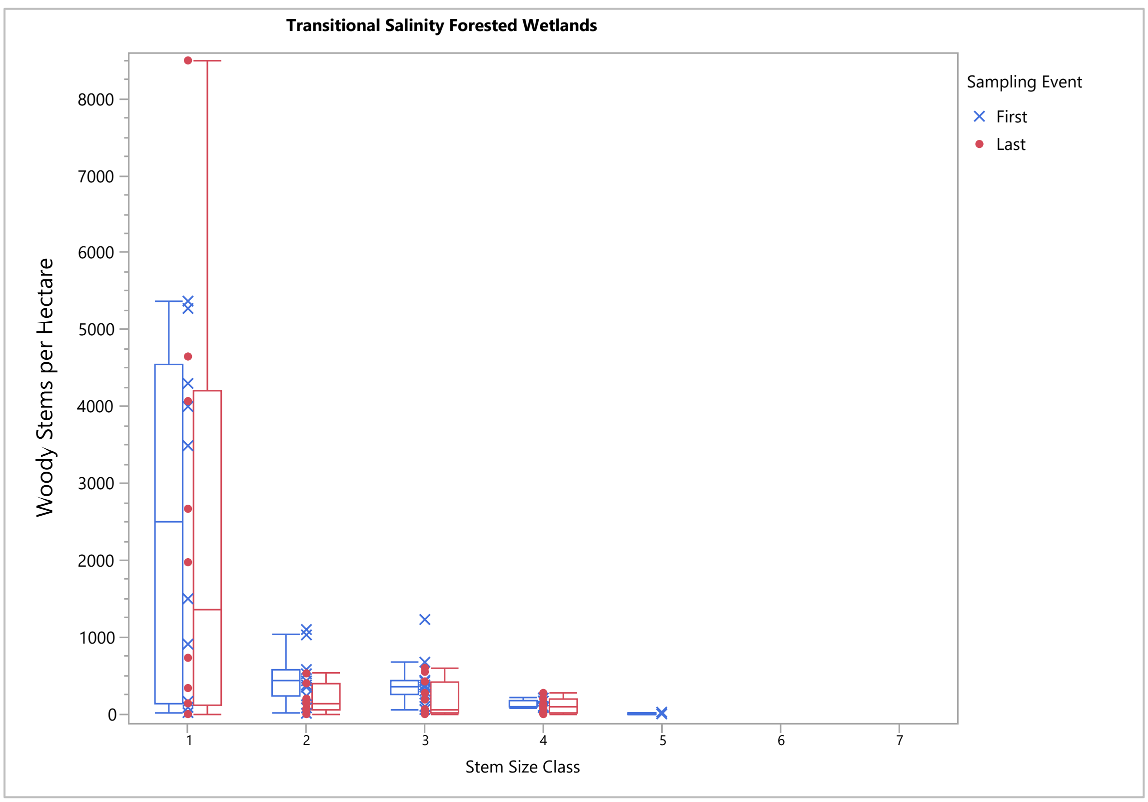


Figure 37. Woody stem size classes by sampling event in forested freshwater and transitional salinity wetlands, plant community at first sampling.

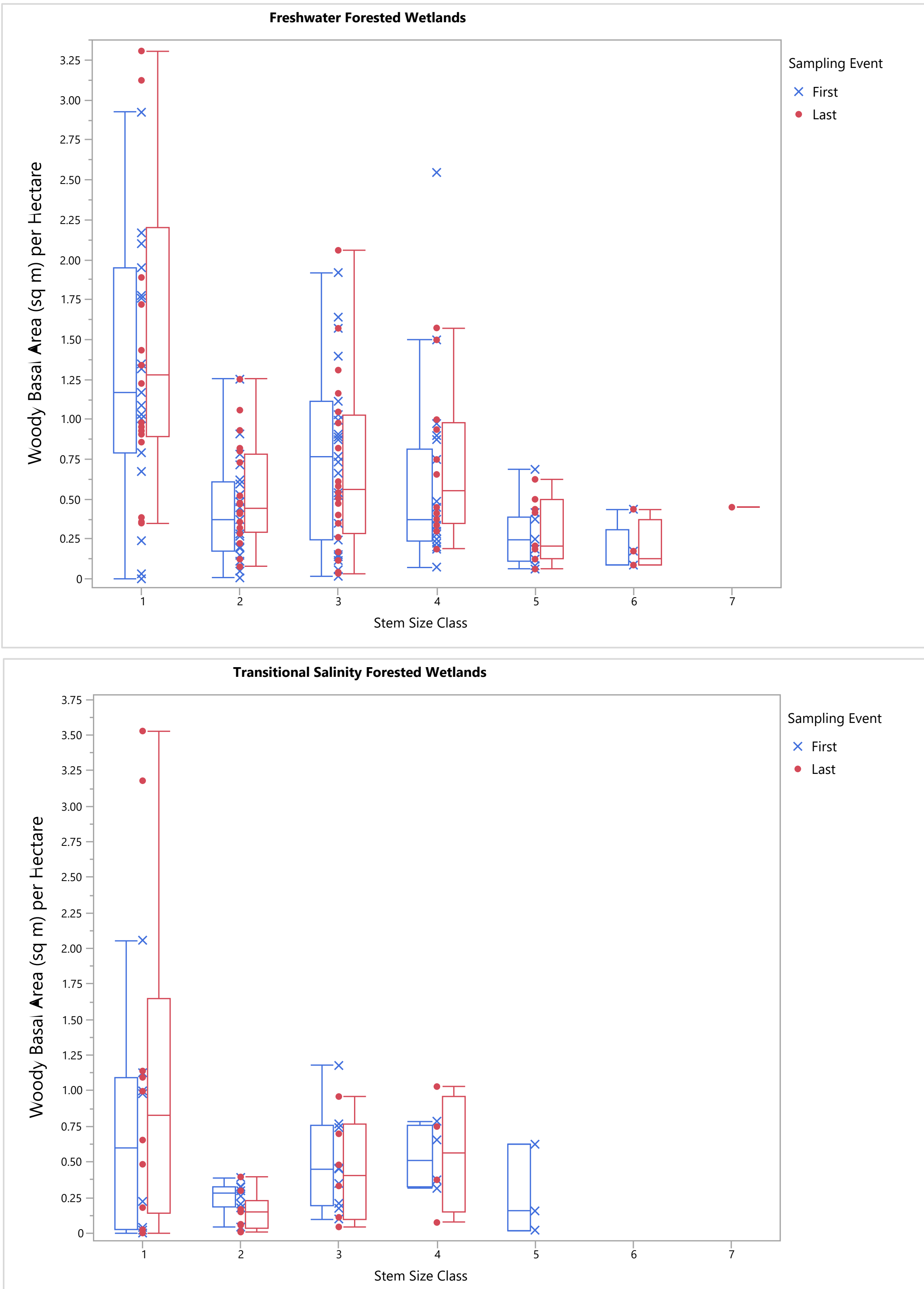


Figure 38. Woody stem basal area per hectare by stem size class and sampling event in forested freshwater and transitional salinity wetlands; plant community at first sampling.

4.3 Multivariate Vegetation Community Analysis Results

NMDS was performed using presence/absence data for all common species across all sites and sampling events (2314 observations). The frequency cutoff for a species to be considered common was > 0.5% of all occurrences, or more than 22 total occurrences across all sites. The result was about 90%, or 483 of the 536 species, being classified as common for the multivariate analyses. Shifts in plant community were inferred from distance between NMDS points from original sampling and resampling, normalized by years between sampling events. The sites with the largest changes in plant community composition tended to be more easterly sites (Figure 39). They are listed in Table 12.

The NMDS on all common species showed the USFWS sites to experience the largest shift in plant community, followed by sites from a variety of sources (Table 12). Two of the of the USFWS sites were freshwater forest wetlands and six were brackish marsh sites. Two of the USFWS brackish marshes had only two and three species observed each sampling event, with only one or two species in common between the two sampling events, so those sites were excluded from Table 12 and Figure 39. Sampling timing and a hurricane could have been factors in producing large changes in species composition. All the USFWS sites (except ALL005) were sampled in late spring (end of April to early May 2013) during original sampling and mid-summer (mid-July to early August 2016) in the resample. Hurricane Arthur made landfall in eastern North Carolina in July 2014 between sampling events, but matched pairs tests on USFWS site resample data from top horizon soil sodium, total exchange capacity (CEC), potassium and Ca+Mg did not show any clear trend.

NMDS was performed a second time using presence/absence data for all woody species (168 species; 2546 observations). The median number of woody species across all site visits was 18 in forested wetlands and 12 in shrub wetlands (maximum across all sites: 44 [HTNCRK 2007]). Most forested and shrub wetland sites did not exhibit large shifts in woody plant communities between sampling events, but those that showed large changes were scattered around the middle (north to south) of the study area (Figure 40).

Table 12. List of top 15 sites with largest changes per year in overall plant species composition (all sites) and woody community composition (non-marsh sites). Rows are colored coded based on whether the Wetness Affinity Index showed an increase or decrease of ≥ 0.25 units per decade.

Site	NMDS Distance Moved per Year (Greatest to Least)	Change in Wetness Affinity Index	Years Between Sampling Events	Source
Presence/Absence Common Species (All Sites)				
MCI026	0.0389	Increased	3	USFWS
CRT026	0.0388	Increased	3	USFWS
ALL030	0.0319	Decreased	3	USFWS
CDR027	0.0287	Decreased	3	USFWS
PNSBAY	0.0208	Slight/None	5	NWCA
RRV013	0.0197	Decreased	3	USFWS
ALLRIV	0.0191	Slight/None	5	DWR-NWCA
BKLKBM	0.0182	Increased	13	DWR-CVS
EASTLK	0.0180	Slight/None	19	DWR-CVS
TLYCRK	0.0176	Slight/None	9	NUTRIEN
MAMAR	0.0173	Slight/None	12	NCSU
SEES2	0.0159	Increased	7	DUKE
SHBGBY	0.0140	Increased	8	DWR-CVS
GRMAR	0.0128	Slight/None	12	NCSU
JKSCRK	0.0128	Slight/None	9	NUTRIEN
Presence/Absence All Woody Species (Non-Marsh Sites)				
EASTLK	0.0106	Slight/None	19	DWR-CVS
SEES4	0.0090	Increased	7	DUKE
BRTTBY	0.0077	Slight/None	15	DWR-CVS
SEES18	0.0074	Decreased	7	DUKE
JCBCRK	0.0070	Slight/None	9	NUTRIEN
TLYCRK	0.0064	Slight/None	9	NUTRIEN
GSCRKN	0.0052	Slight/None	13	DWR-CVS
SEES2	0.0046	Increased	7	DUKE
LSFOR	0.0039	Increased	13	NCSU
SEES13	0.0038	Decreased	13	DUKE
BRCCRK	0.0038	Slight/None	15	DWR-CVS
HDDLWS	0.0037	Increased	8	NUTRIEN
JKSCRK	0.0037	Slight/None	9	NUTRIEN
RRV013	0.0036	Decreased	3	USFWS
HDDLMN	0.0035	Slight/None	9	NUTRIEN

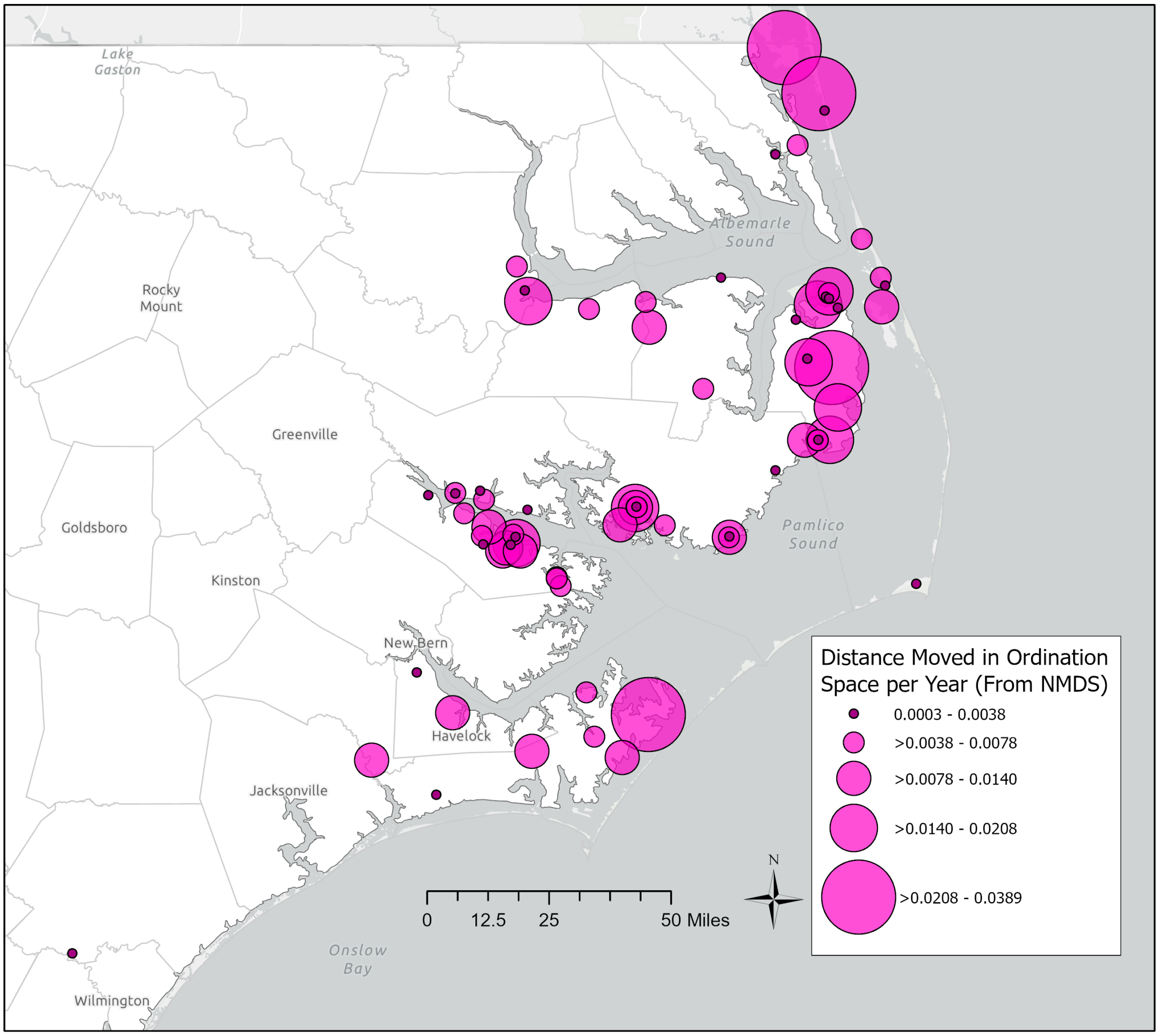


Figure 39. Map of relative change in overall plant community composition per year based on NMDS. Smallest dots represent no appreciable change; larger sized dots represent greater change relative to the rest of the sites.

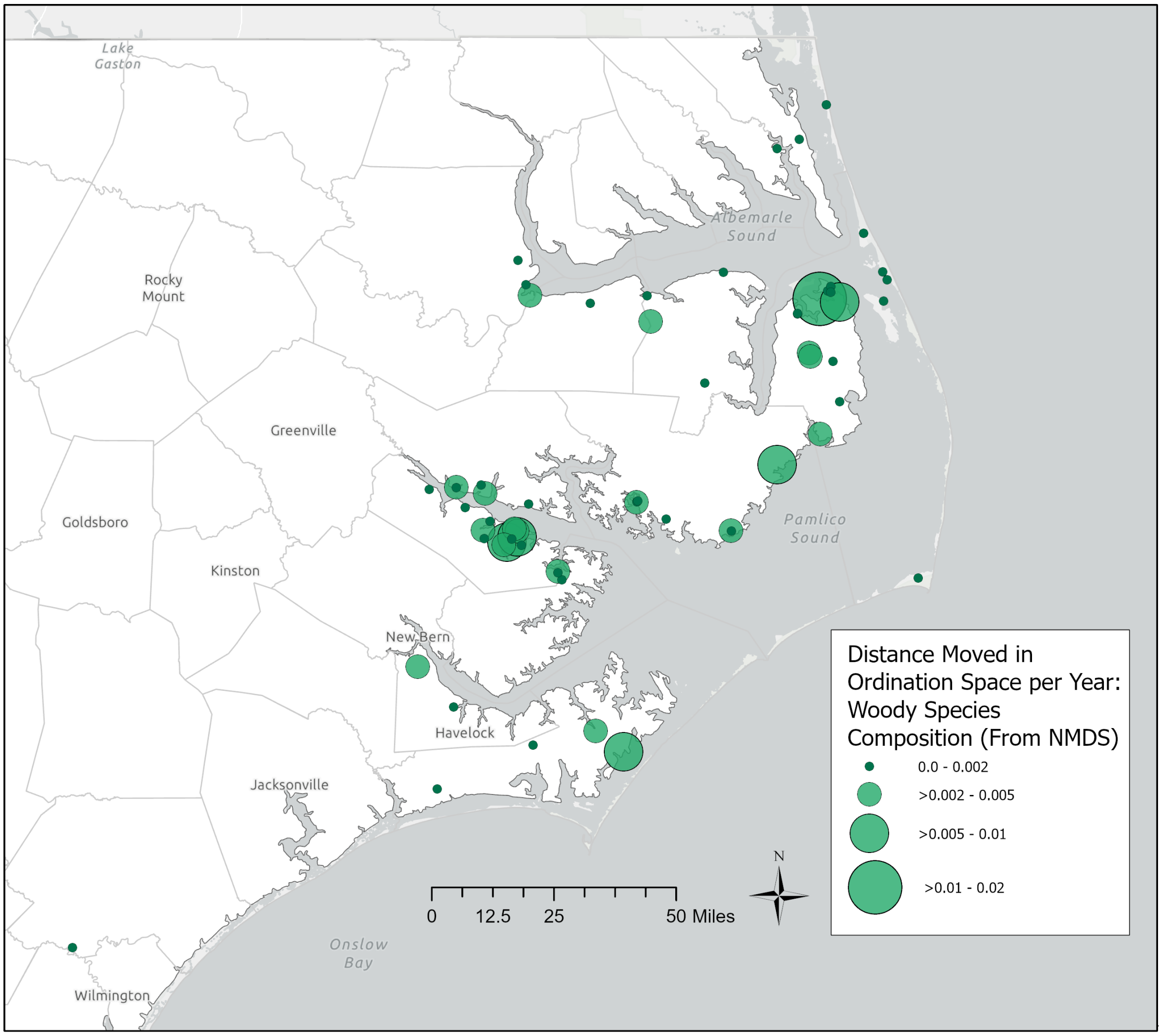


Figure 40. Map of relative change in woody plant community composition per year in forested and shrub wetlands, based on NMDS. Smallest dots represent no appreciable change; larger sized dots represent greater change, relative to the rest of the sites.

4.4 Vegetation Community SIMPER Results

Results of the SIMPER analysis showed that freshwater forest wetland plant community composition was 63% dissimilar/37% similar between original sampling and resampling. A list of the top taxa driving the differences between original sampling and resampling plant communities revealed 15 taxa to be found in increased frequency and 8 taxa found in decreased frequency (Table 13). Oaks (*Quercus* spp.), sedges (*Carex* spp.), sweetgum (*Liquidambar styraciflua*), tupelo (*Nyssa* spp.) and pines (*Pinus* spp.) were the top taxa found in increased frequency in freshwater wetlands, while Virginia creeper (*Parthenocissus quinquefolia*), netted chain-fern (*Woodwardia areolata*), Carolina jessamine (*Gelsemium sempervirens*) and blackberry (*Rubus* spp.) were the major taxa found in decreased frequency across freshwater forested wetland sites. Two top nonnative taxa driving differences in freshwater forested wetland plant communities over time were Japanese honeysuckle (*Lonicera japonica*) and Japanese stiltgrass (*Microstegium vimineum*), found in increased frequency across sites.

Transitional salinity wetlands showed comparable rates of dissimilarity in plant community composition between first and last sampling events (67%). Most of the top taxa found in increased frequency were brackish-tolerant, including sawgrass (*Cladium jamaicense*), saltbush (*Baccharis halimifolia*), and narrowleaf and broadleaf cattails (*Typha angustifolia* and *Typha latifolia*) (Table 14). Compared to freshwater forest wetlands, a much greater number of top taxa were found in decreased frequency across transitional salinity sites between original sampling and resampling. These included red maple (*Acer rubrum*), hollies (*Ilex* spp.), blueberry (*Vaccinium* spp.), oaks (*Quercus* spp.), tupelo (*Nyssa* spp.), sweetbay magnolia (*Magnolia virginiana*) and others.

Brackish wetlands were 69% dissimilar in plant communities between original and resampling events. Non-woody taxa showed increased frequency across sites, including seashore saltgrass (*Distichlis spicata*), the nonnative common reed (*Phragmites australis*), eastern poison ivy (*Toxicodendron radicans*) and southern seaside goldenrod (*Solidago mexicana*) (Table 15). Woody species were in the top taxa showing decreases in frequency across brackish wetland sites, including saltbush (*Baccharis halimifolia*), wax myrtle (*Morella cerifera*), swamp redbay (*Persea palustris*), and loblolly and pond pines (*Pinus taeda* and *Pinus serotina*).

Table 13. List of species driving the main differences in plant community composition between first and last sampling in NC Outer Coastal Plain freshwater wetlands (forested and shrub wetlands), based on presence/absence. Taxa in left hand columns are ranked within their group (Increasing Taxa and Decreasing Taxa) from greatest change in frequency to least change. [B]=brackish tolerant

Freshwater Forested and Shrub Wetlands

Taxa Driving Main Differences Between First and Last Sampling	
Taxa Increasing in Frequency	
Quercus spp. (see list)	
Carex spp. (see list)	
Liquidambar styraciflua	
Nyssa biflora; N. sylvatica; N. aquatica	
Pinus taeda; P. serotina; P. palustris	
Osmunda regalis	Brackish tolerant
Mitchella repens	
Berchemia scandens	
Saururus cernuus	Brackish tolerant
Dichanthelium spp. (see list)	Some spp. brackish tolerant
Osmundastrum cinnamomeum	
Lonicera japonica	
Bignonia capreolata	
Carpinus caroliniana	
Microstegium vimineum	
Taxa Decreasing in Frequency	
Parthenocissus quinquefolia	
Woodwardia areolata	
Gelsemium sempervirens	
Rubus pensilvanicus; R. trivialis; R. hispidus	
Lyonia lucida	
Fraxinus pennsylvanica; F. profunda; F. caroliniana)	
Vaccinium fuscatum; V. formosum; V. crassifolium; V. macrocarpon; V. pallidum; V. stamineum; V. tenellum	
Ilex spp. (see list)	Some spp. brackish tolerant

Carex species
Carex abscondita
Carex amphibola
Carex atlantica
Carex bromoides
Carex comosa
Carex crinita
Carex debilis
Carex elliottii
Carex emmonsii
Carex gigantea
Carex glaucescens
Carex hirsutella (Carex complanata)
Carex intumescens
Carex laevivaginata
Carex lonchocarpa
Carex lupulina
Carex lurida
Carex normalis
Carex oxylepis
Carex seorsa
Carex stipata
Carex striata
Carex stricta
Carex styloflexa
Carex swanii
Carex typhina

Dichanthelium species
Dichanthelium boscii
Dichanthelium clandestinum
Dichanthelium commutatum
Dichanthelium dichotomum
Dichanthelium laxiflorum
Dichanthelium lucidum
Dichanthelium scabriusculum
Dichanthelium scoparium[B]
Dichanthelium sphaerocarpon
Dichanthelium strigosum
***Ilex* species**
Ilex coriacea
Ilex decidua
Ilex glabra
Ilex laevigata
Ilex opaca
Ilex verticillata[B]
Ilex vomitoria[B]
***Quercus* species**
Quercus alba
Quercus falcata
Quercus hemisphaerica
Quercus laurifolia
Quercus lyrata
Quercus michauxii
Quercus nigra
Quercus pagoda
Quercus phellos
Quercus stellata
Quercus velutina
Quercus virginiana

Table 14. List of species driving the main differences in plant community composition between first and last sampling in NC Outer Coastal Plain transitional salinity wetlands, based on presence/absence. Taxa in left hand columns are ranked within their group (Increasing Taxa and Decreasing Taxa) from greatest change in frequency to least change. [B]=brackish tolerant

Transitional Salinity Wetlands			
Taxa Driving Main Differences Between First and Last Sampling		***Carex* species**	***Smilax* species**
Taxa Increasing in Frequency		*Carex abscondita*	*Smilax bona-nox*
Cladium jamaicense	Brackish tolerant	*Carex alata*[B]	*Smilax glauca*
Baccharis halimifolia	Brackish tolerant	*Carex atlantica*	*Smilax laurifolia*
Andropogon virginicus; A. glomeratus[B]	Some spp. brackish tolerant	*Carex bromoides*	*Smilax rotundifolia*
Parthenocissus quinquefolia		*Carex cephalophora*	*Smilax walteri*
Typha angustifolia; T. latifolia	Brackish tolerant	*Carex comosa*	***Vaccinium* species**
Rubus pensilvanicus; R. flagellaris		*Carex debilis*	*Vaccinium corymbosum*
Taxa Decreasing in Frequency		*Carex glaucescens*	*Vaccinium elliottii*
Osmunda regalis	Brackish tolerant	*Carex intumescens*	*Vaccinium formosum*
Acer rubrum		*Carex radiata*	*Vaccinium fuscatum*
Ilex glabra; I. opaca; I. vomitoria[B]*; I. coriacea*	Some spp. brackish tolerant	*Carex stipata*	*Vaccinium macrocarpon*
Hydrocotyle verticillata; H. umbellata		*Carex striata*	*Vaccinium tenellum*
Vaccinium spp. (see list)		*Carex striatula*	
Pinus serotina; P. taeda; P. palustris		*Carex tribuloides*	
Arundinaria tecta		***Quercus* species**	
Woodwardia areaolata		*Quercus falcata*	
Juncus roemerianus[B]*; J. effusus; J. coriaceus; J. marginatus*	Some spp. brackish tolerant	*Quercus hemisphaerica*	
Quercus spp. (see list)		*Quercus laurifolia*	
Nyssa biflora; N. sylvatica		*Quercus lyrata*	
Carex spp. (see list)	Some spp. brackish tolerant	*Quercus nigra*	
Spartina patens; S. cynosuroides	Brackish tolerant	*Quercus phellos*	
Magnolia virginiana		*Quercus velutina*	
Smilax spp. (see list)		*Quercus virginiana*	

Table 15. List of species driving the main differences in plant community composition between first and last sampling in NC Outer Coastal Plain transitional salinity wetlands, based on presence/absence. Taxa in left hand columns are ranked within their group (Increasing Taxa and Decreasing Taxa) from greatest change in frequency to least change.

Brackish Wetlands	
Taxa Driving Main Differences Between First and Last Sampling	
Taxa Increasing in Frequency	
Distichlis spicata	Brackish tolerant
Phragmites australis	Brackish tolerant
Toxicodendron radicans	Brackish tolerant
Solidago mexicana	Brackish tolerant
Taxa Decreasing in Frequency	
Baccharis halimifolia	Brackish tolerant
Morella cerifera	Brackish tolerant
Typha latifolia; T. angustifolia; T. domingensis	Brackish tolerant
Persea palustris	
Pinus taeda; P. serotina	

4.5 Water Quality Results

Water chemistry measurements were obtained during resampling only by DWR for this project, and not by any project partners. Some form of water quality data were available for 28 individual sites in total and from 37 sampling events. In 13 sampling events (from 8 sites), limited water quality data were available for past NWCA sites from 2011 and 2016; historical data were available for certain parameters and not others.

Of the DWR sites, 16 had both surface water and shallow groundwater present at one meter depth, six (6) sites were dry at the surface but had groundwater, and two (2) sites had sampleable surface water in puddles but no groundwater. Two sites with groundwater had an extremely limited amount present during sampling. At one of these sites, there was not enough groundwater present to get samples for all parameters, so a partial parameter set was selected for lab analysis. At the other of these sites, there was enough water for the water quality meter to obtain pH, salinity and conductivity, but not enough to collect for any lab analysis. The result is some inconsistency in the number of sites for each parameter analyzed, except for pH, salinity and specific conductivity, which were obtained from all water available. Two additional sites were dry with groundwater but during sampling, a heavy rainstorm occurred and made the groundwater unsampleable.

Descriptive plots for the various water quality parameters are provided in Figure 41 and Figure 42. Median pH was more acidic in groundwater than surface water. With the exception of sulfate, medians of all parameters (salinity, specific conductivity, sodium, potassium and bromide) were higher at one meter depth than at surface, but sometimes only slightly.

Wetland water quality data from all water samples (surface and shallow groundwater combined) showed strong positive correlations between salinity (ppt) and bromide, chloride, sodium, potassium and sulfate (shown in Figure 43) as well as specific conductivity, calcium and magnesium (not shown; $p<.0001$ for each correlation). These correlations indicate seawater as the origin of salinity in the sampled wetlands.

Surface water salinity was predictive of shallow groundwater salinity at one meter depth ($r^2=0.97$; $p<.0001$) (Figure 44). The relationship was not quite 1:1, with shallow groundwater having slightly lower salinity than surface water across the sites. Shallow groundwater salinity was also highly correlated with other properties of surface water: sodium ($r=.98$), sulfate ($r=.99$), bromide ($r=.95$) and specific conductivity ($r=.98$) ($p<.0001$; N=18 for all).

Across all sites with water quality data, surface water salinity ranged from 0.05 to 17.52 ppt, and shallow groundwater salinity ranged from 0.02 to 13.26 ppt. Those with the highest water salinity were clustered in the Pamlico River and Neuse River areas (Figure 45).

To determine which sites had been affected by seawater, chloride ion concentrations were assessed. In samples from surface water and/or groundwater from 30 samples, chloride was significantly positively correlated with bromide, calcium, potassium, sodium, magnesium and sulfate, indicating a common source of seawater for the chloride (F test; $p<.0001$ for all). Water samples with a chloride ion concentration above the average for freshwater streams and lakes (100 mg/L chloride typical maximum) were considered to be affected by seawater (Goldman and Horne 1983; Essink 1999; Konikow and Reilly 1999; Melloul and Zeitoun 1999; Kelly 2005).

Two-thirds of project sites with water chloride concentration data (67%; 21 of 31 sites) were deemed affected by seawater, based on the chloride ion concentrations (Figure 46). These included eight (8) freshwater forested wetlands, five (5) transitional salinity forested wetlands, three (3) transitional salinity shrub wetlands, two (2) brackish shrub wetlands and three (3) brackish marshes. Sites not in that group ("not affected by seawater") were primarily freshwater forested wetlands (8 sites) along with two (2) transitional salinity forested wetlands.

Sites that were considered affected by seawater and those that were not showed a marked difference in salinities in both surface water and groundwater (Wilcoxon test: $p=<.0001$) (Figure 47). Median salinity in sites unaffected by seawater was 0.11 ppt in both surface water and groundwater while median salinity in sites affected by seawater was 3.58 and 1.78 ppt in surface water and groundwater, respectively. In sites deemed to be affected by seawater, salinity was always above 0.2 ppt, while salinity was always below 0.2 ppt in sites considered unaffected by seawater. Mapping of wetlands affected by seawater shows them to be scattered along the coastline with two sites further inland, in the Albemarle-Pamlico Peninsula and near New Bern (BRCCRK and FUTRLL, both adjacent to creeks subject to storm surges) (Figure 48).

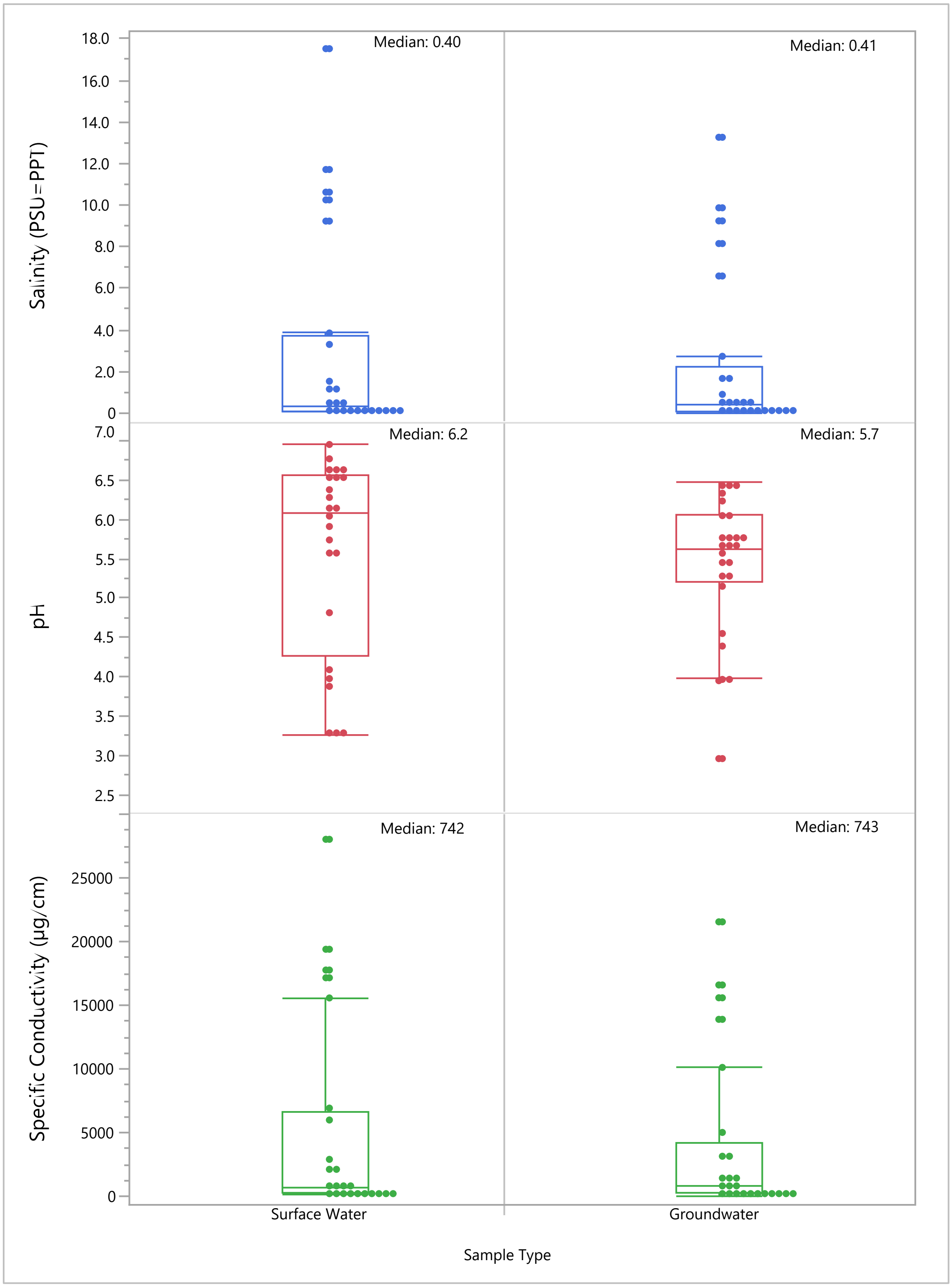


Figure 41. Boxplots for salinity, pH, and specific conductivity for surface water and shallow groundwater (N=25 for each). Some sites had only surface water or groundwater present and water was only collected from DWR and NWCA sites, in 2016, 2021, and 2022.

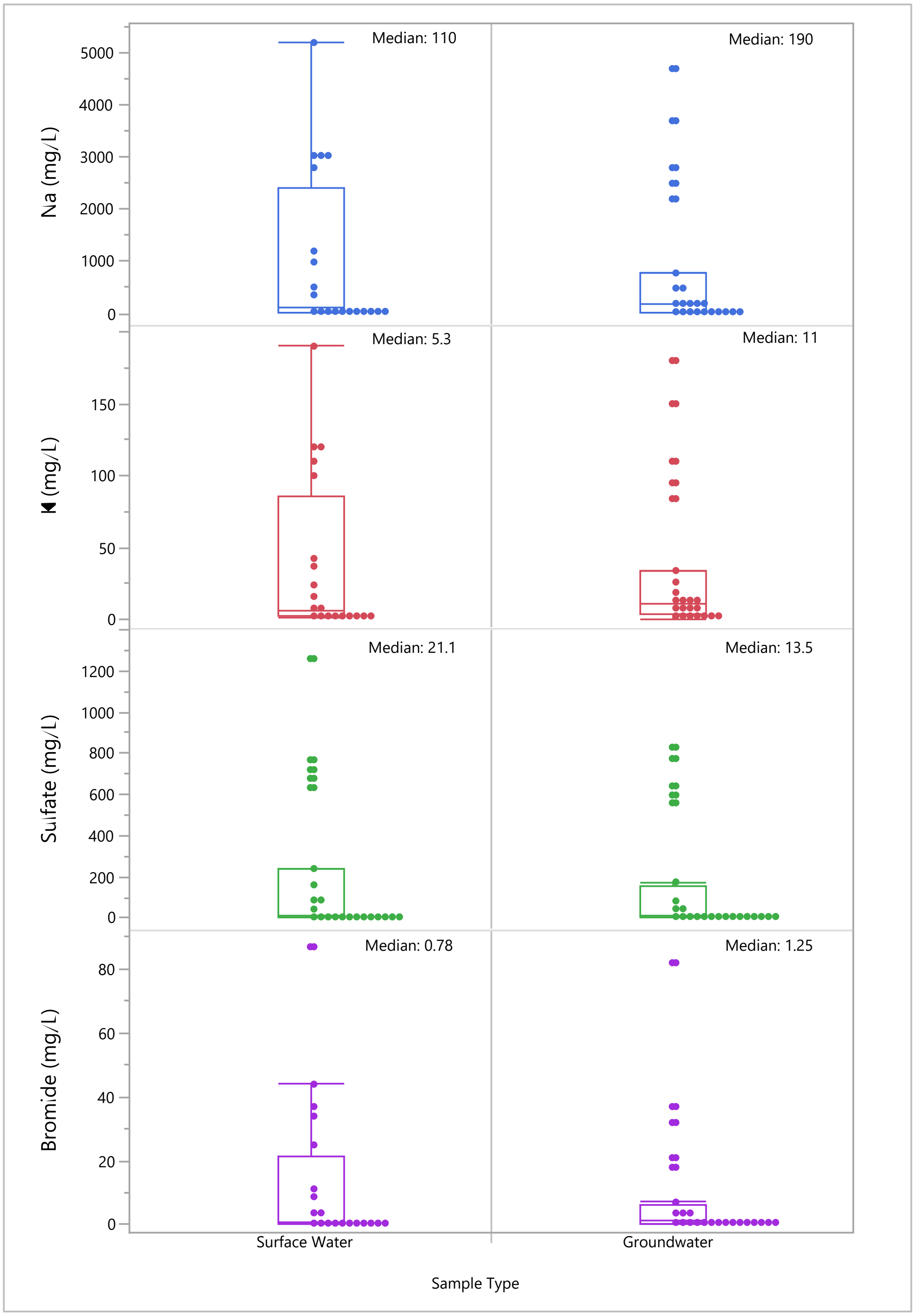


Figure 42. Boxplots for sodium, potassium, sulfate, and bromide in surface water (N=25) and shallow groundwater (N=23). Some sites had only surface water or groundwater present and water was only collected from DWR and NWCA sites, in 2016, 2021, and 2022.

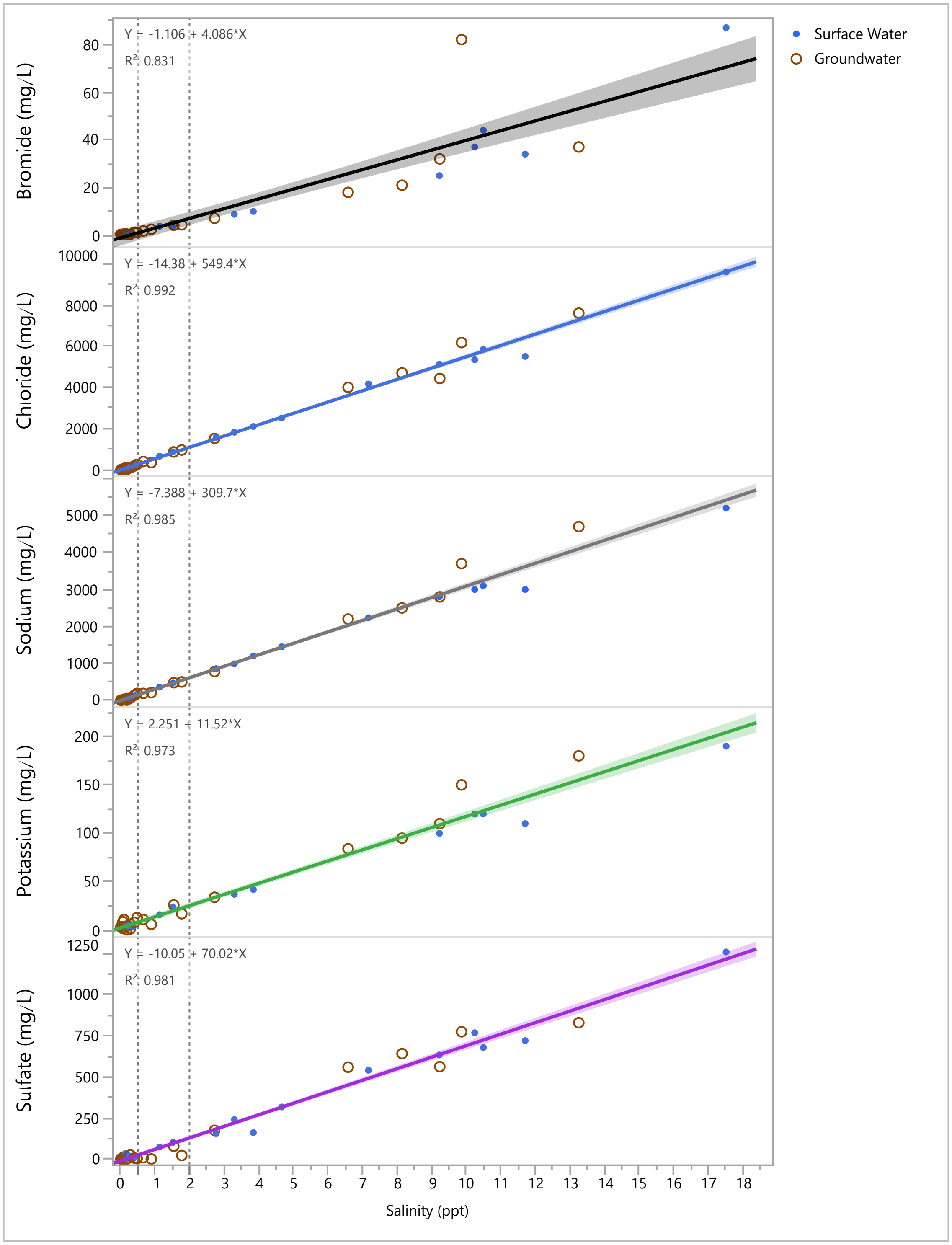


Figure 43. Correlations between water salinity and bromide, chloride, sodium, potassium, and sulfate - surface water and shallow groundwater. All correlations p<.0001. Some sites had only surface water or groundwater present and water was only collected from DWR and NWCA sites, in 2016, 2021, and 2022.

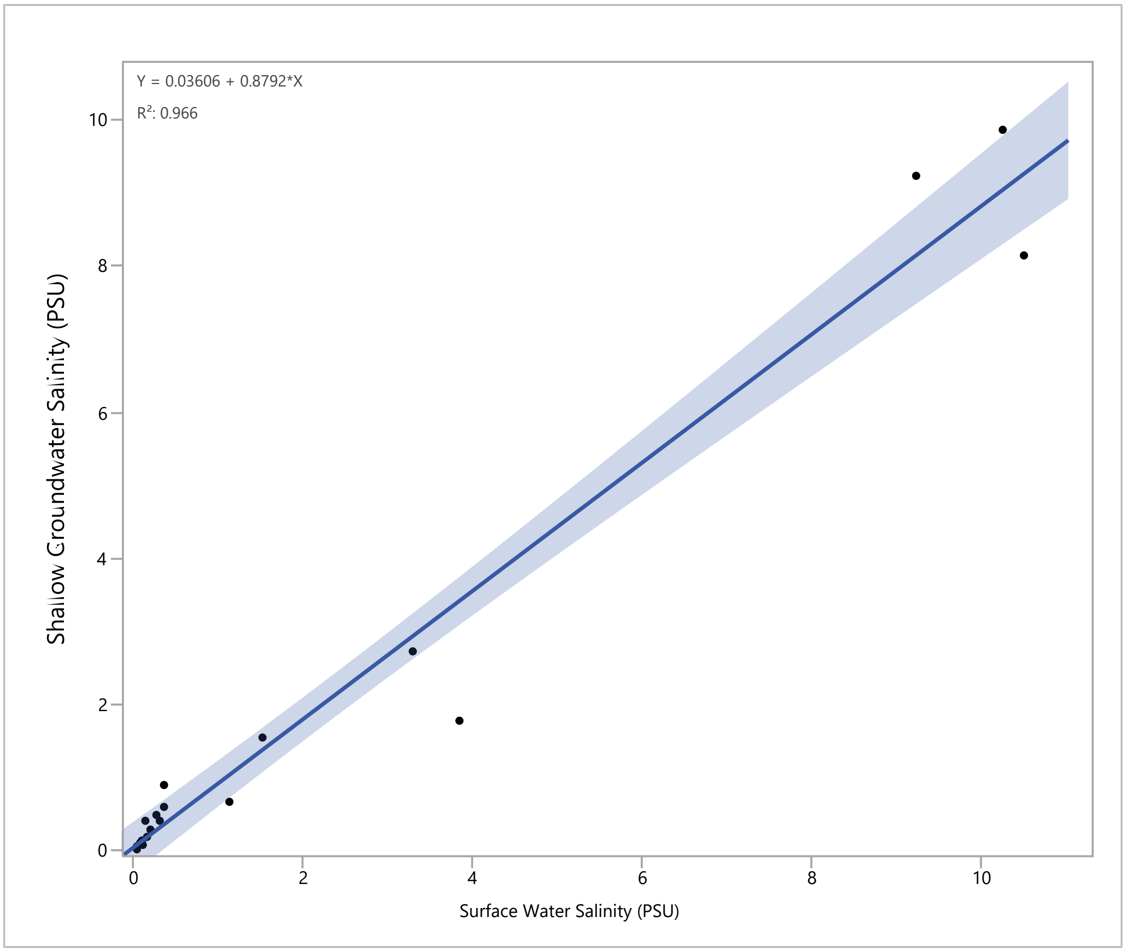


Figure 44. Correlation between surface water salinity and shallow groundwater salinity (1 meter depth) in 18 Outer Coastal Plain wetlands. Some sites had only surface water or groundwater present and water was only collected from DWR and NWCA sites, in 2016, 2021, and 2022.

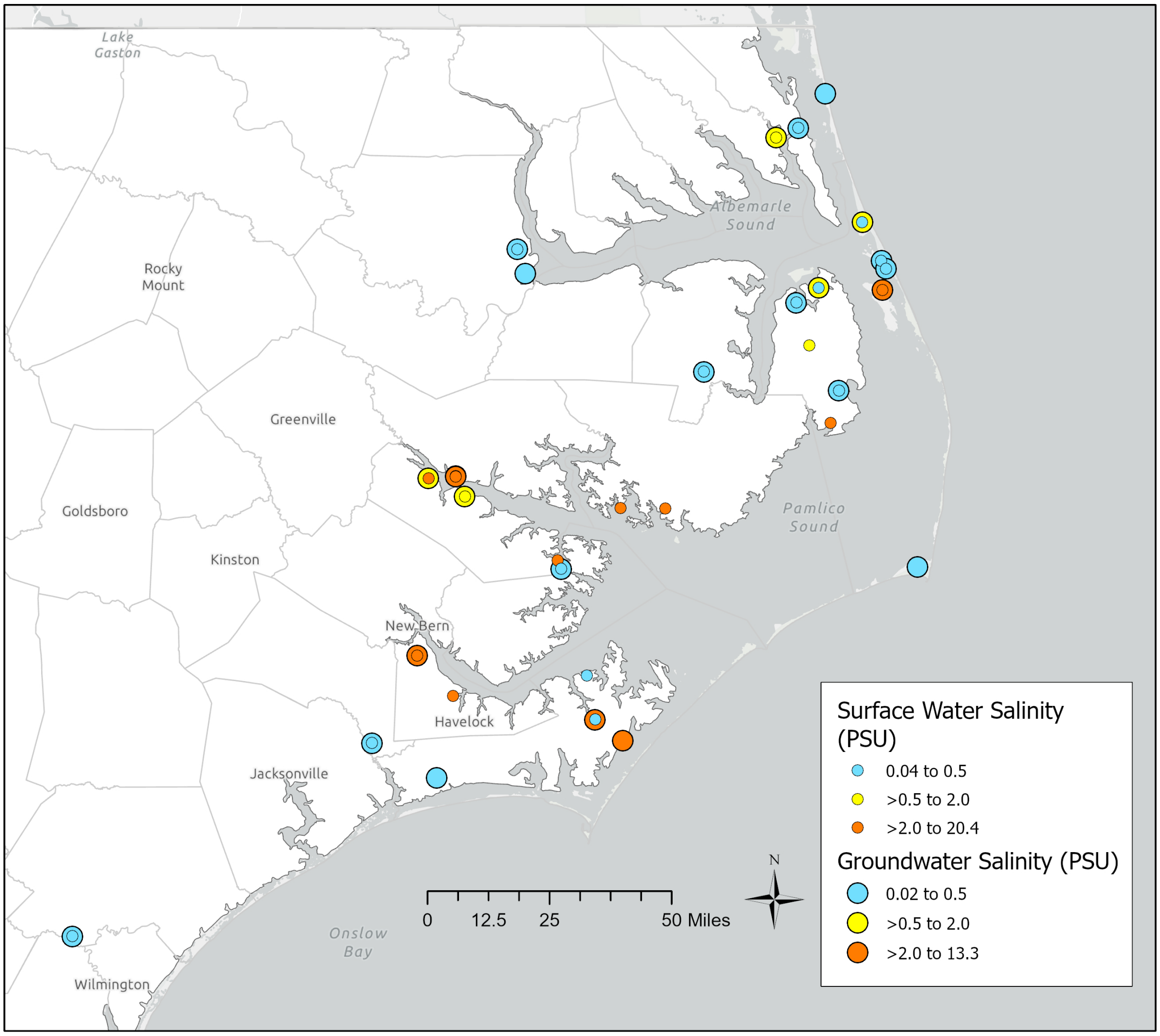
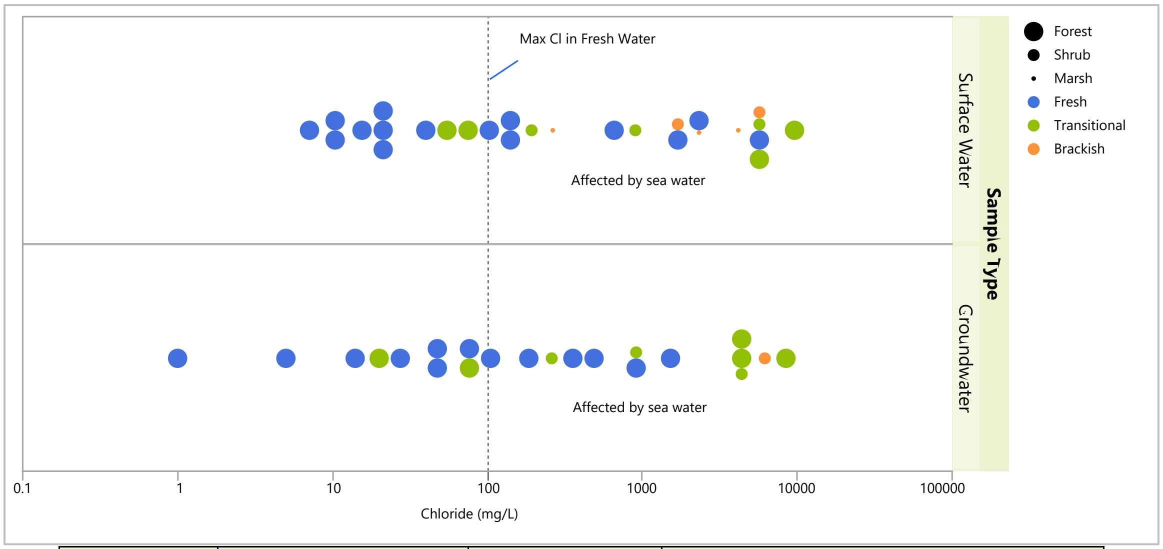


Figure 45. Map of surface water (small dots) and groundwater (larger dots) salinity levels at resampling sites (DWR sites). Water is considered brackish over 0.5 PSU or ppt and the chronic exposure point for plant community change is considered to be 2.0 PSU or ppt. Some sites had only surface water or groundwater present and water was only collected from DWR and NWCA sites, in 2016, 2021, and 2022.



Site	Surface Water	Groundwater	Wetland Type
Sites Deemed Affected by Seawater at Sample Collection			
BRTTBY	Absent	A	Transitional Salinity Forest
BRCCRK	A	A	Freshwater Forest
BNSCRK	A	Not collected	Brackish Marsh
EASTLK	Absent	A	Transitional Salinity Forest
FUTRLL	A	A	Transitional Salinity Shrub
GTMNHS	A	A	Freshwater Forest
GDNCRK	A	Absent	Freshwater Forest
GSCKGM	Present; N (93 mg/L)	A	Freshwater Forest
GSCRKN	A	A	Transitional Salinity Shrub
GSCRKS	A	A	Transitional Salinity Forest
HLSCRK	A	A	Freshwater Forest
HTNCRK	N	A	Freshwater Forest
LWSCRK	A	A	Freshwater Forest
PNSBAY	A	Not collected	Brackish Marsh
PRRDGE	A	A	Freshwater Forest
PBLCRK	A	A	Transitional Salinity Shrub
SHBGBY	A	A	Brackish Shrub
SMCRKN	A	Not collected	Transitional Salinity Forest
STHRIV	Present in 2016; N; absent in 2022	A	Transitional Salinity Forest
STKBAY	A	Not collected	Brackish Marsh
SWQTDK	A	Not collected	Brackish Shrub
Sites Deemed Not Affected by Seawater at Sample Collection			
ALLRIV	N	Not collected	Freshwater Forest
AYDLTT	N	N	Freshwater Forest
BKLKBM	N	N	Freshwater Forest
BUXTON	Absent	N	Freshwater Forest
ERBTIL	N	N	Transitional Salinity Forest
GGMCLL	N	N	Freshwater Forest
RCCNBY	Absent	N	Freshwater Forest
ROANIL	N	N	Freshwater Forest
SMNCRK	N	N	Freshwater Forest
TRPNPT	Absent	N	Transitional Salinity Forest

Figure 46. Sites determined to be affected by seawater based on chloride ion concentration (shown in log scale). A=deemed affected, N=deemed not affected. Some sites had only surface water or groundwater present and water was only collected from DWR and NWCA sites, in 2016, 2021, and 2022.

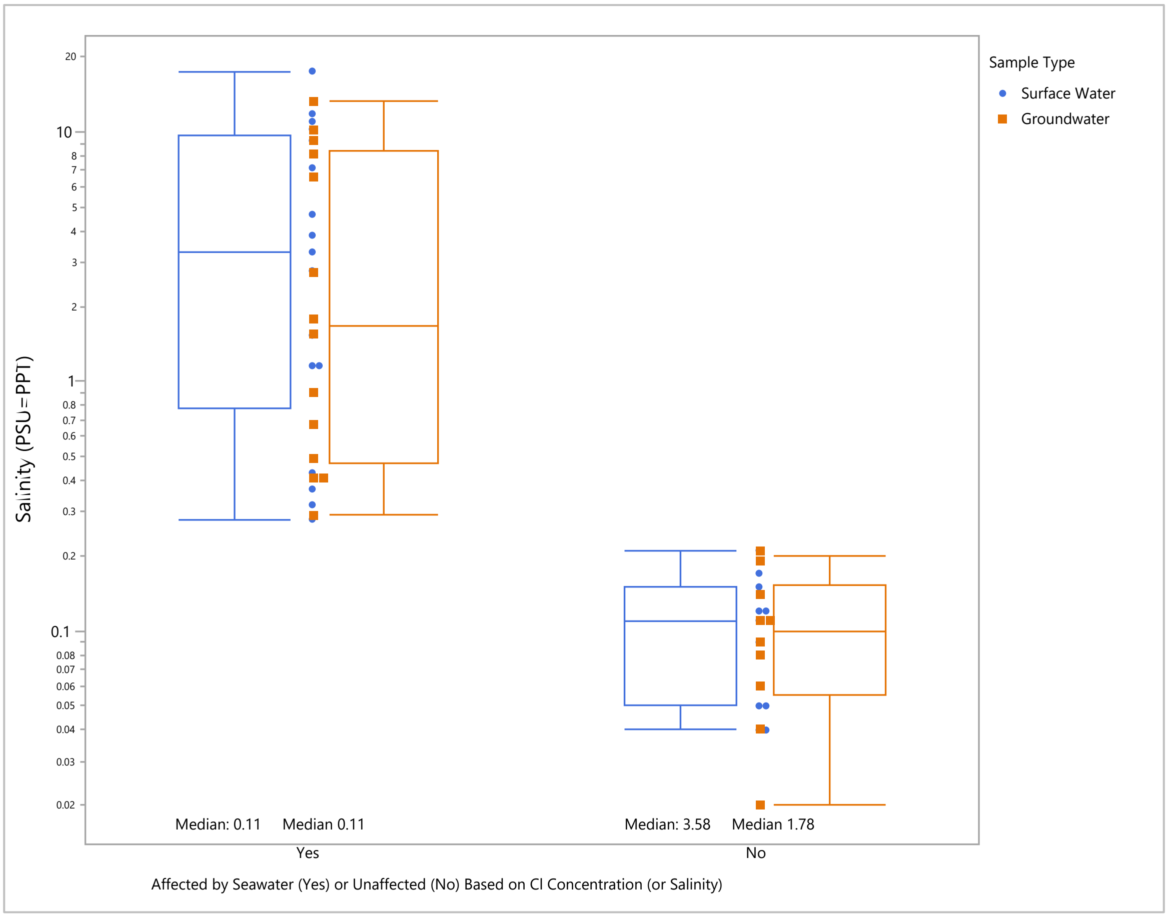


Figure 47. Surface water and shallow groundwater salinity (log scale) in sites determined to be affected by seawater and not affected based on chloride (Cl) ion concentration. Some sites had only surface water or groundwater present and water was only collected from DWR and NWCA sites, in 2016, 2021, and 2022.

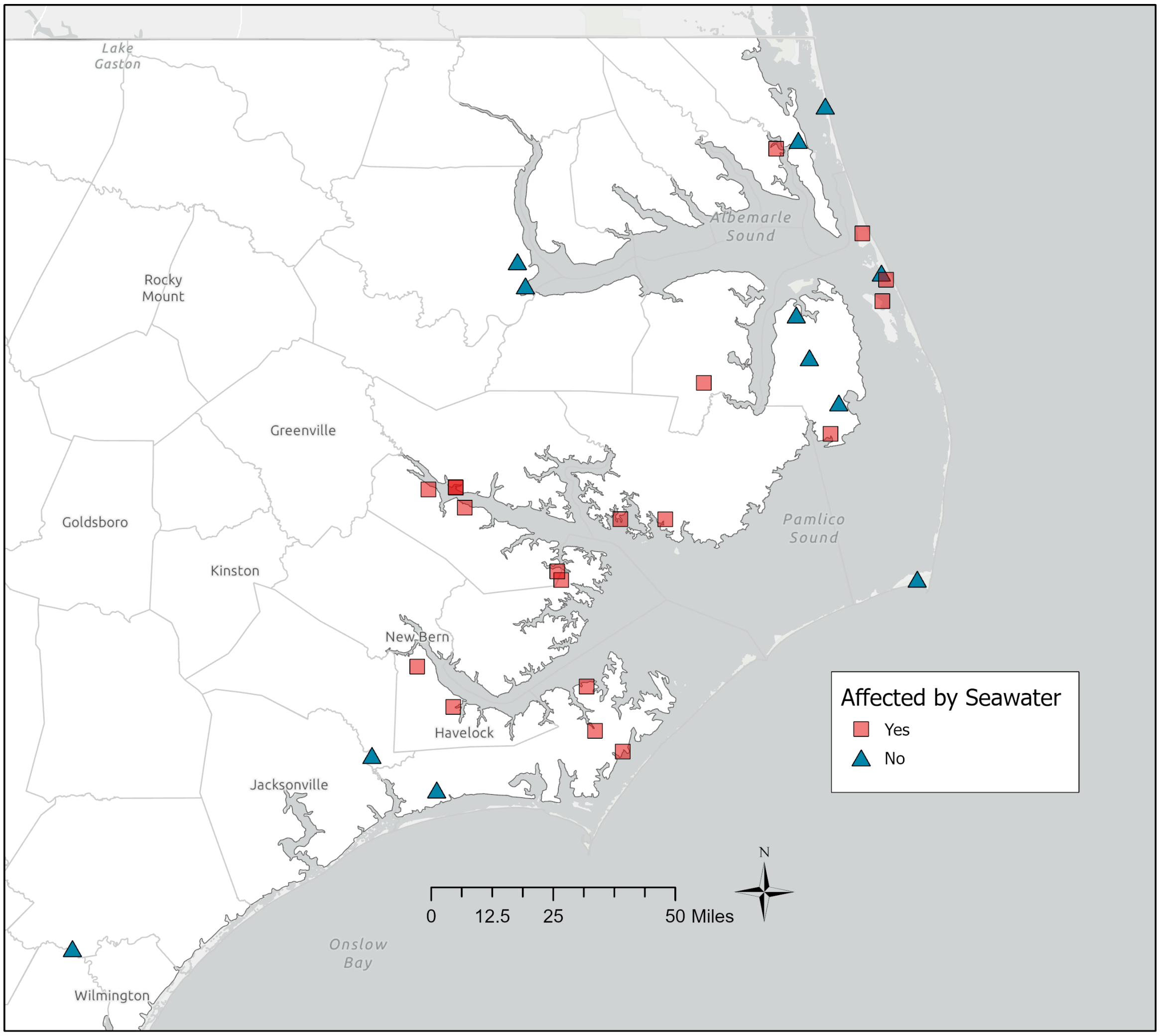


Figure 48. Map of sites with water chemistry data that were determined to be affected by seawater based on chloride ion concentration. Some sites had only surface water or groundwater present and water was only collected from DWR and NWCA sites, in 2016, 2021, and 2022.

4.6 Soil Chemistry Results

Shallow groundwater salinity was predictive of soil sodium levels (r^2=0.81; p<.0001; N=22) (Figure 49) but surface water salinity was not (r^2=.07; p=.17; N=28). Shallow groundwater sodium was also highly correlated with top horizon soil sodium (r=.91; p<.0001; N=22).

Top horizon soil sodium was correlated with surface water sodium (r=.63; p=.003; N=20) and bromide (r=.51; p=.021; N=20), but other soil chemistry parameters (CEC, chloride, potassium, Ca+Mg, pH) were not well correlated with measured surface water parameters.

Freshwater wetland soil sodium levels in the outer Coastal Plain were sometimes high (>500 ppm), depending on their proximity to tidally influenced waters and their vulnerability to storm surges from hurricanes (Figure 50). Wetlands that were not vulnerable to Category 1 hurricane storm surges and those that were non-tidal showed much lower levels of soil sodium in the top soil horizon at the time of sampling. As expected, overall soil sodium levels exhibited an increasing gradient from freshwater wetlands (median = 100 ppm) to transitional salinity wetlands (median = 804 ppm) to brackish wetlands (median = 3,568 ppm) (Figure 50). Analysis of differences in soil sodium levels between first and last sampling showed that freshwater and brackish wetlands were generally stable over time (with some outliers), but transitional salinity wetlands tended to show increases over time (Figure 51).

Soil was most acidic in forested wetlands (median pH = 4.5), mid-range in shrub wetlands (median = 4.9), and least acidic in marsh wetlands which were all brackish (median = 5.5) (Figure 52). CEC and Ca+Mg showed similar increasing gradients from forested to shrub to marsh wetlands, as well as sodium, potassium and sulfate (Figure 53).

In addition to the top horizon samples collected on nearly all study sites, soil samples were collected on DWR sites from each horizon to a one-meter depth and analyzed for soil chemistry. Parameter data by horizon and wetland salinity regime are presented in Figure 54 to Figure 56. Soil sodium, Ca+Mg, ammonium and sulfate all were generally highest in the top horizon and decreased with increasing depth, in freshwater, transitional salinity and brackish wetlands. Drops in sulfate with increasing soil depth were consistent with findings by Hackney et al. (2007) in swamp forests in the Cape Fear region of North Carolina.

The Ca:Mg ratio in soils was graphed against soil chloride concentration, soil sodium and water salinity, because Ca:Mg has been suggested to be an indicator of saltwater intrusion. Project results showed overall inverse relationships between Ca:Mg ratio and chloride concentration, soil sodium and salinity (Figure 57) (chloride: p=.01; sodium: p<.0001; surface water salinity: p=.044; groundwater salinity: p=.09). Soil Ca:Mg ratio was positively correlated with water Ca:Mg ratio (r=.49; p=.001).

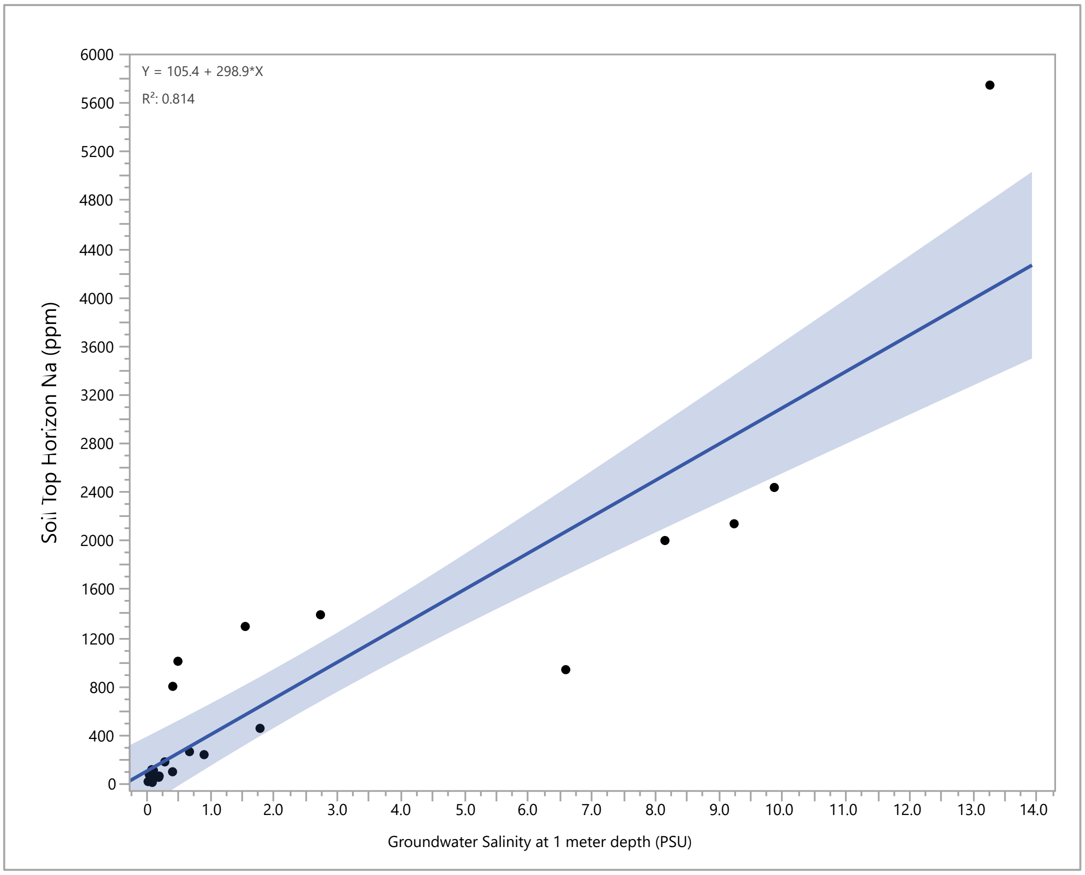


Figure 49. Regression of groundwater salinity against soil top horizon sodium in 22 outer Coastal Plain wetlands. Some sites had only surface water or groundwater present and water was only collected from DWR and NWCA sites, in 2016, 2021, and 2022.

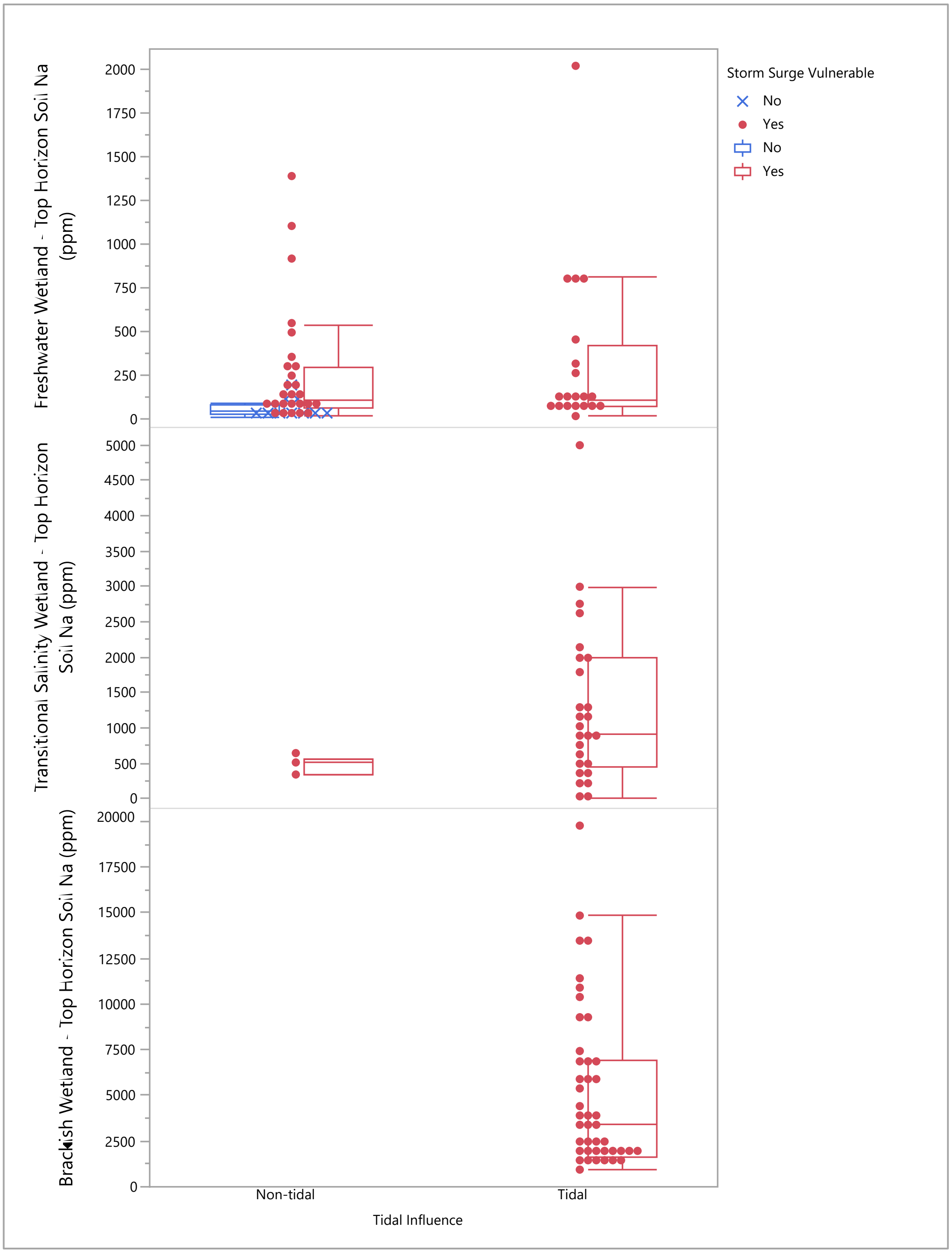


Figure 50. Top horizon soil sodium levels in outer Coastal Plain wetlands by storm surge vulnerability and tidal influence for freshwater, transitional salinity, and brackish wetlands (salinity regime at time of sampling). Original and resampling data included.

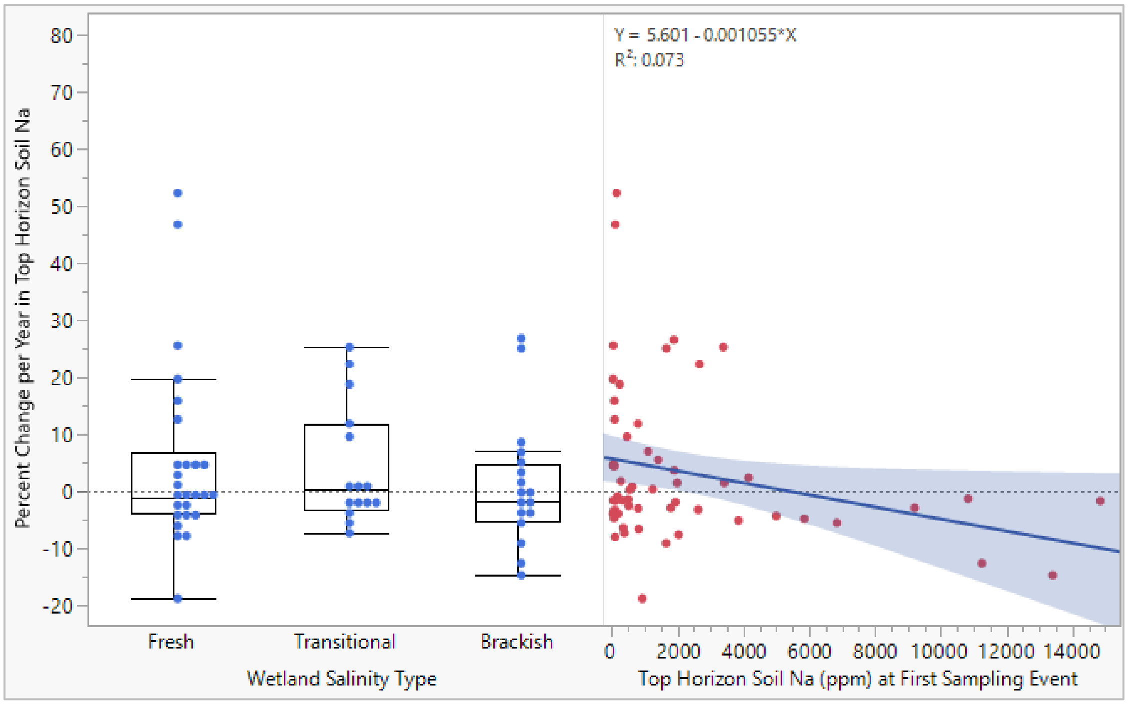


Figure 51. Percent change per year in top horizon soil Na in relation to wetland salinity type and in relation to first sampling top horizon soil Na level.

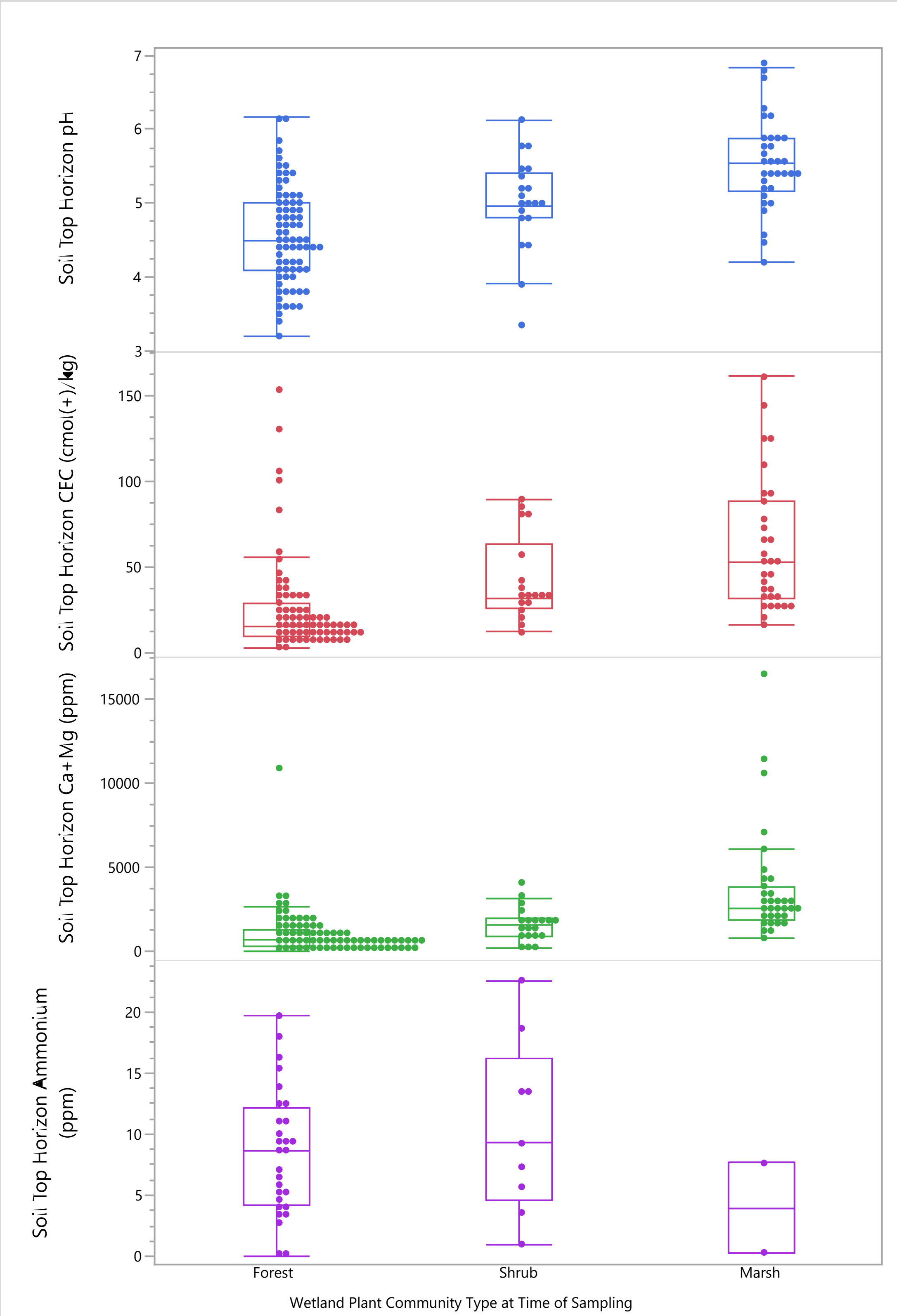


Figure 52. Descriptive boxplots for soil pH, CEC, Ca+Mg, and ammonium by wetland plant community at sampling time. N=127, original and resampling data included.

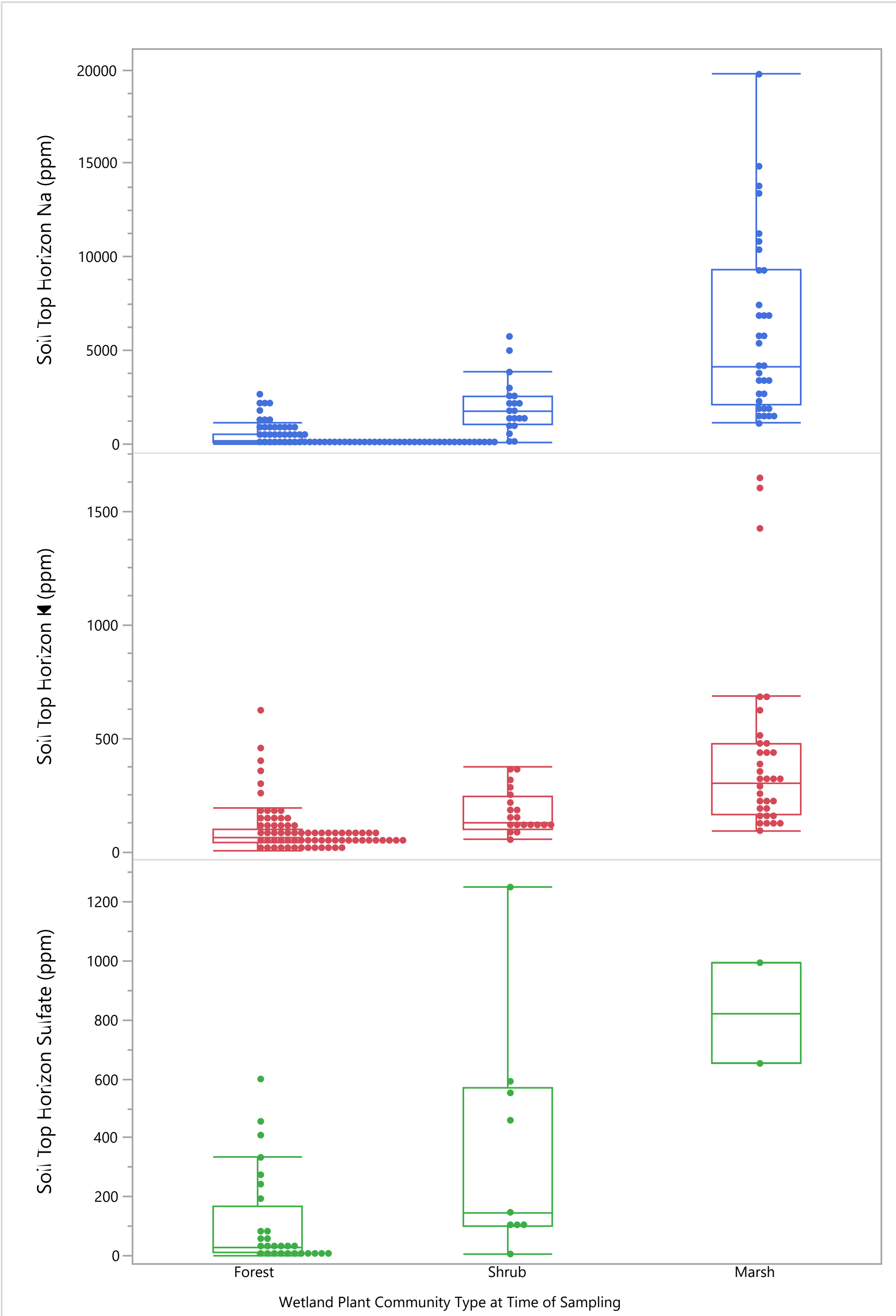


Figure 53. Descriptive boxplots for sodium (Na), potassium (K), and sulfate by wetland plant community at sampling time. N=127, original and resampling data included.

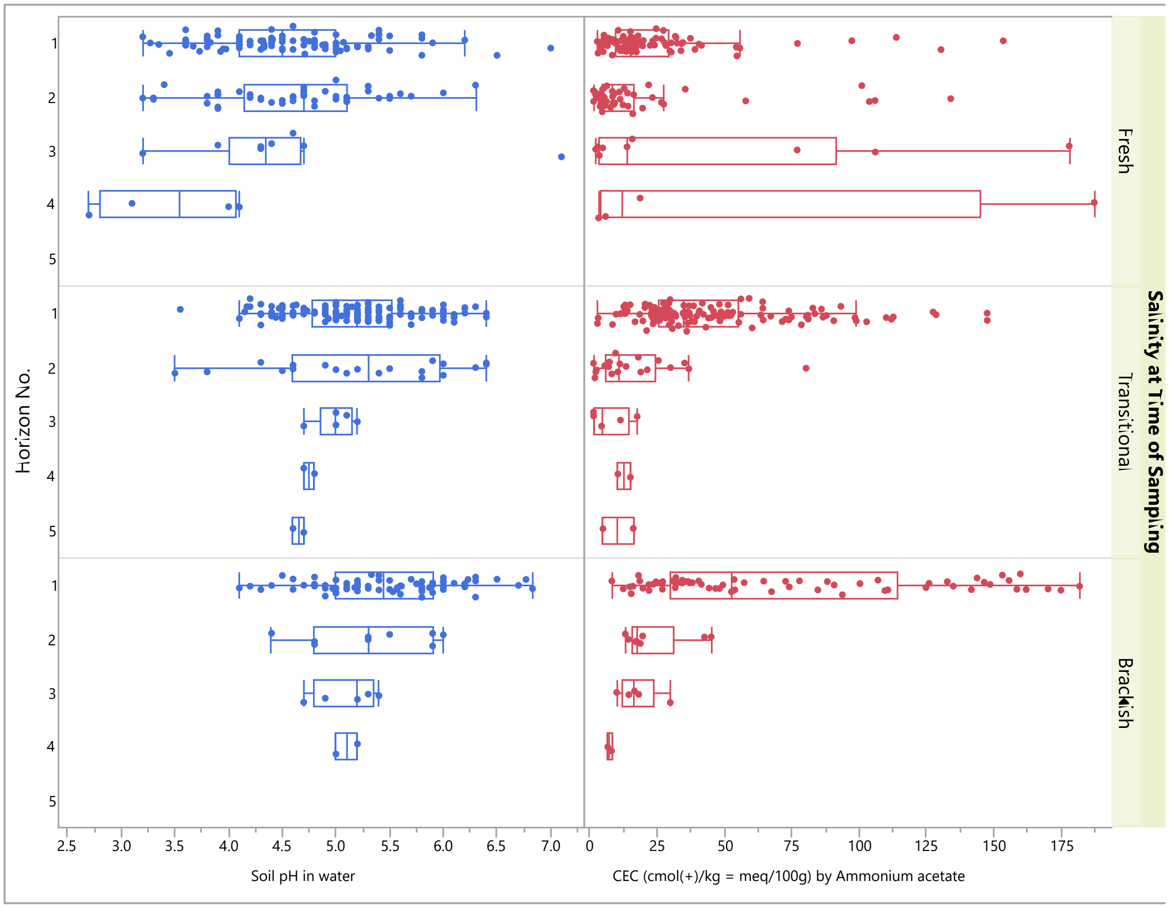


Figure 54. Soil pH and CEC at various horizons in freshwater, transitional salinity, and brackish outer Coastal Plain wetlands. Data from 2021 and 2022 from 241 separate soil samples at various horizons within 1 meter depth.

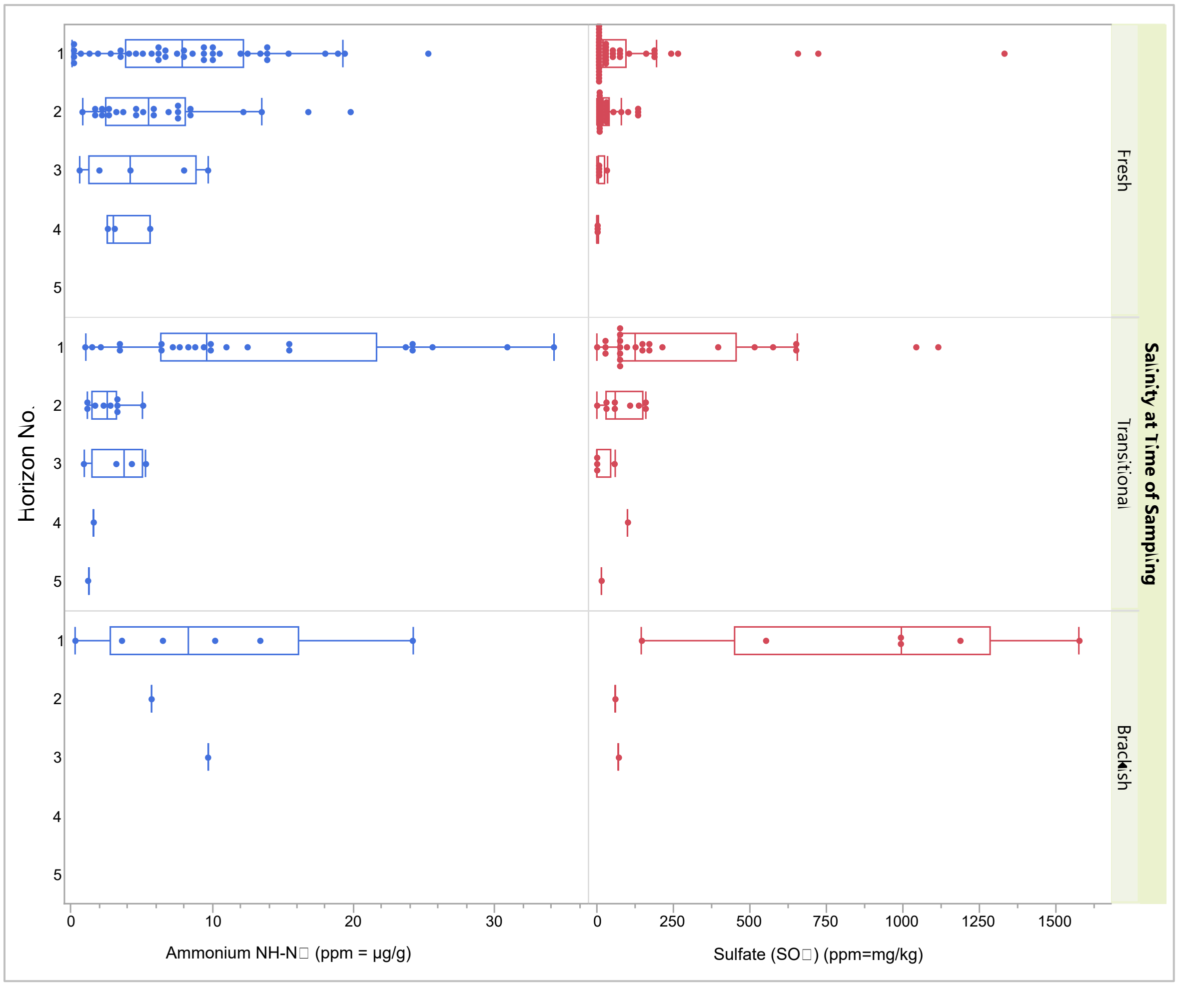


Figure 55. Soil ammonium and sulfate at various horizons in freshwater, transitional salinity, and brackish outer Coastal Plain wetlands. Data from 2021 and 2022 from 241 separate soil samples at various horizons within 1 meter depth.

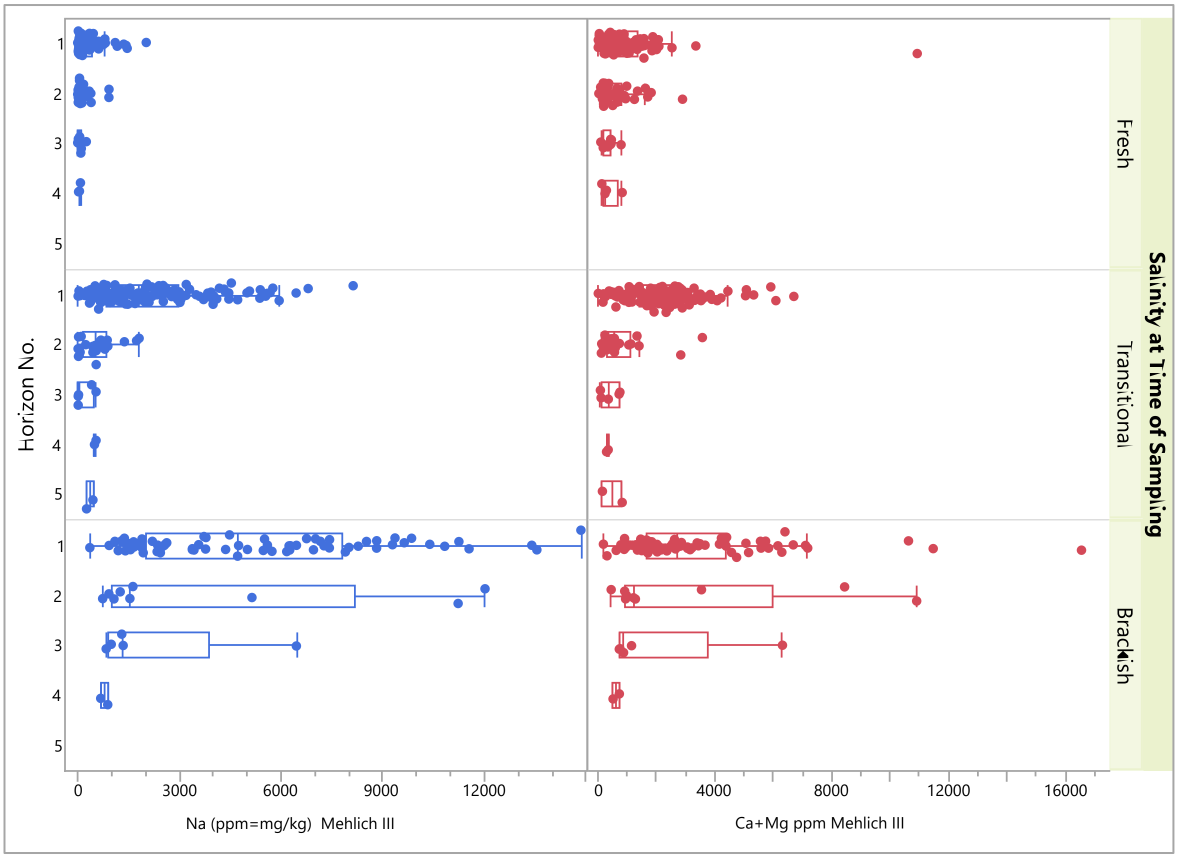


Figure 56. Soil sodium and Ca+Mg at various horizons in freshwater, transitional salinity, and brackish outer Coastal Plain wetlands. Data from 2021 and 2022 from 241 separate soil samples at various horizons within 1 meter depth.

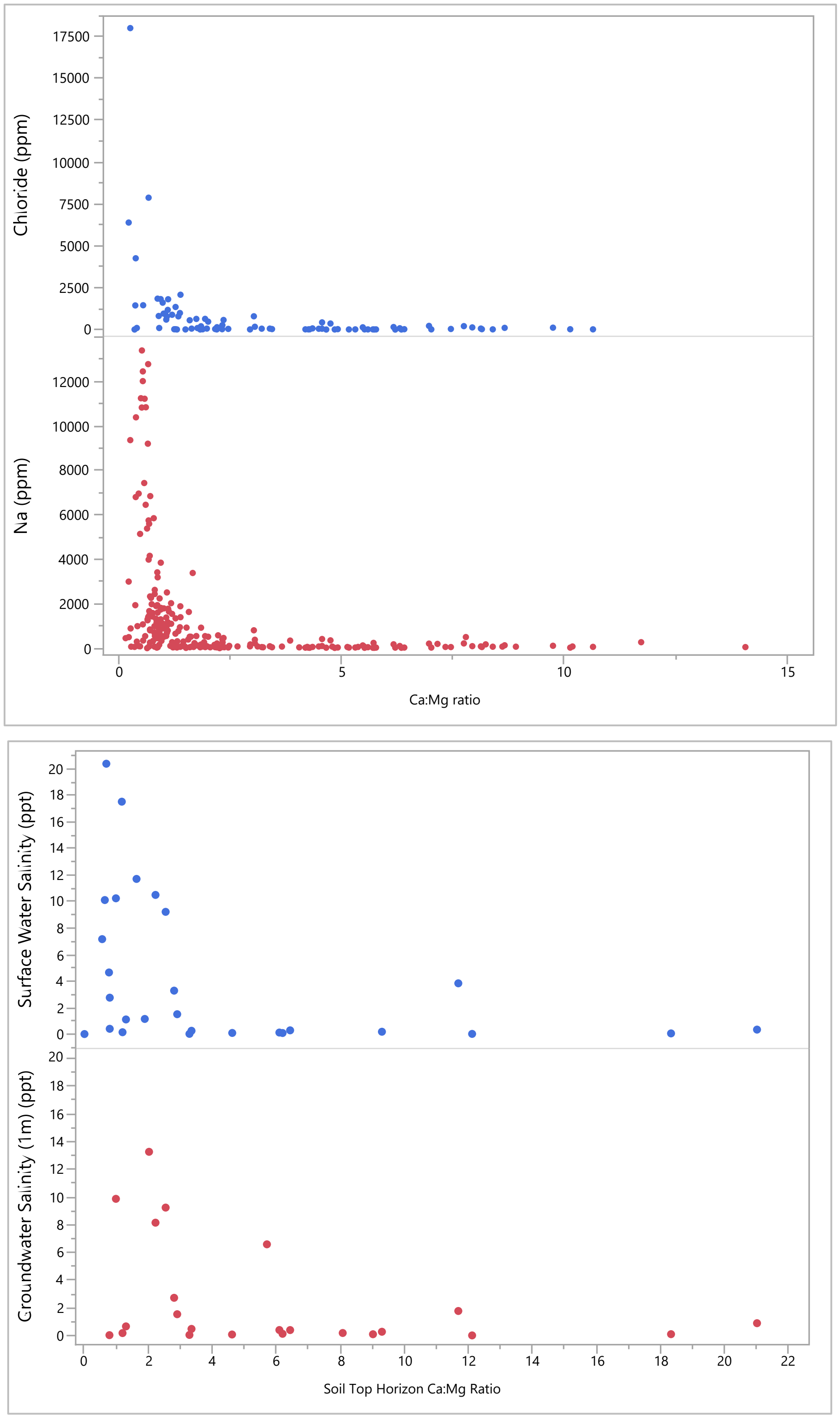


Figure 57. Inverse relationships between soil Ca:Mg ratio and soil chloride and sodium concentrations, and between soil Ca:Mg ratio and water sample salinity.

4.7 Plant Community Changepoints or Thresholds

Changepoint analysis was done using the TITAN analysis method (Baker and King 2010). The TITAN threshold analysis was run with the dataset from this study, which included Anderson et al.'s (2022) dataset with the addition of more sites over a wider geographic area, as well as vegetation data that included mature trees. The TITAN analysis was performed for this project by a subcontractor (S. Anderson) using data from all sites included in this study, plus eight additional partner sites that were excluded from the analysis preceding this section. Although the excluded sites did not meet the site selection criteria for this study, the data on plant species occurrence, cover and soil chemistry were useful for the TITAN threshold analysis. It was judged reasonable to include the extra data to bolster the analysis, rather than exclude them because the sites did not meet the site selection criteria for the rest of this project in terms of resample interval or location. Details of the TITAN threshold analysis process and results are outlined in detail in Appendix E and are summarized here.

Change in plant community composition over time was significantly correlated with top horizon soil sodium, supporting the idea of a change threshold, or "tipping point," related to soil sodium, where sensitive taxa ("decreasers") begin to disappear from samples (Figure 58). The result of the TITAN community change threshold analysis using our dataset of individual plant species occurrences in relation to soil sodium predicted a change threshold of 383 µg/g of soil sodium for sensitive taxa (Table 16). This threshold was estimated by using only the taxa information deemed pure and reliable by the analysis, therefore it was the most conservative estimate possible. Anderson et al. (2022) did the same analysis on data from 34 wetland sites in the Albemarle-Pamlico Peninsula, using occurrence of herbaceous plants, and shrub and tree species <4m in height. The sensitive taxa "understory community" changepoint predicted from their analysis was 265 µg/g of soil sodium.

Half of the project's forested wetland sites (of those with soil chemistry data) had soil sodium levels at or above the whole community "tipping point" threshold predicted by the TITAN analysis for sensitive taxa, at any sampling event (Table 17; Figure 59). An additional five sites were above the sensitive taxa understory community threshold predicted from analysis by Anderson et al. (2022).

Sites that were above the soil sodium threshold at original sampling showed an increase over time in the proportion of plant community that was brackish-tolerant, but sites below the sodium threshold showed no significant change (Wilcoxon matched pairs test: $p=0.024$ sites above threshold; $p>0.05$ sites below threshold). Between sampling events, top horizon soil sodium was generally stable (median change was a decrease of 1.2% per year), but not always. While most forested sites remained stable, some transitional salinity forests showed noticeable differences in top horizon soil sodium between first and last sampling events (Figure 51; Figure 60). Per year increases, however, were as great as 52% per year (SEES13) and decreases as much as 19% per year (ALLRIV). The amount of change per year was not significantly related to community structure, salinity type, elevation, tidal influence, or whether the sodium level at first sampling was above or below either threshold. However, vulnerability to storm surge made a significant difference in the trend observed (Kruskal-Wallis test: $p=.048$); if wetlands were vulnerable to storm surge, median change was positive (0.7% per year), whereas it was negative (-4.0% per year) in wetlands not vulnerable to storm surge.

Enough species and soil sodium data were available to assign each of 184 wetland plant taxa to one of two categories: 1) "decreaser/sensitive" or 2) "increaser/tolerant." The analysis indicated that 142 of those taxa were deemed sodium sensitive, meaning they were found less frequently and with less cover as soil sodium increased across the study sites (Table 18). The remaining 42 taxa were indicated as "increasers/tolerant", meaning they occurred more frequently and with greater cover as soil sodium increased (Table 19).

Utilizing regression of soil sodium against groundwater salinity data (Figure 49), the whole plant community changepoint of 383 μg/g soil sodium corresponds to approximately 0.93 ppt shallow groundwater salinity (1 m depth) or 1666 μS/cm specific conductivity. The understory community changepoint of 265 μg/g soil sodium corresponds to approximately 0.54 ppt shallow groundwater salinity (1 m depth) or 1022 μS/cm specific conductivity. The probability that a site deemed affected by seawater (based on chloride ion concentration; Figure 46) would have soil sodium above the community threshold was significantly greater than the probability that it would be below the threshold (Fisher's Exact Test; $p<.0001$) on the 32 DWR sites with water chemistry data available. Sites deemed affected by seawater had significantly higher top horizon soil sodium levels than those not in that category (Wilcoxon test: $p<.0001$), as well as higher surface and shallow groundwater salinity (Wilcoxon test: $p<.0001$ for both). Sites affected by seawater also had greater proportions of and cover by brackish-tolerant species (Wilcoxon test: $p=.003$). Sites affected by seawater exhibited greater shifts in their woody plant community over time than those that were not (Wilcoxon test: $p=.008$). In summary, the category of "affected by seawater" was significantly associated with higher top horizon soil sodium, higher salinity, greater shifts in the woody plant community, and higher proportions of brackish-tolerant species, as well as cover by them.

Soil sodium was considered to have substantially changed on a site if values changed by more than one standard deviation (standard deviation being calculated using within-site data from one partner [NCSU] which had analyzed 7 soil samples from each of their sites). The majority of freshwater wetland sites did not experience substantial changes in soil sodium between sampling events, particularly if they were below the plant community change thresholds during first sampling (Figure 60; Table 20). Three sites (GNDCRK, SEES13, SEES17) experienced substantial increases in soil sodium, while four (ALLRIV, BTMNHS, HLSCRK, PRRDGE) experienced drops in soil sodium. Of the transitional salinity wetlands, three saw substantial increases (GSCRKS, SEES1, SEES4) while five decreased substantially (ERBTIL, MATRA, GSCRKN, SWFOR, TRPNPT).

Soil sodium can vary across sites and with increasing depths. Examination of soil sodium data within North Carolina State University partner sites sometimes showed wide within-site variation in soil sodium, particularly if levels were over the whole community threshold of 383 μg/g sodium (Figure 61). Brackish marshes showed more within-site variation in soil sodium than transitional salinity or freshwater wetlands. An assessment of soil sodium with increasing depth showed that if the top horizon soil sodium (top 10 cm soil depth) was above the whole community change threshold of 383 µg/g, then sodium generally stayed above that threshold through increasing depth, with a few exceptions (Figure 62). At the approximate limit of root zone (30 cm; Light et al. 2002), soil sodium was above the threshold if the top horizon was above that threshold.

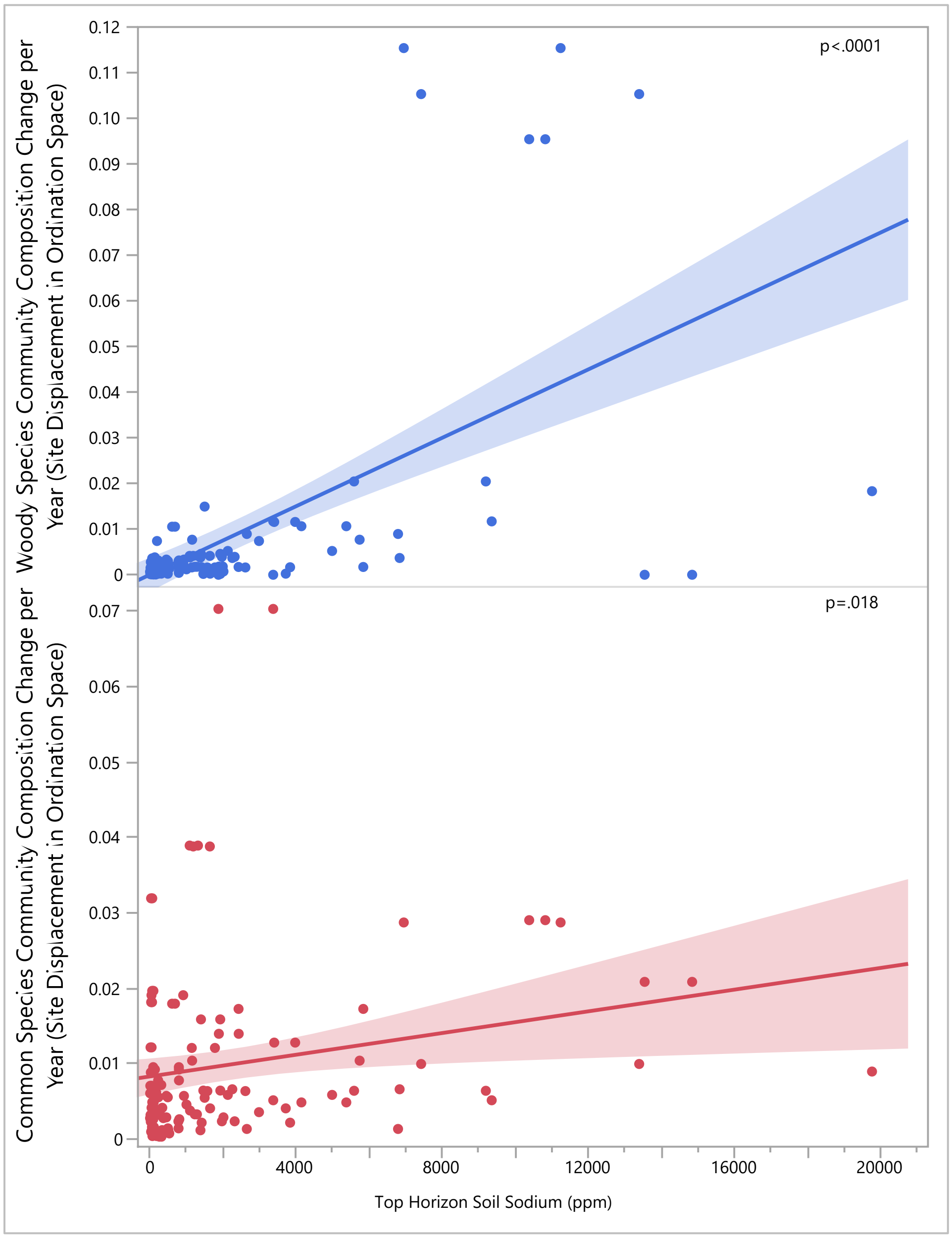


Figure 58. Correlation between plant community compositional changes over time and top horizon soil sodium levels.

Table 16. Community change points from TITAN (Baker and King 2010) for decreasing and increasing taxa for sodium and calcium + magnesium with the 5-95% quartile range, from analysis with this project and from Anderson et al. (2022). Quartiles were determined from 1000 bootstrap runs for the whole community threshold analysis and 500 for the understory community threshold analysis.

			Quartile				
Variables	**Sensitive/ Tolerant Taxa**	**Change Pt.**	**0.05**	**0.1**	**0.5**	**0.9**	**0.95**
Whole Community Threshold Analysis (from this study)							
Sodium (µg/g or ppm)	**Sensitive (Decreasing)**	383	187	308	383	877	930
	Tolerant (Increasing)	930	750	803	963	1107	1180
Ca + Mg (mEq/g)	**Sensitive (Decreasing)**	54	34	39	55	56	57
	Tolerant (Increasing)	88	55	56	87	88	89
Understory Community Threshold Analysis (from Anderson et al. 2022)							
Sodium (µg/g or ppm)	**Sensitive (Decreasing)**	265	264	265	303.5	342	734
	Tolerant (Increasing)	3843	900	991	3843	4388	4459
Ca + Mg (mEq/g)	**Sensitive (Decreasing)**	45	6	6	45	79	81
	Tolerant (Increasing)	236	135	142	236	255	258

Table 17. Number of wetlands above and below the community changepoint thresholds for top horizon soil sodium during any sampling event, by original community type.

Community Type	**No. of Wetland Sites Above or Below the Sensitive Taxa Whole Community Change Threshold for Soil Sodium (383 µg/g)**		**No. of Wetland Sites Above or Below the Understory Community Change Threshold for Soil Sodium (265 µg/g)**	
	Above	Below	Above	Below
Forest	20	20	25	15
Shrub	11	1	11	1
Marsh	13	0	13	0

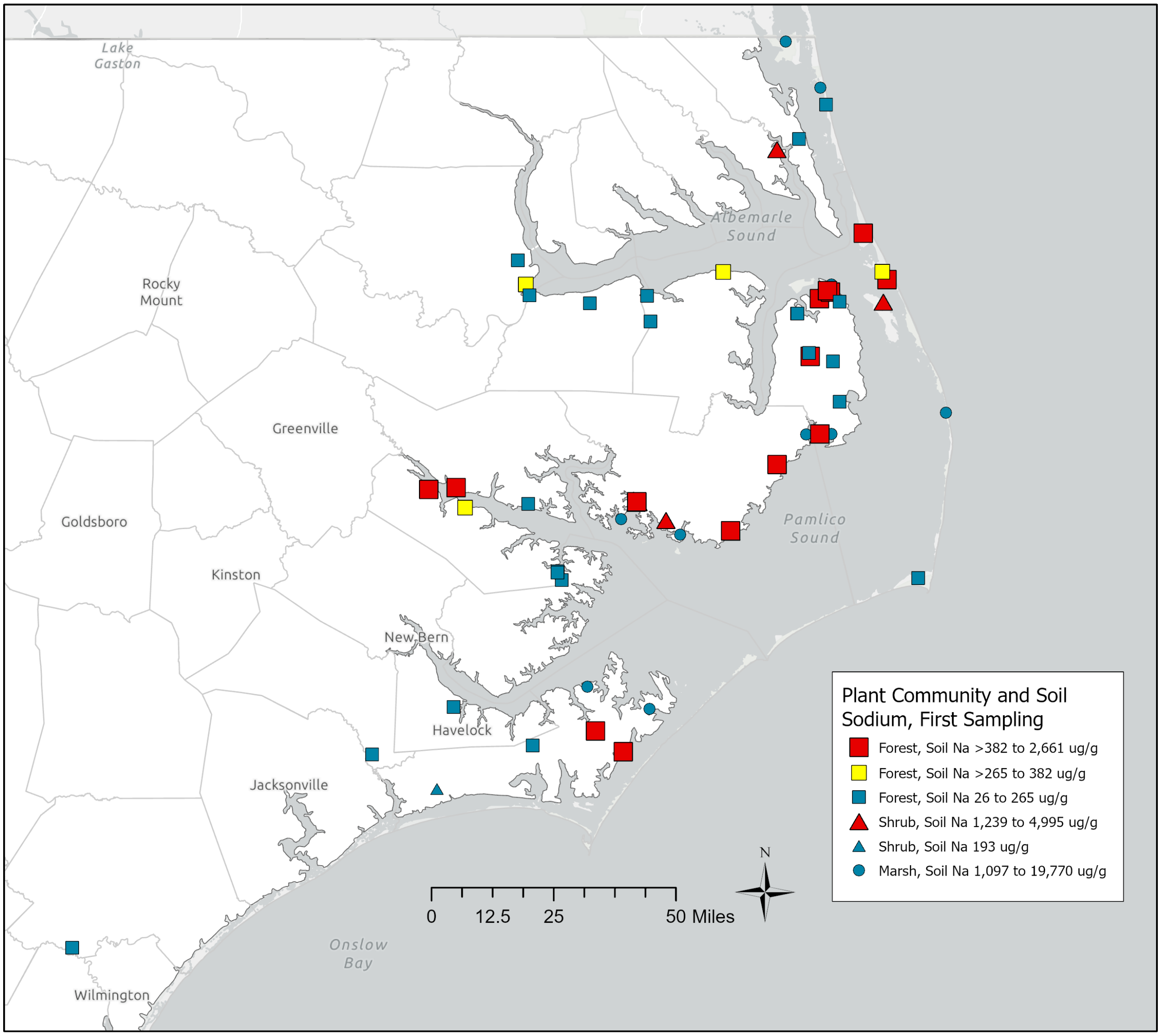


Figure 59. Map of highest top horizon soil sodium levels measured from each site, by plant community at first sampling. Points are grouped in relation to the threshold of 383 µg/g (ppm) sodium predicted by a TITAN plant community changepoint analysis from our dataset in relations to the understory community change threshold of 265 µg/g soil sodium from Anderson et al. (2022). Some partner sites had no soil data available.

Table 18. List of wetland plant taxa deemed to be "decreasers" or "sensitive" species which decrease in coverage and occurrence with higher top horizon soil sodium. When a taxon is identified to the genus level, the designation refers only to those species which occur in the NC Outer Coastal Plain.

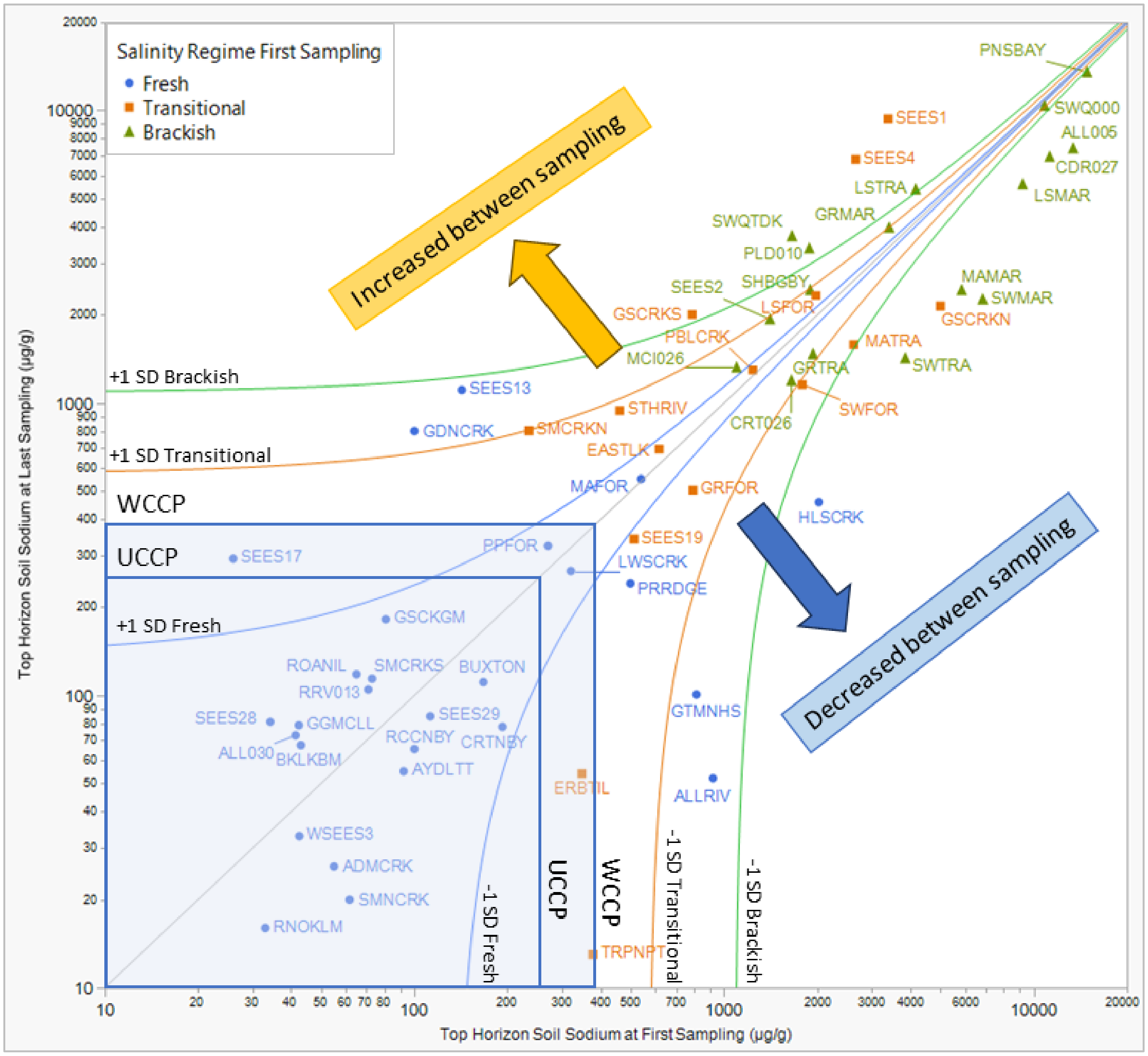
Wetland Plant Taxa Deemed "Decreasers" or "Sensitive" Taxa with Increasing Soil Sodium			
Acer rubrum	*Dichanthelium commutatum*	*Lycopus virginicus*	*Quercus michauxii*
Alnus serrulata	*Dichanthelium dichotomum*	*Lyonia ligustrina*	*Quercus nigra*
Amelanchier canadensis	*Dichanthelium lucidum*	*Lyonia lucida*	*Quercus pagoda*
Apios americana	*Dichanthelium strigosum*	*Magnolia virginiana*	*Quercus phellos*
Aralia spinosa	*Dioscorea villosa*	*Microstegium vimineum*	*Quercus velutina*
Arisaema triphyllum	*Diospyros virginiana*	*Mikania scandens*	*Rhus copallinum*
Aronia arbutifolia	*Erechtites hieraciifolius*	*Mitchella repens*	*Rhynchospora sp.*
Arundinaria tecta	*Eubotrys racemosus*	*Morella caroliniensis*	*Rubus sp.*
Asimina triloba	*Euonymus americanus*	*Morella cerifera*	*Rubus trivialis*
Asplenium platyneuron	*Eupatorium capillifolium*	*Murdannia keisak*	*Sassafras albidum*
Athyrium asplenioides	*Fagus grandifolia*	*Muscadinia rotundifolia*	*Saururus cernuus*
Berchemia scandens	*Fraxinus caroliniana*	*Nekemias arborea*	*Scutellaria lateriflora*
Bignonia capreolata	*Fraxinus pennsylvanica*	*Nyssa biflora*	*Sium suave*
Boehmeria cylindrica	*Fraxinus profunda*	*Nyssa sylvatica*	*Smilax bona-nox*
Callicarpa americana	*Galium tinctorium*	*Onoclea sensibilis*	*Smilax glauca*
Campsis radicans	*Gaylussacia frondosa*	*Osmundastrum cinnamomeum*	*Smilax laurifolia*
Carex abscondita	*Gelsemium sempervirens*	*Oxydendrum arboreum*	*Smilax rotundifolia*
Carex atlantica	*Gordonia lasianthus*	*Parathelypteris noveboracensis*	*Smilax smallii*
Carex bromoides	*Hamamelis virginiana*	*Parthenocissus quinquefolia*	*Smilax walteri*
Carex debilis	*Hexastylis arifolia*	*Peltandra virginica*	*Solidago rugosa*
Carex glaucescens	*Hypericum hypericoides*	*Persea palustris*	*Sphenopholis pensylvanica*
Carex lonchocarpa	*Ilex coriacea*	*Persicaria arifolia*	*Symphyotrichum sp.*
Carex seorsa	*Ilex glabra*	*Persicaria sp.*	*Symplocos tinctoria*
Carex sp.	*Ilex opaca*	*Persicaria virginiana*	*Taxodium distichum*
Carex striata	*Ilex verticillata*	*Phytolacca americana*	*Thelypteris palustris*
Carpinus caroliniana	*Itea virginica*	*Pinus serotina*	*Toxicodendron radicans*
Chamaecyparis thyoides	*Juncus coriaceus*	*Pinus taeda*	*Triadenum walteri*
Chasmanthium laxum	*Juncus effusus*	*Platanthera clavellata*	*Ulmus americana*
Cicuta maculata	*Leersia oryzoides*	*Pontederia cordata*	*Vaccinium corymbosum*
Clematis crispa	*Leersia virginica*	*Prunus serotina*	*Vaccinium formosum*
Clethra alnifolia	*Liquidambar styraciflua*	*Pteridium aquilinum*	*Vaccinium fuscatum*
Cornus florida	*Liriodendron tulipifera*	*Quercus alba*	*Vaccinium sp.*
Cornus foemina	*Lobelia cardinalis*	*Quercus alba*	*Viburnum nudum*
Crataegus sp.	*Lonicera japonica*	*Quercus falcata*	*Viola sp.*
Cyrilla racemiflora	*Lonicera sempervirens*	*Quercus hemisphaerica*	*Woodwardia areolata*
Decodon verticillatus	*Ludwigia palustris*	*Quercus laurifolia*	*Woodwardia virginica*
Decumaria barbara			

Table 19. List of wetland plant taxa deemed "increasers" or "tolerant" species which increase in coverage and occurrence with higher top horizon soil sodium. When a taxon is identified to the genus level, the designation refers only to those species which occur in the NC Outer Coastal Plain. Asterisks denote the fact that this designation conflicts with prevailing plant key information, indicating only freshwater habitats in North Carolina for that taxon.

Wetland Plant Taxa Deemed "Increasers" or "Tolerant" Taxa with Increasing Soil Sodium		
Andropogon glomeratus	*Ilex vomitoria*	*Rosa palustris*
Andropogon virginicus	*Ipomoea sagittata*	*Rubus pensilvanicus**
Baccharis halimifolia	*Iris virginica*	*Sabal minor**
Bolboschoenus robustus	*Iva frutescens*	*Sagittaria lancifolia*
Carex stipata	*Juncus roemerianus*	*Samolus valerandi*
*Centella erecta**	*Juniperus virginiana*	*Smilax sp.**
Cladium jamaicense	*Kosteletzkya pentacarpos*	*Solidago mexicana*
Dichanthelium sp.	*Lycopus sp.**	*Solidago virgata*
Distichlis spicata	*Osmunda regalis*	*Spartina alterniflora*
Erianthus giganteus	*Panicum virgatum*	*Spartina cynosuroides*
Eupatorium serotinum	*Persicaria punctata*	*Spartina patens*
Hibiscus moscheutos	*Phragmites australis*	*Triadenum virginicum**
*Hydrocotyle umbellata**	*Polygonum sagittatum*	*Typha angustifolia*
Hydrocotyle verticillata	*Ptilimnium capillaceum**	*Typha latifolia*

Table 20. Top horizon soil sodium during first and last sampling from sites which experienced a whole plant community type shift between sampling events and sites that crossed the community change threshold of 383 ppm (μg/g) between sampling events. All sites listed were in locations vulnerable to storm surge from Category 1 hurricanes.

Site	Soil Sodium First Sampling (μg/g)	Soil Sodium at Last Sampling (μg/g)	Above/Below TITAN Threshold	Type of Plant Community Change Between Sampling Events
Sites Experiencing Complete Shift in Wetland Plant Community Type				
EASTLK	617.0	695.5	Above	Forested to Open Water
BRTTBY	1164.3	5748.0	Above	Forested to Shrub
MAFOR	539.3	549.3	Above	Forested to Shrub
STHRIV	460.3	940.3	Above	Forested to Shrub
SEES4	2660.8	6799.3	Above	Forested to Marsh
SEES18	203.0	2994.5	Below -> Above	Forested to Marsh
LSFOR	1979.7	2327.9	Above	Forested to Marsh
MATRA	2621.4	1581.3	Above	Shrub to Marsh
SWTRA	3845.4	1425.1	Above	Shrub to Marsh
SWQTDK	1656.3	3726.3	Above	Shrub to Marsh
Other Sites Crossing Community Change Threshold in Top Horizon Soil Sodium Between Sampling Events				
GDNCRK	100.0	801.0	Below -> Above	Forested (no change)
SEES13	142.3	1106.7	Below -> Above	Forested (no change)
SMCRKN	234.5	804.0	Below -> Above	Forested (no change)
ALLRIV	920.3	52.1	Above -> Below	Forested (no change)
GTMNHS	813.8	100.7	Above -> Below	Forested (no change)
PRRDGE	498.1	241.3	Above -> Below	Forested (no change)
SEES19	512.3	342.0	Above -> Below	Forested (no change)



Freshwater Wetland	Top Horizon Sodium: First Sampling / Last Sampling (µg/g) (Yrs Between)	Transitional Salinity Wetland	Top Horizon Sodium: First Sampling / Last Sampling (µg/g) (Yrs Between)
GDNCRK	100 ↑ 801 (15)	GSCRKS	789 ↑ 2002 (13)
SEES13	142 ↑ 1107 (13)	SEES1	3384 ↑ 9362 (7)
SEES17	26 ↑ 293 (13)	SEES4	2661 ↑ 6799 (7)
ALLRIV	920 ↓ 52 (5)	ERBTIL	348 ↓ 54 (13)
GTMNHS	814 ↓ 101 (13)	MATRA	2621 ↓ 1581 (12)
HLSCRK	2022 ↓ 458 (10)	GSCRKN	4995 ↓ 2140 (13)
PRRDGE	498 ↓ 241 (34)	SWFOR	1789 ↓ 1155 (12)
		TRPNPT	377 ↓ 13 (13)

Figure 60. Differences in top horizon soil sodium between first and last sampling events by site, with table of sites with substantial changes; graph axes on log scale. Color coded reference lines represent one standard deviation away from the 1:1 (no change) reference line. Standard deviation values from soil sodium one partner study (NCSU) which sampled multiple locations within each site (Figure 61). Sites outside the appropriate standard deviation reference line can be considered to have substantially changed between sampling events. WCCT = whole community change threshold and UCCT = understory community change threshold.

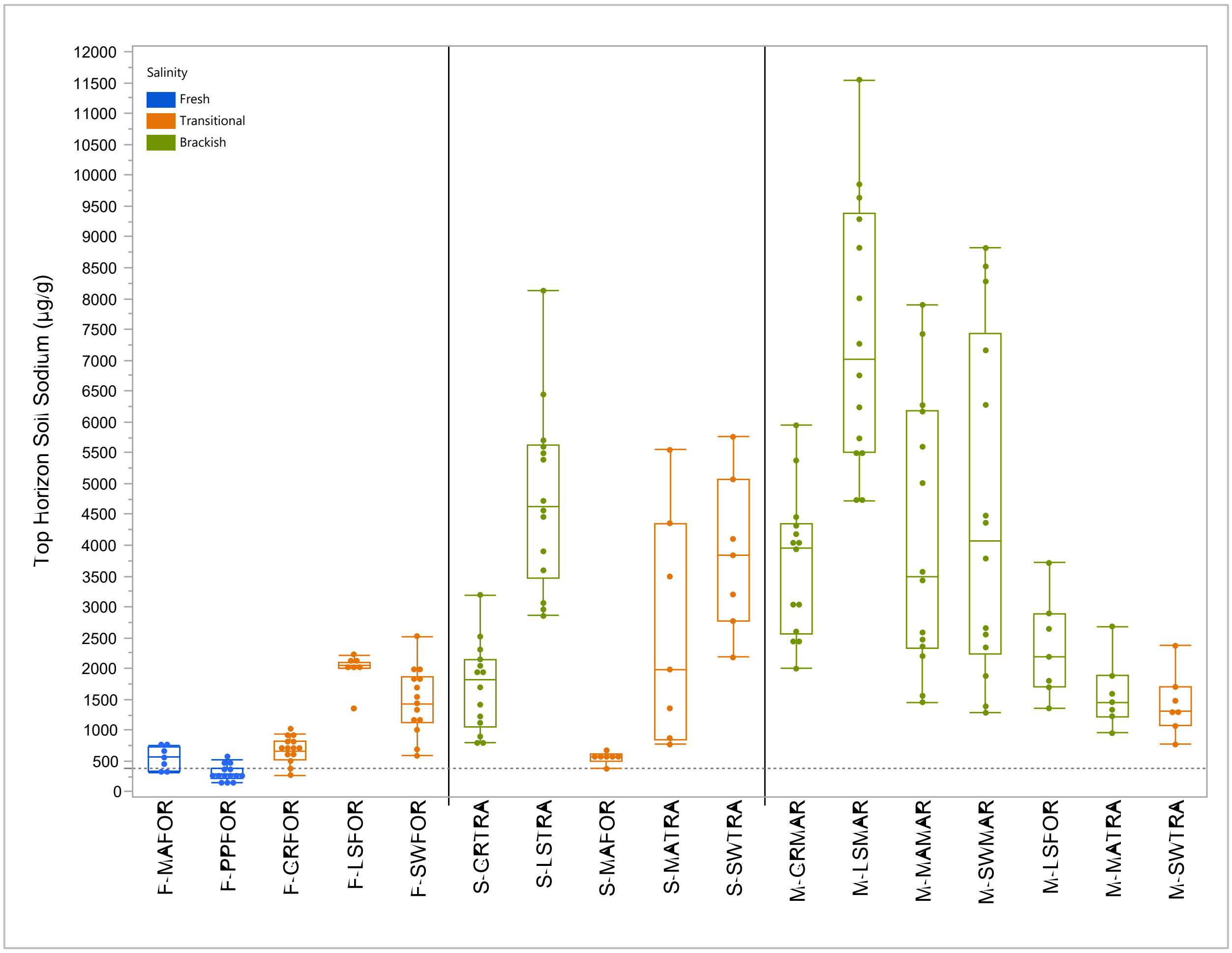


Figure 61. Variation in top horizon soil sodium within sites, from NCSU partner data with seven samples analyzed per site. F = forest, S = shrub, M = marsh. Dotted line on y-axis represents the whole community change threshold of 383 µg/g (=ppm) top horizon soil sodium.

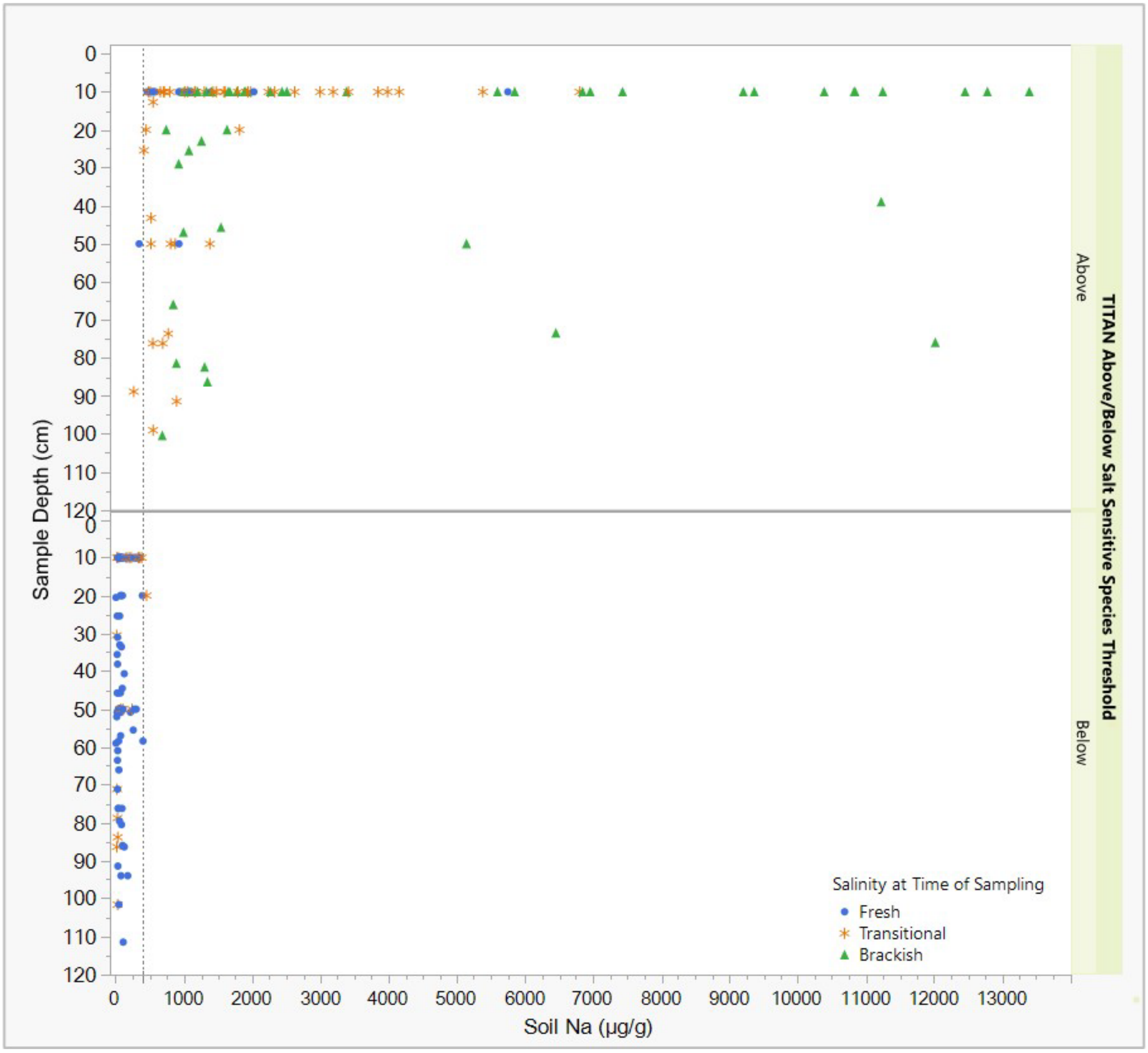


Figure 62. Soil sodium by sample depth for all horizons to one meter depth, where the top horizon soil sodium at that sampling event exceeded ("above") and fell below ("below") the whole plant community change threshold of 383 µg/g, represented by dotted line on x axis. If no depth measurement for top horizon was available, it was assumed to be 10 cm.

5 Discussion

5.1 Successional Processes – Typical and Atypical

Overall, wetland sites in this study slightly gained nonnative species, lost floristic quality, gained brackish-tolerant species and coverage, and lost tree cover while gaining herbaceous species. Freshwater forested wetlands showed the most changes in plant community composition, particularly in terms of greater invasion by nonnative taxa and a reduction in plant community quality (mean C). Overall cover of tree and shrub taxa also decreased significantly, with corresponding increases in herbaceous cover in these freshwater forested wetlands, sometimes leading to conversion to non-forested wetlands.

Unfortunately, increases in frequency and cover of invasive nonnative species in many ecosystems, including high quality wetlands, can be expected in the absence of active management to reduce them. These increases depress mean C, an indicator of plant community quality. Expansion of nonnative species and the coincident reduction in floristic quality measures may happen with and without any influences of climate change, though climate change may favor certain nonnative species and challenge certain native species. The top nonnative species creating changes in wetland plant communities in the outer Coastal Plain were Japanese honeysuckle (*Lonicera japonica*), Japanese stiltgrass (*Microstegium vimineum*) and the common reed (*Phragmites australis*), all of which are difficult to manage.

Studies of floristic quality over time on wetland restoration sites and successional upland sites indicate an asymptotic increase over time, with mature sites at a stable level of floristic quality (Gutrich & Hitzhusen 2004; Matthews et al. 2009; Spyreas et al. 2012). Normal successional processes in forested ecosystems experiencing non-stressful hydrologic conditions result in increased canopy cover and decreased herbaceous cover, as trees mature and further shade the understory (Rheinhardt 2007).

In outer Coastal Plain wetlands, changes in the opposite direction could be attributed to saltwater intrusion killing off intolerant woody vegetation, especially mature trees, with the resulting increase in available light encouraging expansion of cover by herbaceous species. Investigations using aerial imagery from 1949 through 2018 along tidal creeks along the Cape Fear River in North Carolina indicated a 14 to 18% loss of forested wetland from conversion to emergent marsh wetland. These conversions were in part due to higher salinities caused by saltwater access through frequent channel dredging in the Cape Fear River and in part due to rising salinities with higher sea levels (Magolan and Halls 2020). That study area also experienced the conversion of marsh to open water, a total loss of wetland ecosystem. Conversion of freshwater forest to brackish shrub or marsh wetland usually means a loss of species; species richness and diversity were lower in brackish marshes than freshwater forested wetlands in this study. The forested sites in this study experienced a reduction in tree taxa cover with an increase in shrub taxa and herbaceous species cover over time. Data collection from nearly 15% of the sites (11) included notes like "large pines mostly dead", "red cedars mostly dying", "many loblolly pines dying/dead", or data indicating woody stems were present at original sampling and completely absent at resampling. Thirteen (13) percent of sites in this study experienced a complete community shift, usually from a forested to less forested wetland type (shrub or marsh wetland).

In addition to salinity changes, the reduction in canopy species in the current study could also be caused by stressful flooding conditions resulting in woody species mortality. During reconnaissance for this study, the DWR team found several potential resample sites to be impassable due to high water levels and unstable muck. Surviving trees were found on hummocks created by fallen trees and herbaceous species were limited to those hummocks and tree bases (Gianopulos pers. obs.). These unsampleable sites included one site on the White Oak River sampled by DWR crews in 2011 and several sites located on the mouth of the Roanoke River sampled by CVS in the mid-1990s. Data from Surface Elevation Table monitoring for changes in elevation within the Roanoke River National Wildlife Refuge (NWR) from 2013 to 2020 showed that forested wetlands in the Roanoke River NWR have not been accreting while water levels have risen (Ladin and Moorman 2021). Similar monitoring in peatland wetlands of Pocosin Lakes NWR and Alligator River NWR has also shown subsidence which has increased their vulnerability to sea level rise and increased flooding.

Prolonged deep flooding can cause tree and seedling mortality, even for those species adapted to flooding, such as bald cypress and water tupelo (Conner et al. 2007; Light et al. 2007). Sustainability of these ecosystems depends on the recruitment of new individuals (saplings), which often require dry periods to germinate. Submergence of seedlings for just 10 to 20 days is fatal to seedlings of many swamp and bottomland hardwood species (Light et al. 2007). Freshwater wetlands in this study showed recruitment of woody species, but transitional salinity wetlands did not; stems counts in smaller size classes decreased between original and last sampling. Within the Albemarle-Pamlico Peninsula, Smart et al. (2020) found that aboveground biomass significantly decreased in forested, shrub and marsh wetlands between 2001 and 2014, using remote sensing and field data collection. Conner et al. (2007) also found recruitment to be lacking in many tidal freshwater forests in coastal Louisiana and northeastern South Carolina; long term inundation has caused conversion to shrub or marsh systems, or open water. Few tree species are adapted to deep flooding by brackish or saline waters. However, Conner et al. (2007; p. 237) stated that "experiments suggest that species already well-adapted to long flood durations handle the additional stress of low-level salinity better than do species somewhat less tolerant to flooding."

Transitional salinity wetlands are the most vulnerable to conversion to non-forested wetland types through loss of recruitment as well as mortality of larger trees. Brackish marsh area in the outer Coastal Plain of North Carolina has been expanding into previously forested areas, wetland or upland. Gray et al. (2021) estimated that between 1989 and 2011, emergent marsh wetland area in the North Carolina Coastal Plain more than doubled, increasing by 111%, located mostly in low-lying and fringe areas along the state's estuaries. Their results showed land cover spatial dataset pixels classified as forest or agriculture in 1989 converting to this emergent wetland by 2011. The largest conversion of agricultural land was to forest, some of which was to wetland forest, part of "an ongoing trend of farm abandonment in coastal areas in response to field salinization and the rising costs of active drainage" (Gray et al. 2021; p. 16). "In areas where the saltwater intrusion is less intense, [sea level rise] is still causing farms to be abandoned as they become too wet and turn into swamp forests" (Gray et al. 2021; p. 5). Gray et al. (2021) also stated that "a large number of pixels that changed to [emergent] wetland…appear to be along field boundaries as well as ditches and canals that provide connectivity to saltwater" (p. 14). This is what Ury et al. (2020) found in the Albemarle-Pamlico Peninsula, where on-the-ground data collection indicated that forested sites with high soil sodium content or low elevation have experienced a loss in woody biomass over time. Our data showed a similar trend; higher soil sodium was correlated with drops in woody stem basal area but also with greater species compositional changes in woody plant communities, particularly in forested wetlands. In a study on Swan Quarter NWR, Poulter et al. (2008) found that loblolly pine (*Pinus taeda*) seedlings were only observed at top horizon soil sodium levels below 400 µg/g; recruitment of new seedlings was negatively affected by higher soil sodium levels and competition with sawgrass. Transitional salinity sites in the current study are likely to show reduced recruitment of loblolly pines, as their median top horizon soil sodium level was 742 µg/g, with few sites falling below 400 µg/g (range 235 to 4,995 µg/g; n=15).

Sea level rise itself has the potential to cause more frequent and longer lasting flooding in freshwater or transitional forested wetlands. The flow of fresh water through North Carolina's streams and rivers through the Coastal Plain out to estuaries is slowed or impeded by higher sea levels, thereby keeping more water in the wetlands longer. On the other hand, in situations of low flow from drought, freshwater withdrawals, or low rainfall, higher mean sea levels also increase the potential for brackish or saline waters to reach higher into swamps and marshes, flooding them with saline waters longer than in the past.

Although North Carolina's existing Coastal Plain freshwater forested wetlands may still generally be in good condition and not yet changed as much as transitional wetlands, the fact that most of them are vulnerable to hurricane storm surge is important, because storm surges can bring large volumes of saline water into these important wetlands. An event like this can kill trees immediately and for years after a hurricane, because the elements of saltwater can persist in the soil, raising soil sodium levels and suppressing seedling germination and growth (Conner and Inabinette 2003). Prior freshwater flooding in wetlands experiencing storm surges can limit saline waters infiltrating the soil and allow trees to survive. However, low rainfall times, such as autumn in the Southeast US, droughts, and high levels of growing season evapotranspiration mean less available fresh water to buffer the effects of storm surges. Hurricanes tend to make landfall in North Carolina more often in the fall (NOAA's National Hurricane Center 2023). Storm surges and high tides during these times raise the probability of overland or underground saltwater intrusion into freshwater wetlands along the coast. Saltwater intrusion or storm surges resulting in salinities of just 2 to 5 ppt can alter carbon cycling in tidal freshwater forests, by suppressing methane production but increasing CO_2 production (Marton et al. 2012; Wang et al. 2017). Increasing salinity in wetland soils increases CO_2 production through reduction of sulfates by microbes, while reducing methane produced by methanogen microbes (Jakobsen et al. 1981; Chambers et al. 2013).

In addition to changes in forested wetlands, brackish marshes in this study have experienced a reduction in cover by herbaceous species, which was expected to remain stable. This could be attributed to increased flooding by higher sea levels and higher tides. Brackish and salt marshes exist in a balance between accretion/drying out and flooding, where too much of one or the other causes degradation or disappearance of the marsh (Linthurst and Seneca 1980). Reductions in plant cover could be signaling impacts of greater flooding by higher sea levels. High salinity could potentially cause reductions in plant cover as well, if saltwater is trapped and evaporation creates concentrated areas of salt, or salt pans with little vegetation (Srivastava and Jefferies 1995).

In this study, increases in soil salinity over time did not necessarily appear to be the cause of the reduction in herbaceous cover in marshes. Brackish marshes overall showed a reduction in median top horizon soil salinity from approximately 6,800 μg/g to 4,000 μg/g, which could indicate greater flooding by freshwater input into the estuaries during the study period. Increased flooding has been associated with die-back of North Carolina and Louisiana salt marshes due to ponding and the associated increase in sulfide concentrations and other factors (Linthurst and Seneca 1980; Flynn et al. 1990).

However, soil sodium data that could be found in the literature on other brackish marshes in North America suggest that the brackish marshes in this study had higher levels than others even during initial sampling (median 6,800 μg/g; range 1,097 to 19,769 μg/g soil sodium). A study of Louisiana marshes reported mean top horizon soil sodium levels of 90 to 650 μg/g in unmanaged sites (Boumans and Day 1994). Delaune et al. (2008) reported approximately 400 to 1,600 μg/g soil sodium in other natural Louisiana coastal marshes. Top horizon soil sodium in brackish marshes on Swan Quarter NWR in North Carolina ranged from 527 to 1,180 μg/g (Poulter et al. 2008).

5.2 Indicators of Saltwater Intrusion

Some have suggested that the Ca:Mg ratio in water can be used to categorize sites as affected by seawater, where those affected would have a ratio greater than 1.0 (Sudaryanto and Naily 2018). Chloride ion concentrations have also been used for detecting seawater intrusion into freshwater drinking wells, when the chloride is correlated with other elements of seawater (bromide, calcium, potassium, magnesium, sodium, sulfate) (Konikow and Reilly 1999; Kelly 2005; McSwain et al. 2014; Abdalla 2015; Asare et al. 2021). The category of "affected by seawater" based on either the Ca:Mg ratio or chloride ion concentration was associated with higher top horizon soil sodium, greater changes in the species composition of the woody plant community, and higher proportions of brackish-tolerant species, as well as cover by these species. However, there were conflicts between categorization based on the Ca:Mg ratio and categorization based on the chloride ion concentration. Sometimes, sites that were very obviously influenced by seawater based on the presence of highly salt-tolerant species (e.g., BRTTBY, FUTRLL, GSCRKN, SHBGBY) were categorized as not affected by seawater using the Ca:Mg ratio cutoff of 1.0 (Sudaryanto and Naily 2018). Overall, the chloride ion concentration-based categorization corroborated on-the-ground observations much more often than the Ca:Mg ratio-based categorization. Moreover, Lebrato et al. (2020) have reported wide regional variation in the Ca:Mg ratio in seawater, including river-influenced areas and coastal seas. Thus, the Ca:Mg ratio in water samples may not be a good indicator of intrusion by seawater. Surface or groundwater sample chloride ion concentration over 100 mg/L seems to be a better indicator of the presence of seawater, when it is also correlated with other ions found in seawater. A chloride ion concentration of 100 mg/L corresponds to approximately 0.3 ppt salinity, based on the close relationship between the two.

Baldwin (2007) suggested that the Ca:Mg ratio in soils may be a valuable tool for indicating intrusion by seawater, citing unpublished data indicating the ratio to be inversely related to chloride and sulfide concentrations in some coastal wetlands. Results from the current study also showed inverse relationships between soil Ca:Mg ratio and soil chloride, soil sodium and water salinity (sulfide data were unavailable, preventing a comparison with Baldwin's assertion). However, the potential unreliability of water Ca:Mg as an indicator and the positive correlation between water Ca:Mg and soil Ca:Mg means further study is needed to test whether the Ca:Mg ratio in soils could indicate seawater intrusion, particularly in a variety of wetlands and watershed situations. Threshold analysis could also be extended to the Ca:Mg ratio to determine what levels could possibly indicate seawater influence.

Sulfate, along with potassium, has been noted as being drastically higher in seawater than in freshwater (Mitsch and Gosselink 2000). However, sulfate can be broken down biologically, so is unlikely to be a good indicator of saltwater intrusion in wetlands.

5.3 Tipping Points/Community Change Thresholds

A number of scientists have concluded that woody species in wetlands are sensitive to water salinities of 2 ppt or higher (Pezeshki and Chambers 1986; Pezeshki et al. 1989; Conner et al. 1997; Kozlowski 1997). In a study by Hackney et al. (2007) in the Lower Cape Fear River, tidal freshwater wetlands exposed to chronic salinity levels of approximately 3.5 ppt experienced major plant community changes. They estimated a threshold for chronic salinity exposure of 2 ppt, which they suggested sets into motion conversion of forested wetland plant communities to primarily herbaceous, or marsh, wetland communities. They believed that the cause was salinity itself coupled with microbial-mediated results of biochemical processes associated with the components of seawater.

However, impacts are probably felt at much lower salinity levels than 2 ppt. Conner et al. (2007) and Krauss et al. (2007) found losses in tree diversity began in swamps along the lower Savannah River in Georgia as salinity increased from zero to 1.2 ppt. The type and number of canopy tree species decreased as salinity increased to 1.2 ppt (water tupelo [*Nyssa aquatica*], green ash [*Fraxinus pennsylvanica*] and sweetgum [*Liquidambar styraciflua*] died out). At more saline tidal forested wetlands (between 1.2 and 2.0 ppt), Conner et al. (2007) found that overstory species were of a different species composition than understory woody species, suggesting an impending conversion to a new plant community. They also found woody stem basal area to be negatively correlated with salinity between zero and 3 ppt. Within the current study's forested and shrub wetlands, data showed a similar relationship, but it was not statistically significant.

Anderson et al. (2022) analyzed soil sodium in relation to plant species occurrence from 34 wetland sites in the Albemarle-Pamlico Peninsula, using occurrence of herbaceous plants along with shrub and tree species <4m in height. The "understory community" changepoint predicted from their analysis was 265 μg/g of soil sodium. With data on the entire wetland plant community in this study, the same changepoint threshold analysis predicted a "whole community" change threshold of 383 μg/g (ppm) of soil sodium. Taken together, a top horizon (top 10 cm) soil sodium range of 265 to 380 μg/g could be considered a "red flag" zone, where a wetland could be vulnerable to whole plant community level shifts, especially under chronic exposure. The whole community changepoint of 383 μg/g soil sodium corresponds to approximately 0.93 ppt shallow groundwater salinity (1 m depth) or 1660 μS/cm specific conductivity, based on regression analysis. The understory community changepoint of 265 μg/g soil sodium corresponds to approximately 0.54 ppt shallow groundwater salinity (1 m depth) or 1022 μS/cm specific conductivity. Groundwater salinity and specific conductivity are easily measured with water quality meters and could potentially be used as proxies for lab soil analysis of soil sodium. Surface water salinity was not well correlated with soil sodium, so should not be used as a proxy for soil analysis.

Based on chloride ion concentrations, sites with surface or shallow groundwater salinities at 0.3 ppt or above could be considered impacted by seawater, if chloride ion concentrations are correlated with other elements of seawater such as bromide, calcium, potassium, magnesium, sodium and sulfate. If chloride ion concentrations are not correlated with these other elements, it may have a non-seawater source. Vegetative communities are potentially able to tolerate chronic exposure to salinities between about 0.3 and 0.5 ppt, but the threshold analysis suggests that chronic exposure to salinities above 0.5 ppt could precipitate changes in the understory community in freshwater wetlands, where sensitive species clearly decrease in frequency of occurrence and more tolerant species increase.

5.3.1 Species Needing Further Investigation

The community threshold analysis generated lists of species deemed "sensitive" to and "tolerant" of increasing soil sodium. However, the categories some species were placed in conflicted with information available in botanical keys and resource websites created by expert field botanists. The particular species with TITAN-assigned categories conflicting with expert botanical judgement should be targeted for experimental studies on salinity tolerances (Table 21).

Table 21. List of species categorized by the threshold analysis in conflict with information from recognized botanical resources (resources listed in Appendix F).

Scientific Name	Growth Form/Duration	Botanical Resources Brackish Tolerant?	TITAN Analysis Brackish Tolerant?
Andropogon virginicus	Herbaceous Perennial	No brackish habitat listed	Yes
Carex stipata	Herbaceous Perennial	No brackish habitat listed	Yes
Centella erecta	Herbaceous Perennial	No brackish habitat listed	Yes
Hydrocotyle umbellata	Herbaceous Perennial	No brackish habitat listed	Yes
Rubus pensilvanicus	Shrub Perennial	No brackish habitat listed	Yes
Sabal minor	Shrub Perennial	No brackish habitat listed	Yes
Triadenum virginicum	Herbaceous Perennial	No brackish habitat listed	Yes
Ilex verticillata	Shrub Perennial	Yes	No; sensitive
Mikania scandens	Vine Perennial	Yes	No; sensitive
Morella cerifera	Shrub Perennial	Yes	No; sensitive
Peltandra virginica	Herbaceous Perennial	Yes	No; sensitive
Persicaria arifolia	Herbaceous Annual	Yes	No, sensitive
Saururus cernuus	Herbaceous Perennial	Yes	No; sensitive
Sium suave	Herbaceous Perennial	Yes	No, sensitive
Toxicodendron radicans	Vine Perennial	Yes	No; sensitive

5.3.2 Challenges with Using TITAN Threshold Analysis with Soils Data

One challenge with using top horizon soil sodium in calculating changepoints is that soil sodium is not necessarily static over time or space. While changes measured in top horizon soil sodium on project sites usually ranged from a 20% increase to a 10% decrease, some individual sites experienced enormous changes between sampling events, including in wetlands that were freshwater wetlands at first sampling (Figure 60). At those sites whose top horizon soil sodium appeared to change drastically, changes could have been brought about by significant freshwater flooding or storm surges between sampling events. However, they could also be a result of resampling in slightly different areas from the original soil sampling. It is possible that soil sodium can vary spatially within a relatively small area, and sampling a different location from the original sampling could potentially produce wide variation between sampling events not necessarily indicative of change over time. One project partner collected and analyzed multiple soil samples from each of their sites, and soil sodium sometimes showed wide variation, particularly if levels were over the whole community threshold of 383 µg/g sodium. In sites affected by seawater, microtopography may play an important role in determining top horizon soil properties, by affecting the flow of saline water across a site. More study is needed on spatial variation of soil properties within wetlands.

The TITAN threshold analysis was also used to produce a Ca+Mg threshold for whole plant community change (55 mEq/g) (Appendix E). On project sites, the properties of soil sodium and soil

Ca+Mg were significantly correlated with each other ($p<.0001$; $r^2=0.62$); however nearly all sites with soil chemistry data had Ca+Mg levels above the predicted whole community change threshold. The validity of the Ca+Mg threshold figure is uncertain because all sites included in this study had top horizon soil Ca+Mg above the threshold, and in the full dataset used for the threshold analysis, very few data points existed below that threshold (nine sites or 6%). In contrast, the data were split nearly 50:50 in relation to the soil sodium threshold (46% below; 54% above). Despite the suggestion by Baldwin (2007) that calcium and magnesium could be good indicators of seawater intrusion into wetlands, top horizon soil sodium levels may be a better indicator of changes in plant communities than Ca+Mg or the Ca:Mg ratio. A dataset with wider variation in Ca+Mg is needed to test the stability and validity of the threshold predicted by the TITAN analysis using the current project's dataset.

5.4 Resampling Challenges

Error could have been introduced by the varying difficulty of locating original sampling plots. Despite best efforts to locate the original sampling plots, DWR scientists and partners acknowledge difficulties in finding permanent markers from the original sampling events if they were left. Sometimes, permanent markers were not placed during the original sampling. Additionally, latitude/longitude values obtained or estimated decades ago had different levels of accuracy than that during resampling times. However, sampling teams always had the benefit of historical information, and if they were unable to locate the original sampling plot markers, they corroborated other historical information such as the presence of certain large trees, original plant community descriptions and site drawings to locate the resample plots as accurately as possible.

5.5 Closing Notes

Although rate of change over time is the best standardized measurement available with this dataset, it does imply a constant rate of change, especially for long interval sites. Without continual monitoring, it is impossible to know whether some sites changed quickly from one community type to another, for example from shrub wetland to brackish marsh, and then stabilized as the second community type after the transition. A rapid large change like this could appear slow when calculating rates of change over long resample periods, when in fact it could have been the opposite.

Given the changes occurring in transitional salinity forested wetlands in particular, detection of freshwater coastal wetlands that are vulnerable to change, or affected by seawater, is imperative. Loss of forested freshwater wetlands comes with a loss of the tremendous value they provide to humans and wildlife, economically, ecologically, culturally and recreationally (Isbell et al. 2011). Freshwater forested wetlands showed recruitment of new individuals of woody species between original and resampling, but there was a drop in recruitment between sampling events in transitional salinity forested wetlands. Additionally, larger size classes of trees were represented in the freshwater forested wetlands, but they were generally missing in the transitional forested wetlands during original sampling and more so during resampling.

Decreasing recruitment in transitional salinity forested wetlands is a concern that warrants attention, through further research, management of existing areas, restoration of historical wetland areas, or creation of new wetland areas. Indicators of seawater intrusion could be used to detect sites that have been minimally impacted or are unimpacted by seawater and efforts could be made to conserve, protect and restore these existing coastal forested wetlands. Priority should also be put on conserving land upslope of these important wetlands, as well as proactively managing them for future sea level rise.

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