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Elizabeth Anne Bruen
eab1340@rit.edu

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Effect of Simulated Canopy Cover on Created Vernal Pools in the Northeastern US

by

Elizabeth Anne Bruen

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Master of Science in Environmental Science

Thomas H. Gosnell School of Life Sciences

College of Science

Rochester Institute of Technology

Rochester, NY

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Committee Approval:

Anna Christina Tyler, Ph.D. Date
Thesis Advisor

Elizabeth N. Hane, Ph.D. Date
Committee Member

Solon Morse, Ph.D. Date
Committee Member

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Abstract

Vernal pools in the Northeastern United States are small, forested wetlands characterized by ephemeral flooding. These unique ecosystems provide critical habitat for both terrestrial and aquatic organisms, abate seasonal flooding, and are biogeochemical hotspots. Vernal pools may be created to maintain key ecosystem services associated with loss of natural wetlands. In many instances, however, created pools do not mimic the ecological functions and services of extant vernal pools due to shortcomings associated with the surrounding land use and canopy cover, hydrology, or invasion by opportunistic plants. Because of the importance of these systems to regional biodiversity, understanding the conditions required for adequate restoration of ecosystem function is imperative. The goal of this thesis was to determine differences in physico-chemical and biological properties between created and natural wetlands and to experimentally manipulate light availability and litter input in created pools to evaluate the importance of canopy cover in establishing desired ecosystem functions in created vernal pools. This study took place in natural (n=4) and created (n=8) vernal pools at High Acres Nature Area in Perinton, NY. Natural pools received over three times more carbon input from leaf litter and had greater light availability than created pools. The macroinvertebrate and plant communities were also markedly different, with a striking lack of invasive plants in natural pools. There were no significant differences in soil phosphorous or ammonium, but there were differences in surface water concentrations of the respective nutrients. Invasive species regrowth was limited in pools with experimental canopy shade, suggesting the potential for artificial canopy cover to limit invasion by undesirable plants, help to promote biodiversity, and aid in successful wetland restoration.

Introduction

Vernal pools in the Northeastern United States are small, forested wetlands characterized by periodic flooding, typically in spring. Wetlands provide the largest value of ecosystem services on an areal basis relative to other terrestrial ecosystems (Costanza, 2014). These include flood control, drought prevention, water quality protection, nutrient removal and provision of habitat for numerous species (Mitsch and Gosselink, 2000; Schlesinger, 1997). Vernal pools in particular are key components in flood protection following spring snowmelt and rains, and can be important regional sinks for nitrogen (Brooks, 2009; Capps et al., 2014). These unique ecosystems also support both terrestrial and aquatic organisms, acting as local hotspots for biodiversity (Colburn et al., 2007). To combat extensive loss of wetlands, vernal pools may be created to mitigate losses in area and function of existing vernal pools (Lichko and Calhoun, 2003). In many instances, however, created pools do not mimic the natural ecological functions found in extant vernal pools due to shortcomings in the restoration process, especially susceptibility to invasive plant species, lower soil organic matter, altered carbon cycling, an altered hydroperiod, lack of canopy cover, and lower diversity of macroinvertebrates (Korfel et al. 2010; Lichko and Calhoun, 2003). Because of the importance of these systems for regional biodiversity of both terrestrial and aquatic species, gaining a better understanding of conditions required for adequate ecosystem function is imperative to enable more successful vernal pool creation.

Vernal pools are depressional, often ombrotrophic, wetlands characterized by periodic flooding, typically during the spring and fall as a result of increased precipitation and snow melt (Mitsch and Gosselink, 1993). Small isolated pools are fed primarily by precipitation, whereas more complex systems of pools may be temporarily connected to other water bodies via groundwater or surface water (Rains et al., 2006). The limnology of vernal pools is extremely variable as a result of the small volume of water and temporal fluctuations (Bonner et al., 1997; Carrino and Swanson, 2007) that are controlled in part by precipitation, bathymetry and loss to groundwater (Black and Zedler, 1998; Brooks 2004). It is this hydroperiod, or temporal pattern of flooding, that is a key driver of biogeochemistry and biotic community structure (Batzer et al., 2005). However, the surrounding landscape may be equally important. In California, vernal pools are most often found in open grasslands, whereas in the Northeastern United States and other temperate regions, vernal pools are typically found in forested landscapes. The surrounding forest canopy has an indirect effect on hydroperiod, by influencing light availability, temperature, evaporation and water uptake and transpiration by vegetation (Black and Zedler, 1998; Brooks, 2004).

In seasonal pools, there is substantial variation in water chemistry, including nutrient concentrations and pH, associated with the length and frequency of the hydroperiod (Bonner et al., 1997). As a result, vernal pools may be hotspots of biogeochemical activity. The rates of decomposition and denitrification are greater in flooded zones than in the surrounding landscape (Capps et al., 2014) and there is evidence of mercury methylation and bioaccumulation in seasonal forest pools relative to the length of the hydroperiod (Brooks et al. 2012). However, these factors are also driven by the canopy cover, which controls temperature, evaporation, light availability and delivery of labile carbon (Schiesari, 2006), resulting in characteristic differences in the biogeochemistry of closed canopy versus open canopy pools. In an open canopy pool, high light availability promotes growth of algae and floating and emergent vascular plants that then supply higher trophic levels. In closed canopy pools, however, light is intercepted by the trees and organic matter delivery to the pools is delayed until leaves are shed in the autumn. As a result of this more refractory source of organic matter, closed canopy pools tend to have a higher carbon: nitrogen ratio in the soils (Schiesari, 2006).

Hydroperiod and canopy cover are also driving factors in the biological community of vernal pools, which includes macroinvertebrates, vascular plants, algae, amphibians, migratory birds, and some mammals. Vernal pools serve important nursery functions for many species of amphibians because of the lack of fish and other desiccation-intolerant predators (Bonner et. al, 1997). Obligate vernal pool amphibians, including the endangered tiger salamander (*Abystoma tigrinum*), Jefferson salamander (*Ambystoma jeffersonian*) and wood frogs (*Rana sylvatica*), spend their lives near their natal vernal pool. During the flooded period, a variety of opportunistic species colonize the pools (Bonner et al., 1997). These species often have adaptations that allow them to leave or survive desiccation later in the season. As a result of the unique adaptations required to survive the varying quantities of water in a vernal pool, the diversity of rare species may be higher in vernal pools than in permanently flooded systems (Collinson et al., 1995), though pools also provide habitat for common permanent pond species

(Nicolet, 2004). Thus, at the landscape level, vernal pools contribute to overall forest community biodiversity (Nicolet, 2004).

R. sylvatica and *A. Jeffersonian* have higher growth rates in an open canopy pool than in a closed canopy pool (Schiesari, 2006). This is due to factors associated with canopy cover, such as leaf litter, temperature, hydroperiod and light availability. An experimental canopy removal over vernal pools in Connecticut showed that certain canopy intolerant species, the green tree frog (*Hyla versicolor*) and spring peepers (*Sudacris crucifer*) were more abundant after cutting part of the canopy (Skelly et al., 2014). As indicated by these studies, certain amphibian species require some direct sunlight for development within vernal pools, though some cover is still necessary to provide carbon, and reduce water loss through evapotranspiration. Thus restoring the pools and the surrounding area is of utmost important for declining amphibian populations and we are in need of a better understanding of the factors that lead to successful vernal pool restoration (Brooks, 2005).

While the duration of the hydroperiod is important for amphibians, pool hydrology has less of an effect on the presence and abundance of macroinvertebrate species than the predominant food source, whether it is detritus in the form of leaf litter from the above canopy or autochthonous production (Bischof et al., 2013). The food web of closed canopy vernal pools requires an external source of carbon to function properly (Rubbo et al., 2006). The type of leaf litter, i.e. what species it is from, is not as important to the food web as the presence or absence of litter. Removing litter may lower respiration rates in vernal pools but won't affect primary production (Rubbo et al., 2006). The algal assemblages in vernal pools are not affected by the tree species in the canopy above, and thus the leaves they shed (Verb et al. 2001). Similarly, for decomposition rates in the pools, the chemistry of the leaf litter is of secondary importance, primarily decomposition rates are affected by climatic region (Aerts, 1997).

Plants in vernal pool ecosystems must also be adapted to undergo the transitions from wet to dry periods. Many species undergo metamorphosis in their photosynthetic pathways based on what resource is limiting, i.e. carbon dioxide during the wet period and water during the dry period (Keeley, 1999). Up to 50% of carbon fixation in vernal pool plants demonstrating crassulacean acid metabolism may occur in the dark, suggesting an advantage of this type of metabolism in these intermittently flooded systems (Keeley and Sandquist, 2014). Vernal pools with a longer hydroperiod were found to have lower exotic plant cover as a longer hydroperiod allows for native plant species adapted to flooding (Gerhardt and Collinge, 2003). Hydroperiod length and tussock height of the sedge *Carex stricta* are directly related (Lawrence and Zedler, 2011) meaning that in years when there is more water available aquatic plant species have more growth potential.

Vernal pool restoration and creation

Creating vernal pools to replace those lost to human development may enhance regional biodiversity, key biogeochemical processes, and flood protection. Creation and restoration of vernal pools requires attention to numerous factors, including canopy cover, hydrology, soil type and geology, organic matter, and substrate for organisms. Failure of restoration projects often results from sitting on unsuitable soil or geology, failure to understand effects of surrounding land use (including canopy cover), failure to establish appropriate hydrology, or invasion by opportunistic plants (Sutter and Francisco, 1998; Zedler and Kercher, 2004). In vernal pool restoration projects there is a lack of understanding about the relationships between depth, precipitation, and hydroperiod (Black and Zedler, 1998). It is also particularly unclear what the role of canopy cover, and subsequent leaf litter input, is during vernal pool creation. In addition, many projects have site specific performance goals, often geared towards replacing extant functions of destroyed wetlands. Thus, vernal pool design must account for the functions of the pool being replaced, i.e. if amphibian breeding habitat is desired, substrate for attachment of egg masses must be provided (Lichko and Calhoun, 2003). There are, however, a variety of challenges including difficulty meeting lost functions in creation and restoration projects due to our lack of understanding the differences between created and natural systems (Brinson, 1993).

The disturbance associated with wetland creation frequently opens the door for opportunistic plant invasion (Zedler and Kercher, 2004), such that restored or created wetlands often have more invasive species than reference wetlands (Balcombe, 2005). Invasive plants are problematic because they can alter biogeochemical characteristics of ecosystems they invade

(Ehrenfeld, 2003; Zedler and Kercher, 2004). They decrease biodiversity and increase susceptibility to further invasion through interactions with microorganisms and large animals (Zedler and Kircher, 2004). Because created vernal pools may also have higher light availability due to the lack of a full canopy, these systems are highly susceptible to invasion by herbaceous plants that impact the creation of functionally equivalent wetlands. However, it is not clear what role the tree canopy has on created vernal pool function and whether pool restoration may be improved by additional attention to factors associated with the surrounding landscape.

The overall goals of this study were to establish the differences in physio-chemical and biotic attributes between natural and created vernal pools in Western, NY and to determine if simulated canopy cover will limit invasive plant cover and influence the trajectory of vernal pool development. I predicted that natural vernal pools would receive greater inputs of leaf litter and as a result have higher soil organic matter, more diverse amphibian and macroinvertebrate communities, lower algal biomass and lower cover by invasive plants than created pools. In addition, I hypothesized that experimental leaf litter and shade creation would bring organic matter, nutrient and biodiversity levels closer to those of natural pools. Leaf litter will provide an energy source in the form of organic carbon, and shade creation will increase hydroperiod by reducing evapotranspiration; thus, providing more development time for macroinvertebrate and canopy tolerant amphibian larvae. The shade was also expected to deter shade-intolerant invasive wetland plants.

Methods

Site Description

This study was conducted at High Acres Nature Area (HANA) in Perinton, NY. HANA, adjacent to the High Acres Landfill, is owned by Waste Management of New York, LLC and is a certified Corporate Lands for Learning site through the Wildlife Habitat Council. The land cover has changed multiple times throughout its history. The original wetlands were drained for agriculture in the 1820s, followed by gravel mining and agriculture. The property was purchased by Waste Management of New York, LLC in the 1980s. Between 2009 and 2012 a series of wetlands were created to mitigate neighboring landfill expansion. Currently, HANA contains forested upland, forested wetlands, emergent wetlands, shrub scrub and open water ponds.

Natural vernal pools exist in the forested upland (Figure 1). These are complex pools that are hydrologically connected through surface water during peak flooding. The canopy cover is comprised of predominantly Maple species (*Acer spp.*), with contributions from Oaks (*Quercus spp.*), Eastern Cottonwood (*Populus deltoides*), Beech (*Fagus grandifolia*), and Basswood (*Tilia americana*). Four discrete pools were randomly selected for this study.

In 2009, eight vernal pools were created. Most of the existing trees were removed during construction, leaving an expanse of land with little tree cover (Figure 1). In addition, excavation removed top layers of soil, likely removing the native plant seed bank and reducing organic matter in the soil. Trees and shrubs, including Maples (*Acer spp.*), Red Osier Dogwood (*Cornus sericea*), and Sycamore (*Platanus occidentalis*), were planted during the original construction. These plantings were augmented in July 2014 with live stakes of four species, Silky Dogwood (*C. amomum*), Red Osier Dogwood (*C. sericea*), Common Elderberry (*Sambucus canadensis*), and Common Buttonbush (*Cephalanthus occidentalis*). The pools were also rapidly colonized by herbaceous plants, including True Sedges (*Carex spp.*), Water Plantain (*Alismo plantago aquatica*), and Broadleaf Arrowhead (*Saggitaria latifolia)* in addition to a number of invasive plants. The herbicide glyphosate was applied in 2013 to the entire area of the created vernal pools to remove Cattail (*Typha spp.*), Reed Canary Grass (*Phalaris arundinacea*), and Purple Loosestrife (*Lythrum salicaria*). Spraying in the area surrounding the pools continued in 2014 and 2015, but a 2 m buffer zone was established around each pool where no herbicide was applied. Invasive species were removed from all created pools in June 2014, and cattail seed heads were cut again in July 2015.

Pool bathymetry was measured at the peak of spring flooding in 2014 for created pools and 2015 for natural pools. I measured the length at the longest axis of each pool, and the width at the center of the length axis. Water depth was measured by overlaying a grid of 50 points over the rectangle formed by the length and width axes and measuring the depth at the intersection of the grid lines. The points without standing water were marked as 0 cm to delineate the boundary of each pool. Because all pools were approximately elliptical, the maximum pool surface area (SA) was calculated using the formula SA=$\frac{LW\pi}{4}$, where L is the length of the long axis and W is the width of the pool at the center. The pool volume was calculated using the equation $V = \frac{A*d}{(1+2)/p}$

from Brooks and Hayashi, 2002, as adapted from Hayashi and van der Kamp, 2000. Where V is the maximum volume, at maximum area, A, and maximum depth, d, and $p = \frac{\log\frac{d}{do}}{log\frac{r}{ro}}$, where d_0 corresponds to radius of 0.5 m and r_0 corresponds to the radius at y_0 (Hayashi and van der Kamp, 2000). To monitor maximum water level changes over time, a 2 m section of PVC pipe was placed at the deepest point depth and depth was measured biweekly throughout the hydroperiod from April to October of 2014 and 2015 (natural pools in 2015 only). Heterogeneity of the bottom of the pools was calculated by finding the standard deviation of the difference between depth point and 8 surrounding points for all depth points (n=50) and was averaged for the bathymetry grid.

To assess differences in canopy cover between natural and created pools, ambient light availability was measured using a LiCOR 2pi quantum light sensor following the installation of the shade cloths. Litterfall was also measured in both natural and created pools to determine the amount of organic carbon supply from the tree canopy. Leaf litter was collected using 53 L plastic laundry baskets. Five holes were drilled in the bottom of each basket to allow water to drain. Three or six baskets were placed in each created or natural pool, respectively, with the larger number of baskets in the natural pools reflecting their larger size. Litter was collected bi-weekly from mid-October through December 2014, and late August through December 2015. Leaves were air dried, sorted by species and weighed. The organic carbon content was calculated using average leaf carbon values (Cornell, 1992).

Experimental Design

To simulate the impact of canopy cover on created vernal pools, I implemented four treatments (n=2 pools): leaf litter addition, shading, leaf litter + shading, and a control (Table 1). The low replication within treatments is acceptable because there is manipulation of a whole ecosystem as opposed to a small part (Hurlbert, 1984). The manipulations for each pool were randomly selected. Prior to the initiation of the treatments, the baseline vegetation cover was assessed, and then all invasive plants were clipped below the water level. Shade cloth (Easyshade BLK40 Sunblock 40% in black), a permeable material that allows rainwater to flow through but blocks UV light was used to reduce light availability in Shade and Both treatment pools. In early June, 2014, Four 3 m X 7 m cloths were suspended across each pool and secured to 1.5 meter sections of PVC piping. Shade cloths did not cover the entire surface area of the pool, but were sufficient to shade approximately 20-30% of the pool area with a reduction in light availability of approximately 80% (Table 1). Shade cloths were taken down prior to a significant snowfall event during the winter of 2015 and reinstalled in April 2015. Leaf litter was applied to the Litter and Litter + Shade treatments in June 2014 and June 2015. Leaves were supplied by Waste Management's High Acres Landfill municipal composting operation. Leaves were collected from local area residents during the previous fall and maintained in large composting rows over the winter. Approximately 3 m^3 of leaf litter was applied to each treatment pool and spread evenly using a garden rake, resulting in a litter depth of approximately 7 cm.

Sampling & Analytical Methods

Water column nutrients (ammonium, nitrate and phosphate), soil organic matter and extractable nitrogen and phosphorus, and macroinvertebrate and plant communities were measured in the created vernal pools between May 22 - June 2, 2014 prior to the implementation of experimental manipulations , and twice during each summer (2014 and 2015) after treatments were imposed. The natural pools were sampled during 2015 only, but the created pools were sampled in 2014 and 2015. In 2014, water column samples were collected once from the created pools and were collected monthly during the flooded season of 2015, resulting in May and June samples. Soil samples were collected twice, once during the hydroperiod in June and once after desiccation in August in 2014 and 2015. Macroinvertebrates were sampled using the kicknet once during the duration of the hydroperiod, and soil cores were taken once. Benthic microalgae and periphyton was assessed twice during the 2015 hydroperiod.

Water column nutrient samples were collected using a 60 ml syringe and immediately filtered through a 0.45 um PES syringe filter into Whirlpak bags. The samples were placed immediately on ice prior to freezing at -20°C until analysis. In the lab, water samples were analyzed for NO_3^-, NH_4^+, and PO_4^{3-}. NO_3^- and PO_4^{3-} were analyzed using Lachat Quickchem 8500 autoanalyzer with a cadmium-reduction. NH_4^+ was measured using the phenol-hypochlorite method (Solarzano, 1969).

Soil samples for organic matter and extractable nitrogen and phosphorous (n=3 per pool) were collected with a metal soil auger to a depth of 10 cm. These samples were frozen at -20°C until analysis. To extract NO_3^- and NH_4^+, 5 g subsamples were shaken with 2M KCL, centrifuged and then filtered. NO_3^- was measured using a Lachat Quickchem 8500 autoanalyzer with a cadmium reduction (Lachat, 2003) and extractable NH_4^+ was measured using the phenol-hypochlorite method (Solarzano, 1969). Soil phosphorous was determined using two 0.1 g subsamples, one for total phosphorous (TP) and inorganic phosphorous (IP). The samples were mixed with 0.5 mL of $Mg(NO_3)_2$ and combusted for two hours at 550°C. Ten ml of 1N HCl was added to all samples, they were shaken, allowed to settle, were filtered, and analyzed using the ammonium molybdate method (Murphy and Riley, 1962), and a Shimadzu 1800 spectrophotometer.

The plant community within each pool was determined by measuring the percent cover of each species in the entire pools. This was done prior to implementation of treatments on June 2 2014, and again in August 2014, and three times in 2015, May 26, July 7, and August 16. In 2015, three data points within each pool were added to assess vegetation cover at a smaller scale. A 1 m^2 quadrat was placed down in three locations within each pool and percent cover of all species was determined.

Microalgal biomass was assessed using two methods that were designed to determine the benthic algae on the soil surface and the periphyton colonization on structures within the pools. Benthic chlorophyll *a* was measured as a proxy for benthic microalgal biomass. Surface sediment samples were taken on June 3, 2014, and June 1 and August 15, 2015 with a 5 cc syringe corer to a depth of 1 cm, immediately placed in 15 ml centrifuge tubes, wrapped in aluminum foil, and placed on ice in the dark. Samples were stored at -80°C and analyzed within 30 days. Six mL of 90% acetone was added to each tube which was then shaken, prior to sonication for three - one minute intervals. Following a 24 h extraction at -20°C, samples were shaken to resuspend and centrifuged for five minutes. Absorbance at 665 nm and 750 nm was measured on a Shimadzu 1800 spectrophotometer before and after acidification and the chlorophyll *a* concentration calculated according to Lorenzen, 1967.

Periphyton was measured based on EPA Water: Bioassessment Chapter 6, Periphyton Protocol using the adaptation of Rosemond et al., 1993 and Stevenson and Bahls, 1999. While natural substrate is preferred for colonization, it is much more difficult to accurately assess biomass on a heterogeneous natural surface (Stevenson and Bahls, 1999). As a result, I used 2.5 cm x 2.5 cm clay tiles, which were chosen over other artificial substrates as they harbor the highest biodiversity of periphyton (Tuchman and Stevenson, 1980). I placed 3 tiles randomly in each pool, in pools with shade treatment the tiles were placed beneath the cloth. After 3 weeks, the entire tile was collected and placed in a 60 ml centrifuge tube, wrapped in aluminum foil, and placed on ice until storage at -80°C. Chlorophyll *a* was measured as above, except that 10 mL of 90% acetone was necessary to cover the tile completely.

Infaunal and epifaunal macroinvertebrates were sampled in June 2014 and May 2015. Infaunal invertebrates were collected using a 9.5 cm polycarbonate core tube inserted 10 cm into the sediment. After sieving the sediment through 1 mm mesh, animals were removed and preserved in 100% ethanol. Epifauna were sampled by agitating the water with a kicknet for 1 minute while traveling along a 5 meter transect (Le Viol, 2009). Invertebrates were isolated and preserved as above. Individuals were identified to the lowest practical taxonomic level using guidebooks of freshwater macroinvertebrates in the northeastern US, with the aid of a dissecting microscope (Merritt et al., 2008; Peckarsky, 1990). Both samples from each pool were combined and the Shannon-Weiner Diversity Index was calculated. Amphibian populations were estimated using egg mass observation and quantifying tadpole presence. For eggs, a two person observation, as adapted from the technical reports of Faccio, 2010, and Paton, Timm, and Tupper, 2003, was performed. In the field, an assistant wrote down all egg masses I observed for half of each pool, and then for the second half the roles were reversed to eliminate observer bias. Tadpoles were identified and counted concurrent with macroinvertebrate kicknet sampling. The presence of amphibian egg masses or tadpoles during invertebrate sampling was noted in 2014 and tadpoles collected while using the kicknet were counted, identified and released.

Statistical Analysis

To statistically interpret the differences between natural and control treatment created vernal pools a repeated measures ANOVA was performed for all measured parameters, except leaf litter parameters which were analyzed using a one way ANOVA. To statistically analyze the differences between the experimental treatments I performed repeated measures ANOVA and paired t post hoc test. Macroinvertebrate community was also analyzed using a discriminant canonical correlation analysis. All data was tested for normality using JMP 12 software.

To see how the treatments are effective at bringing the created pools closer to the function of natural pools, a principal component analysis was conducted with JMP 12 software. One PCA was performed which included environmental variables, such as chlorophyll *a*, soil and water nutrient concentrations and soil organic matter, plant community, including total cover of invasive species, woody species, and emergent cover, and macroinvertebrate community, which included all macroinvertebrate diversity and. The data was separated by year and a row-wide estimation was used.

Results

Using JMP 12 there were differences found in the physiochemical and biological variables of each pool, created (Table 2; Table 3) and natural (Table 3). All created vernal pools were found to be approximately elliptical on the surface and the depth profile was semi-circular. The natural vernal pools had varying depths and shapes with more heterogeneous bottom ($p=0.025$). The surface area (SA) and volume (V) were smaller in created pools than in all natural pools, but the SA: V ratio was comparable between both sets of pools (Table 1). The water depths of all vernal pools vary greatly over time (Figure 2).

Leaf Litter

The natural pools had greater inputs of leaf litter. There was significantly more carbon per basket ($p<0.001$) than created pools (Table 4), as a result of the greater overall mass of leaves falling into the natural pools. The average carbon falling into to the natural pools was 0.15 kgm^{-2} $^{\cdot}$C, but in the created pools this number was less than 0.004 kgm$^{-2\cdot}$ C (Table 4).

The natural pools also had leaves from significantly more ($p=0.0052$) species than the created pools (Table 4). The created pools averaged 2 species per basket, but the natural pools were more than double at 5 species per basket.

Nutrients

Nitrate concentrations in surface water were significantly different among the treatments (p=0.016) and between natural pools. Surface water nitrate was significantly higher in control pools (Figure 3A). Phosphate concentrations were also significantly different (p<0.0001) among the treatments (Figure 3B). Shade Cloth pools, and Both pools were not significantly different, and Control pools and Both pools were not significantly different, but Shade Cloth pools and Control pools were different. Natural pools and control pools were not significantly different. Ammonium concentrations were significantly different (p=0.0384) among treatments, but there was also a significant interaction between time and treatment (p=0.0045). May 2014 was different than May 2015 (p=0.0041) and June 2015 (p=0.0038). May and June of 2015 were not significantly different than each other (p=0.8051).

There were no differences in soil phosphorus or extractable ammonium among treatments or between natural and created pools. Soil phosphorous was not significantly different among any treatment (p=0.49 and p=0.22, respectively; Figures 4B and 4C).

Organic Matter

Soil organic matter was found to be significantly different among treatments, p=0.020 (Figure 5). In the created pools, there were also temporal differences in organic matter. From June 2014 to August 2014, all treatments nearly doubled the amount of soil organic matter. The natural pools had lower levels of organic matter than control pools. Organic matter content in natural pools was more constant than any of the created pools.

Invasive Plant Cover

The regrowth of invasive plants was significantly greater in Control pools than Shade Cloth or Both treatments, but not Leaf Litter pools (p =0.039; Figure 6). Shade Cloth and Both treatments had similar regrowth, and Leaf Litter treatments were not significantly different from any other treatment. No invasive plants were found in any of the natural pools (Table 5).

For *Typha latifolia* (Figure 7A) and *Phalaris arundinacea* (Figure 7B), there were no significant differences among treatments (p=0.124 and p=0.562, respectively). For *Lythrum salicaria*, however, (Figure 7C) Shade and Control pools are significantly greater than Both and Leaf Litter pools (p=0.001). There is a time*treatment interaction where Shade Cloth pools are significantly greater during May 2015(p=0.002).

Chlorophyll *a*

There was substantial temporal variability in benthic microalgal chlorophyll *a* in the created pools that was not observed in the natural pools, which were consistently lower than the Control created pools (p=0.0035; Figure 8A) and similar between sampling dates. There was a significant difference (p=0.0449) among treatments in benthic microalgae biomass, where control pools were higher (Figure 8B). Periphyton biomass was also temporally variable in the created pools, but there were more consistent differences among treatments (p=0.0015; Figure 8B). The control pools were again significantly higher than the natural pools (p<0.001).

Macroinvertebrates

The macroinvertebrate species richness was marginally greater (p = 0.054) in created (S =3.9) than natural pools (S=3; Table 6; Figure 9). In general, from 2014 to 2015 there was a decrease in individuals and in total number of species of macroinvertebrates (Figure 9). I found no differences in total abundance (p=0.3993) or diversity (p=0.3001) between created and natural pools.

While there were no statistical differences in individuals, species richness, or diversity, there were differences in the community structure (Figure 10). Taxa found in both sets of pools include Amber Snails (*Succinea sp.)* and case-maker Caddisflies *(Limnephilus sp.).* Amber snails were the most prevalent taxa found in the created pools, but rare in the natural pools. In contrast, Caddisflies were the third most prevalent taxa in the natural pools but the sixth most in the created pools.

Amphibians

In 2014, prior to the implementation of the amphibian study, two egg masses from Wood Frogs (*Rana sylvatica*) and one from the American Toad (*Bufo americanus*) were observed in pool C8. In 2015, no egg masses were observed in any of the created or natural pools. Tadpoles were found in pools C1, C3 and C5, but not in any natural pools (Table 8). *Rana sylvatica* tadpoles were found in C5, a Leaf Litter pool (3 tadpoles), and in C3, a Shade Cloth pool (2 tadpoles). *Bufo americanus* tadpoles, 2, were found in C1, a control pool. I found no tadpoles in the remaining five pools.

Principal Components Analysis

In Figure 11, the score plots from the PCA are shown. The plant community (Figure 11A) in the natural pools has very little variability, but all created pools have a lot of variability, based on how spread the points are on the figure and none of the treatments are closest to the natural pools. Both pools are closest to the natural pools, with Shade Cloth and Leaf Litter pools following. Control pools were the farthest from the natural pools, indicating the most differences in environmental variables. For macroinvertebrate community (Figure 12C), there was a temporal difference, where in general all 2015 points were closer than 2014 points. In 2015, all pools were negative in component 1 but in 2014, there was much more variation and most were positive in component 1.

The loading matrix (Table 9) shows how heavily each variable in the overall PCA is weighted for the first four components and whether it is positive or negative. Variables in the first component that are weighted heavily are organic matter, emergent plant cover, periphyton, soil phosphorus, soil ammonium, *T. latifolia*, *P. arundinacea*, *L. salicaria, S. putris,* and *P. armigera* in the positive direction, in the negative direction is surface heterogeneity, water ammonium, *Limnephilus* sp., *E. tenax*, and *S. occidentale*. In the second component, variables heavily weighted in the positive direction includes Prionocyphon sp., Macromia sp., *A. plantago-aquatica*, emergent plant cover, invasive plant cover, macroinvertebrate diversity and number of flooded days. In the negative direction, *P. armigera*, macroalgae, *Cornus sp*., and woody cover are heavily weighted.

Discussion

This study examined the nutrient and organic matter levels within the soil and water, the animal diversity, and plant cover of eight created vernal pools and four natural pools. The combined effect of Leaf Litter and Shade Cloth had the greatest effect, bringing these pools to a more similar functional state to the natural pools s. The treatments were also successful at prohibiting regrowth of invasive species, which impacts the physicochemical and biological characteristics of the pools.

Impact of Hydrology on Factors within Pools

The depth of the natural pools varied spatially within each pool, while the created pools were more regular and bowl shaped. The greater heterogeneity of the natural pools creates more diversity of habitats and small-scale hydrological differences, leading to greater biodiversity (Bauder, 2005). The slightly larger SA:V ratio indicates that there is more interaction between the benthic environment and the water column when flooded, although this effect was minimal. However, this can impact nutrient cycling within the pool, as levels of oxygen and water may be more variable.

The month of May 2015 was anomalously warm and dry relative to typical western New York state weather (NOAA Physical Sciences Division). The reduced precipitation combined with greater evaporation likely resulted in less water in the pools, in the surrounding hyporheic zone and in the groundwater, accounting for the drop in water levels that occurred towards the end of May 2015. In contrast to the hot and dry May, June 2015 was cooler and wetter than average (NOAA Physical Sciences Division), resulting in a refilling of pools from heavy rains and low evaporation. The pools were inundated until the end of July when they all desiccated within a week and remained dry for the remainder of the season. Differences in inundation length among different vernal pools are greater in years with greater precipitation (Bauder, 2005). In 2014, all of the created pools dried completely only once, as opposed to 2015 where many dried completely, and then flooded again. This had implications on nutrient dynamics as well as living organisms within the pool. The decreased diversity of macroinvertebrates and smaller numbers of amphibians in the created pools between 2014 and 2015 is likely a result of these interannual weather patterns between years. Since many of the pools nearly dried up, or were less than 0.1 m deep at this critical time for macroinvertebrates and amphibians, many aquatic organisms within the pools may have been unable to survive.

Organic matter and nutrient dynamics

The natural pools had significantly more carbon input in the form of leaves. This input of carbon can provide nutrients and organic matter to the pools, and serve as habitat for organisms such as macroinvertebrates and amphibians. As a result, the leaf litter input is the base of the food web in the natural pools. In contrast, the created pools were dominated by herbaceous plants and algae, which provide a different food source and structurally different habitat. The results of this are seen in the macroinvertebrate communities found in the two types of pools. *Eristalis tenax* larvae, found only in the natural pools, are known to inhabit waters with high levels of organic matter (Aguilera et al., 1999). Similarly, case maker caddisflies (*Limnephilus* sp. larvae), were found in both natural and created pools, but were nearly 2x more abundant in natural pools. This taxa requires organic materials to build a protective case and are likely more abundant in natural pools because of the greater leaf litter inputs; these organic casings are better able to retain water and thus, they are often found in temporary pools (Zamora-Munoz and Svenson, 1996). In Leaf Litter treatment pools, there wasn't a consistent effect on this species. It is likely that there are interactions with other variables, such as light, water depth, and nutrient concentrations, limiting the growth of these animals, as well as low recruitment to the new pools. *Gammarus,* common taxa found in the created pools but not in the natural pools, is less susceptible to pollution than *Limnephilus* (Schulz and Liess, 1999). The high prevalence of *Gammarus* and lower prevalence of *Limnephilus* in the created pools may indicate more polluted waters in the created pools.

Paired with the greater inputs of carbon, there were leaves from more species in the natural pools than in the created pools. This indicates that there is a more developed canopy above the natural pools, which is to be expected since much of the canopy was removed upon creation of the eight vernal pools. Leaves of different tree species will contain varying ratios of C:N:P, which can alter the chemistry of the pool below. A more diverse canopy can also support greater diversity of animals which rely on trees for food and shelter.

Within vernal pools the amount of organic matter relative to nutrients, like nitrogen, affects the community within the pool. The sensitivity of vernal pools to disturbance, particularly nutrient loading, complicates restoration efforts aimed at achieving lost ecosystem functions. Such high and varied levels of organic matter in the created pools (Figure 6) may be the result of greater levels of benthic microalgae, or other photosynthetic organisms, such as *Lemna minor* (Duckweed) captured within the soil sample. Many of the created pools were covered in *L. minor* and also had more benthic microalgal biomass (Figure 8). The accumulation of this organic matter is a primary form of pedogenesis in vernal pools (Hobson and Dahlgren, 1998). This has implications for the soil types of the vernal pools in the future, and the high levels of organic matter may indicate that the created vernal pools are in the process of forming soils closer to the natural pools.

The potential for eutrophication can cross through from the aquatic phase to the terrestrial phase of vernal pools (Kneitel and Lessin, 2009). This can alter biological community, and can account for some of the differences among the pools. Land use surrounding ephemeral wetlands, including the upland forest, has a significant impact on vernal pool ecology and biogeochemistry (Bedard-Haughn et al., 2006). In general, the natural pools were less variable between sampling dates for nitrate, phosphate and ammonium because there is less anthropogenic disturbance immediately surrounding them. There is a golf course adjacent to the created pools at HANA, and there is potential for nutrient-enriched runoff from surface and groundwater, which can cause some of the variability in nutrient levels over time. Created pools 1-3 are also located closest to a road, which can result in polluted runoff.

Natural pools had lower levels of ammonium, which may contribute to the low levels of plant cover, benthic microalgae and periphyton relative to the created pools where nutrients were higher. Paired with higher ammonium levels, the created pools in general received more light, even with the shade cloth treatment. Higher levels of irradiance allow for direct photosynthetic activity within the pools, as opposed to in the canopy above as is seen in the natural pools, and may support algae and herbaceous plants with high light requirements in contrast to the low-light ferns and saplings found in the natural pools.

Biodiversity & Invasive Species

Addition of shade and leaf litter may inhibit invasive species colonization during vernal pool restoration. Although the invasive plant regrowth was relatively high initially after cutting and implementation of the treatment, over time there was a steady decline in invasive cover in shade treatments suggesting an important role of shade in reducing invasive species colonization.

Individual invasive species responded differently to the treatments, though. There was an increase in *L. salicaria* in all treatments between 2014 and 2015, which was also observed in nearby systems (Tyler et al. unpub. data). However, the two Leaf Litter treatments demonstrated some resistance to regrowth of this species, suggesting that addition of organic material may help to deter colonization. *Typha latifolia* and *Phalaris arundinacea* did not respond to any of the treatments significantly. *T. latifolia* cover was low prior to removal, and the small cover that regrew is not likely a threat. *P. arundinacea* was also not different among any treatments because it was highly variable among pools. Pools with the highest cover of this species were located closest to a large emergent wetland that has high cover of many invasive species, such as *L. salicaria, T. latifolia* and *P. arundinacea* (Tyler et al. unpub. data) and likely serves as a seed source.

Noninvasive plants found within both complexes of pools differed. The natural pools had ephemeral plants in the surrounding land area, including *Erythronium americanum* (Trout Lily), *Podophyllum* spp. (*Mayapple*), and *Arisaema triphyllum* (Jack-in-the-pulpit). Ephemeral plants have the potential to alter system-level nutrient fluxes at rates higher than previously expected (Blank et al., 1980). These plants support the vernal dam hypothesis, in which nutrients that would have been lost from the system are kept there through temporary plants which initially take in nutrients but then release them back when they stop growing after the spring. This is another potential cause for steady nutrient levels within the natural pools.

In the created pools, there were more herbaceous and emergent plant species, as well as mats of macroalgae and *Lemna minor*. There is potential for aquatic plants to remove up to 75% of N and P from surface water (Reddy and de Busk, 1985). The greater percent cover of plants in the created pools, all of which had an open canopy, has implications for nutrient cycling. An open canopy pool, as a result of greater amounts of light which lead to more emergent plants, can remove excess nutrients from the water column better than a closed canopy pool. In future creation projects, this should be taken into account based on the desired functions of the pool.

Interactions among Variables

The PCA illustrates clear differences between the natural and the created pool that are characterized by organic matter, invasive species, emergent plant cover, periphyton, soil phosphorus and ammonium, were loaded positively in the first component. Invasive species were found with high levels of soil nutrients in the created pools, which support findings in previous studies linking invasive species to high levels of nutrients (Ehrenfeld, 2003). The high organic matter levels may be a result of invasive species, where their litter has high decomposition rates (Ehrenfeld, 2003). It is likely that the organic matter values are high also because the soil contained high levels of periphyton, as demonstrated by the loading values. The high nutrient levels in the soil allowed for periphyton to colonize an area. Negatively in component 1, were heterogeneity, water ammonium, *Limnephilus* sp., *E. tenax*, and *S. occidentale*, all characteristic of the natural pools. The more heterogeneous surface of the natural pools, allowed for more macroinvertebrate species, as demonstrated by the loadings.

In principal component two, loaded heavily in the positive direction were *Prionocyphon* sp., *Macromia* sp., *A. plantago-aquatica,* emergent plant cover, invasive species cover, macroinvertebrate diversity, and number of flooded days. Emergent plant cover is likely linked to invasive species cover as a result of other variables within the pools that allow for greater plant growth. Invasive species growth and cover has been linked to improper hydroperiod, as they are more adaptable to variations in water level (Miller and Zedler, 2003). Macroinvertebrates are also related to number of flooded days, as it allows more time for development from larvae to adult. Negatively in component 2, *P. armigera, Cornus* sp., macroalgae, and woody cover were heavily weighted. The high cover of macroalgae may prevent emergent plant species, which is why there is only woody species cover, including *Cornus.* This component suggests a trajectory of development related to flooding duration and the dominant vegetation type, with flooding promoting macroalgal growth and woody species.

There was far less variation in environmental variables or plant communities in natural pools than created pools, in general and among treatments. Both treatment pools, followed by Shade Cloth and Leaf Litter pools had the most similar environmental variables, plant and macroinvertebrate communities to the natural pools in 2015. This shows that the treatments were effective at driving the function of created pools towards that of natural pools. The interaction of shade and organic matter inputs were the most effective, showing that in open and closed canopy pools the differences in light and organic matter are both important, and neither factor alone is enough.

Overall, vernal pool creation is a viable option for restoring these critical ecosystems that have been lost. Better management strategies and practices are needed to ensure they function more similarly to an undisturbed natural vernal pool. Canopy cover is a main determinant of the function of vernal pools, and thus, it is of utmost importance in managing these vital ecosystems. Implementing treatments to imitate a forest canopy can alter the biogeochemistry and biotic community composition of a created vernal pool. The findings from this study can be used in future vernal pool creation or restoration projects. Installing treatments that simulate canopy cover may also facilitate invasive species removal in created pools. Invasive species removal has been a great challenge as the threat of invasive species is high in wetland creation projects and there are few known ways to completely remove invasive species. The findings from this study can be used for invasive species removal, where shade, organic matter inputs, or a combination of the two can be used as a supplement to manual removal. This has implications for biodiversity, as well as various other environmental factors that may be impacted by the presence of invasive plants.

Conclusions

There are many important factors that must be considered when creating a vernal pool; some of the most important are light availability, organic matter, and bathymetry. When choosing a location for a vernal pool, it is important to look at the surrounding land for the topography, tree cover, and soil type. A vernal pool should be more heterogeneous on the bottom, as it creates microhabitat availability. If tree cover is limited, consider planting trees such as Maples, Oaks, Basswood and Cottonwood, all of which were prevalent above the natural pools and installing shade cloth while the trees are developing to prevent invasive plant species from colonizing the pools. However, when present, the canopy should be left intact and pools developed within the existing forest matrix.

Tables

Table 1: Maximum depth and surface area of each pool measured at the peak of spring flooding on May 22, 2014 for the created pools and April 24, 2015 for the natural pools.

Pool	Depth (m)	Area (m^2)	Volume (m^3)	SA:V (m^{-1})	Heterogeneity	Irradiance	Treatment
C1	0.42	71.5	9.99	7.2	0.08	51.99	Control
C2	0.57	130.6	13.27	9.8	0.05	38	Shade Cloth
C3	0.39	76.6	9.94	7.7	0.11	4.96	Shade Cloth
C4	0.34	73.6	8.56	8.6	0.09	10.41	Both
C5	0.51	51.1	8.51	6	0.1	127.56	Leaf Litter
C6	0.2	31.4	2.09	15	0.09	70.61	Control
C7	0.27	42.9	3.86	11.1	0.07	130.61	Leaf Litter
C8	0.32	66.4	7.07	9.4	0.1	16.59	Both
N1	0.34	598.9	67.89	8.8	0.09	1.6	Natural
N2	0.37	304.7	38.22	7.9	0.14	1.36	Natural
N3	0.58	100.9	19.53	5.2	0.11	2.61	Natural
N4	0.36	914.5	110.58	8.2	0.19	6.31	Natural

Table 2: Repeated measures ANOVA results for the comparisons among treatments, time and the interaction between treatment and time.

	Treatment			**Time**			**Treatment*Time**		
	DF	F	P	DF	F	P	DF	F	P
Water Column NO_3^-	6	2.87	**0.016**	1	0.03	0.855	24	1.38	0.27
Water Column PO_4^-	6	10.08	<**0.001**	2	19	<**0.001**	24	2.00	0.11
Water Column NH_4^+	6	2.37	**0.038**	2	0.77	0.546	24	5.84	**0.005**
Organic Matter	8	3.69	**0.0286**	1	3.83	0.056	24	3.48	0.058
Soil NH_4^+	8	1.48	0.222	1	0.21	0.649	24	0.45	0.74
Soil Phosphorus	4	0.9	0.493	1	0.02	0.898	24	0.09	0.99
Invasive Species	6	2.67	**0.039**	3	1.36	0.277	24	3.97	**0.019***
Typha latifolia	6	1.88	0.124	3	3.4	**0.033***	24	0.36	0.79
Phalaris arundinacea	6	0.82	0.562	3	0.68	0.579	24	0.68	0.72
Lythrum salicaria	6	5.23	**0.001**	3	0.57	0.642	24	9.89	**<0.001***
Microalgae	3	4.94	**<0.001**	2	2.59	0.084	14	6.03	**<0.001***
Periphyton	3	11.19	<**0.001**	1	54.92	**<0.001**	14	47.13	<**0.001***
Macroinvertebrate Richness	3	2.94	0.054	1	8.35	**0.013**	9	0.51	0.73
Macroinvertebrate Abundance	3	1.12	0.399	1	3.08	0.103	9	0.62	0.66
Macroinvertebrate Diversity	3	1.36	0.3	1	0.61	0.626	9	0.9	0.48

Table 3: Results of the one-way ANOVA comparing natural and created pools.

	dF	F	P
Heterogeneity	5	7.10	**0.024**
LL Carbon (2014)	23	25.78	**<0.001**
LL Carbon (2015)	23	61.47	**<0.001**
LL Richness (2014)	23	9.39	**0.005**
LL Richness (2015)	23	78.67	**<0.001**
Number of Flooded Days (2015)*	5	1.04	0.451
Water Column NO_3^-	11	10.19	**0.007**
Water Column PO_4^-	11	1.33	0.826
Water Column NH_4^+	11	1.067	0.245
Organic Matter	11	7.18	**0.034**
Soil Total Phosphorous	11	1.43	0.115
Extractable NH_4^+	11	3.47	**0.016**
Microalgae	17	6.59	0.298
Periphyton	17	3.38	**0.007**
Macroinvertebrate Richness	5	30.9	0.805
Macroinvertebrate Abundance	5	19.89	0.693
Macroinvertebrate Diversity	5	4.89	0.287

*Analyzed among all treatments and natural pools, but only for 2015 values.

Table 4: Species richness of leaf fall and calculated carbon input (g m^{-2}) in created and natural pools (Mean ±SE).

		Created	**Natural**
2014	S	2.3±0.3	4.8±0.8
	Carbon	3.4±1.2	39.3±7.3
2015	S	2.4±0.2	6±0.4
	Carbon	64.6±18.8	540.6±20.4

Table 5: Occurrence of herbaceous and woody plants in created (n=8) and natural (n=4) vernal pools at High Acres Nature Area. Values are the percent of pools in which the species was found. Created pools were assessed in June 2014, prior to cutting of invasive plants and implementation of experimental treatments; natural pools were assessed monthly from May through August 2015.

Species	**Created**	**Natural**
Ash saplings (*Fraxinus pennsylvanica*)	0%	100%
Dogwood (*Cornus spp.*)	50%	0%
Purple Loosestrife (*Lythrum salicaria*)*	87.50%	0%
True Sedges (*Carex spp.*)	75%	75%
Water Plantain (*Alisma plantago-aquatica*)	37.50%	0%
Reed Canary Grass (*Phalaris arundinacea*) *	25%	0%
Broadleaf Arrowhead (*Sagittaria latifolia*)	12.50%	0%
Broadleaf Cattail (*Typha latifolia*)*	12.50%	0%
Narrow Lady Fern (*Athyrium angustum*)	0%	25%

*Denotes an invasive species.

Table 6: Total number of individuals of macroinvertebrate taxa present in the created (n=4) and natural pools (n=4). Created pools contain pooled data from the two pools for 2014 and 2015, n=4, and four natural pools only from 2015, n=4.

	Both	**Shade Cloth**	**Leaf Litter**	**Control**	**Natural**
Succinea sp.	38	32	28	23	3
Plerocera sp.	0	12	16	0	0
Enallagma sp.	9	16	18	2	0
Gammarus sp.	2	37	6	4	0
Planorbula armigera	8	9	4	8	0
Limnephilus sp.	5	4	2	10	19
Macromia sp.	2	2	0	0	0
Prionocyphon sp.	1	10	0	2	0
Haplotaxida sp.	0	0	0	16	0
Sphaerum occidentale	0	0	0	0	47
Psorophora ciliate	0	0	0	0	25
Eristalis tenax	0	0	0	0	4

Table 7: Bray Curtis dissimilarity values comparing all treatments, where C is control, SC is shade cloth, LL is leaf litter, B is both, and N is natural.

	Plant	Macroinvertebrate
C-SC	0.64	0.46
C-LL	0.40	0.47
C-B	0.58	0.33
SC-LL	0.48	0.67
SC-B	0.62	0.51
LL-B	0.22	0.52
C-N	**0.83**	**0.89**
SC-N	**0.85**	**0.93**
LL-N	**0.96**	**0.95**
B-N	**0.83**	**0.97**

Table 8: Amphibian tadpoles and adults observed within each treatment.

	Control	Shade Cloth	Leaf Litter	Both	Natural
American Toad (*Bufo americanus)*	2				*
Wood Frog *(Rana sylvatica*)		2	3		
Northern Leopard Frog*(Rana pipiens)*		*	*		
Gray Tree Frog *(Hyla versicolor)*		*	*		
Bullfrog *(Rana catesbeiana)*					*

*Adults were observed within the pools at time of tadpole collection

Table 9: Loading matrix from principal components analysis for the first five components with eigenvalues greater than 1.

	PC 1	PC 2	PC 3	PC 4	PC 5
Eigenvalues	4.28	3.21	2.48	1.88	1.75
Percent Variance	20.4	15.3	11.81	8.93	8.34
Flooded Days	-0.23	0.44	0.27	0.47	0.31
Heterogeneity	-0.39	0.33	0.03	0.29	0.25
Macro Diversity	-0.19	0.72	0.37	-0.19	0.28
Invasive Species	0.35	-0.31	0.77	0.00	0.11
Emergent Cover	0.34	-0.36	0.76	0.14	0.17
Woody Cover	-0.12	-0.19	-0.27	0.23	-0.25
Organic Matter	0.61	0.44	0.11	0.25	-0.20
Microalgae	-0.31	-0.23	0.49	-0.48	0.21
Periphyton	0.72	-0.02	0.02	0.07	-0.22
WC Ammonium	-0.49	-0.44	-0.40	-0.01	0.37
WC Nitrate	0.02	-0.35	0.34	0.29	-0.15
WC Phosphate	0.30	-0.19	-0.11	-0.83	-0.12
Extractable Phosphorous	0.54	0.23	-0.11	-0.42	0.04
Extractable Ammonium	0.66	-0.30	0.21	0.24	-0.05
Trichoptera	-0.47	-0.54	-0.25	0.22	0.30
Diptera	-0.60	0.52	0.27	-0.08	-0.42
Amphipod	0.29	0.41	-0.16	-0.11	0.32
Bivalve	-0.63	0.53	0.26	-0.06	-0.42
Gastropod	0.65	0.38	-0.28	0.21	0.25
Odonata	0.33	0.44	-0.10	-0.11	0.59
Lumbriculus	0.38	0.16	-0.33	0.19	-0.39

Figures

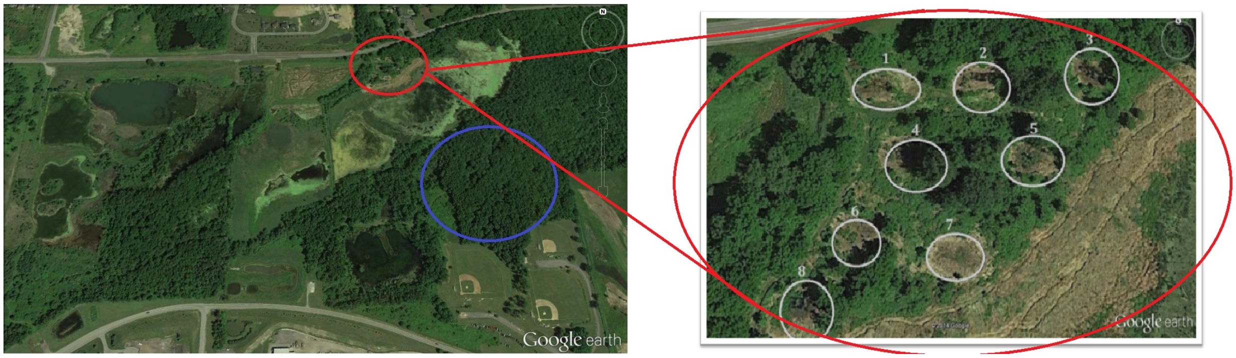

Figure 1: Aerial imagery of High Acres Nature Area with the created pools outlined in red, and natural pools outlined in blue. Each individual created pool is outlined (right) where the lack of canopy cover is evident.

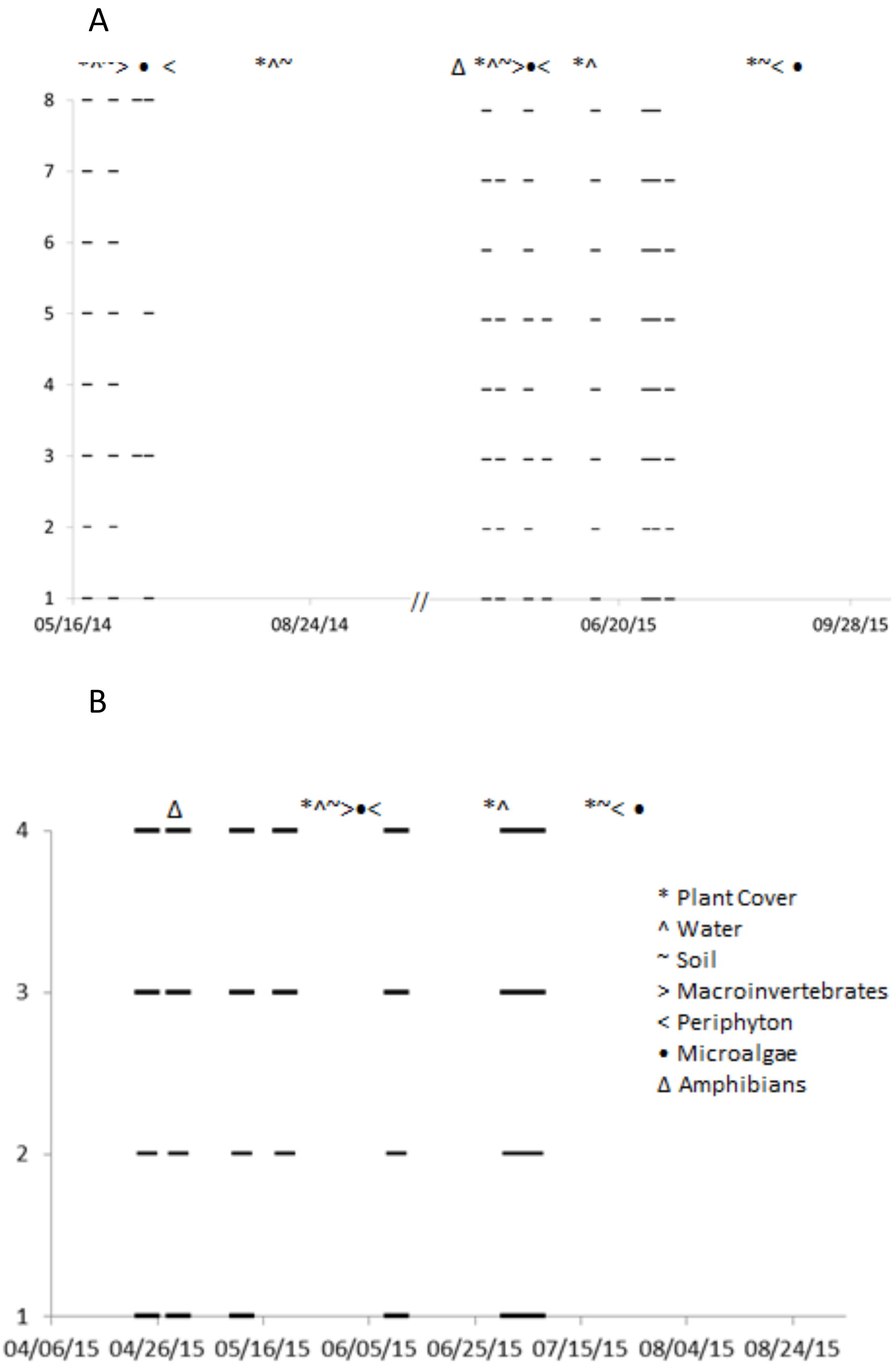

Figure 2: Length and frequency of flooding in A), created and B) natural pools paired with frequency and timing of sampling.

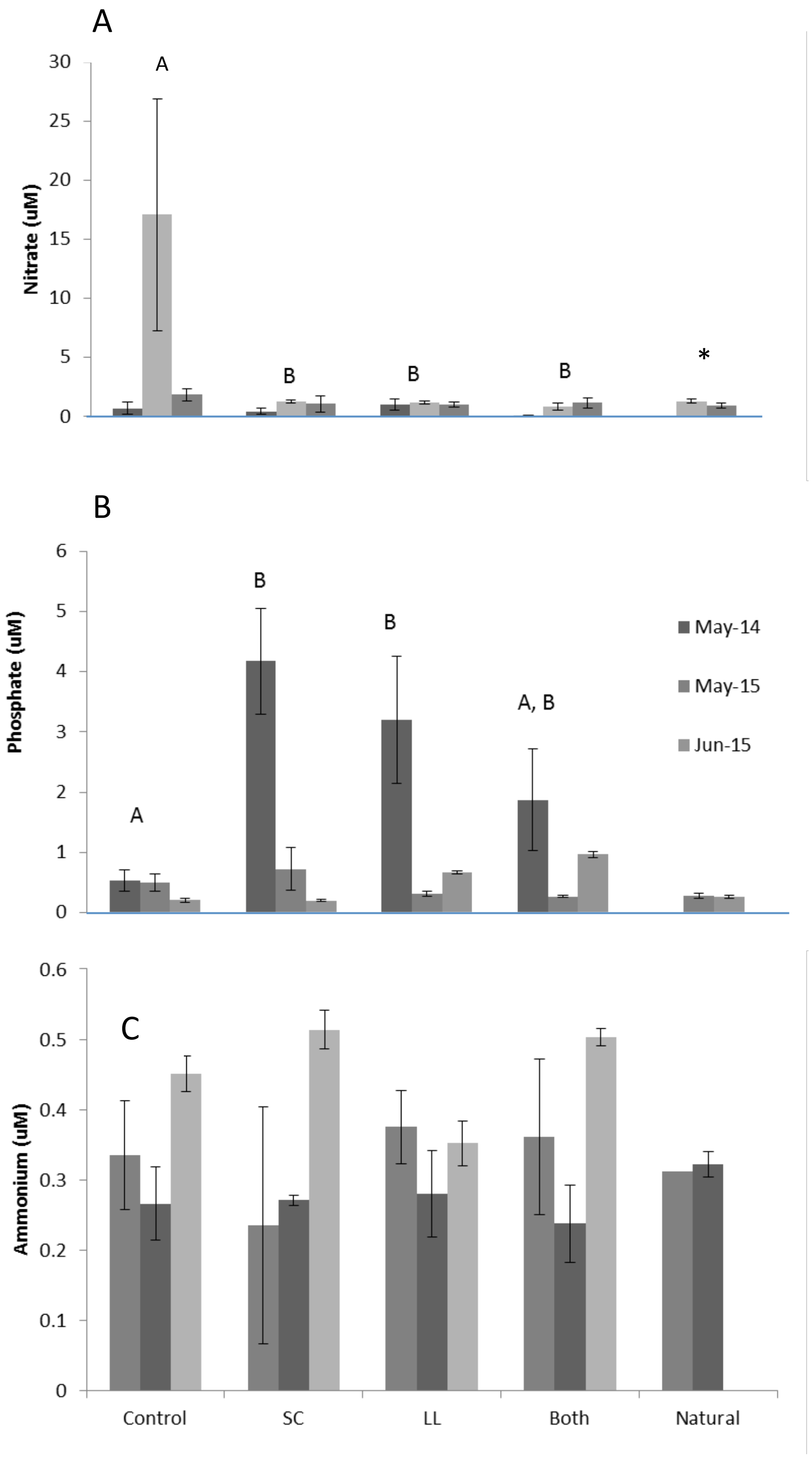

Figure 3: Surface water concentration of A) nitrate, B) phosphate, and C) ammonium over time in all treatments.

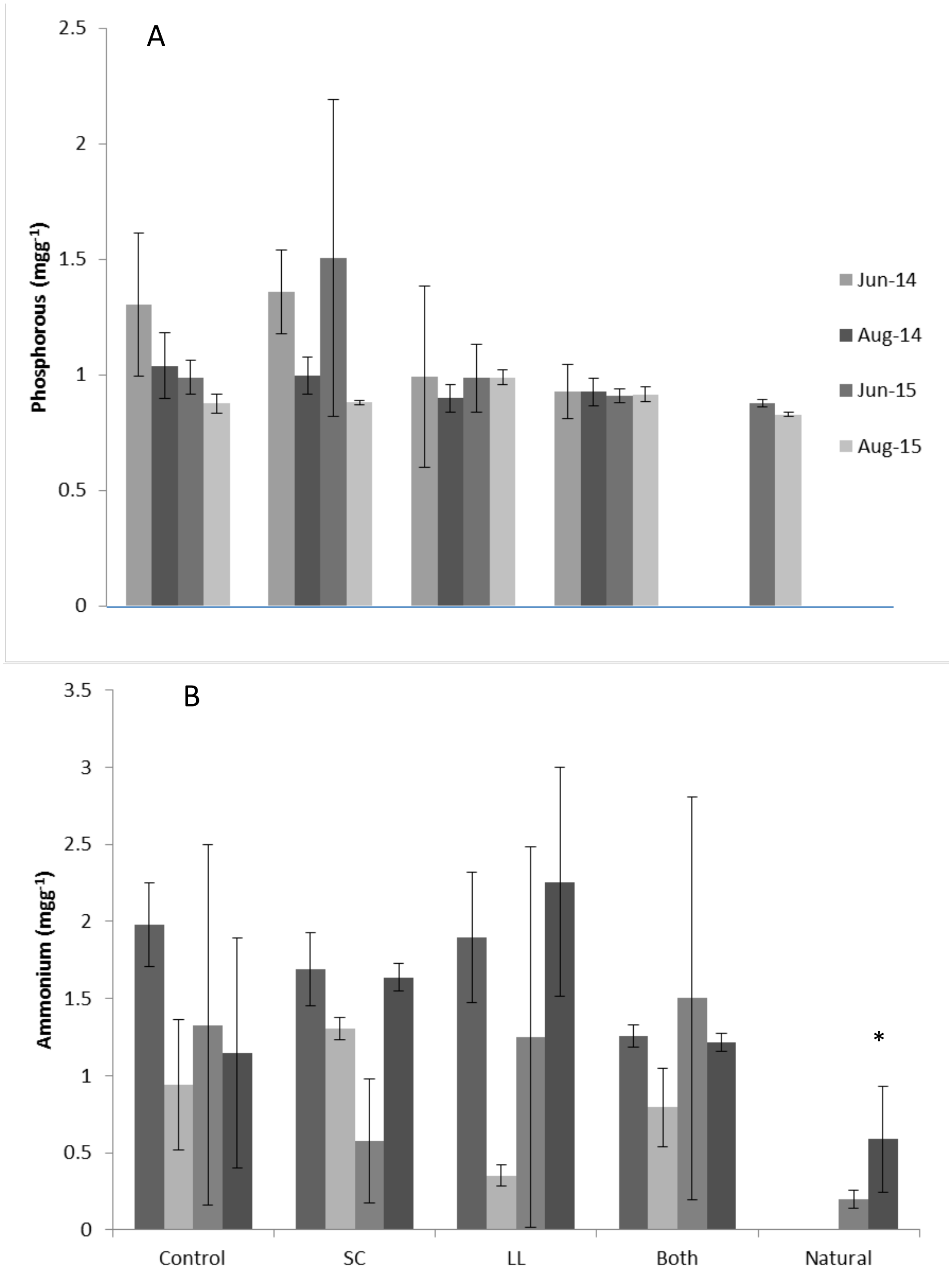

Figure 5: Extractable A) Phosphorus, and B) Ammonium concentrations per gram of soil at each sampling date among all treatments.

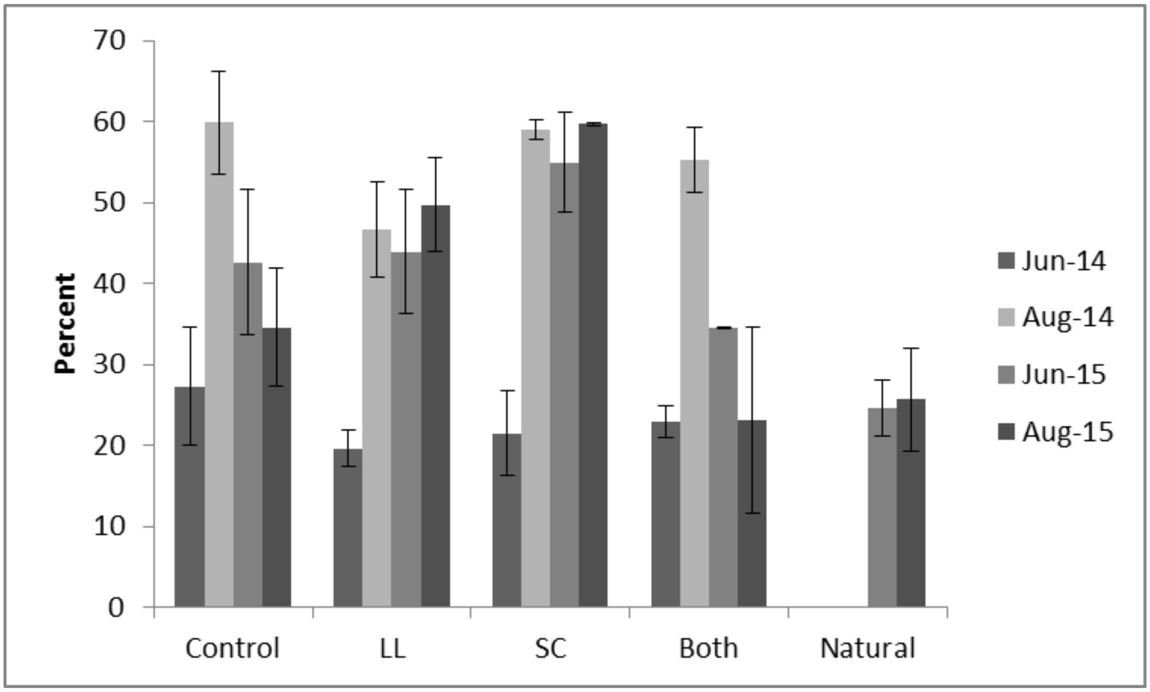

Figure 6: Percent organic matter in soils from all pools at different sampling times.

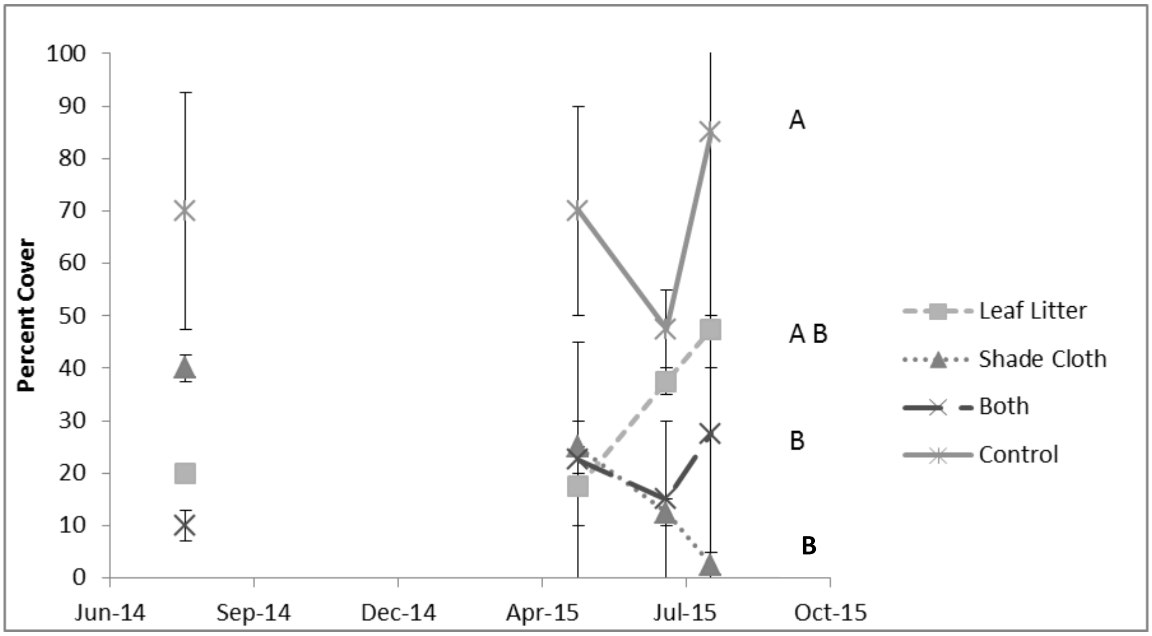

Figure 7: Invasive species percent cover over time for each treatment

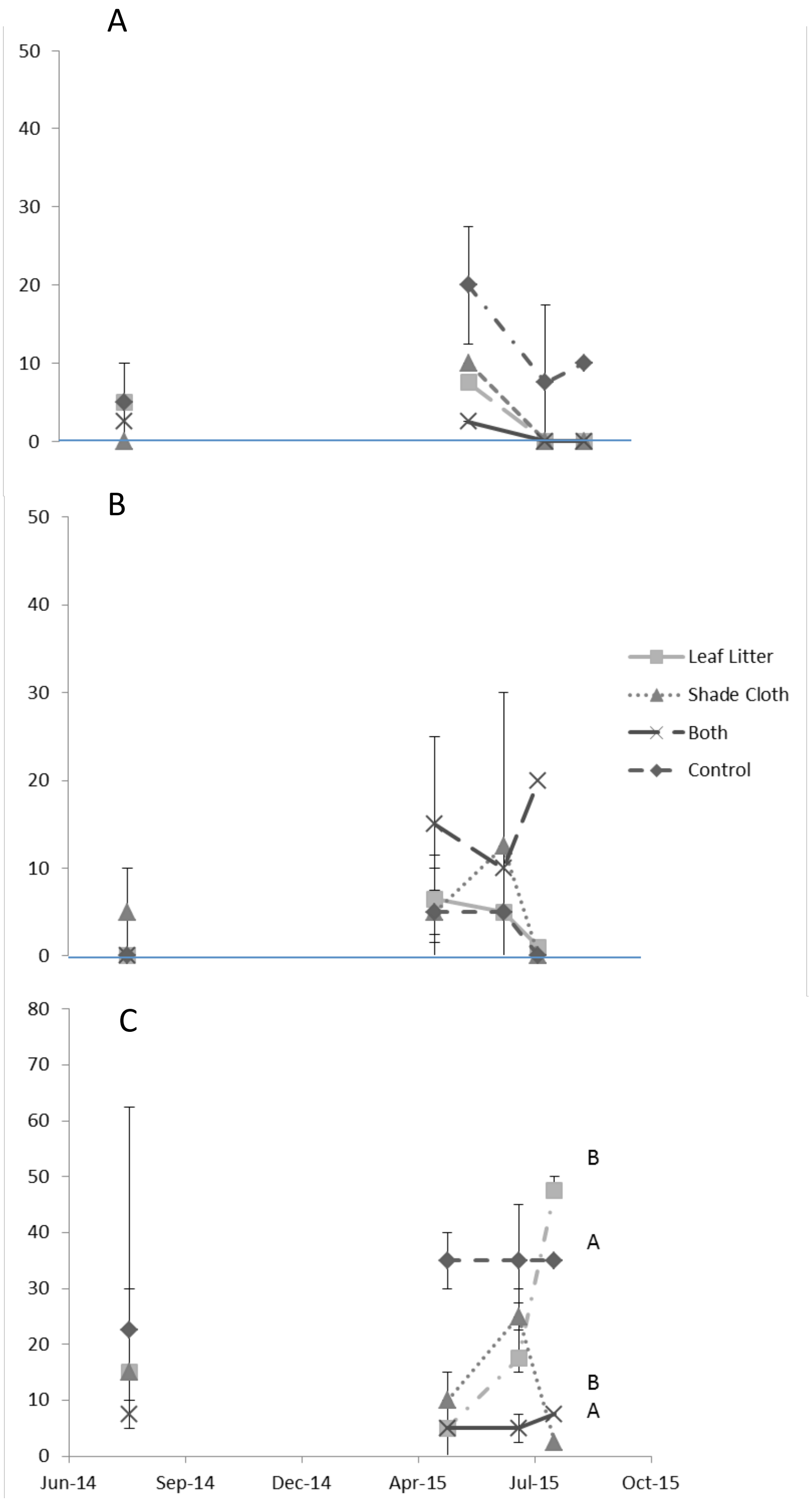

Figure 8: Individual cover of A) *Typha*, B) P*halaris arundinacea*, and C) *Lythrum salicaria* over time. Note different y-axes.

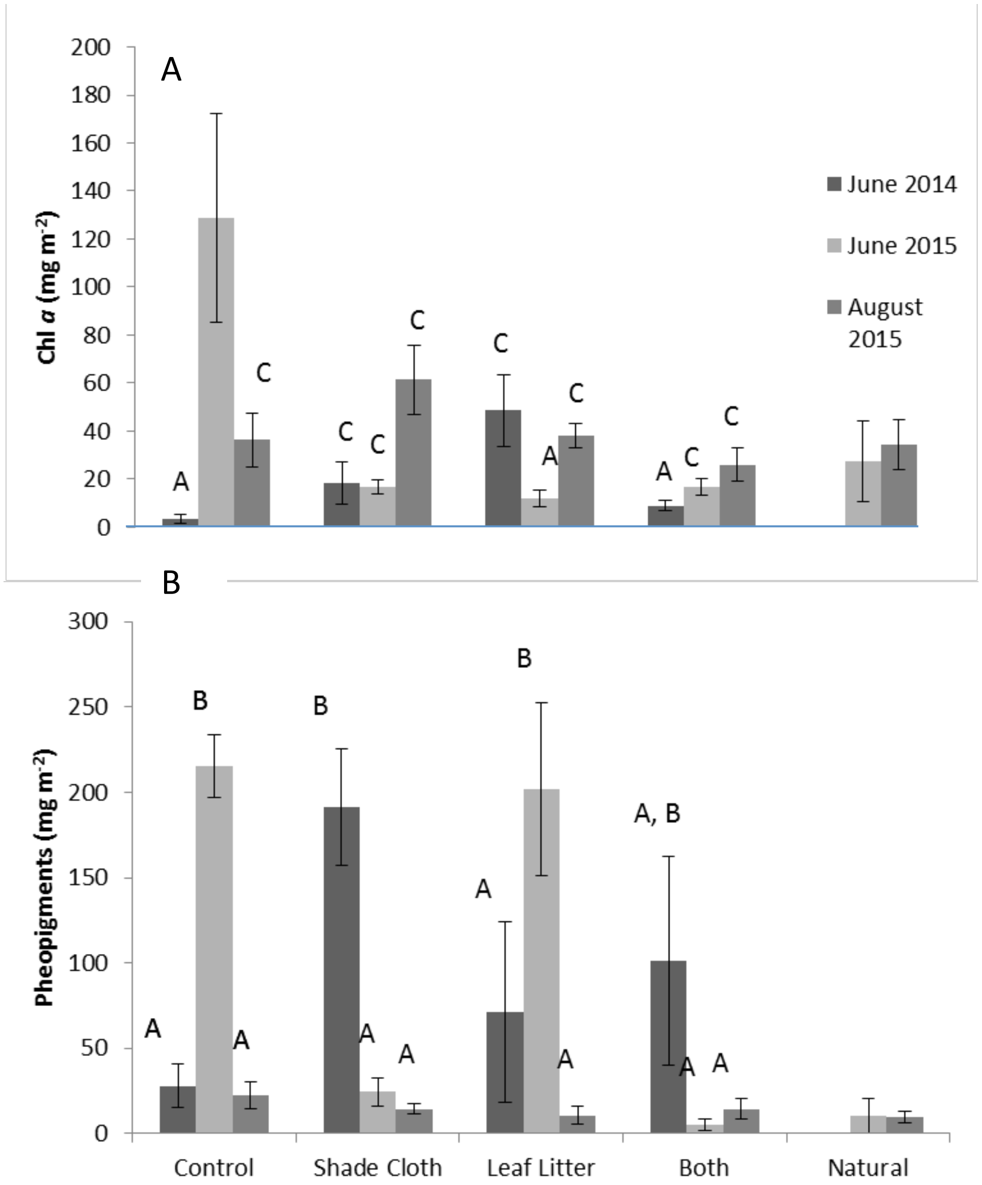

Figure 9: A) Benthic micro-algal biomass using chlorophyll *a* as a proxy B) Periphyton biomass using chlorophyll *a* as a proxy in experimental created pools and natural pools.

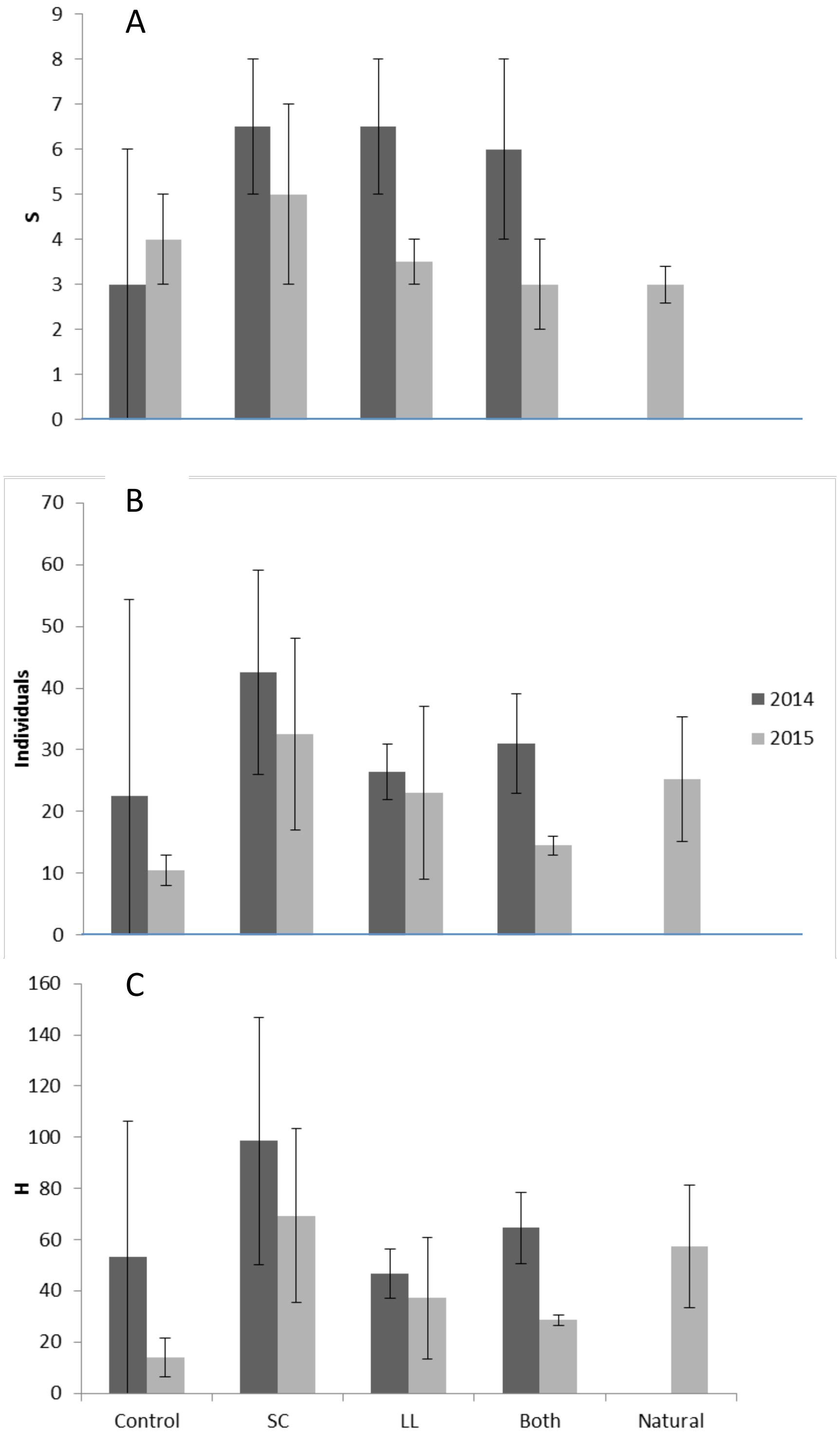

Figure 10: Macroinvertebrate results among all treatments showing A) species richness (S), B) total number of individuals, and C) Shannon-Weiner diversity index results.

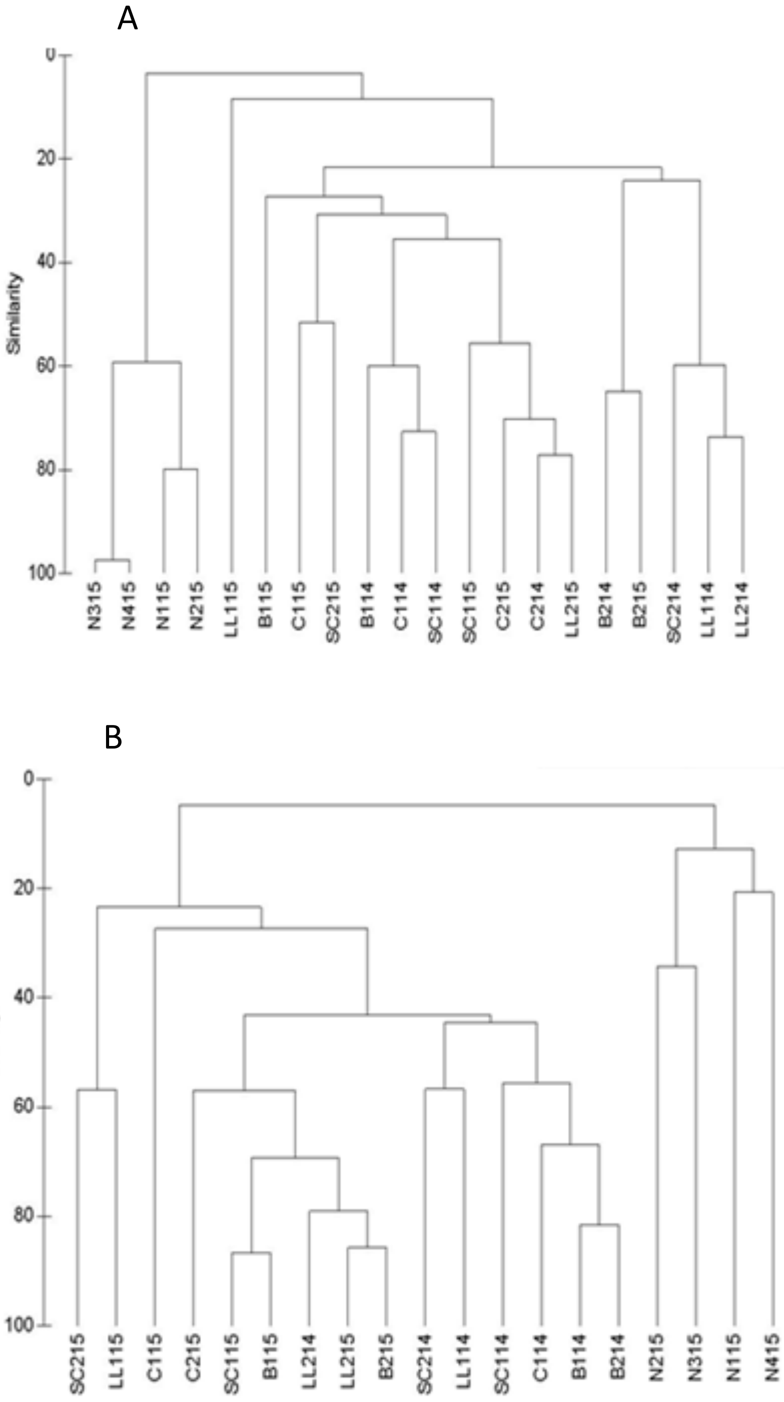

Figure 11: Cluster diagram showing similarity of A) plant and B) macroinvertebrate communities in all pool, by treatment and year.

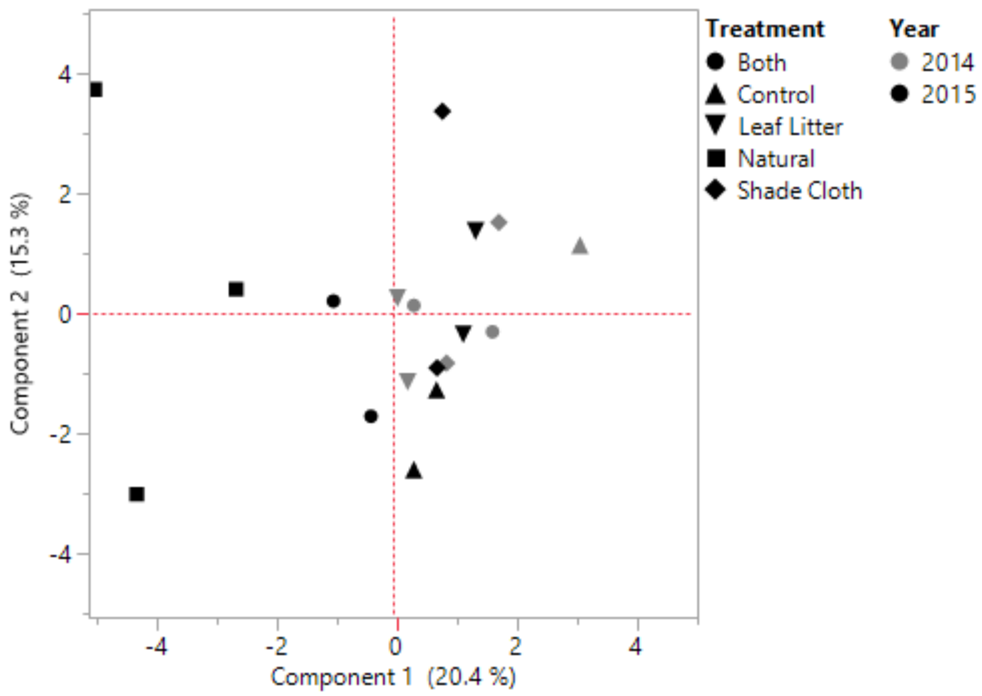

Figure 12: Principal components analysis score plot of variables showing where each treatment falls in the first two components in 2014 and 2015.

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