Assessing the Effects of Agriculture on Plant Diversity in Ephemeral Wetlands

Alexis Jackson and Mackenzi Hallmark

Introduction

Wetlands function as important economic and ecological resources, providing habitat for unique plant and animal species, fresh water, natural upstream nutrient filtration, flood abatement, and are habitat for the production of major crops such as rice (Zedler and Kersher 2005). However, wetlands are facing many obstacles because of global change factors such as increased nutrient inputs from agriculture, invasive plant species, destruction for infrastructure, increased temperature and droughts (Adame et al. 2019, Xu et al. 2019 and Lamsal et al. 2019). Despite their importance, about one-half of wetlands in the contiguous United States have been converted to human development use since European colonization, primarily for agricultural use (Brinson and Malvárez, 2002). A study conducted in the Midwestern United States even found that protected wetlands on agricultural-retired land are being converted back to agricultural uses indicating that wetlands are being wholly destroyed or are within close proximity to farming (Morefield et al. 2016). Although the replacement of wetlands with farms is one of the biggest threats to their existence, there is still much to be understood about the effects of nutrient runoff from agricultural areas on wetland health (Carolina et al. 2016).

Nutrients, soil and clay content, pH, and surrounding land use are the most prominent factors suggested to affect species diversity (Ehrenfeld 2008). Soil provides essential nutrients for optimal plant growth. Wetlands have limiting nutrients, which can affect whether invasive or noninvasive plants will grow. Nitrogen is typically the limiting nutrient, and once invasive species are removed, native plants have a greater capacity to take in more nitrogen (Wang et al. 2015). When nitrogen- and phosphorus-rich agricultural runoff finds its way into wetlands, plant biodiversity and an environment's susceptibility to invasion are affected (Kercher and Zedler 2004). According to the fluctuating resource availability hypothesis, an abundance of nutrient inputs into wetland water is a form of disturbance that invasive plant species seize as an opportunity to establish and take over native species leading to decreased species richness (Davis et al. 2000).

Plant biodiversity is a defining characteristic of wetlands, and it is well known and documented that invasives and nonnatives threaten to take over native plant species (Alpert et al. 2000). However, the conditions under which invasives are able to establish is less understood, especially in ephemeral wetlands. Ephemeral wetlands are a type of wetlands that occasionally hold water, but are seasonally dry and can be characterized by their smaller size, inundation in the spring, and dry conditions during the growing season (Little and Church 2018, Boeckman and Bidwell 2015). Many studies observe wetlands during their wet season; however, this leaves little understanding of the interactions occurring during the dry season. As temperatures increases because of environmental change, dry seasons will become more prominent, and the efficiency and productivity of wetlands in this condition offers a different environment for plant establishment and growth.

In this study, we ask: does ephemeral wetland proximity to agricultural land affect its plant biodiversity? Six dry ephemeral wetlands in Montgomery County, Virginia were surveyed for species richness and soil nitrogen and phosphorus concentrations. We hypothesize that wetlands surrounded by a greater area of agricultural land will have higher nutrient concentrations and lower plant diversity due to decreased interspecies competition for resources. Specifically, we predicted that: 1.) Nutrient concentrations will be higher in wetlands that are surrounded by a higher percentage of agricultural land, 2.) There will be lower plant biodiversity in wetlands that have a higher percentage of surrounding agriculture, and 3.) There will be a higher percentage of invasive plants in wetlands with a higher percentage of surrounding agricultural land.

Methods

Study Sites

This study used an observational approach for data collection. All sites were located within the Christiansburg and Blacksburg area (Montgomery county). Wetlands were found with ArcGIS mapping software using the National Wetland Inventory data. Google Earth Pro satellite imaging and mapping software was then used to confirm wetland location and accessibility. Sites were included in the study if there was no standing water (i.e. dry), and if they were publicly accessible. The sites include **STrEAM Lab** (*37°12'53.4"N 80°26'27.9"W*), **Kentland Farm** (*37°19'56"N, 80°59'03"W*), **Merrimac** (*37°11'15.7"N 80°25'45.6"W*), **Heritage Park** (37°14'25.4"N 80°27'32.3"W) **Corporate Research Center Wetland** (*37°12'14.01"N, 80°25'0.59"W)* and **Pandapas Pond** (*37°16'55.65"N, 80°28'0.05"W*).

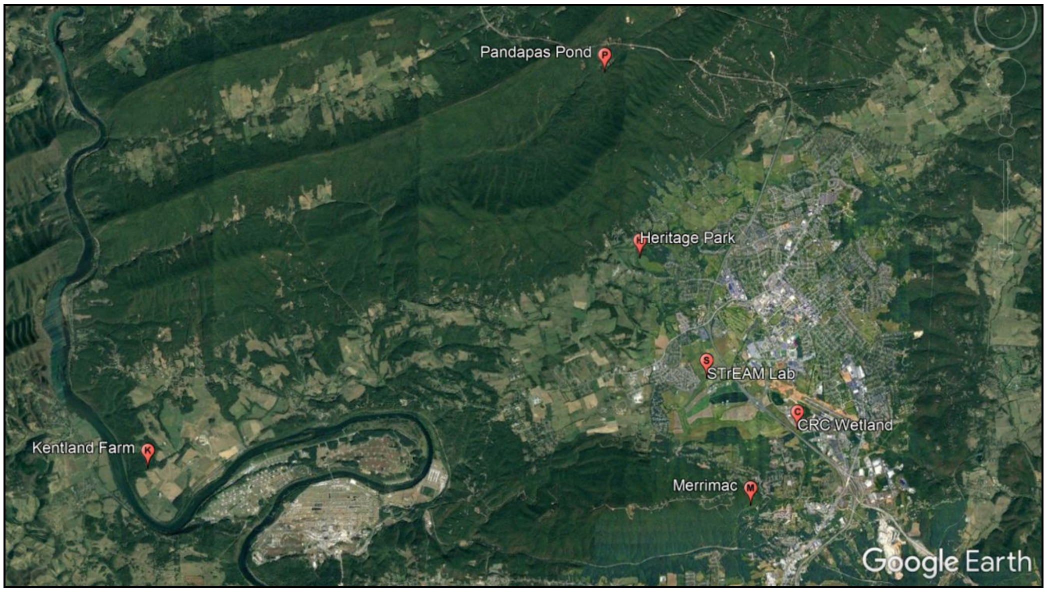

Figure 1. Satellite image location of all wetland sites *Credit: Google Earth Pro*

Field Sampling

At each site, pictures were taken from the North, South, East and West direction. Before laying the transect, wetland delineation was assessed using all of the following observations: moss growth on trees that was above contact with the ground, water lines on trees, the presence of hydric soil, hydrophilic vegetation, and ArcGIS data that supported wetland presence (U.S. Army Corps of Engineers 2010). Once the wetland boundaries were ascertained, a transect was laid through its longest length (Little and Church 2018). A random number generator was then used to produce five random points for quadrat placement and two more random points where soil samples would be taken (Little and Church 2018). If any two random numbers fell within 111 cm of each other, a new random number was generated to avoid the quadrats overlapping and double counting of plants. Flags were then placed at each point to mark and center a Daubenmire quadrat frame (55.5cm x 55.5cm). The vegetation was surveyed within the dimensions of the quadrat frame.

A plant was determined to be within the confines of the quadrat if the point at which the stem met the ground was also within the quadrat. Vegetation was closely examined to differentiate between different species, and each apparently unique type was assigned a morpho-species identification code. Taxonomic identification was determined after surveying using notes and pictures taken of each plant. Each individual plant was counted for each species. This process was repeated for every square area.

Two soil samples were taken approximately seven centimeters below the surface at each wetland. The soil was placed in centrifuge tubes to be taken back to the lab for analysis of pH and nitrogen and phosphorus concentrations.

Laboratory Analyses

Soil samples were taken back to the lab for analysis of nitrogen and phosphorus concentrations and pH using the Model-EL LaMotte Soil Test Kit (Maryland, United States). Samples were frozen and tested within 24 hours after being collected. Results for the nitrogen and phosphorus concentrations were determined using a scale provided in the kit ranging from “trace”, “medium”, and “high”. Each results category has an assigned approximation of the specified nutrient per pound per acre (lbs/acre).

Species Identification

To identify the plants found in the quadrats, pictures were uploaded into *iNaturalist*, a public citizen science project used to identify and locate organisms around the world. The suggestions offered by this application were confirmed using field guides and the USDA Plant Database. 61.22% of plants were identified to the species level, 30.61% were identified to the family level, and 8.16% were unable to be identified.

Once species were identified, the Virginia Department of Conservation and Recreation Native and Invasive Species Lists were used along with the USDA Plant Database to determine if the plant was invasive or native. A plant was deemed invasive if it was on the Virginia DCR list of invasive Species. If a plant was listed as native or introduced it was deemed native. Plants that were unable to be identified to the species level were not assigned a category of invasiveness.

Data Analysis and Model Statements

Simpson's Diversity Index was calculated for each wetland using the equation **(1-D)** (Mangurran and McGill 2011). Crop agriculture data was derived from the USDA GIS layer for the year 2018 and was laid over Montgomery County. Percent area agriculture was calculated within five square kilometers surrounding each wetland using Google Earth Pro. Cells in the grids containing crops were counted and then divided by the total number of cells within the five kilometer radius to calculate percent agriculture. Each cell within the grid was approximately one square kilometer.

To test the effects of percent agriculture on nutrient concentrations (Prediction 1), we used a series of linear regression models with percent agriculture as the predictor and phosphorus concentration or pH as the response. To test the effect of percent agriculture on plant biodiversity (Pediction 2), we used a series of linear models with percent agriculture as the predictor and Simpson’s diversity or total plant abundance as the response. In addition, a nonparametric muti-deminisonal scaling (NMDS) analysis was used to visualize the relationship between species composition and site. To test the effect of surrounding agriculture on percent invasives (Prediction 3), we used linear models with percent agriculture as the predictor and either percent invasives or percent natives as the response. Finally, we used a series of linear models to examine the effects of percent natives or percent invasives on the richness, abundance, or Simpson's diversity of plants.

Results

Nitrogen was observed within the soil samples, but there was no variation between sites as only "Trace" amounts were found .This lack of variation meant that we did not run any statistical tests on nitrogen concentrations effects on plant diversity. However, phosphorus concentrations did vary between sites. After running a linear regression between phosphorus concentration and percent agriculture, we found no effect of percent agriculture on phosphorus concentration (p=0.333, R^2=0.5193, F=1.62).

Since there was no effect found between percent agriculture and phosphorus or nitrogen, we decided to see if pH had any effect. The relationship between soil pH and species richness was found to be marginally significant (*p*=0.0506; Fig. 2).The linear regression model was then used to determine if there was an effect of pH on abundance; however, there was no significant relationship (*p*=0.9661).

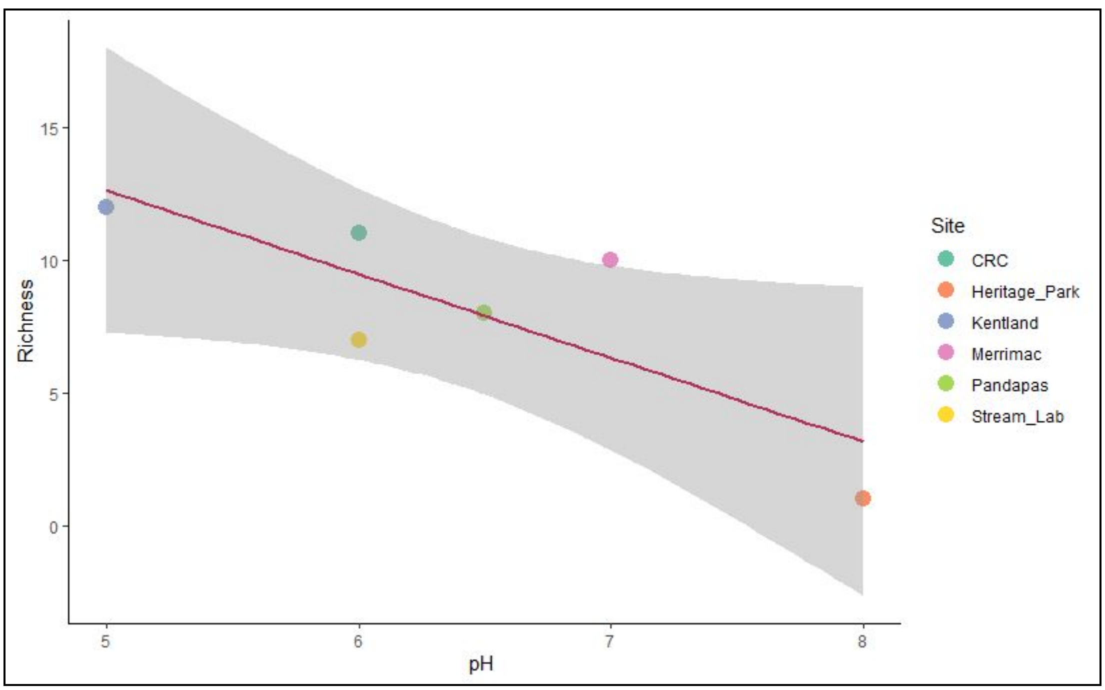

Figure 2. Relationship between soil pH and species richness among six sites. P values reported there was a significant relationship between the two variables.

In order to determine if percent agriculture in a surrounding landscape affected plant biodiversity in wetlands, we used a linear regression model with percent agriculture as the predictor variable and Simpson's Diversity Index as the response variable (Figure 3). There was no significant effect of percent agriculture on Simpson's Diversity Index (p = 0.4103, R^2=0.1742, F=0.8436).

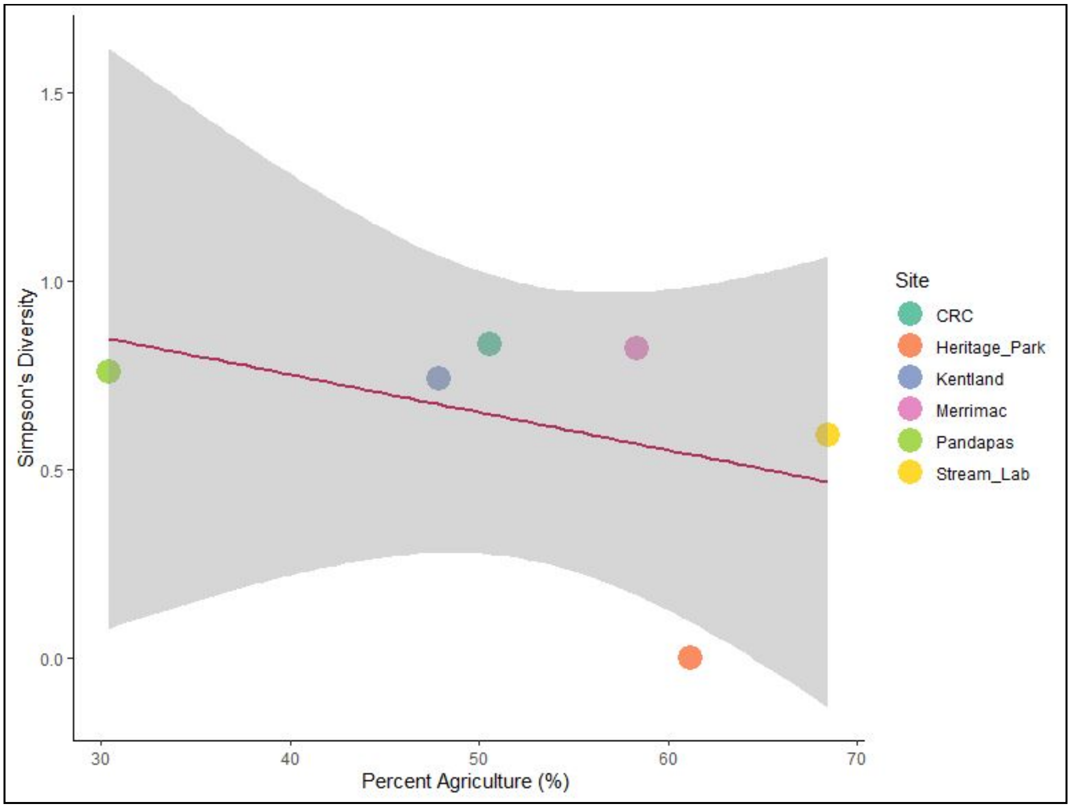

Figure 3. Percent agriculture and **Simpson's Diversity** Index model for six wetland sites near Blacksburg,VA

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There was also no significant relationship between percent agriculture and species richness between the sites (p = 0.5074). However, there was a significant relationship between percent agriculture and plant abundance. Across an increase in agriculture from approximately 30-70%, there was a ten-fold increase in plant abundance (p = .04243; Figure 4).

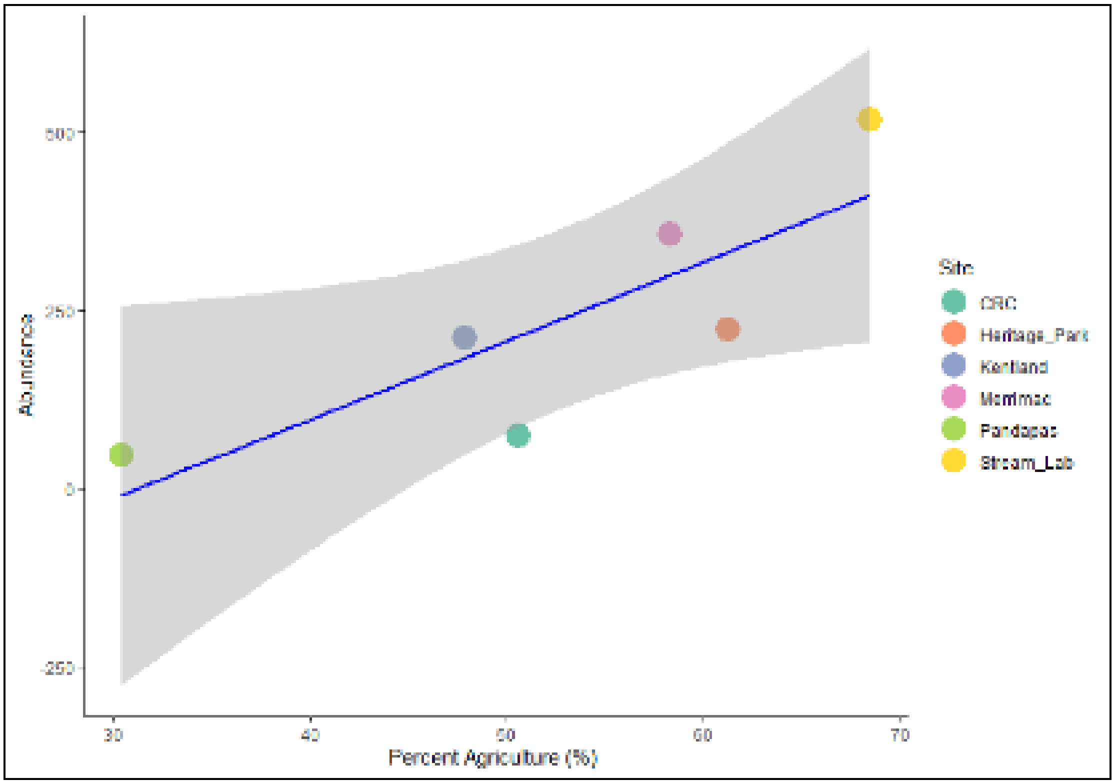

Figure 4. Relationship of percent agriculture and abundance between sites.

The NMDS plot was used to determine whether sites differ in their overall species composition (stress = 0.0006; Fig. 6). Based on the spatial relationships of the clusters in the plot, we found that Merrimac, CRC, Pandapas, and Kentland have the most overall similar species composition to each other. Heritage Park and Stream Lab are most dissimilar from each other and from the other clusters as indicated by their placement within the plot (Fig. 6).

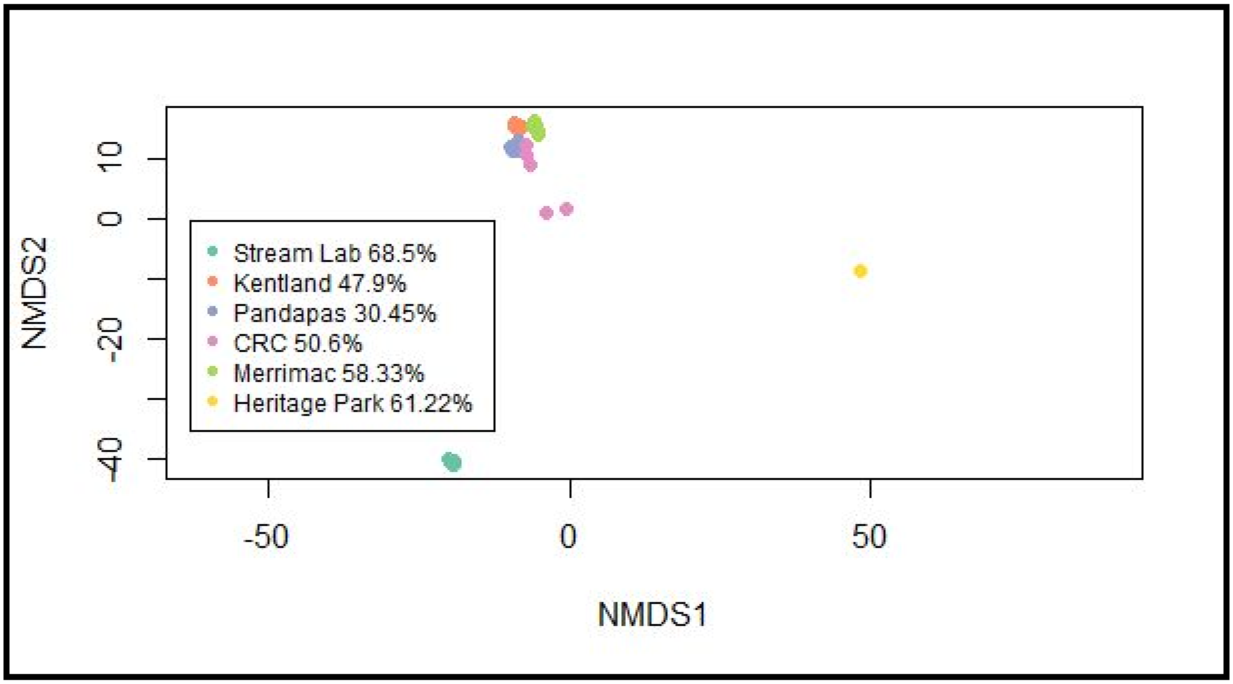

Figure 5. NMDS ordination plot for species composition at each site. Note: Heritage Park only contained the same single species for each of the five quadrats; therefore, each plot point overlays one another appearing to be a single point when in fact it is all five points on top of each other.

The effect of percent agriculture on percent invasive species had no overall significance (p=0.1693, R^2 =0.4122, F=2.805,Figure 6). However, it is interesting to note that three of the four wetlands sites with the highest percent agriculture (CRC, Heritage, and Stream Lab) all had no invasive species found. There was also no effect of percent agriculture on the percent of native species (p=0.2691, R^2=0.2912, F=1.644) (Figure 7). The three sites with the highest percent agriculture values (Heritage, Stream Lab, and Merrimac) also had the highest abundance values; so we ran the linear regression on percent natives and abundance but there was no significant effect (p= 0.2586, F=1.731, R^2= 0.3021) Linear regression models were also run to test the effect of invasives and natives on Simpson's Diversity Index, and richness. The only significant result from these tests was the negative effect of percent natives on species richness (Figure 8; Table 1).

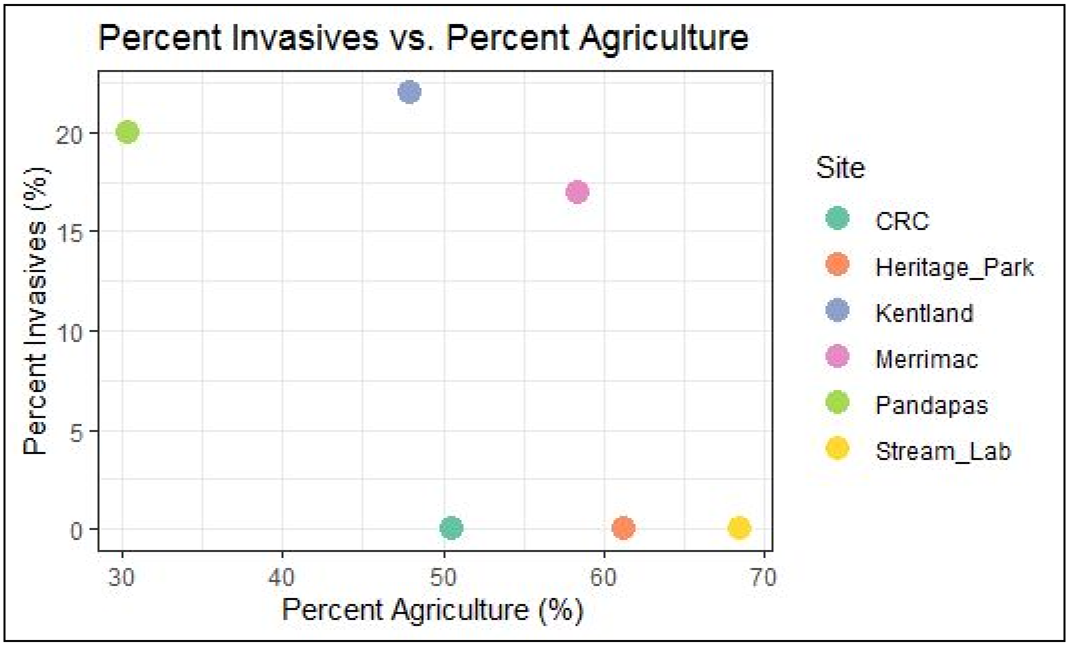

Figure 6. The effect of Percent Agriculture on Percent Invasive Species for each site.

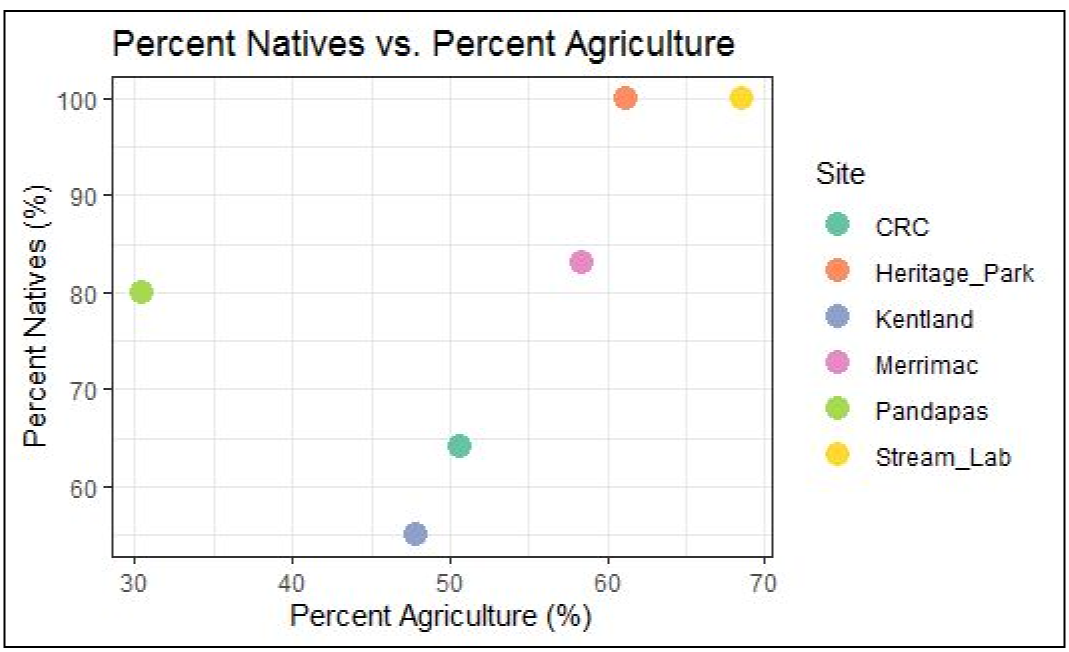

Figure 7. The effect of Percent Agriculture on Percent Native Species for each site.

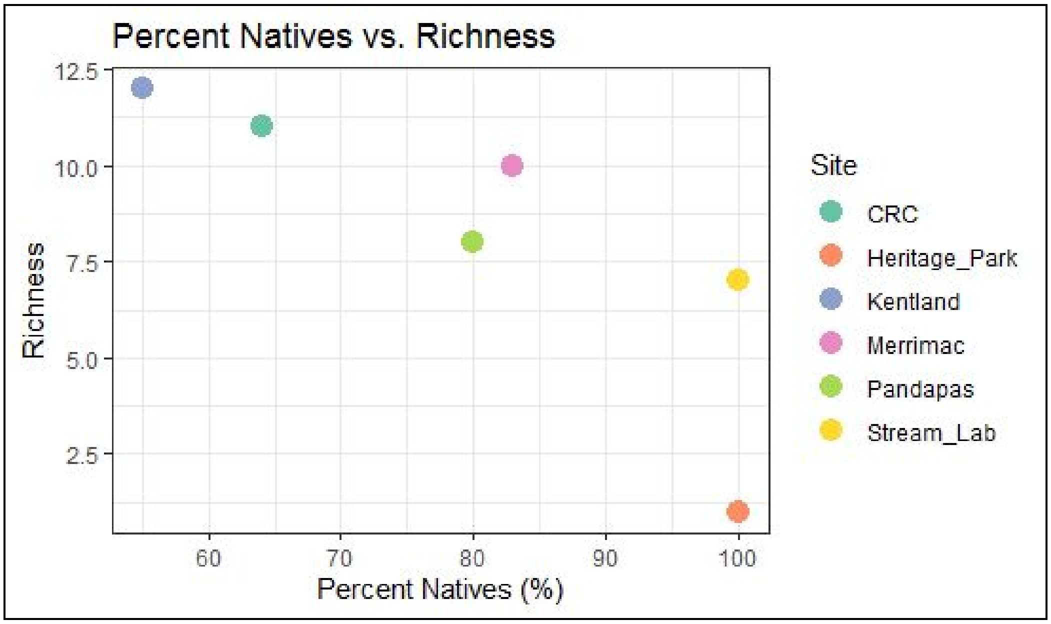

Figure 8. Effect of Percent Natives on Richness.

Table 1. Linear Regression Results for Percent Invasives and Percent Natives

Variable X	Variable Y	p-value	F-statistic	R-squared
% Invasives	Abundance	0.6344	0.2641	0.06193
% Natives	Abundance	0.2586	1.731	0.3021
% Invasives	Richness	0.2916	1.474	0.2692
% Natives*	Richness	0.04161	8.753	0.6864
% Invasives	Simpson's Diversity	0.3121	1.336	0.2504
% Natives	Simpson's Diversity	0.1679	2.828	0.4142

*Indicates significant result

Discussion

In this study, we sought to determine the effect agricultural areas surrounding ephemeral wetlands had on plant biodiversity during the dry season. Past studies have illustrated that higher nutrient concentrations of nitrogen and phosphorous can decrease plant biodiversity in inundated wetlands (Kercher and Zedler 2003). However, these studies have not evaluated the effects of nutrients where water is not present continuously in small and hydrologically variable ephemeral wetlands. In high agricultural areas such as Montgomery County, Virginia, where our study took place, it is important to evaluate the effects of nutrient runoff from neighboring croplands on these vulnerable habitats. Our results indicated that there was no effect of percent agriculture on Simpson's Diversity Index, phosphorus or nitrogen concentrations, and neither invasive or native species percentages. We did, however, find an effect of percent agriculture on abundance.

Overall, the results of this study do not support the main hypothesis that wetlands surrounded by a greater area of agricultural land will have higher nutrient concentrations and lower plant diversity due to decreased interspecies competition for resources during the dry season. It was determined from our results that percent area agriculture had no effect of either phosphorus or nitrogen using the coarse nutrient measurements of the LaMotte Soil Test Kit. Nitrogen levels were consistently low at every site; therefore, no discernable conclusion can be made regarding its effect on plant diversity. This does, however, suggest that even in areas of higher percent agriculture (i.e. Stream Lab and Heritage Park with 68.5% and 61.22%, respectively) the nitrogen levels do not vary from areas of lower percent agriculture. However, these low nitrogen observations could be in relation to the ending of the agricultural season and a rain event happening after a record of continuous drought. This could have caused the very few leftover nutrients from the growing season to be washed away before soil samples were taken. Although phosphorus levels did vary, it was only slightly, and also had no relationship to percent agriculture.

Despite no relationship being observed between nitrogen and phosphorus concentrations and percent agriculture, there was, however, a relationship between plant abundance and the percent area agriculture. Those sites with the most crops within a five kilometer radius were found to have higher number of individual plants along the transect. Since phosphorus and nitrogen levels did not increase as percent agriculture increased, phosphorus and nitrogen cannot be attributed to the increase of plant abundance. In previous studies, it has been shown that lowering the phosphorus loading levels, will increase the chance that the wetland will be able to maintain its biodiversity (Braskerud 2019). Therefore it could be assumed that the wetlands that were observed within this study are less impacted by invasive species because of its low phosphorus levels and its “trace” amounts of nitrogen detected. This is directly supported by more native species than invasive species being located within the six wetlands we observed. We decided to test the relationship between pH, a control variable of this study, and abundance in an effort to explain a mechanism for increased abundance, but there was no significant relationship that could offer an explanation. Soil pH was, however, correlated to the richness of species at each site. The pH was observed to be mostly neutral within each wetland. Stormwater can cause pH to become neutral and plants have the ability to acidic drainage into nutrients it can use to create optimal growth ph despite acidic conditions (Nyquist and Greger, 2009). Plants also have the ability to change the soil pH surrounding it which may be correlated to why we were observing these neutral pH. This leads to the question of if soil pH is a driver of richness, why is it not a driver of abundance?

The NMDS analysis represents the relationships between all of the quadrats we sampled throughout the study. Kentland, Merrimac, Pandapas, and CRC sites were clustered relatively close to one other indicating that there was similar species composition between those sites and within their respective quadrats. The CRC site was the only one of that cluster with slightly more variation among two of the quadrats. These four sites also had the highest species richness and the lowest percent area agriculture calculated. Stream Lab and Heritage Park had the highest percent area agriculture and the lowest species richness and were very distant from each other and the other cluster on the NMDS plot. Although species richness and percent agriculture do not have a relationship according to our results, the NMDS plot suggests that there could be a relationship between percent agriculture and species composition and evenness.

The fluctuating resource hypothesis led us to our prediction that the wetlands surrounded with higher percent agriculture and thus more nutrient concentrations would have more invasives due to increased disturbance and an abundance of resources that decreases interspecies competition (Davis et al, 2000). This was not the case, however. In fact, far fewer invasive species were found than expected. Although this is good news, the environment is constantly changing and ephemeral wetlands are at increased risk of invasive species due to their dry state. The filling and drying of water acts as a disturbance and invasive species have been shown to have a competitive advantage to native species during these conditions when compared to permanent wetlands (Price et al. 2011). This disturbance coupled with the disturbance of agriculture could act together to increase the risk of invasion in temporary wetlands. More research is required to understand the complex interactions of hydrologic regimes of wetlands and anthropogenic effects.

It is also important to discuss the issues concerning plant identification in this study when discussing the percent invasives and percent natives. We were able to identify approximately 61% of plants with confidence despite being in their vegetative state. However, the information used to determine if a plant was native or invasive was somewhat lacking. The Virginia Invasive Plant Species List provided by the Virginia Department of Conservation and Recreation has not been updated since 2014. Also, neither the VA DCR or the USDA Plant Database had much information on the status of plants that were considered to be *introduced.* Because of this, introduced species were considered to be natives but this could have skewed our results . More accurate, clear, and up-to-date information is needed to bolster studies trying to understand plant biodiversity and the ecological and economic threats of invasive species.

Overall, the results found in this study conclude that percent agriculture does not affect the biodiversity of plants in ephemeral wetlands and that they may be less susceptible to invasive species due to having lower phosphorus and nitrogen levels, which does not support our hypothesis. The mechanisms behind why there were consistently low nutrient levels in the ephemeral wetlands despite being surrounded by varying percentages of agricultural land are not clear. Continued research will offer a better understanding of the relationship between wetland nutrient concentrations and agriculture within different temporal seasons.This extension will compose of sampling the same wetlands in a period where rainfall is more consistent, standing water is present and runoff is more prevalent. We believe that more studies examining these complex relationships will provide better agricultural and invasive species management practices in order to protect and preserve the vital economic and ecological services of wetlands.

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