Comparison of vernal pool characteristics in an old-growth forest and a disturbed urban forest in Southern Ontario

By

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ABSTRACT

There is a large incentive to understand vernal pool ecosystems in Southern Ontario, as they serve essential ecological functions within forests – including providing critical habitat for at-risk amphibians like Jefferson salamanders. Additionally, while deforestation has drastically reduced the quantity of vernal pools in this region, it is unclear how these landscape alterations have affected the quality and characteristics of vernal pools in remaining woodlots. This study addresses this gap in knowledge, by evaluating whether or not vernal pool characteristics vary between an old-growth forest (Backus Woods) and a more disturbed forest (Hamilton) in Southern Ontario. Using fall field surveys, I recorded characteristics of 10 vernal pools in the Hamilton region and 13 vernal pools in the old-growth forests of Backus Woods, and used GIS to evaluate their landscape context. Vernal pools in the Hamilton region had less shaded canopies, shallower depths, and higher water conductivities and total nitrate nitrogen concentrations than those in Backus Woods. Hamilton vernal pools occurred nearer to forest edges and adjacent urban lands, whereas those in Backus Woods were located within larger regions of forest and were surrounded by agricultural land. Land use was not a significant predictor of vernal pool characteristics; instead, forest structure (e.g., disturbed vs old-growth, coniferous vs deciduous) likely played a key role in determining how vernal pool characteristics responded to the surrounding landscape. My results support that human disturbances such as land use alteration can influence vernal pool characteristics, which may influence habitat suitability of at-risk amphibians.

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1. INTRODUCTION

1.1 Overview

Vernal pool ecology is a field of research that has gained increasing traction in recent years, as vernal pools have become increasingly recognized as important targets for conservation due to their large ecological value and threatened nature as landscape features. Much of the published literature has evaluated vernal pool ecology within the United States, where an intense and rapid landscape conversion has reduced areas of vast forest to small, isolated forest patches within a matrix of developed urban and agricultural land cover (Evans et al., 2017; Stokes et al., 2021). This urbanization has threatened both the quantity and quality of remaining vernal pool habitats throughout the region, motivating interest in understanding and preserving what remains of North American vernal pool ecosystems (Baldwin and deMaynadier, 2009). While there is comparatively little literature evaluating the state of vernal pools within Canada, a similar pattern of forest conversion for urban and agriculture development has occurred in Southern Ontario (S. ON) (Bradford, 2016). This presents an urgent need for vernal pool research in S. ON, to evaluate the state of vernal pools in remaining forests and promote their effective conservation.

1.2 Large ecological value of vernal pools as landscape features

In North America, vernal pools are defined as geographically isolated, temporary wetlands occurring in shallow topographic depressions throughout forested landscapes (Schrank et al., 2015; Rothenberger et al., 2019). They have a seasonal hydrology, filling with water in the fall and spring but drying in the summer to create a vital habitat for a specialized biota whose life history strategies often incorporate these aquatic and terrestrial transitions. This includes obligate amphibian species that require vernal pools to complete their lifecycles; approximately 56% of frog, toad, and salamander species in the northeastern United States require vernal pools for breeding, development, foraging, or hibernation (Rothenberger et al., 2019). This list includes at-risk species such as the Jefferson salamander (*Ambystoma jeffersonianum*), that is designated as provincially and federally endangered in Ontario and Canada (Ministry of the Environment, Conservation and Parks, 2014; Government of Canada, 2021). In the advent of a global amphibian decline, one of the most severe and well-documented examples of the ongoing biodiversity crisis, the loss of vernal pool habitats and surrounding forest areas are particularly prominent threats to these species' survival (Angelini, Bielby and Costa, 2020).

Vernal pools also provide essential ecological services to many semi-aquatic and terrestrial species such as birds, mammals, and turtles, facilitating these animals' migrations and providing places to find shelter, food, and water (Evans et al., 2017; Eakin, Hunter and Calhoun, 2018; Rothenberger et al., 2019). Vernal pools support a diverse community of plants and shrubs, many of which are also adapted to the wet-dry cycles (Deil, 2005; Kneitel and Lessin, 2010; Schrank et al., 2015). Additionally, vernal pools serve several larger roles within forest landscapes, by functioning as biogeochemical hotspots for denitrification and organic matter decomposition, and maintaining watershed integrity by providing services such as aquifer recharge and water filtration (Capps et al., 2014; Evans et al., 2017). These various ecological roles provide value to vernal pools as small natural features (i.e., small ecosystems that play larger roles in ecosystem processes than their small size would indicate) (Rothenberger et al., 2019).

1.3 Variability of vernal pool characteristics

Vernal pools are highly variable in their physical, hydrological, and chemical characteristics. Generally, they are known to be precipitation-driven and hydrologically maintained by local weather conditions and ground-water exchange (Rothenberger et al., 2019). Their unique wet-dry hydrological cycles create a shifting community assemblage that varies with their dry and wet phases (Kneitel and Lessin, 2010). In California, vernal pools first undergo a dry phase, dominated by few species presenting aboveground, followed by a wet phase, where pools fill with standing water and an aquatic microbe, algae, invertebrate, and vascular plant community dominates. As rainfall ceases and temperatures increase in the late spring, vernal pools desiccate and move into a terrestrial phase, where a vascular plant community typically dominates.

The composition and diversity of a vernal pool's faunal community are strongly affected by its hydroperiod, or the timing and duration of its wet phase (Brooks, 2004). The timing, length, and frequency of inundation can each affect the relative abundance, species richness, and community composition of vernal pools (Kneitel and Lessin, 2010). As hydroperiod increases, species richness also tends to increase, but this relationship is complex since biotic factors such as predation also increase in importance with increasing hydroperiod (Brooks, 2004). Amphibians in particular are often highly sensitive to hydroperiod, as they require standing water to remain long enough for their eggs to develop and for their larvae to metamorphose, but need dry conditions during other parts of the season to prevent predator colonization of breeding pools (Schrank et al., 2015). The duration and timing of phases in vernal pool cycles can also be highly variable from year to year, which may promote species coexistence since species differ in their niche requirements and response to environmental conditions (Kneitel, 2014). While hydroperiod is a critical component of vernal pool ecology, it is a very difficult feature to predict and model. The relationships between pool size, depth, area and volume to hydroperiod are often weak and inconsistent, since they are influenced by a variety of complex factors – such as local weather conditions, precipitation, ground-water exchange, soil properties, vegetation assemblages, and overhead canopy shading (Schrank et al., 2015; Luymes and Chow-Fraser, 2022).

In addition to hydroperiod, amphibian communities can also shift in response to changes in canopy cover. Canopy cover can affect pool temperature, and support detrital food webs through inputs of leaf litter, which can impact amphibians' developmental rates and species interactions (Calhoun et al., 2014). Decreases in canopy cover may result in increases of light, temperature, and primary productivity, attracting a broader array of amphibian and invertebrates species that may either compete with or prey upon pool breeding specialists (Calhoun et al., 2014). Different amphibian species show discrepancies in their responses to variations in habitat characteristics such as canopy cover and vegetation cover, likely due to their differences in resource requirements: for example, many anurans are found in open canopy pools since these provide higher nutritional quality food sources (e.g., algae, decaying non-woody plants) than the decaying leaf litter that covers closed canopy pools (Luymes and Chow-Fraser, 2022).

Additionally, microhabitat cover types within pools can impact amphibian communities in other ways, since vegetation can provide shade, refuge, and potential egg attachment sites (Rothenberger et al., 2019). In pools devoid of vascular plants, amphibians may also use fine and coarse woody material deposited in pools from adjacent trees and shrubs for these purposes (Rothenberger et al., 2019). Certain amphibians may also have preferences for leaf litter type; Feuka et al. (2017) observed that while wood frogs showed no leaf litter preference, unisexual blue-spotted salamanders (*Ambystoma laterale* × *jeffersonianum*) showed a preference for deciduous leaf litter in dark conditions and coniferous litter in more illuminated conditions.

Water chemistry in vernal pools can also vary widely, and is influenced by water sources, bedrock and soils, surrounding plant community, and watershed land use (Colburn and Calhoun, 2018). Groundwater is likely to provide more constant thermal and chemical water quality than precipitation and runoff, since runoff carries sediments, organic detritus, and dissolved materials transported by snowmelt and storm runoff from the surrounding watershed (Colburn and Calhoun, 2018). Changes in water chemistry have been well-documented to affect vernal pool amphibian species richness and abundance. For example, Calderon et al. (2019) observed species richness and abundance to correlate negatively with water chemistry variables such as phosphate, nitrogen, and total coliform concentrations, and correlate positively with other variables, such as electrical conductivity and dissolved oxygen in freshwater amphibian habitats. Other studies show negative relationships between electrical conductivity and amphibian embryonic and larval survival (Karraker, Gibbs and Vonesh, 2008). Since most anuran amphibians spend their first developmental stages in aquatic environments, and have complex adult life cycles that incorporate both aquatic and terrestrial elements, both larvae and adults are usually susceptible and sensitive to declines in water quality and the presence of pollutants dissolved in water (Calderon et al., 2019; Eakin, Calhoun and Hunter, 2019).

Thus, research in temporary aquatic systems should consider the complex interactions between amphibian habitat suitability, vernal pool characteristics, and features of the surrounding landscape, as these can all influence spatiotemporal patterns of biodiversity within a region (Kneitel, 2014).

1.4 Vulnerability of vernal pools as landscape features

Some of the largest threats to vernal pools may be caused by human modifications to the landscape, which have the power to dramatically alter vernal pool characteristics. European settlement of eastern North America initiated broad-scale forest disturbances, which resulted in more than three centuries of forest clearing for agricultural production, commercial logging, and urban development (Parker et al., 2008). This dramatically reduced the total area and fundamentally altered the composition, structure, and distribution of remaining forests throughout these lands (Parker et al., 2008). These land modifications resulted in numerous threats to vernal pools; occurring directly, through habitat loss or fragmentation due to deforestation, as well as indirectly, since the replacement of natural cover with impervious surfaces results in major changes to hydrological, chemical, and physical characteristics of urban waterways (Eakin, Hunter and Calhoun, 2018). For example, Carrino-Kyker and Swanson (2007) found that while physiochemical variables of vernal pools (including nutrient levels and conductivity) varied within a region, these water quality parameters were correlated with increases in nearby urban and agricultural land uses.

While vernal pools are typically classified as oligotrophic (unproductive) water bodies, patterns of shifting diversity suggest that nutrient inputs and other associated anthropogenic activities may have negative effects on vernal pool biodiversity as these systems move towards higher productivity levels (Kido and Kneitel, 2021). Eutrophication (i.e., an excessive input of nitrogen and phosphorus into ecosystems that results from human activities) is a prominent and well-documented threat to many terrestrial and freshwater ecosystems (O'Hare et al., 2018; Wurtsbaugh, Paerl and Dodds, 2019). However, excessive nutrient inputs may be particularly damaging to vernal pools since they are largely reliant on both precipitation and surface runoff, and are thus prone to rapid changes in water quality (Kneitel and Lessin, 2010). Studies on California vernal pools have found nutrient addition to alter water quality by increasing turbidity, chlorophyll-a, and nutrient levels (especially phosphorus), in addition to causing larger shifts in dissolved oxygen concentrations and in pH (Kido and Kneitel, 2021). Since these are vernal pool characteristics that are often determinants of habitat suitability, alterations of these characteristics may adversely impact local amphibian species.

Urbanization can have significant negative effects on amphibian community structures. Species richness, density and diversity have been shown to have negative relationships with increasing urban land cover, which strongly affects anurans with short hydroperiods, early breeding activity, and substantial upland habitat use (Calderon et al., 2019). Generally, increasing urban cover results in an increase of pollutants from largely non-point sources (e.g., salts from roadways, sediments from construction sites, runoff of lawn fertilizers and pet wastes, and inputs from unsewered developments; Carpenter et al., 1998) that are then carried by surface runoff into nearby vernal pools. These additions result in large changes in water chemistry, including high values of electrical conductivity, high concentrations of nitrates, nitrites, total phosphates, chloride, un-ionized ammonium, and trace metals, extreme values of pH, high concentrations of organochlorine and organophosphate pesticides, high concentrations of pharmaceuticals, high levels of organic matter, and low concentrations of dissolved oxygen (Calderon et al., 2019). The effects of these pollutants on amphibians have been widely documented, and can include low densities of egg masses, low hatching success, and low larvae survival and development rates (Calderon et al., 2019).

As well as affecting amphibians through changes to vernal pool water chemistry, urbanization of forests can also impact amphibian communities directly. Since the life cycle of pool-breeding amphibians includes spending parts of the year in adjacent upland forest, many amphibians require several hundred meters of forest buffer around their breeding pools for migration and for completing the terrestrial components of their lifecycles (Semlitsch and Bodie, 2003; Powell and Babbitt, 2015). While the required size of forest buffer around a vernal pool may vary between species, the critical threshold of surrounding forest habitat for spotted salamanders has been suggested to be between 100m and 300m, and that of wood frogs to be around 300m (Homan, Windmiller and Reed, 2004). Other species may require even larger forest buffers to encompass their overwintering movement patterns – for example, studies have suggested that the critical habitat threshold for Jefferson salamanders extends at least 175m from a pool's edge (Faccio, 2003), and possibly much further – up to 400 to 450m from breeding pools (Drunen et al., 2020). Thus, forest modifications can both directly (e.g., by increased mortality from road kills; Puky, 2005) and indirectly (e.g., by decreased reproductive success due to high water conductivity from road salts entering pool basins; Karraker, Gibbs and Vonesh, 2008) affect vernal pool amphibians.

Changes to vernal pool species assemblages have been documented due to forest disturbances or nearby modified land uses within 1000m of pools, demonstrating that these species can respond to land cover changes at even greater distances than they have been observed to move from breeding pools (Eakin, Hunter and Calhoun, 2018; Eakin, Calhoun and Hunter, 2019). This has strong implications for vernal pool conservation – since vernal pool protection policies should clearly extend beyond their relatively small surface watershed limits (Larocque et al., 2018).

Interestingly, the responses of amphibians to changes in forest structures in urbanizing landscapes have been observed to vary between regions (Eakin, Calhoun and Hunter, 2019), highlighting the need for specific studies investigating how land use changes will influence the quality and distribution of vernal pool habitats in specific regions. This is particularly true since the effects of vernal pool degradation due to human activities may be compounded by effects such as climate change, which also have the potential to alter vernal pool characteristics (especially hydroperiod) (Brooks, 2009; Montrone et al., 2019). Continued degradation of the matrix in which protected habitat patches are located, climate change, and the interactions between climate change, habitat loss, and habitat fragmentation, are all likely to be significant future challenges for vernal pool conservation (Stokes et al., 2021).

1.5 Vernal pool conservation and challenges in Southern Ontario

There is an urgent need for research on vernal pool ecosystems within Canada, and particularly within S. ON, where similarly to forests in the northeastern United States, there has been very rapid and intense land conversion of forested regions for agriculture and urban development (Parker et al., 2008; Bradford, 2016). As a result of this land conversion, few woodlots remain, and they are embedded in an urban and agricultural matrix that makes them particularly vulnerable to degradation. S. ON is also recognized as a global epicentre of amphibian biodiversity, and so these forests represent an important component of regional natural heritage (Evans et al., 2017; Nature Conservancy Canada, 2020; Stokes et al., 2021). However, there is a 'lag' in research on vernal pools in Canada, with very few studies evaluating vernal pool characteristics and whether (and to what extent) human activities have altered their quality and ecosystem functions (e.g., Larocque et al., 2018). Additionally, there has been very little mapping of vernal pools in Ontario, and no comprehensive dataset for vernal pools in the S.

ON region (Luymes and Chow-Fraser, 2021). It is very likely that this lack of information has detrimentally prevented the effective conservation of Ontario's vernal pools.

A myriad of challenges hinders conservation efforts for preserving vernal pools in S. ON, including finding, assessing and categorizing, and protecting these pools in policy. Additionally, there is a growing body of literature providing evidence that vernal pools across North America are inadequately protected (e.g., Carrino-Kyker and Swanson, 2007; Rothenberger et al., 2019). However, there is yet very little research published on whether (and to what extent) remaining vernal pools in S. ON have been affected by the large forest disturbances and landscape modifications that have occurred in this region (e.g., Bradford, 2016). While there have been a few studies evaluating vernal pool characteristics in Michigan in the United States, where vernal pools are also located near the Great Lakes, these studies are of limited use for comparisons to S. ON, due to inherent differences between regions. For example, variations in ecological functions observed between these regions could be attributable to the inherent differences in location (e.g., differences in soil topography, climate) rather than to the vernal pool features themselves (Rothenberger et al., 2019). As a result, true reference sites, which minimize confounding factors and act as paired controls, are crucial for quantitatively measuring performance variation of vernal pools in different regions.

Consequently, there is an urgent need for vernal pool research in S. ON, including comparison studies that examine correlations between biological function and a variety of vernal pool characteristics, including hydrology, slope, water quality, within-pool vegetation, canopy cover, soil characteristics, and landscape context for forested regions (Mina et al., 2017; Rothenberger et al., 2019). Important steps for effective management and conservation in this area include identifying the target areas in need of protection (i.e., by inventorying small wetlands and characterizing the surrounding terrestrial habitats), and identifying potential constraints (e.g., the current state of surrounding terrestrial habitat, degree of degradation, etc.) that may hinder successful implementation of such plans (Evans et al., 2017).

This study evaluates vernal pool characteristics within S. ON using a comparison of two study regions, with the objective to investigate whether vernal pool characteristics in this area have been affected by urbanization of the surrounding forest landscape. The first study region included the old-growth and relatively intact forests of Backus Woods, which provided good reference conditions for vernal pool characteristics in S. ON. The second study region included forests in the Hamilton region, where forests were in a more disturbed state and were located near a large urban centre.

1.6 Study hypotheses

I hypothesized that vernal pool characteristics would vary predictably between Backus Woods and Hamilton, and predicted that differences in water quality, canopy cover, and microhabitat assemblages would be determined by several key features of the surrounding landscape – including the distance from vernal pools to the edge of the forest, and the proportions of urban land uses within 1000m from each pool (**Figure 1**). More specifically, due to Hamilton's higher proportion of urban land use, I predicted vernal pools in the Hamilton region to have smaller distances to the forest edge, smaller degrees of canopy cover, higher water conductivity and water nutrient (total phosphorus; TP, and total nitrate nitrogen; TNN) concentrations, as well as different microhabitat assemblages – including higher proportions of algae and herbaceous vegetation cover types than vernal pools in Backus Woods.

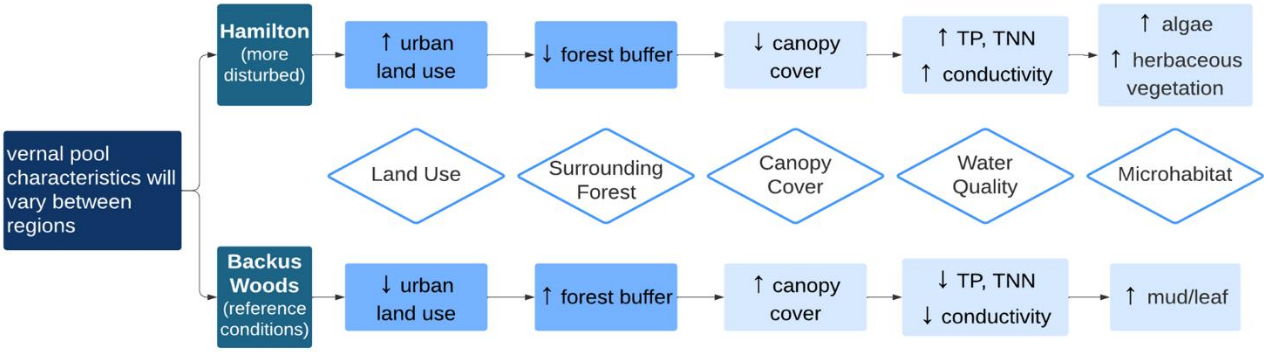

Figure 1: Study hypotheses, predicting differences in vernal pool characteristics and landscape context between Backus Woods and Hamilton vernal pools.

2. METHODS

I conducted two site surveys to observe and record vernal pool characteristics. The first survey was conducted in early fall (October 2021), while the foliage was still on the trees, and the second survey was conducted in late fall (November 2021), after several weeks of frequent rainfall had allowed the vernal pool basins time to fill with standing water.

2.1 Study regions

The study regions selected for comparison were the old-growth forest of Backus Woods, and the more disturbed forest near the Hamilton region. These are both forests located in S. ON that lie within the Carolinian Life Zone – a small but very highly biodiverse ecoregion within Canada. Because of their similar climates and location within Ontario (near the Great Lakes, and within the Carolinian Life Zone), I expected vernal pool characteristics to vary similarly between the two forests, expect for differences resulting from the regions' different forest structures and nearby land uses.

Backus Woods is a property owned by the Nature Conservancy of Canada, and is one of the highest quality old-growth hardwood forests in all of Ontario, and the best remining example within the Carolinian Life Zone (Nature Conservancy Canada, 2020). Thus, Backus Woods provided good reference conditions for vernal pool characteristics in S. ON, and was selected as the control region for my comparison. Hamilton also features a forested landscape, but one with a higher degree of forest disturbance (i.e., not old-growth). It is also directly adjacent to the city of Hamilton, a very large urban centre in S. ON. During site selection, is important to consider that searching for study sites in a completely disturbed forest area is impractical, since vernal pools cease to exist without their surrounding forest. Thus, the forests in Hamilton were a good choice for the second study region, since they are relatively more disturbed than those of the reference region, but are still intact enough to contain several vernal pools. It is also important to note that because vernal pools are frequently located on private lands, site selection for this study was restricted to sites with public access, or where permission was given to sample.

In total, 23 vernal pools were included in this study: 13 in Backus Woods, and 10 in Hamilton (**Figure 2**). These were located either by hiking along trail networks, or using latitude and longitude coordinates obtained by a former member of the Chow-Fraser lab group, Dr. Nick Luymes. Vernal pools were identified by the presence of a basin (either dry or containing standing water depending on the timing of the site visit) within a forest matrix. I conducted site surveys in the 2021 fall season: once in early October, and a second time in early November.

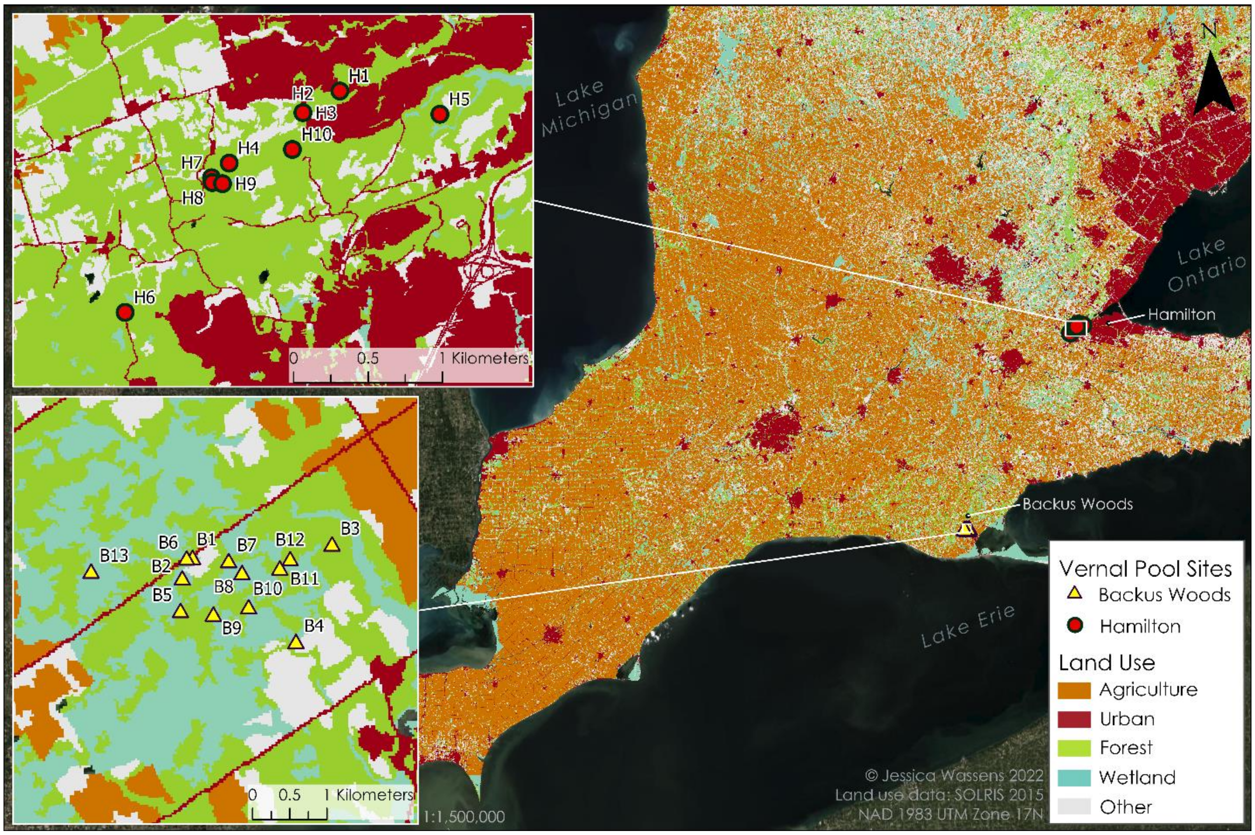

Figure 2: Locations of vernal pool study sites in S. ON, and their surrounding land uses (SOLRIS 2015).

2.2 Pool basin morphology

I determined the surface area of vernal pool basins by measuring the maximum length and width of the pool basin, and by using the formula of an ellipse to approximate the pool's surface area. Maximum length was recorded along the longest transect, and maximum width was recorded along the next longest transect occurring perpendicular to the length. Since vernal pools were relatively small, I used a measuring tape to measure the length and width of each pool, and visually approximated the 90° placement of the width transect against the length transect. Vernal pool perimeters were recognized by abrupt changes in the herbaceous layer, or by a micro-topography break.

I measured vernal pool depth during my second site survey, by measuring the depth of the standing water at the deepest point in the pool basin (usually near the pool center). Since pools were generally shallow, vernal pool depth was also measured with a measuring tape.

2.3 Pool microhabitat composition

I estimated vernal pool microhabitat composition visually, as a percentage of different cover classes. These classes included: mud and leaf litter, herbaceous vegetation, thicket, moss, and algae. The abundance of sticks and woody debris was also noted, as a qualitative observation. Differences in the microhabitat compositions observed between the two site surveys were noted, as the microhabitat composition tended to shift for many of the pools as their basins filled with water later in the fall. However, I only used microhabitat composition observations from the second site survey in my subsequent analyses.

2.4 Canopy cover

Canopy cover was measured during the first site survey (while the trees still had their leaves), using a convex spherical densiometer. To estimate the canopy cover over a particular pool's basin, I calculated an average value from 20 readings. Individual readings were taken at each of the four cardinal directions at five points within the pool's basin: at the center of each pool, and at each of the ends along the length and width transects across the pool's basin (**Figure 3**). This method is used by other vernal pool studies to estimate over-story density (e.g., Eakin, Hunter and Calhoun, 2018; Rothenberger et al., 2019).

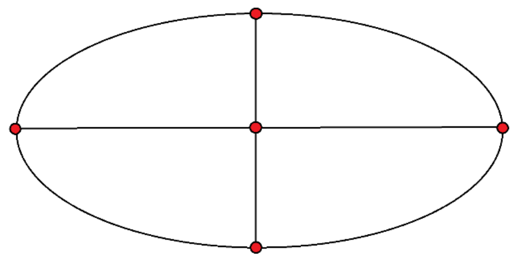

Figure 3: Example of densiometer sampling locations (red circles) within an elliptical vernal pool basin, to estimate the over-story canopy cover.

During my site surveys, I also observed general differences in the type of trees surrounding vernal pools (e.g., deciduous, mixed, coniferous).

2.5 Water chemistry

To measure water conductivity, I used a multiparameter sonde (AquaTroll 500). For these measurements, data from each pool was gathered approximately 1m from the shoreline, and at mid-depth, as was done by Rothenberger et al. (2019).

I also collected grab samples of water in bags, and froze these samples for later nutrient content analysis in the lab (TP and TNN). While collecting these water samples, I tried to avoid stirring up sediment and debris from the bottom of the vernal pool basin, to avoid capturing this within my sample. Water samples were collected from the middle of the standing body of water, or near the vernal pool’s edges if there was dense vegetation in the center of the pool. Samples were kept chilled in a cooler until they could be frozen, and samples were processed within three months of freezing. I conducted analyses of these samples using the molybdenum blue method to process TP (Murphy and Riley, 1962), and the cadmium reduction method using Hach protocols (Nitrate, MR) and reagents to process TNN (Hach Company, 2014).

2.6 Surrounding land use

I classified the land use surrounding each vernal pool in ArcGIS Pro v.2.9.2 (Esri Inc., 2021), using land cover data obtained from the SOLRIS v.3.0 land use inventory (Ministry of Natural Resources and Forestry, 2019). Land use was re-classified into five general categories for analysis: urban, agriculture, forest, wetland, and other (**Table 1**). I included tree cultivated plantations and hedgerows within the forest category, as regions within Hamilton and Backus Woods forests that were classified within the SOLRIS land use raster as these land use types appeared to be forest when viewed using Google Earth satellite imagery. While I classified many of the other SOLRIS land classes into the other category, this was mainly for ease of analysis – many of these land use classes did not appear within the regions of interest. Most of the areas within Backus Woods and Hamilton that classified into the other general land use category were from the undifferentiated class of the SOLRIS land use raster, which were generally fields without a crop type.

Land cover types were categorized within 50m, 500m, and 1000m buffer circles around each vernal pool, to assess differences in land use at different spatial contexts. Each of these spatial scales is ecologically relevant; wetland protection and timber-harvesting policies often invoke a 30m buffer size (Veysey Powell and Babbitt, 2015), 500m encompasses the critical habitat of most vernal pool amphibians (Faccio, 2003; Freidenfelds, Purrenhage and Babbitt, 2011), and 1000m includes landscape-scale effects of forest urbanization (Eakin, Calhoun and Hunter, 2019).

Table 1: Summary of the re-classification of SOLRIS land use raster data into five more general land use categories

Classification	SOLRIS class name	Value	Description
Urban	Transportation	201	Highways, roads
	Build-Up Area - Pervious	202	Urban recreation areas (i.e., golf courses, playing fields)
	Built-Up Area - Impervious	203	Residential, industrial, commercial, and civic areas
Agriculture	Tilled	193	Agricultural fields managed as row crops, that may also be rotated with perennial crops
Forest	Forest	90	Tree cover > 60%, > 75% canopy cover, > 2m in height
	Coniferous Forest	91	Tree cover > 60%, > 75% canopy cover of upland coniferous tree species, > 2m in height
	Mixed Forest	92	Tree cover > 60%, >25% canopy cover of both upland coniferous and deciduous tree species, > 2m in height
	Deciduous Forest	93	Tree cover > 60%, > 75% canopy cover of upland deciduous tree species, > 2m in height
	Plantations – Tree Cultivated	191	Tree cover > 60%, > 2m in height, linear organization, uniform tree type. Mostly coniferous species, can be confused with upland forest
	Hedge Rows	192	Tree cover > 60%, trees > 2m in height, linear arrangement. Confusion may exist with trees under 2 meters in height
Wetland	Treed Swamp	131	Treed communities. Water table at, near, or above substrate surface. Tree cover > 25%, dominated by hydrophytic tree and shrub species
	Thicket Swamp	135	Open and shrub communities. Water table at, near, or above substrate surface. Tree cover <= 25%, hydrophytic shrubs > 25%, dominated by hydrophytic tree and shrub species
	Fen	140	Mineotrophic peatland. Open, shrub and treed communities. Water table at, near, or above substrate surface. Tree cover <= 25%, dominated by edges, grasses, and low shrubs dominate
	Bog	150	Ombrotrophic peatland. Open, shrub and treed communities. Water table at, near, or above substrate surface. Tree cover <= 25%
	Marsh	160	Open and shrub communities. Water table at, near, or above substrate surface – tree and shrub cover <=25%, dominated by emergent hydrophytic macrophytes

Other	Open Beach/Bar	11	Unconsolidated mineral substrates
	Open Sand Dune	21	Exposed sands; <25% vegetative cover
	Treed Sand Dune	23	Exposed sands; 25% < tree cover < 60%
	Open Cliff and Talus	41	Vertical exposed bedrock or slopes of rock rubble at the base of cliffs; < 25% vegetative cover
	Treed Cliff and Talus	43	Vertical exposed bedrock or slopes of rock rubble at the base of cliffs; 25% < vegetative cover < 60%
	Open Alvar	51	Level, unfractured limestone bedrock, or patchy mosaic of bare rock pavement and shallow substrates over bedrock; vegetative cover < 25%.
	Shrub Alvar	52	Level, unfractured limestone bedrock, or patchy mosaic of bare rock pavement and shallow substrates over bedrock; tree cover < 25%; shrub cover => 25%
	Treed Alvar	53	Level, unfractured limestone bedrock, or patchy mosaic of bare rock pavement and shallow substrates over bedrock; 25% < tree cover < 60%
	Open Bedrock	64	Primarily non-calcareous bedrock features, confined to the Canadian Shield in ecoregion 5E; tree cover < 10%.
	Sparse Treed	65	60% < tree cover < 10%. Confined to the Canadian Shield in ecoregion 5E
	Open Tallgrass Prairie	81	Prairie graminoids; tree cover <= 25%; shrub cover <= 25%
	Tallgrass Savannah	82	Prairie graminoids; 25% < tree cover < 35%
	Tallgrass Woodland	83	Prairie graminoids; variable cover of open-grown trees/ 35% < tree cover < 60%
	Open Water	170	Water depth > 2 meters. Lake trophic status. No macrophyte vegetation, trees or shrub cover
	Extraction – Aggregate	204	Pits, quarries
	Extraction – Peat/Topsoil	205	Peat and topsoil extraction
	Undifferentiated	250	Includes some agricultural features not included in tilled (i.e. orchards, vineyards, perennial crops and idle land > 10 years – out of agricultural production), as well as urban brown fields, hydro and transportation right-of- ways, upland thicket, and openings within forests.

2.7 Mean distance to forest edge

I calculated the mean distance to the forest edge using the re-classified SOLRIS land use data, by measuring the distance from the center of each vernal pool to the forest edge in the 16 cardinal and intercardinal directions, and averaged these values to determine the mean distance to forest edge. For this analysis, I included forest and wetland classes as ‘inside’ forest zones, and included urban, agriculture, and other classes as ‘outside’ forest zones. I considered the boundary between these zones as the forest edge.

2.8 Data analyses

I used t-tests to evaluate differences in vernal pool characteristics between Hamilton and Backus Woods. I also performed a principal components analysis (PCA), to examine clustering within the dataset and explore variables driving the largest differences between Hamilton and Backus Woods vernal pools. Due to small sample sizes, I transformed continuous variables to achieve a more normal distribution of the data. Finally, correlations between vernal pool characteristics and landscape features were tested with a Spearman’s rank correlation coefficient. These data analyses were all conducted using JMP v.16 statistical software (SAS Institute Inc., 2022).

3. RESULTS

3.1 Regional differences in pool characteristics and landscape features

Many vernal pool characteristics varied significantly between Hamilton and Backus Woods (**Table 2**). Compared to Backus Woods, the water in Hamilton's vernal pools had TNN nutrient values that were 11.8 times larger (Hamilton, H: 0.320 ± 0.0270 mg/L, Backus Woods, BW: 0.027 ± 0.0152 mg/L, **Figure 4b**), and conductivity values that were 3.1 times larger (H: 779.0 ± 46.00 μS/cm, B: 249.8 ± 34.03 μS/cm, **Figure 4c**). In terms of pool basin characteristics, Hamilton's pools were 33% shallower (H: 35.1 ± 6.32 cm, BW: 52.3 ± 4.93 cm, **Figure 4d**), and on average, had 44% less of the leaf/mud microhabitat cover type (H: 53.8 ± 11.92 %, BW: 81.9 ± 1.45 %, **Figure 5**). On average, Hamilton's pools also had 21% less canopy cover shading the pools' basins (H: 73.7 ± 5.63 %, BW: 93.9 ± 1.31 %, **Figure 4f**). There were also differences observed in the surrounding landscape between the two regions, with Hamilton pools having 41% smaller mean distances from vernal pools to the forest edge (H: 373.7 ± 41.40 m, BW: 632.9 ± 57.16 m, **Figure 6**), and 21% more urban land use within a 1000 m buffer circle around vernal pools (H: 23.2 ± 6.57 %, BW: 2.0 ± 0.27 %, **Figure 7**). Regional differences were also observed in other land use classes at the 1000m spatial scale, including the proportions of agriculture (H: 0.0 ± 0.00 %, BW: 5.9 ± 2.19 %), wetland (H: 1.2 ± 0.43 %, BW: 40.4 ± 2.74 %), forest (H: 59.7 ± 5.31 %, BW: 41.4 ± 0.92 %), and other (H: 10.3 ± 1.11 %, BW: 10.3 ± 1.11 %) land use classes (**Figure 7**). The proportion of urban land use class also differed significantly between the two regions at the 500m spatial scale (H: 17.1 ± 6.76 %, BW: 1.8 ± 0.25 %, **Table 2**), but not at the 50m spatial scale.

Other vernal pool characteristics did not vary significantly between Hamilton and Backus Woods (**Table 2**). Specifically, no significant regional differences were observed between the pools' mean surface area (**Figure 4e**), and the proportions of thicket, moss, algae, and herbaceous vegetation microhabitat cover types (**Figure 5**). Interestingly, there was also no significant difference observed between the TP values of vernal pools' water in Hamilton and Backus Woods (**Figure 4a**).

Table 2: Summary of means ± standard error (SE) for vernal pool characteristics in Backus Woods (n=13) and Hamilton (n=10). p-values represent results of t-test, with asterisks denoting significance.

Feature	**Backus Woods Mean ± SE**	**Hamilton Mean ± SE**	**p value**
TP (µg/L)	84.2 ± 9.60	171.4 ± 54.65	0.15
TNN (mg/L)	0.027 ± 0.0152	0.320 ± 0.0270	<0.0001*
Conductivity (µS/cm)	249.8 ± 34.03	779.0 ± 46.00	<0.0001*
Length (m)	56.3 ± 8.01	36.7 ± 4.06	0.04*
Width (m)	27.7 ± 4.26	19.3 ± 3.71	0.15
Area (m^2)	1473.4 ± 394.23	630.9 ± 180.83	0.07
Depth (cm)	52.3 ± 4.93	35.1 ± 6.32	0.05*
Canopy Cover (%)	93.9 ± 1.31	73.7 ± 5.63	0.006*
% Leaf litter/mud	81.9 ± 1.45	54.3 ± 12.10	0.05*
% Herbaceous vegetation	8.7 ± 1.23	28.6 ± 9.28	0.06
% Thicket	5.6 ± 1.13	4.9 ± 0.99	0.63
% Moss	3.7 ± 0.53	3.3 ± 0.99	0.68
% Algae	0.0 ± 0.00	9.0 ± 5.47	0.13
Mean distance to forest edge (m)	632.9 ± 57.16	373.7 ± 41.40	0.002*
% Urban within 1000m	2.0 ± 0.27	23.2 ± 6.57	0.01*
% Agriculture within 1000m	5.9 ± 2.19	0.0 ± 0.00	0.02*
% Forest within 1000m	41.4 ± 0.92	59.7 ± 5.31	0.02*
% Wetland within 1000m	40.4 ± 2.74	1.2 ± 0.43	<0.0001*
% Other within 1000m	10.3 ± 1.11	16.0 ± 1.73	0.01*
% Urban within 500m	1.8 ± 0.25	17.1 ± 6.76	0.05*
% Agriculture within 500m	1.2 ± 1.20	0 ± 0.00	0.34
% Forest within 500m	46.0 ± 1.83	68.8 ± 6.36	0.006*
% Wetland within 500m	44.2 ± 2.79	2.0 ± 0.84	<0.0001*
% Other within 500m	6.7 ± 2.52	12.0 ± 2.25	0.13
% Urban within 50m	4.4 ± 2.96	3.0 ± 2.54	0.74
% Agriculture within 50m	0.0 ± 0.00	0.0 ± 0.00	---
% Forest within 50m	36.8 ± 9.45	88.0 ± 4.59	0.0001*
% Wetland within 50m	56.0 ± 8.50	3.9 ± 2.58	<0.0001*
% Other within 50m	2.8 ± 2.00	5.0 ± 3.06	0.56

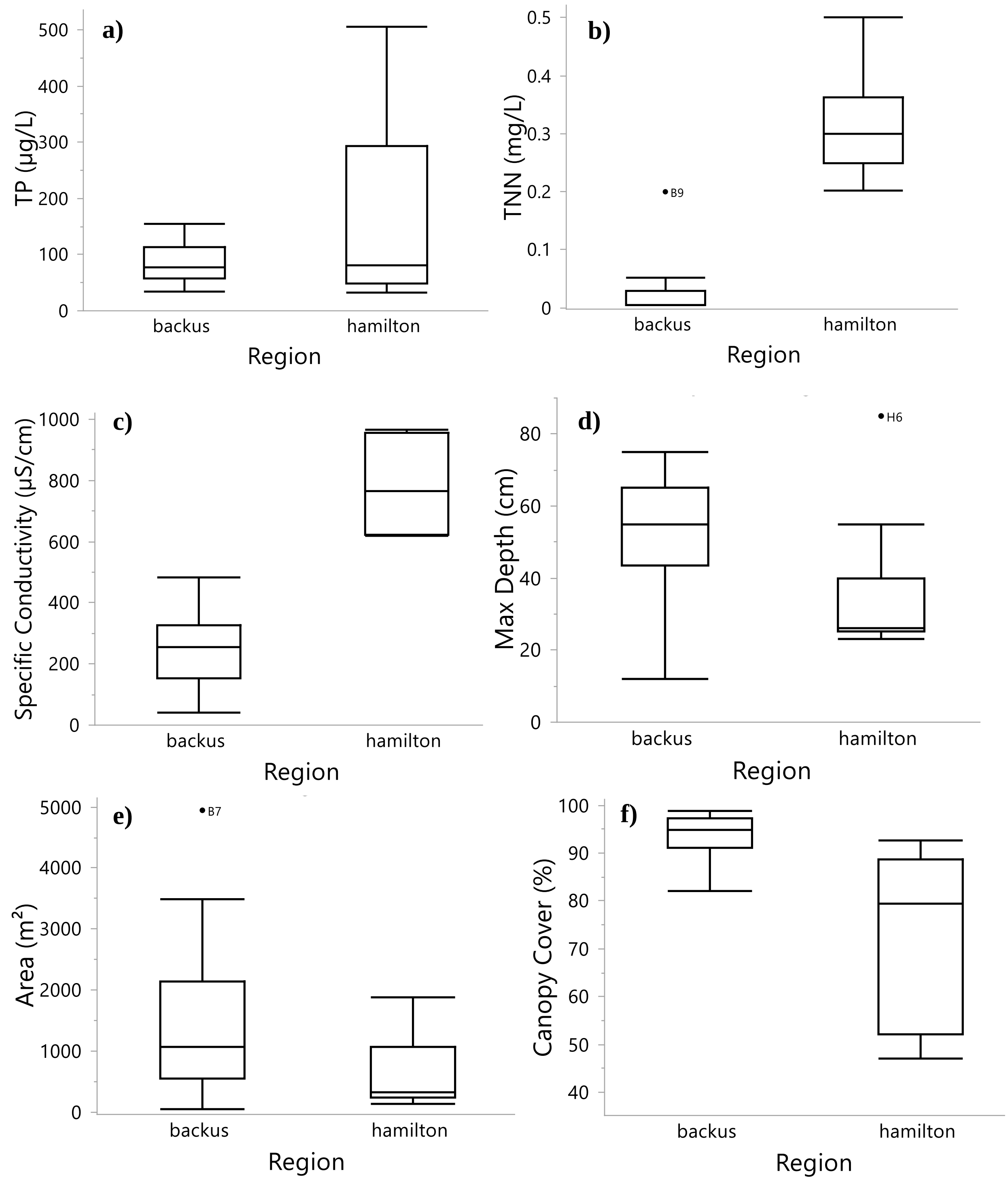

Figure 4: Regional differences in vernal pool characteristics between Backus Woods and Hamilton, including: **a)** TP, **b)** TNN, **c)** conductivity, **d)** maximum depth, **e)** area, and **f)** canopy cover. Middle horizontal line represents the median, with the adjacent two horizontal lines representing the interquartile range (25th and 75th percentiles).

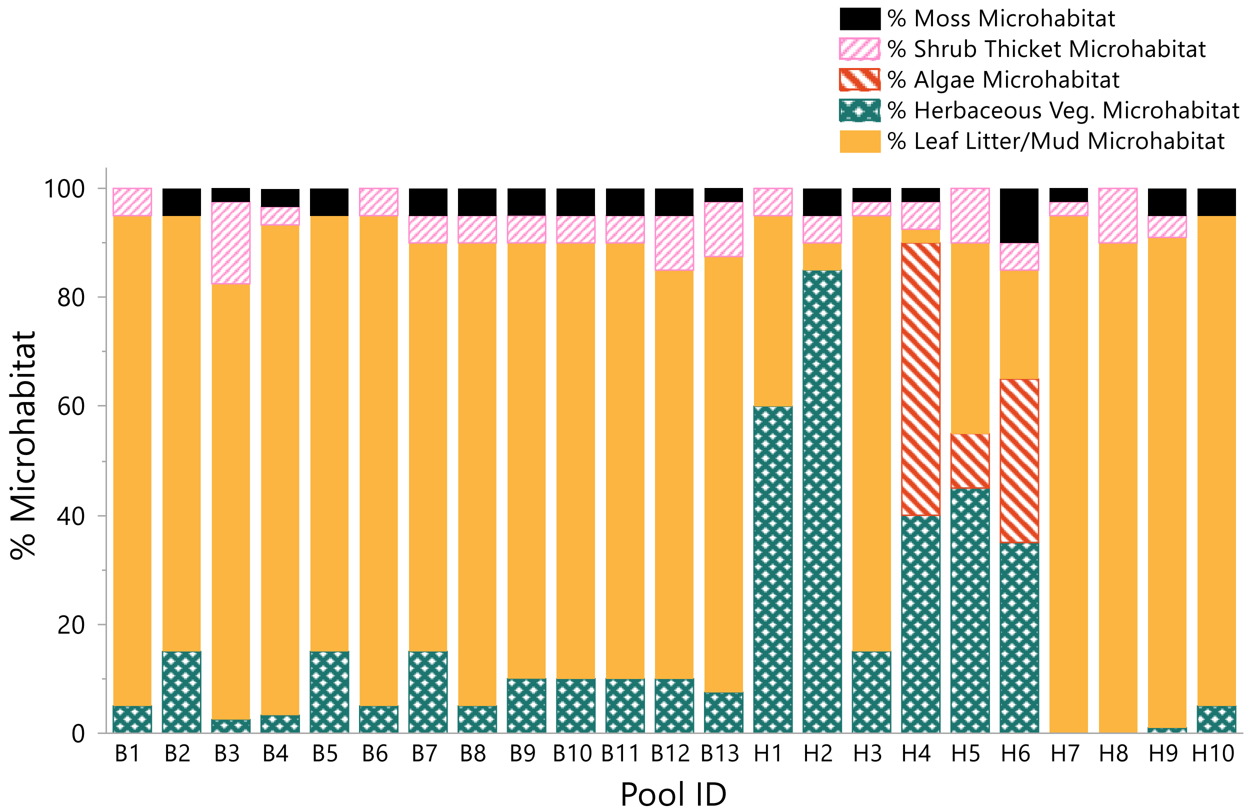

Figure 5: Regional differences in microhabitat compositions between Backus Woods and Hamilton vernal pools. Pool IDs starting with B and H represent vernal pools in Backus Woods and Hamilton, respectively.

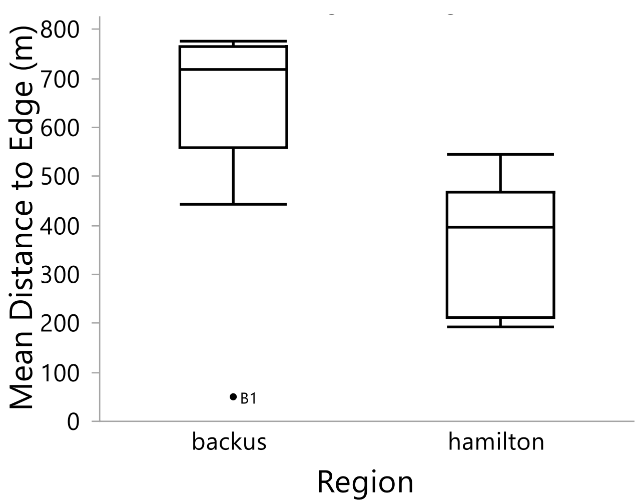

Figure 6: Regional difference in the mean distance from vernal pools to the forest edge between Backus Woods and Hamilton. Middle horizontal line represents the median, with the adjacent two horizontal lines representing the interquartile range (25th and 75th percentiles).

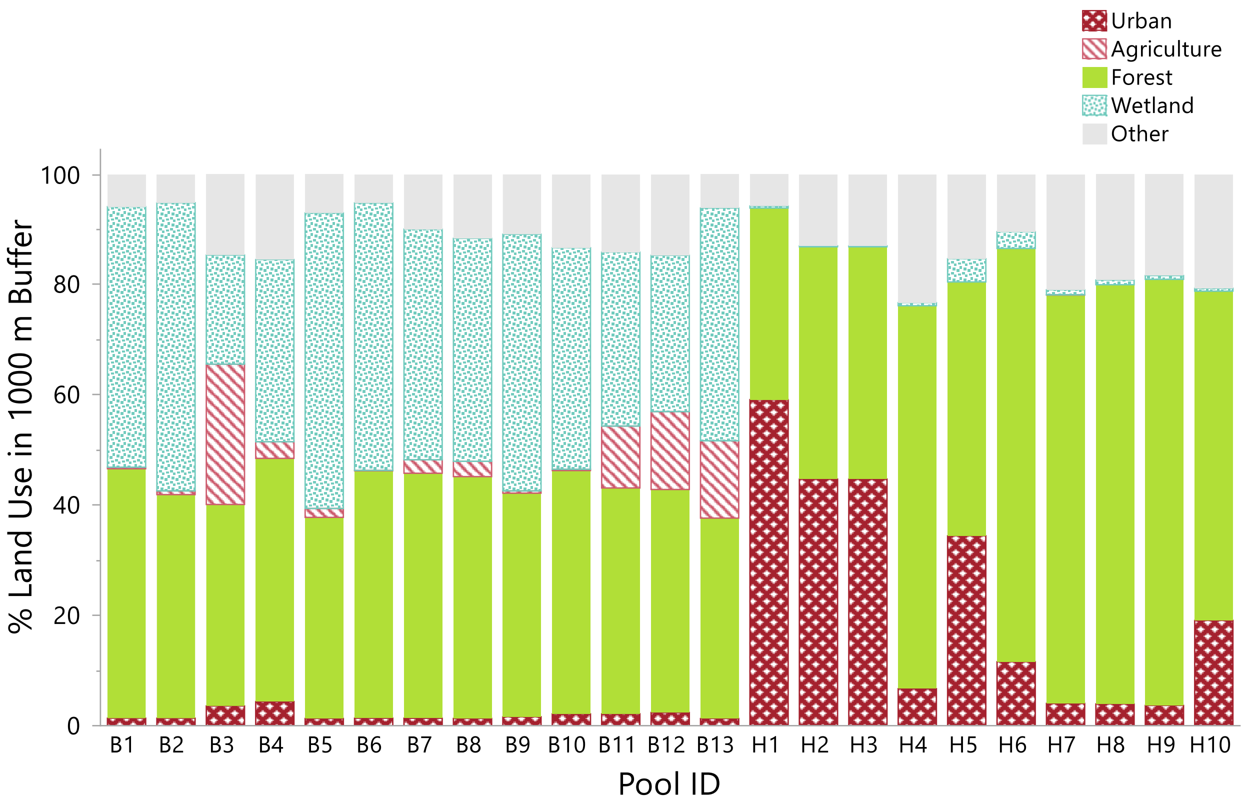

Figure 7: Regional differences in land use within 1000m of vernal pools between Backus Woods and Hamilton. Pool IDs starting with B and H represent vernal pools in Backus Woods and Hamilton, respectively.

3.2 Variables driving the largest regional differences

In total, seventeen variables were included in the PCA. The first four axes explained 77% of all the variation in the dataset, with the first two axes explaining approximately 50% of this variation (**Table 3**, **Figure 8**). PC1 (which explained 35% of the variation) ordinated vernal pools mainly according to their degree of water-quality impairment and canopy openness, since it was strongly correlated with TNN, conductivity, proportion of wetland land uses within 1000m, and canopy cover. Notably, the proportion of urban land use also correlated positively along PC1. PC2 (which explained an additional 17% of the variation) was significantly correlated with the proportions of moss and shrub microhabitat cover types, the proportions of agriculture and other land uses within 1000m, and pool basin area. There was significant clustering of Hamilton and Backus Woods vernal pool sites within the PCA biplot, which seemed to be primarily driven by PC1. The more disturbed Hamilton sites were located at the right on Axis 1, while the older-growth Backus Woods sites were located to the opposite end, at the left. Sites within each region seemed to vary similarly along PC2.

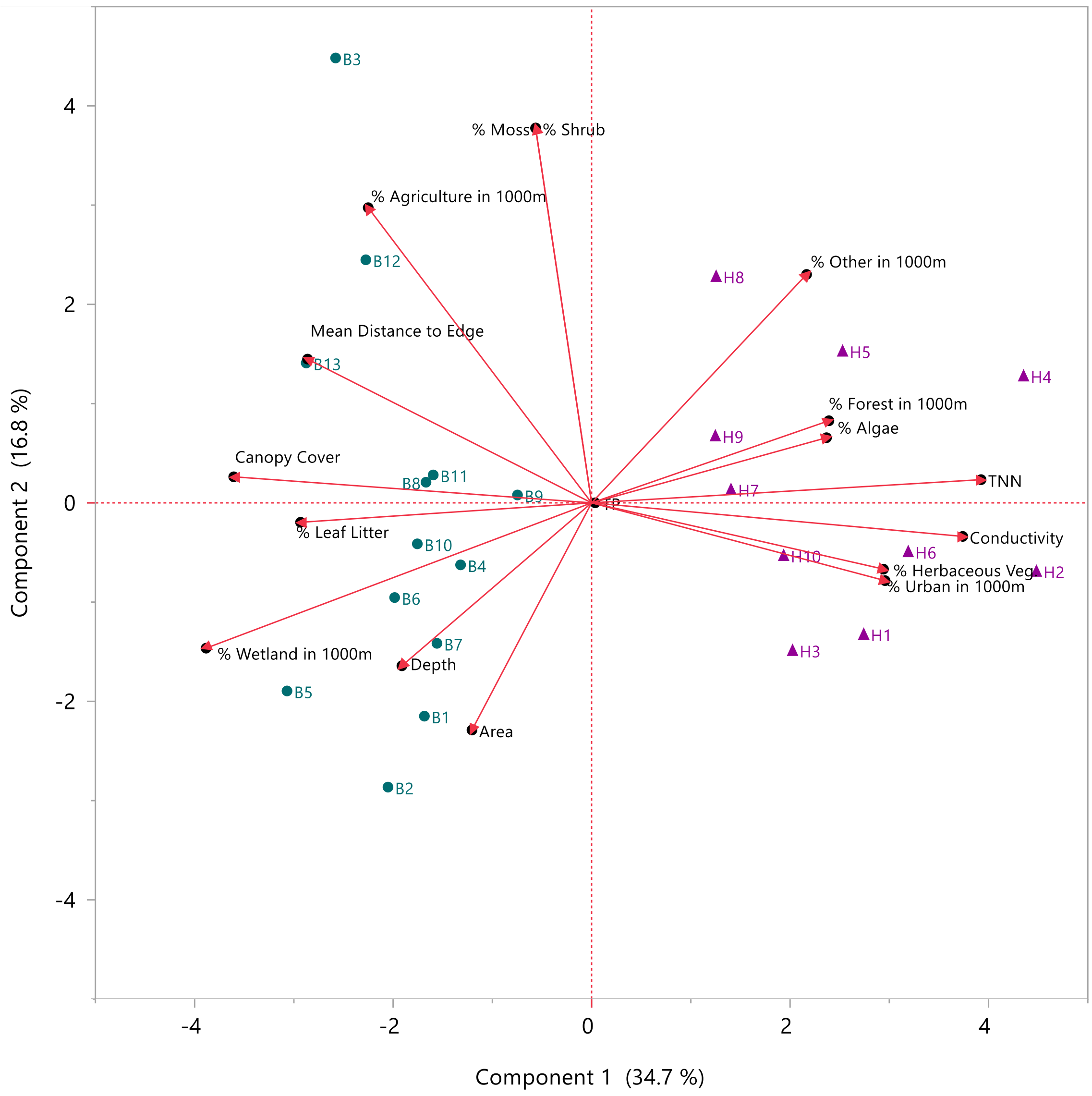

Figure 8: PCA biplot of PC2 vs PC1, showing variables (vernal pool characteristics and landscape features), as well as vernal pool sites for Backus Woods (turquoise circles, n=13) and Hamilton (purple triangles, n=10). An arcsine transformation was used for % Leaf Litter, Herbaceous Veg., Moss, Thicket, Algae, as well as % Urban, Agriculture, Forest, Wetland, Other in 1000m variables. A log transform was applied to TNN, TP, Conductivity variables.

Table 3: Summary of the loadings and proportions of variation explained by the first four PCA axes.

PC axis	Vernal Pool Characteristic Variable	Loading	Percent Explained	Cumulative Percent Explained
1	TNN % Wetland Conductivity Canopy Cover	0.36 -0.36 0.34 -0.33	34.7	34.7
2	% Moss % Shrub % Agriculture % Other Area	0.50 0.50 0.39 0.30 -0.30	16.8	51.5
3	% Forest % Herbaceous Veg. % Leaf Litter % Other	0.40 -0.40 0.40 0.34	15.2	66.7
4	% Algae TP % Urban Canopy Cover	-0.53 0.41 0.38 0.29	10.2	76.8

3.3 Relationships between pool characteristics and landscape features

In general, many significant correlations were observed between vernal pool characteristics and surrounding landscape features. For example, mean distance to the forest edge correlated positively with canopy cover (Spearman ρ: 0.6; **Figure 9e**), and negatively with both TNN values (ρ: -0.6; **Figure 9c**) and conductivity values (ρ: -0.7; **Figure 9a**). The proportion of urban land use within 1000m of vernal pools correlated positively with both TNN values (ρ: 0.7; **Figure 9d**) and conductivity values (ρ: 0.8; **Figure 9b**), and correlated negatively with canopy cover (ρ: -0.6; **Figure 9f**). Unsurprisingly, the proportion of urban land use within 1000m also correlated negatively with the mean distance to the forest edge (ρ: -0.7; **Figure 10a**).

There were also interesting relationships observed between vernal pool characteristics to other vernal pool characteristics. For example, within a vernal pool basin's morphology, there was a positive relationship observed between pool depth and surface area (ρ: 0.4; **Figure 10b**). Generally, TNN values correlated positively with conductivity values (ρ: 0.8; **Figure 10d**). Also, water conductivity values correlated negatively maximum pool depth (ρ: -0.4; **Figure 10c**). In terms of microhabitat composition, the proportion of algae correlated negatively with the degree of canopy cover (ρ: -0.5; **Figure 10f**), and positively with TNN (ρ: 0.5; **Figure 10e**).

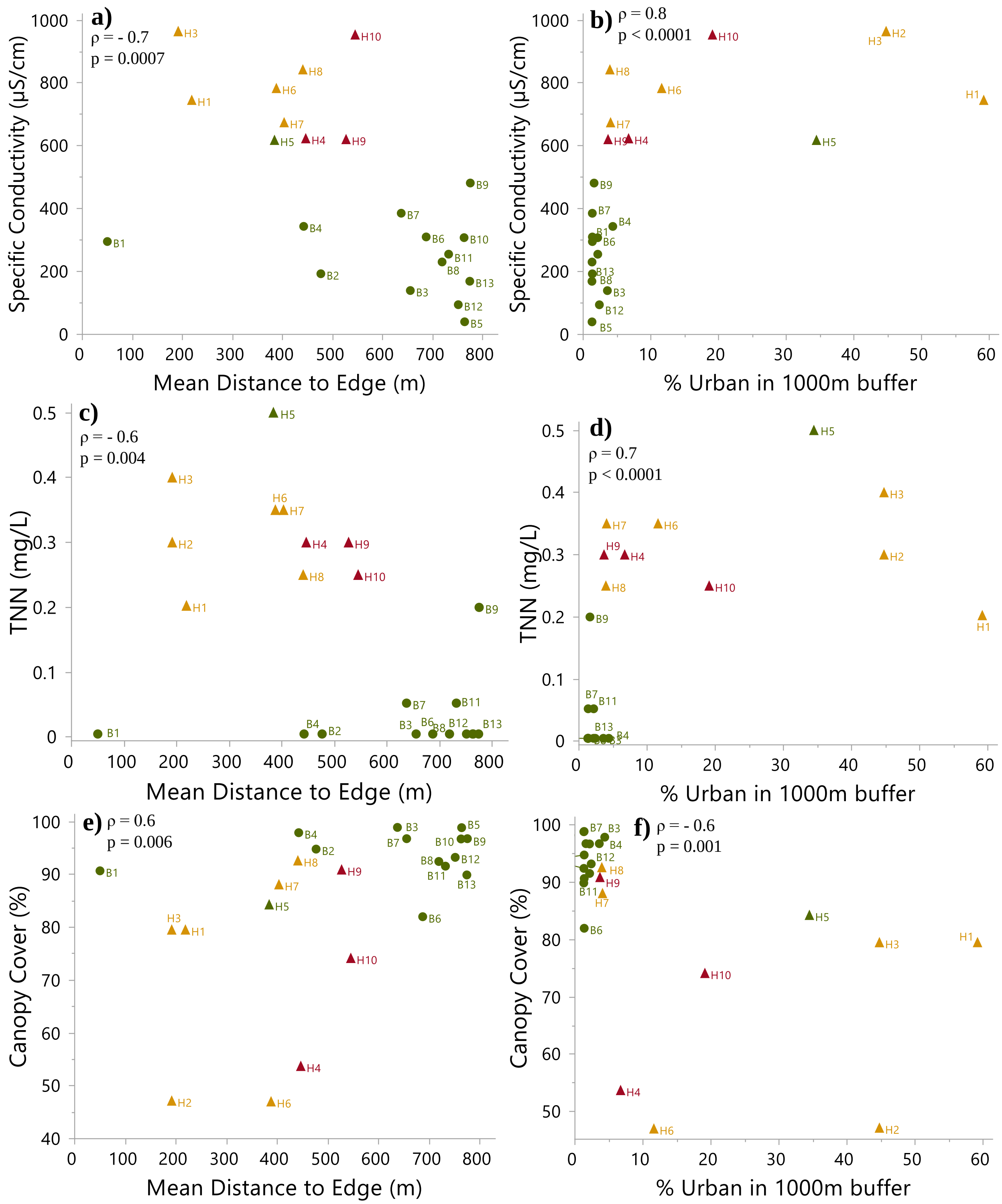

Figure 9: Relationships between vernal pool characteristics and surrounding landscape features, including: distance to the forest edge to **a)** conductivity, **c)** TNN, and **e)** canopy cover; as well as the % urban land use within 1000m to **b)** conductivity, **d)** TNN, and **f)** canopy cover. Circles represent Backus Woods pools, and triangles represent Hamilton pools. Points are coloured by forest type, as either deciduous (green), mixed (yellow), or coniferous (dark red).

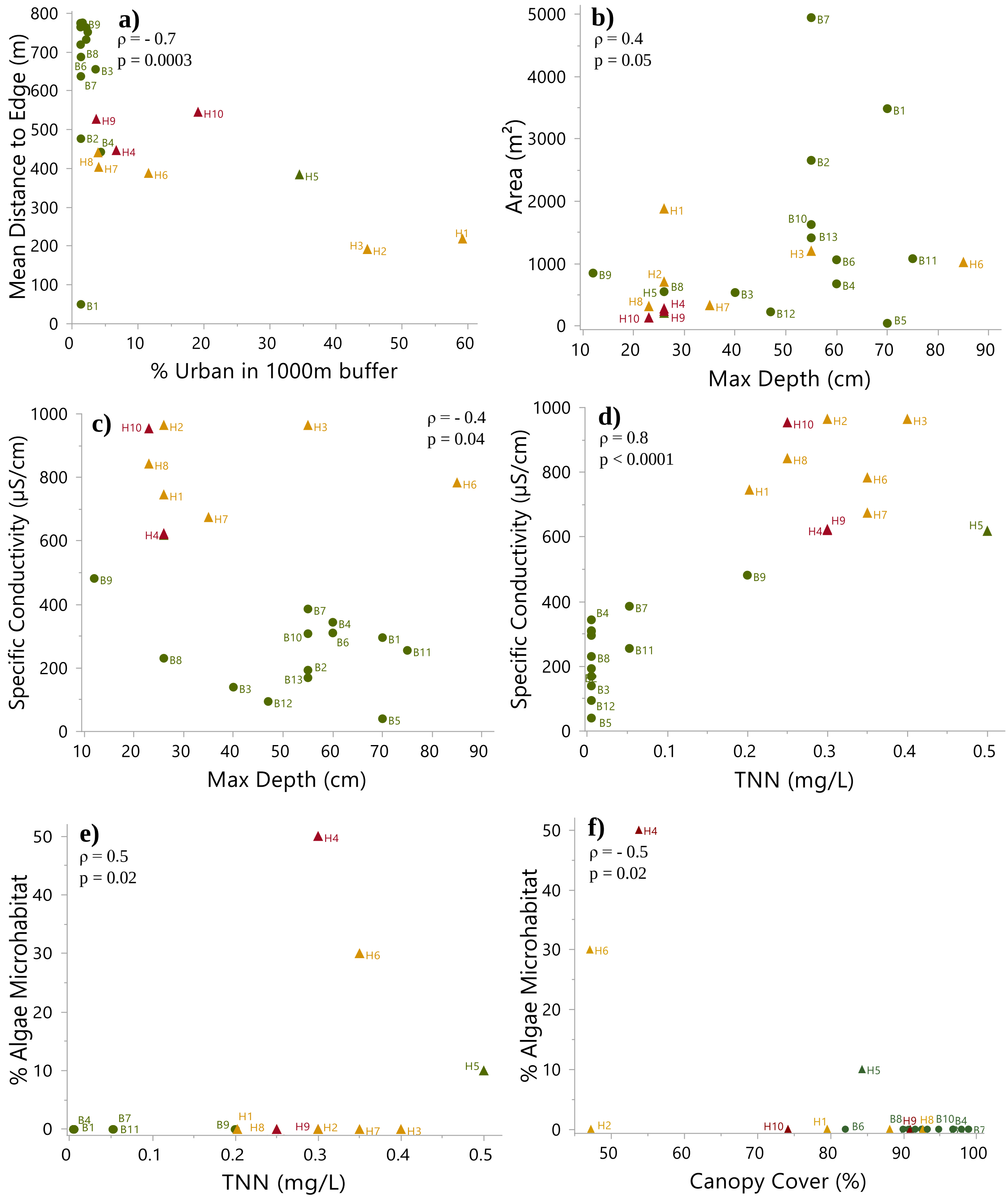

Figure 10: Relationships between vernal pool characteristics and surrounding landscape features, including: **a)** distance to edge and % urban land use within 1000m, **b)** pool area and depth, **c)** conductivity and pool depth, **d)** conductivity and TNN, and **e)** % algae and TNN. Circles represent Backus Woods pools, and triangles represent Hamilton pools. Points are coloured by forest type, as either deciduous (green), mixed (yellow), or coniferous (dark red).

4. DISCUSSION

The goal of this study was to investigate whether vernal pool characteristics varied between a disturbed region (Hamilton) and a reference region (Backus Woods) in S. ON, to better understand which pool characteristics are affected by urbanization of a forest landscape. There were several distinct differences observed between vernal pools in Backus Woods and in Hamilton. The pool characteristics with the greatest regional differences included water TNN and conductivity values, pool basin depths, proportions of leaf and mud microhabitat types, and the degree of overhead canopy shading. There were also significant differences observed in the landscape context between the two regions, with smaller mean distances to the forest edge and much higher proportions of urban land use observed within 1000m of vernal pools in Hamilton.

Correlations between these vernal pool characteristics suggest many of the observed regional differences were likely attributable to landscape context, and therefore that modifications of forest landscapes may result in predictable changes to pool characteristics. Since many of these changes may also threaten vernal pools' biological communities, it is imperative to understand how and why these changes occur, and to eventually establish thresholds that limit their potential for adverse ecological impacts (e.g., determining a minimum size of forest around a vernal pool that is required to buffer against the effects of urbanization and preserve the quality of threatened species' critical habitats). This information will be essential for more effectively prioritizing vernal pool conservation against competing stakeholders' interests (e.g., better balancing conservation efforts against continued economic growth and urban expansion).

4.1 Urbanization and land use differences

Since forests in Hamilton are adjacent to the City of Hamilton, one of the large urban centers in S. ON, Hamilton vernal pools generally had higher proportions of urban land use and smaller proportions of 'unmodified' land uses (either forest or wetland) within 1000m of pool centers compared to pools in Backus Woods (**Figure 7**). As hypothesized, Hamilton's vernal pools also had smaller mean distances to the forests' edge (**Figure 6**), most likely due to the higher rates of forest disturbance, fragmented nature of the forest structure, and occasional forest clearings in Hamilton forests (**Figure 2**). In comparison, vernal pools in Backus Woods had smaller proportions of surrounding urban land use and larger mean distances to the forests' edge, due to the old-growth and relatively undisturbed state of the forest. Other than a small proportion of urban land use observed within Backus Woods due to occasional roadways, Backus Woods' pools had significantly less of this external urban influence (2.0 ± 0.27%, as opposed to Hamilton's 23.2 ± 6.57%; **Table 2**). However, since Backus Woods is also surrounded by a sea of agriculture, there was a higher proportion of agricultural land use observed at the 1000m spatial scale than there was in Hamilton – although these proportions were still relatively small (5.9 ± 2.19%, as opposed to Hamilton's 0.0 ± 0.00%; **Table 2**).

While this proportion of agricultural land use is small, its presence points to the fact that Backus Woods is not a perfect reference region; at the 1000m spatial scale, it too starts to show signs of degradation. This points to one of the major limitations of this study, since there are no truly undisturbed reference regions remaining in S. ON. The inclusion of this agricultural land use in the analyses also created several spurious relationships, where increases in the proportion of agricultural land correlated with changes in vernal pool characteristics that I would not expect; for example, in the PCA, increases in agricultural land use correlated positively with increases in the mean distance to forest edge, and negatively with variables such as TNN and conductivity (**Figure 8**). Logically, I would expect the opposite trends, since conversion of forest to agricultural fields would generally decrease the mean distance to the forest edge, and increase the concentrations of TNN and conductivity due to the inputs of field run-off (via non-point source nitrate inputs from fertilizers, manure, leguminous crops, and irrigation return-flows; (Carrino-Kyker and Swanson, 2007; Mahanta et al., 2022). Additionally, since Backus Woods had high higher proportions of its area classified as wetland within the SOLRIS dataset, the proportion of forest land use when analyzed was actually higher in Hamilton than in Backus – producing other spurious relationships (e.g., proportion of forest land use correlating positively with increases in TNN, conductivity, proportion of urban land use, proportion of algal cover, etc.) (**Figure 8**).

Also, while the proportions were numerically similar, there was a significantly higher proportion of other land use around Hamilton vernal pools (**Table 2**). When these ‘other’ areas are examined more closely (e.g., during site visits, or using Google Earth imagery), these included a variety of landscape features, such as clearings in forests and urban brown fields (which seemed to be more common in Hamilton forests), idle land that’s been out of agricultural production for over ten years (which seemed to be more common in Backus Woods), and areas of dense thicket (which seemed common to both regions). While these differences were interesting to note, they were not the focus of this study.

It is also interesting to note that there was no observable difference in the proportions of agricultural and other land uses at the smaller spatial scales (50m, and 500m buffers) (**Table 2**). The proportion of urban land use was significantly different at the 500m, but not the 50m scale; and the proportion of forest and wetland land uses were significantly different at all three spatial scales. However, while the proportions of many of the land use classes varied between regions at the 500m and 50m spatial scales, I chose the 1000m scale as the focus for subsequent analyses, since this is where I observed the largest differences in the proportions of urban land use between the two regions (**Table 2**). Additionally, since urbanization has been documented to affect vernal pool communities within 1000m of pool basins (Skidds et al., 2007; Eakin, Calhoun and Hunter, 2019), I believed the inclusion of this larger landscape context to be ecologically relevant, and thus important to consider in my analyses.

4.2 Mean distance to forest edge

As predicted, there was a smaller mean distance to the forest edge observed for pools in Hamilton than for pools in Backus Woods (**Figure 6**, **Table 2**), with a negative relationship observed between the mean distance to forest edge and the proportion of urban land use within 1000m (**Figure 10a**). This is an intuitive relationship, since generally, the presence of nearby land uses within 1000m suggests that they may cut into forested regions, shrinking the forest's area and making the forest's boundaries closer to vernal pools. Within Backus Woods, vernal pool site B1 was an outlier with a very small mean distance to forest edge compared to the median. This was likely a product of chance, since this pool happened to be located between two urban and other land use features (**Figure 2)**. The urban feature consisted of a roadway that was not paved or maintained, and did not seem to receive much vehicle traffic. It was also quite a narrow linear feature, so it also did not contribute significantly to the proportion of urban land use near this vernal pool (**Figure 7**).

It is worth considering the potential ecological implications of reductions in mean distance to forest edge sizes, as they may create both direct and indirect effects on vernal pool characteristics and vernal pool communities. For example, smaller sizes of forest surrounding vernal pools can pose multifaceted dangers for amphibians; for example, features such as roadways may directly (e.g., road mortalities) or indirectly (e.g., eutrophication effects) increase mortality or affect the development or reproductive success of pool-breeding amphibians (Eakin, Calhoun and Hunter, 2019). Smaller sizes of forest surrounding vernal pools may also create insufficient upland habitat to support these populations during the overwintering stages of their lifecycles (Veysey Powell and Babbitt, 2015). Notably, reductions in the mean distance to forest edge and other disturbances to the forest's structure may also directly modify other vernal pool characteristics – such as reducing the degree of canopy cover over a vernal pool's basin. Thus, Hamilton may less suitable than Backus Woods as habitat for threatened amphibian species.

4.3 Canopy Cover

I hypothesized that due to their proximity to large proportions of urban land use and due to the more disturbed structure of their surrounding forest, vernal pools in Hamilton would have less overhead canopy cover than vernal pools in Backus Woods. Consistent with this hypothesis, I observed a 20% decrease in the average canopy cover for vernal pools in Hamilton (**Table 2**, **Figure 4f**). Canopy cover was also one of the vernal pool characteristics driving the largest variations observed between Hamilton and Backus Woods vernal pools (**Figure 8**, **Table 3**). This difference was fairly intuitive, since Backus Woods consisted primarily of old-growth forests, with larger and taller trees, and generally a fuller overhead canopy structure – producing more complete shading of vernal pool basins. Spearman rank correlation coefficients were negative between canopy cover and proportion of urban land use within 1000m ($\rho = -0.6$; **Figure 9f**), and positive between canopy cover and the mean distance to forest edge ($\rho = 0.6$; **Figure**

9e), suggesting these landscape features could be driving differences in Hamilton vernal pool's canopy cover.

Changes in canopy cover are also ecologically significant, since they can strongly influence species performance due to their effects on light availability and leaf litter input (Schiesari, 2006; Earl et al., 2011). Both of these factors can result in bottom-up effects, greatly altering primary productivity and decomposition. Vernal pools with closed canopies receive lower incident solar radiation, and therefore have lower temperatures and dissolved oxygen levels than pools with open canopies. The degree of canopy openness can also support different food chains (grazing food chains with nutrient-rich algae, or detritus food chains starting with nutrient-poor leaf litter) (Werner and Glennemeier, 1999; Skelly, Freidenburg and Kiesecker, 2002). These gradients in canopy cover therefore influence freshwater species distributions, and change vernal pools' suitability as habitat for different species (Schiesari, 2006). For example, changes in canopy cover may induce a shift in the amphibians emerging from ponds, from primarily anurans in open canopy ponds to primarily salamanders in closed canopy pools (Earl et al., 2011). Declines in salamander populations resulting from timber removal and its associated loss of canopy cover are most pronounced in clear-cut areas, but have also been observed in partial harvest areas (Tilghman, Ramee and Marsh, 2012). Thus, decreases in canopy cover risk threatening vernal pool species composition and suitability as habitat.

4.4 Vernal pool basin morphology

I observed no regional differences in vernal pool basin's area, however, vernal pools in Hamilton were significantly shallower (**Table 2**, **Figure 4d**). Spearman rank correlation coefficients did not reveal a significant relationship between proportion of urban land use or mean distance to forest edge to pool depth, suggesting these surrounding landscape features were likely not the factors driving the observed regional differences in vernal pool depth. Interestingly, other studies have observed that sub-watersheds with high urban land use tend to have shallower vernal pools, that dried earlier in the season (Carrino-Kyker and Swanson, 2007). While the exact relationship between the urbanization and pool depth was unclear, Carrino-Kyker and Swanson (2007) hypothesized the differences in pool depths may instead have been due to the variable origin of the pools, since previous studies have found that pools of anthropogenic origin tend to dry more rapidly than those of natural origin (Calhoun et al., 2003). In the Hamilton vernal pools, it is possible that a similar effect was occurring, since vernal pools in Hamilton were often located near trail networks and roadways that may have modified pre-existing or created new vernal pools.

Conversely, human manipulations of the local water table level could be responsible for the shallower pool depths observed in Hamilton; for example, studies have found that in New Jersey, urban areas containing dams, drainage ditches and channelized streams have a ground water table that was 43% lower than at comparable sites without intensive water management (Carrino-Kyker and Swanson, 2007). Many of the vernal pools sites in Hamilton did show potential evidence of anthropogenic modification of the water level, since I occasionally observed plastic piping installed near vernal pools adjacent to trailways, likely with the purpose of keeping the trails unflooded. It seems possible that these modifications may have contributed to the shallower average vernal pool depths in Hamilton. The Hamilton vernal pool site H6, which was an outlier in terms of pool depth (**Figure 4d**), was likely much deeper than the other Hamilton pools for a similar reason – this pool appeared to have been created from the construction of an elevated section of ground that was part of a trail road, which crossed a sloped area of ground in such a way that it allowed water to collect and form a vernal pool. If this trail had not been constructed, it seems likely that the water from this pool would have continued to flow downhill.

It is also worth noting that generally, vernal pool area and depth were positively correlated in both the PCA (**Figure 8**) and Spearman's rank correlation coefficient test ($\rho = 0.4$; **Figure 10b**) – although this was not as strong of a correlation. While the relationship between vernal pool depth, area, and volume to hydroperiod is often weak and inconsistent across regions (Schrank et al., 2015), it is possible that smaller and shallower vernal pools within S. ON may have shorter hydroperiods. Within a region, many of the other factors that tend to affect hydroperiod may be similar – such as local weather conditions, precipitation, ground-water exchange, and soil characteristics (Schrank et al., 2015) – meaning that depth could be an indicator of hydroperiod within a local scale. Thus, regions in S. ON with shallower pools (such as Hamilton) could be more susceptible to the effects of climate change, whose effects are generally predicted to shorten vernal pool hydroperiods (Brooks, 2009). These hydroperiod changes may also shift vernal pools' suitability as habitat for local species (Bauder, 2005), and are likely to affect vernal pool communities at all levels – including populations of microorganisms (Celewicz and Gołdyn, 2021), invertebrates (Pyke, 2005), vertebrates (Chalmers and Loftin, 2006; Skidds et al., 2007; Kneitel, 2014), and plants (Montrone et al., 2019).

4.5 Vernal pool microhabitat

I had predicted that the vernal pools in Backus Woods and Hamilton would have different proportions of microhabitat cover, with increased proportions of algae and herbaceous vegetation observed for Hamilton's vernal pools, and increased proportions of mud and leaf litter for

Backus Woods' vernal pools. I predicted these differences would be primarily driven by the regions' differing degrees of canopy cover over vernal pools' basins, since canopy cover gradients have direct effects on light intensity and therefore also primary productivity (Schiesari, 2006). Also, previous studies have observed the type of available resources for consumers to shift from algae and macrophytes in open-canopy pools, to detritus (i.e., decomposing leaf litter) in closed-canopy pools, supporting this hypothesis (Schiesari, 2006). Additionally, urban and industrial activities are known to dramatically increase aquatic nitrogen and phosphorus pollution from eutrophication, which can provide algae with required nutrients that would otherwise be limiting and facilitate their growth (Wurtsbaugh, Paerl and Dodds, 2019). Thus, excessive nutrient input can result in shifts within open-canopy pools from vascular plant dominance to algal dominance (Kneitel and Lessin, 2010).

While the proportion of herbaceous vegetation and algae did tend to be higher in Hamilton's vernal pools (**Figure 5**), only the proportion of mud and leaf showed a statistically significant difference between regions (**Table 2**) – although more of these categories may have been statistically significant with a larger sample size. The proportions of moss and shrub did not contribute largely to the makeup of vernal pools' microhabitats in either region (**Figure 5**). Consistent with the study hypotheses, Spearman rank correlation coefficients did reveal a strong positive correlation between the proportion of algae to TNN concentrations ($\rho = 0.5$; **Figure 10e**), and a strong negative correlation between the proportion of algae to the degree of canopy cover ($\rho = -0.5$; **Figure 10f**), suggesting these pool basin characteristics could be driving the observed trends in algae microhabitat cover, even if their differences were not significant between Hamilton and Backus Woods.

Changes in vernal pool microhabitat assemblages may have significant ecological implications for vernal pool species, including obligate vernal pool amphibians. For example, several species use woody and nonwoody vegetation as egg attachment sites (Egan and Paton, 2004; Skidds et al., 2007), while species such as the four-toed salamander (*Hemidactylium scutatum*) demonstrate nesting preferences for mossy hummocks along vernal pool shorelines (Chalmers and Loftin, 2006). Herbaceous vegetation may also represent important food sources for anuran tadpoles (Williams, Rittenhouse and Semlitsch, 2008), as well as protective cover for developing larvae (Hecnar and M'Closkey, 1997). Thus, there may have been shifts in Hamilton vernal pools' suitability as habitat for a number of amphibian species.

4.6 Water chemistry

Due to Hamilton vernal pools' higher proportions of urban land use within 1000m, and smaller mean distances to the forest edge, I hypothesized these pools would have higher nutrient inputs (of both TP and TNN) and higher conductivity values than vernal pools in Backus Woods. As urban land cover increases, the incidence of non-point source pollutions of phosphorus and nitrogen (e.g., from fertilizers applied to lawns and golf courses, failing septic systems, pet waste, etc.) into the surrounding environment can rise significantly (Roberts et al., 2009). Also, urban features such as roadways tend to result in runoff with high conductivity values due to deicing agents such as road salts, which are then transported as solutes into adjacent habitats where they influence biotic and abiotic aspects of the environment (Karraker, Gibbs and Vonesh, 2008). Additionally, larger mean distances to the forest edge may help to shield vernal pools from the eutrophication effects of urban land uses outside the forest, since forests are known to operate as riparian buffers zones that can remove nutrients (TP and TNN) from surface runoff as it passes through (Cao et al., 2018).

Consistent with this hypothesis, vernal pools in Hamilton were observed to have higher TNN and conductivity values (**Table 2**, **Figures 4b** and **4c**), with differences in these variables also explaining a large percentage of the variations observed between Hamilton and Backus Woods vernal pools (**Figure 8**, **Table 3**). Spearman rank correlation coefficients revealed strong positive correlations between the proportion of urban land use within 1000m to both conductivity ($\rho = 0.8$; **Figure 9b**) and TNN ($\rho = 0.7$; **Figure 9d**) values, and strong negative correlation between the mean distance to forest edge to both conductivity ($\rho = -0.7$; **Figure 9a**) and TNN ($\rho = -0.6$, **Figure 9c**) values. It appears that even small increases in the proportion of urban land within 1000m of vernal pools can result in very large increases in both conductivity and TNN concentrations – even a 5% increase in urban land use produced a dramatic increase in both TNN (to about 0.3mg/L) and in conductivity (to about 600µS/cm), after which these values seemed to plateau with further increases in urban land use. This data suggests that for vernal pool conservation strategies, maintaining a proportion of urban land use within 1000m that is less than 5% may be a threshold for preventing degradations in water quality due to forest urbanization.

Surprisingly, despite the very large differences in TNN between Hamilton and Backus Woods vernal pools, TP values did not vary significantly between regions (**Table 2**). However, Hamilton vernal pools did demonstrate a wider range of TP values than Backus Woods vernal pools (**Figure 4a**), and values at both sites tended to be quite high (up to 500µg/L at one site), however it is possible this may have been due to sediment contamination in the water samples. Since Hamilton vernal pools were generally shallower, it was more difficult to avoid the inclusion of sediments and leaf litter debris in the water samples collected from these pools.

While it is not clear why the B9 vernal pool site outlier had such a large TNN value compared to the other sites in Backus Woods (**Figure 4b**), one possible explanation could be that this site was also very shallow (12cm in depth), and had nearly complete overhead canopy coverage (nearly 97%) compared to other pools in the region. Thus, this site may have had less of a dilution effect to its TNN values, if its standing water originated primarily from precipitation and surface runoff instead of from groundwater sources. Interestingly, the Spearman rank correlation test also revealed a negative relationship between pool depth and conductivity ($\rho = -0.4$; **Figure 10c**), although this relationship was relatively weak and may have resulted by coincidence (since most Hamilton pools had high conductivities and shallow depths, while most Backus Woods pools had low conductivities and were deeper. Further studies may be required to elucidate whether there is a dilution effect between vernal pool water conductivity and depth.

The increases in Hamilton vernal pools' TNN and conductivity values may pose numerous adverse effects to its vernal pool communities. For example, different water conductivity values have been observed to correlate consistently with different microbial communities (Carrino-Kyker, Swanson and Burke, 2011). The exposure to contaminants from urban runoff is also a possible cause of population decline in amphibians, since degradations to water quality (including high values of electrical conductance, high concentrations of nitrates, nitrites, and total phosphates, etc.) have been associated with low densities of egg masses, low hatching success, low larvae survival, and low development rates (Calderon et al., 2019). While there is some disagreement on the effects of phosphate on amphibian populations, some literature suggests that, at environmentally-relevant concentrations, phosphate effects on amphibians are likely indirect and related to nutrient enrichment and anoxia due to eutrophic conditions (Calderon et al., 2019).

4.7 Other features of potential interest

Several vernal pool characteristics did vary significantly between Backus Woods and Hamilton, and correlations between these pool characteristics and surrounding landscape features suggest many of these observed differences were attributable to urbanization of the surrounding landscape. However, while land use and the distance to the forest edge play clear roles in determining vernal pool characteristics, these relationships are likely even more complex. For example, forest characteristics, such as forest structure (e.g., old-growth vs disturbed) may also play a key role in determining how vernal pool characteristics respond to their surrounding landscape. Specifically, old-growth forests, such as those in Backus Woods, are recognized for their structural complexity, which allows them the ability to support landscape biodiversity, create substantial carbon stores, and provide resilience to climate change (Price, Holt and Daust, 2021). In contrast, in forest ecosystems with more frequent disturbances, such as in Hamilton, trees tend to have more uniform age ranges, with veteran large trees representing legacies from past disturbances (Price, Holt and Daust, 2021). This relative lack of structural complexity does not allow these forests to fulfill the same range of ecological roles on the landscape.

It seems likely that these differences in forest structure could have a similar effect within forested vernal pool ecosystems – for example, for a given size of forest surrounding a vernal pool, an old-growth forest structure may be better able to ‘buffer’ against the effect of converted land uses outside the forest compared to a disturbed forest structure. This trend is seen in **Figure 9a**, where for a given distance to the forest edge, vernal pools surrounded by deciduous forests seem to generally have lower values of conductivity than vernal pools surrounded by mixed or coniferous forests. Since Hamilton’s disturbed forests consisted principally of mixed and coniferous forests, while Backus Woods’ old-growth forests consisted principally of deciduous forests, it is possible that this difference in forest structure affected how vernal pool characteristics (e.g., conductivity) responded to their surrounding landscape (e.g., mean distance to forest edge). Further studies may be warranted to investigate these relationships with larger sample sizes, as this relationship would have significant ecological implications. Namely, it would suggest that it is not only the amount of forest surrounding a vernal pool that is important for its protection from nearby urbanization, but also the nature and type of forest that is essential. Thus, preserving what remains of old-growth Carolinian forests in S. ON is likely critical to vernal pool conservation in this region, since it seems unlikely that their ecological functions can be replaced by simply planting more trees in deforested or disturbed areas.

5. CONCLUSION

In summary, several regional differences in vernal pool characteristics were observed between Backus Woods and Hamilton. As a result of higher proportions of nearby urban land uses and smaller mean distances to the forest edges, vernal pools in Hamilton had more open canopies, shallower depths, and higher water conductivity and TNN values than pools in Backus Woods. Ultimately, these differences suggest that it is highly probably that a large number of the remaining vernal pools in S. ON have been adversely affected by urbanization and deforestation, and that current legislative measures for their protection are insufficient for preservation of high quality vernal pool habitat in this region. In addition, urbanization within 1000m of vernal pools did not necessarily account for all of the observed variations in pool characteristics; in reality, these relationships are likely more complex, with forest structure (e.g., disturbed vs old-growth) likely playing a key role in determining how vernal pool characteristics respond to the surrounding landscape.

These relationships have strong implications for vernal pool conservation, since alterations to vernal pool characteristics can have adverse effects on species richness, the suitability of vernal pools as habitat for species at risk, and vernal pools' resiliency and adaptability to climate change. Thus, maintaining, and where possible, restoring the integrity and amount of intact old-growth forest in S. ON is an urgent priority for current efforts to preserve this region's rich biodiversity, manage the effects of climate change, and achieve sustainable goals for forested vernal pool ecosystems in this region. The results of this study corroborate and add to a growing body of literature that is recognizing that retaining the integrity of intact forest ecosystems should be a central component of proactive global and national environmental strategies, alongside current efforts aimed at halting deforestation and promoting reforestation (Watson et al., 2018).

As conservation efforts for vernal pool habitat have been largely ineffective at federal, provincial, and state levels of government, regulating land use in and around vernal pools in the United States and Canada remains largely in the hands of local governments. Given the challenges surrounding vernal pool research and protection, involvement of citizens to conserve vernal pool resources may be an essential strategy going forward (Oscarson and Calhoun, 2007). Citizens are often motivated to become involved at the local level since today, many local governments play a lead role in land use planning. Thus, raising awareness about these important issues, and working towards building community efforts using citizen-scientists may be a grassroots catalyst for change that can promote responsible management of these important environmental issues at the local scale (Oscarson and Calhoun, 2007). Complementing "top-down" resource management with a "bottom-up" participatory approach may be a necessary strategy for bridging the gaps between local needs and the agendas of other stakeholders, and working towards those conservation goals to more effectively preserve S. ON's vernal pool ecosystems.

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