IDENTIFYING BROAD-SCALE ABIOTIC AND BIOTIC HABITAT CHARACTERISTICS OF *ANEMONE AMERICANA*, *CONOPHOLIS AMERICANA* AND *GOODYERA PUBESCENS* IN NOVA SCOTIA, CANADA

by

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ABSTRACT

The forest understory is the most biodiverse vegetation layer in temperate forests and it plays a major role in nutrient cycles, energy flow and succession following disturbance. It is often marginalized in research in favour of studying more charismatic canopy species despite its roles in a forest ecosystem and its importance in supporting many forest plants at risk of extinction. Global biodiversity is declining and plant communities are facing greater threats because of human activity. Despite the urgency, many rare plants have not been studied in enough detail to have a sufficient understanding of the factors causing their rarity, including a basic understanding of their habitat requirements. Our work tackled this problem by investigating the habitat of three rare forest plant species, *Anemone americana*, *Conopholis americana* and *Goodyera pubescens* in Nova Scotia, Canada. Specifically, we studied their vegetation community, soil and environment by sampling sites where our target species had been reported and comparing them to randomly selected upland forests without the target species. Our analysis was done using non-metric multidimensional scaling models, contingency table Chi-squared tests, principal component analyses and generalized linear models. We have shown that *Conopholis americana* is associated with shade intolerant forests with medium to fine textured soils of medium fertility that were in an early successional stage and therefore closely matched the habitat preferences of its red oak host. *Goodyera pubescens* mostly grew in shade tolerant deciduous forests, with medium to fine soils of medium to rich fertility in late successional stages; its preferences likely influenced by its mycorrhizal host. Finally, we identified that *Anemone americana* predominately grew in shade tolerant deciduous forests with medium to fine-textured, medium fertility soils in a late successional stage. These results likely reflect its need to sustain the high metabolic demands of a typical spring ephemeral. Our research has been the first to broadly investigate the habitat of our target species, especially in a Nova Scotian context where they have not been studied before. Previously, research had only focused on specific aspects of these species' habitats and not an overall picture. More work needs to build upon our project to develop a detailed overview of these and other rare plant species so that both their habitat and the mechanisms responsible for their rarity are understood. Only then will we be able to effectively conserve them.

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CHAPTER 1 INTRODUCTION

Temperate Forests and Their Understory

Temperate forests are seasonal ecosystems that cover about 25% of the Earth's land (Tyrell et al., 2012). In Eastern North America, the temperate forests are shaped by the warm summers and cool winters that have encouraged a diverse mix of broadleaf deciduous and evergreen needle-leaved conifer trees (McGinley, 2008). Like all forests, they are dominated by tree species that form the overarching forest canopy and the numerous shrub and herbaceous plants that comprise the understory (Gilliam et al., 2011; Moffett, 2000).

The forest understory can be a harsh environment for plants to grow in because of nutrient limitations, frequent disturbances and heavy shading. Understory plants have adapted to these conditions by being stress tolerant, having large seeds, underground nutrient storage organs, long lifespans and heavy shade tolerances (Antos, 2009). Many understory plants recover from forest disturbances by regrowing from underground tissues, such as rhizomes or roots, quickly producing new shoots in the less competitive environment (Antos, 2009). These adaptions have allowed the understory to become diverse and include a wide range of life strategies, growth forms and functional groups. The understory contains about 90% of the forest plant biodiversity (Antos, 2009; Gilliam, 2007; Gilliam & Roberts, 2003; Mark R. Roberts, 2004; Whigham, 2004).

The understory plant community contributes little to a forest's biomass, less than 1% of above-ground material, but it plays a significant role in a forest's biodiversity, nutrient and energy cycling, canopy regeneration and succession following a disturbance (Gilliam, 2007; Gilliam & Roberts, 2003; Mark R. Roberts, 2004). Understory plants account for about 4% of a forest's net primary productivity (Gilliam, 2007; Muller, 1978), captures up to 7% of gained carbon (Neufeld & Young, 2014) and comprises 20% of annual litter (Welch et al., 2007). The herbaceous plants that dominate the understory have higher concentrations of nitrogen, phosphorus, potassium and magnesium compared to tree foliage but tend to shed their tissue more quickly and have faster decomposition rates; therefore, enabling more efficient recycling of nutrients in a forest. Some understory species, like spring ephemerals, can decrease the loss of nutrients, particularly nitrogen, from a forest by growing before trees become active and absorbing the nutrients that would otherwise be lost (Muller & Bormann, 1976). Many herbs are better competitors than canopy seedlings for soil nutrients and sunlight and they can alter their germination and growth, therefore influencing regeneration in a forest for centuries (Lyon & Sharpe, 2003). The understory reacts quite differently to disturbances than canopy species because of their different growth patterns and reproductive strategies. Although this area of research is difficult to study considering the legacy and frequency of disturbances in North American forests, some clear patterns are apparent. Most understory species are robust to disturbance. Most of the understory's species composition and diversity quickly return to pre-disturbance levels after a disturbance (Gilliam, 2007). However, not all understory plants can recover as well. Rare understory plants are often scattered throughout a forest and a disturbance can more easily eradicate them from an area.

The understory contains most plants in a forest and therefore it also contains most of its plant species of conservation concern. In this paper, conservation concern indicates that a species is under suspicion of being threatened with extinction and has a conservation subnational status rank between S2, imperiled, and S3, vulnerable (Atlantic Canada Conservation Data Centre, 2018). A subset of species of conservation concern is classified as being species at risk. Species at risk designation indicates that an organism has been formally protected under legislation (Government of Canada, 2002; The Honourable Kenneth MacAskill & Minister of Natural Resources, 1999). Herbaceous understory plants have higher extinction rates, around three times that of deciduous trees and five times that of conifers (Jolls & Whigham, 2014; Levin & Wilson, 1976; Spyreas & Matthews, 2006). Threats to forest ecosystems are often threats to the understory layer (Gilliam, 2007).

While the understory community is critical for forest ecosystems, its rare species can be marginalized by research that favours studying more charismatic canopy tree species (Gilliam, 2007). Even when research focuses on rare understory plants, they often concentrate on species in specific threatened habitats rather than more general forest ecosystems. For instance, studies have focused on rare plant species associated with forested floodplains (Davis et al., 1996; Knutson & Klaas, 1998; Ross E. Freeman et al., 2003), alpine communities (Fancy et al., 2020; LaRue, 2016) and Atlantic coastal plains (Dazé Querry et al., 2017). Although researching obligate plant species is important, there are many other rare forest plants that don't grow in these habitats and who haven't been robustly studied.

Across the globe, plant species richness is threatened because of human activity (Butchart et al., 2010; Díaz et al., 2006; Reich et al., 2001; Tilman et al., 2006) and rare plants are no exception (Wamelink et al., 2014). A species' rarity is linked to its niche and if an organism is unable to colonize the niche it has adapted to grow in it will die. Grubb (1977) outlines four aspects of a plant's niche; habitat, life-form, phenology and regeneration. If a factor interferes in any of these aspects, it will impact survival and could result in the plant becoming rare. Many factors can cause rarity and for most rare species they are poorly understood (Gravel et al., 2011; Poot & Lambers, 2003; Prendergast et al., 1993; Tittensor et al., 2010). However, some of the most common restraints to plants are linked to intrinsic restraints, dispersal capabilities and establishment, herbivores, disturbances, and abiotic factors (Bricker & Maron, 2012; Diane M. Thomson, 2005; DiTommaso et al., 2014; Emily H. Mooney & James B. McGraw, 2009; Maron et al., 2014; Olsen & Klanderud, 2014; Wade A. Wall et al., 2012; Wamelink et al., 2014). Increasingly, more factors are related to human activities (Andrea Dávalos et al., 2014; Brook et al., 2008; Sala et al., 2000; Tylianakis et al., 2008) and the largest threats are from habitat loss, invasive species and nutrient loss (Bohlen et al., 2004; Collard et al., 2010; Corlett, 2016; Côté et al., 2004; Fisichelli et al., 2013; Thuiller et al., 2005). Many species are facing several threats at once and these factors often interact in synergistic ways, exacerbating their overall impact (Dávalos et al., 2014; Didham et al., 2007). Habitat specificity can also influence a plant's rarity and many forest understory plants are limited by their microsite habitat (Clark et al., 2007; Lindsay A. Turnbull et al., 2000).

Understanding the factors causing species rarity is critical for their conservation (Dávalos et al., 2014; Evans et al., 2011). If we want to slow down and eventually reverse humanities' impacts on rare forest plants, we have to secure their populations by mitigating stressors so they can grow and spread (Rodríguez-Pérez & Traveset, 2007; Rother et al., 2013). Depending on the species and area, this could require management and artificially establishing new populations. Starting new populations can be a difficult process for plants (Holl & Hayes, 2006) and requires a detailed understanding of their habitat (Kwit et al., 2004). Very few rare plants have been researched enough to produce such an overview of their habitat and researchers need to address this gap.

Study Area

Our study area, Nova Scotia, lies roughly halfway from the equator to the North pole, it is a peninsula, dominated by forests and surrounded by the Atlantic Ocean (A. R. Taylor et al., 2020). The western continental winds are moderated in the region by the Atlantic Ocean and the North Atlantic gulf stream which gives the province high humidity, mild winters, cool summers and frequent changes in weather (Environment and Climate Change Canada, 1990; A. R. Taylor et al., 2020). The mean temperature ranges from 22 °C to 23°C in July and -8°C to -12°C in January, the warmest and coldest months. Nova Scotia receives a mean total annual precipitation of 1396 mm (Environment and Climate Change Canada, 2019).

Nova Scotia is dominated by a temperate forest subtype, the Acadian forest ecoregion. The Acadian forest is biodiverse and ranges from the Northeastern United States into Canada encompassing the Maritime provinces, including the majority of Nova Scotia (Nova Scotia Department of Lands and Forests, 2016; Rowe, 1972). Nova Scotia's forests have a varied mixture of roughly 32 conifer and deciduous tree species and are considered a transition zone between the Southern deciduous dominated forests and the Northern boreal, taiga (Loo & Ives, 2003; Loucks, 1962; Nova Scotia Department of Lands and Forests, 2016; Rowe, 1972; Saunders, 1996).

The Acadian forest developed in Nova Scotia roughly 10,000 years ago when the Wisconsin Glaciation ended. This and other glaciation events have shaped the geography and topography of Nova Scotia leaving it with a variety of geological features. The province is characterized by acidic soils, but this can vary substantially across the region and some areas, such as uplands and floodplains, can be much richer (Basquill & Baldwin, 2020; Loucks, 1962; Neily et al., 2013).

The most common canopy forming trees species in Nova Scotia are red, white and black spruce (*Picea rubens* Sarg., *Picea mariana* Mill. and *Picea glauca* Moench), red and eastern white pine (*Pinus resinosa* Ait. and *Pinus strobus* L.), eastern hemlock (*Tsuga canadensis* L.), sugar and red maple (*Acer saccharium* Marsh. and *Acer rubrum* L.), yellow and white birch (*Betula alleghanienis* Britt. and *Betula papyrifera* Marshall) and American beech (*Fagus grandifolia* Ehrh.). Balsam fir (*Abies balsamea* L.), eastern larch (*Larix laricina* K Koch), white ash (*Fraxinus americana* L.), red oak (*Quercus rubra* L.) and trembling aspen (*Populus tremiloides* Michx.), as well as other species, are also frequently encountered across Nova Scotia (Basquill & Baldwin, 2020; Loucks, 1962; Munro et al., 2014; Rowe, 1972; Saunders, 1996).

Forest disturbances play an important role in the forest ecosystems of Nova Scotia and vary considerably in scale and frequency. The major disturbances in the province are from hurricanes and other windstorms, tree-species specific pest outbreaks, such as spruce budworm (*Choristoneura spp.*), forest fires, mostly in the western region, and ice storms. The types of disturbances that can impact a forest are determined by the dominant tree species, climate and physiography of the area (Smith & Smith, 2015; White & Pickett, 1985).

Although ecological disturbances are a normal part of a forest ecosystem, human caused disturbances in Nova Scotian forests are having adverse effects. Several centuries of forestry, agricultural land clearing, human ignited forest fires, more recent forest fire suppression and the introduction of non-native invasive species have heavily impacted the province by altering its species composition and structure (Fernow, 1912; R. S.

Johnson, 1986; Loo & Ives, 2003). Regenerating abandoned farmland, clear-cutting and short-term forest harvesting rotations have made Nova Scotia's forests younger, more fragmented and with a higher proportion of fast-growing, early successional trees, including balsam fir, white spruce, red maple and trembling aspen (Loo & Ives, 2003; Simpson, 2008; A. R. Taylor et al., 2020).

The complex and varied provincial landscape, diversity of tree species and an assortment of disturbances have developed many diverse ecosystems in Nova Scotia (Loo & Ives, 2003). The flora of Nova Scotia is one of the most biodiverse groups of organisms and together form many communities (Munro et al., 2014; Roland & Smith, 1969).

Plant Communities of Nova Scotia

Woodland understory plants are common in the province and typically grow under red spruce, eastern hemlock, eastern white pine, American beech, red and sugar maple and yellow and white birches (Roland & Smith, 1969). This community includes many species such as wild lily of the valley (*Maianthemum canadense* Desf.), starflower (*Trientalis borealis* Raf.), yellow clintonia (*Clintonia borealis* Aiton.), wild sarsaparilla (*Aralia nudicaulis* L.) and partridgeberry (*Mitchella repens* L.) (Munro et al., 2014; Roland & Smith, 1969).

Some areas of the province, such as the Cobequid Hills, support a much richer basic soil dominated by deciduous trees such as sugar and red maple, ironwood (*Ostrya virginiana* Correll.), black cherry (*Prunus serotina* Ehrh.), yellow birch and white ash (Clayden et al., 2010; Roland & Smith, 1969). These communities support many species of shrubs and herbs. Some of the most common shrubs are chokecherry (*Prunus virginiana* L.), American hawthorn (*Crataegus chrysocarpa* Ashe) and highbush cranberry (*Viburnum opulus* L.). The rich soil can also support spring ephemerals, such as yellow violet (*Viola pubescens* Aiton) and round-lobed hepatica (*Anemone americana* Pursh) (Hill & Garbary, 2011; Munro et al., 2014). These communities have been heavily impacted by agriculture in Nova Scotia and several plants that grow here are threatened (Munro et al., 2014).

There are also communities of calcium-loving plants, between Windsor and Cape Breton Island in gypsum outcrops (Munro et al., 2014). There are no distinctive tree species in this community, but it includes shrubs such as round-leaved dogwood (*Cornus rugosa* Lam.) and shepherdia (*Shepherdia canadensis* L.) as well as rare orchids such as yellow lady's slipper (*Cypripedium calceolus* Salisb.) and ram's head lady's slipper (*Cypripedium arietinum* W.T. Aiton). It also includes several unique herbs including hyssop-leaved fleabane (*Erigeron hyssopifolius* Michx.) and balsam ragwort (*Packera paupercula* Michx.) (Munro et al., 2014; Roland & Smith, 1969). Most of the plants that require calcium rich soil to grow are restricted to unmined areas in the gypsum rich landscape and are under threat from mining activities (Jeziorski et al., 2008).

In the southern Nova Scotian tri-counties of Digby, Yarmouth and Queens there is a unique group of over a hundred species of plants found along the Atlantic coast. These Atlantic Coastal plain florae are disjointed from their main distribution, highly threatened and mostly found in wetlands and along some rivers in the above mentioned counties (Hill & Keddy, 1992; Mersey Tobeatic Research Institute, 2011; Munro et al., 2014).

In the northern counties of Victoria and Inverness, Cape Breton, there are populations of artic-alpine plants that are also disjointed from their main populations in the northern boreal forests. These cold-adapted species are rare in Nova Scotia and include some of the most at-risk plants in the province (Munro et al., 2014; Roland & Smith, 1969).

Nova Scotia is home to many more plant assemblages, including those found in coastal and jack pine barrens, floodplains, coastal forests, wetlands and saltmarsh communities (Munro et al., 2014; Roland & Smith, 1969).

Considering the diversity of forest communities in Nova Scotia it is unsurprising that there are thousands of forest dependent species (Bush et al., 2019). Many of them are understory plants that are of conservation concern because of their rarity. Yet few of these plants have been investigated in enough detail to understand the mechanisms behind their rarity, including their habitat requirements.

Research Objectives

Our research set out to identify broad habitat characteristics of three rare forest plants, *Anemone americana*, *Conopholis americana* L. and *Goodyera pubescens* Willd. with three objectives.

Objective One: To identify if there were differences in the canopy, subcanopy, shrub and herb vegetation layers of our target species habitat compared to forests without the target species, i.e. control sites.

Objective Two: To identify if there were differences in the soil texture, fertility, forest vegetation type and forest successional stage of our target species habitat compared to controls.

Objective Three: To identify whether site aspect, slope, elevation, topographic position, soil drainage or soil moisture were influencing the target species populations.

CHAPTER 2 METHODS

Target Species Selection

The first step of this project was to select forest plant species of conservation concern in Nova Scotia for study. To accomplish this, we first extracted all plants ranked as S2 or S3 in the Nova Scotia vascular plant list (Atlantic Canada Conservation Data Centre, 2018). We then extracted and retained all plants that were rated as being facultative or obligate upland according to the Nova Scotia wetland indicator plant list (Blaney, 2012). We added details of the remaining species' habitats from Flora of North America Association (2021), Munro et al., (2014) and the Native Plant Trust (2021) and removed any plants that had a habitat outside of a closed canopy upland forest. From this procedure, we identified seven candidate forest-dependent plants of conservation concern. They were *Anemone americana*, *Carex rosea*, *Conopholis americana*, *Goodyera oblongifolia*, *G. pubescens*, *Platanthera hookeri* and *P. macrophylla*. Finally, we reviewed these seven species and choose three that had the most known sites in the province and were the easiest to accurately identify. This gave us our three target species: *Anemone americana*, *Conopholis americana* and *Goodyera pubescens* (Appendix 1).

Target Species Background

All three target species are native to Central and Eastern Canada and the Eastern United States (United States Department of Agriculture, 2021). In Nova Scotia, *Anemone americana* (round hepatica) and *Conopholis americana* (cancer root) have a subnational rank of S1S2, indicating they are imperilled or critically imperilled. *Goodyera pubescens* (downy rattlesnake plantain) has a subnational rank of S2 in Nova Scotia indicating it is imperilled (Atlantic Canada Conservation Data Centre, 2018). All three species have a Nova Scotian general status rank of orange designating that they are sensitive to threats and at risk of extinction (Munro et al., 2014).

Round leaved hepatica is an herbaceous plant of the *Ranunculaceae*, buttercup, family. It produces mauve coloured, 5- 7 petaled flowers and is one of the earliest plants to bloom in the province, usually in April (Munro et al., 2014). It has a rhizome and can be found in large, clustered colonies. The leaves are simple, basal, have three round lobes and the underside is densely hairy throughout the year. Its fruit is an ovoid-shaped achene (Native Plant Trust, 2021).

Cancer root is an herbaceous, achlorophyllous, parasitic plant of the *Orobanchaeae*, broomrape, family. Although there have been conflicting reports of its host, most have found that it only derives its nutrients from oak (*Qurecus*) trees (Flora of North America Association, 2021). The above-ground plant structure, the spike, reassemble a yellow to white coloured fleshy pinecone made up of a simple stem covered in tight clusters of flowers. Cancer root does not have true leaves, instead, its leaves have been reduced to tiny scales under the flowers. The plant emerges in spring, April to July in Nova Scotia, and starts to turn dark brown than black in late fall/ winter (Munro et al., 2014). The dried spikes can persist into the following spring. Each flower produces an ovoid capsule filled with seeds (Native Plant Trust, 2021).

Downy plantain is an evergreen plant of the *Orchidaceae*, orchid, family. It forms a tight cluster, rosette, of 3- 8 basal oblong leaves with a unique netlike pattern of white veins against a dark green leaf. All parts of the plant are densely pubescent. It forms a rhizome and can usually be found in large colonies (Munro et al., 2014). Each rosette will grow a spiked white inflorescence between July and August that produces a seed capsule (Native Plant Trust, 2021).

Target Species Site Selection

Due to the rarity of our target species, we had to take steps to maximize the chances of capturing them during field sampling. We did this by revisiting sites where other researchers had reported them since the year 2000. Sean Blaney, Executive Director and Senior Scientist at Atlantic Canada Conservation Data Centre provided these data to us.

Control Site Selection

To achieve the aims of our project we needed to sample forested areas that did not contain our target species and would be control sites. We choose these sites by using the Nova Scotia forest inventory (Nova Scotia Department of Lands and Forests, 2021). The forest inventory is a data rich geographic information system (GIS) layer containing information on many forest related attributes (e.g. landforms and vegetation defined forest types). For this procedure, we defined a forest stand in GIS by the dominant canopy tree species in the main species type (SP1) attribute of the forest inventory. To identify control sites, we first extracted all natural (e.g. unharvested) forest stands in Southwest Nova Scotia. Natural stands in the forest inventory had a value of 0 in the forest inventory forest grouping (FORNON) attribute. This excluded areas that had been harvested, were a wetland or were dominated by dead trees or trees shorter than 3 m. We targeted Southwest Nova Scotia (Annapolis, Digby, Kings, Lunenburg, Queens, Shelbourne and Yarmouth counties) because the majority of our target species were recorded in this part of the province and it reduced the time and cost of sampling. Next, we extracted and retained forest stands that were closer than 5 km from a roadway with the GIS buffer tool so the sites would be accessible. We also removed forest stands that were closer than 500 m from the coastline, 250 m from a lake and 50 m from a river to help reduce the potential influence these ecosystem edges would have on the controls. After assigning each stand a number, we used a random number generator (Random.org, 2021) to pick 15 random numbers and their associated forest stand. Finally, we created a random point inside each of the 15 stands by using the GIS random points inside polygons tool. This point represented the control site that would be field truthed. If upon visiting a site we determined it was not appropriate (e.g. it was a wetland or had been harvested), we selected an entirely new site with the above procedure and repeated until we had 15 sites. Figure 1 outlines the locations of the control and sampling sites.

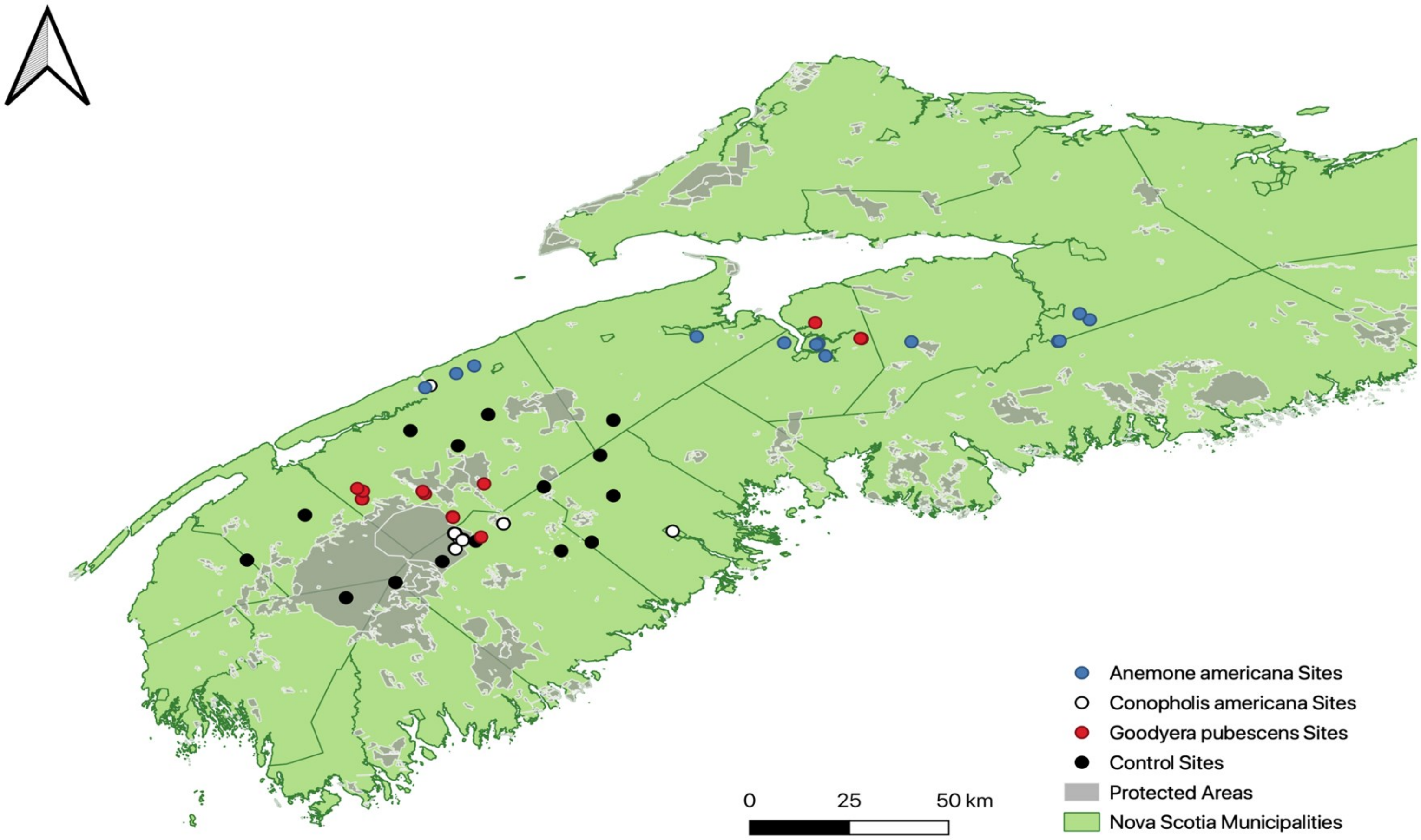

Figure 1 Sampled control, *Anemone americana*, *Conopholis americana* and *Goodyera pubescens* sites in Nova Scotia, Canada (2019).

Field Sampling

We completed all field sampling for this project in October of 2018 and between May and November of 2019. We used the same sampling protocol for the control and target species sites.

We performed an initial survey at each target species site to confirm that the species were still present. We started at the GPS point where the target species was previously reported and radiated outward in a spiral pattern until the target species was no longer encountered or the vegetation type, as defined by the Nova Scotia Forest Ecosystem Classification (FEC) (Neily et al., 2013) changed from the original GPS point. A new survey would begin if we found the target species in a separate vegetation type and the new area would be used as an additional sampling site if it was farther than 60 m from the first sampling site. If we could not locate the target species in a site, we still recorded our sampling variables, and the site was labelled as the target species being undetected.

Our initial field protocol was a linear transect design that crossed areas where the target species had been reported. After a brief field trial in October of 2018, we found this method needed to cover an unworkably long distance to find the target species and we changed the protocol to be a quadrat-based approach. We chose a 20 x 20 m square for the size of the quadrat after reviewing the literature (Cameron, 2019; Neily et al., 2013; Roberts-Pichette & Gillespie, 1999) and consulting with Nova Scotia Environment and Lands and Forestry staff. We concluded that 20 m^2 was small enough to be practical and large enough to capture a representative snapshot of the botanical community and target species.

Depending on if an area was a control or target species site, we placed the quadrat in the same vegetation type as the target species or the random GPS point specified in our control site selection. For the controls the center of the plot was placed at the GPS point. The target species quadrats were placed randomly by starting at the GPS points (were the species was previously encountered) and walking in a prespecified direction and distance (0 - 20 m) for three distinct increments. We choose these directions and distances randomly by using a random number generator (Random.org, 2021). If these directions led us outside of the original vegetation type, then we choose a different direction and distance and continued until we completed three increments.

Once a quadrat was laid, we began to record abiotic and biotic conditions of the sites from procedures modified from Nova Scotia Environment sampling protocols (Cameron, 2019) and the Nova Scotia FEC (Neily et al., 2013).

At each site we recorded six environmental condition measurements that included site aspect, elevation, slope, topographic position, soil drainage and soil moisture (Table 1) and the categorical percent cover of all plant species in the canopy, subcanopy, shrub and herb vegetation layers, as described by Cameron (2009). We also recorded the count of the target species and classified the forest ecosystem according to the Nova Scotia FEC, including vegetation and soil type variables (Table 1) using protocols outlined in the FEC guide.

We identified all botanical specimens by using Munro et al (2014) and Rolland and Smith (1996). All identification was completed in the field unless we needed a microscope. In that case, we collected, dried and pressed the specimens for later identification.

Table 1 An outline of the sampling variables, their definition, units and the tools used to record them.

Sampling Variable	**Type of Variable**	**Definition**	**Units and Equipment Used**
Environmental Variables			
Aspect	Interval (Continuous)	The compass bearing that a site's slope is facing.	Compass degrees (0-360°). Measured with a standard compass.
Slope	Ratio (Continuous)	The angle of the land's surface.	Clinometer degrees (0-90°). Measured with a Suunto brand clinometer.
Elevation	Ratio (Continuous)	The height of the land's surface above or below sea level.	Meters above sea level. Measured with a Garmin brand 64s model GPS.
Topographic Position	Ordinal (Categorical)	The relative position of a site's slope within the landscape.	Categories were level, depression, toe, lower slope, middle slope, upper slope and crest. Measured with researcher's discretion and categories defined by Nova Scotia FEC (Neily et al. 2013).
Soil Drainage	Ordinal (Categorical)	The length of relative time it takes water to be removed from the site's soil in relation to water supply.	Categories were very poor, poor, imperfect, moderately well, well and rapid. Measured with researcher's discretion and categories defined by Nova Scotia FEC (Neily et al. 2013).
Relative Soil Moisture	Ordinal (Categorical)	The average moisture available for plant growth. It is combination of a site's moisture supply, soil drainage and water retention.	Categories were wet, moist/ wet, moist, fresh/ moist, fresh, dry and very dry. Measured with researcher's discretion and categories defined by Nova Scotia FEC (Neily et al. 2013).
FEC Related Variables			
Soil Texture	Ordinal (Categorical)	The percentage of sand, silt and clay in a soil (Neily et al. 2013).	Categories were talus, coarse to medium and medium to fine, adapted from Nova Scotia FEC (Neily et al. 2013) and based on the field recorded soil type.

Sampling Variable	**Type of Variable**	**Definition**	**Units and Equipment Used**
FEC Related Variables			
Soil Fertility	Ordinal (Categorical)	The amount of nutrients and moisture available to plants (Neily et al. 2013).	Categories were very poor to poor, poor, poor to medium, medium, medium to rich and rich, adapted from Nova Scotia FEC (Neily et al. 2013) and based on field recorded soil type.
Forest Vegetation Group	Nominal (Categorical)	Groupings of forests with similar species, conditions and successional paths (Neily et al. 2013).	Categories were cedar, intolerant hardwood, karst, mixed wood open field, spruce – hemlock, spruce – pine and tolerant hardwood, adapted from Nova Scotia FEC (Neily et al. 2013) and based on field recorded vegetation type.
Forest Successional Stage	Ordinal (Categorical)	Succession is the orderly process of community development over time following a disturbance. It involves changes in species structure and community processes (Neily et al. 2013; Odum 1971).	Categories were early, middle and late, adapted from Nova Scotia FEC and based on field recorded vegetation type.

Nova Scotia Forest Ecosystem Classification

The following was adapted from Neily et al (2013). We utilized the Nova Scotia FEC in this project because it is the most extensive catalogue of the province's forests and its corresponding guide is easy to use in the field. The Nova Scotia FEC was developed by the provincial Department of Natural Resources to identify and describe forest ecosystems and to provide a structure for landscape analysis that could be linked to forest management. In 2010, ten years of results from the project were compiled into a key-based, FEC guide that features sections on vegetation, soil and ecosites. The vegetation section is broken down into different forest groups and subcategories called vegetation types; both are characterized by their overstory trees and select understory plant species. The FEC upland forest groups most relevant to this project were cedar, intolerant hardwood, karst, mixedwood, old field, open woodland, spruce- hemlock, spruce-pine and tolerant hardwood. Similar to the vegetation section, the soil section classifies a site into soil types based on its soil texture and soil horizon layers.

Data Transformation

Site aspect needed to be transformed into a new categorical variable to analyze it. We did this by converting it into a measure of how south-facing a site aspect was, which we described as the south-facing index. We took the original 360° range of a compass and divided it into eight equal sections, corresponding to N, NE, E, SE, S, SW, W and NW. We gave the S section a value of five, SW and SE four, W and E three, NE and NW two and N a value of one.

Software

We analyzed all GIS data with Quantum GIS (Quantum GIS, 2021), an open-source platform developed to view, manage and analyze geospatial data. We collected our field data with EpiCollect 5 (Epicollect 5, 2021), a cloud-based web and mobile app used to generate and fill data management forms. All of our statistical tests were done with SPSS statistical software (International Business Machines Corporation, 2021), produced by IBM and under license from Dalhousie University.

Statistical Analysis

Our research set out to identify broad habitat characteristics of three rare forest plants: *Anemone americana*, *Conopholis americana* and *Goodyera pubescens*. We analyzed each target species' data separately and applied three main approaches, each related to different aspects of the plants' habitat (vegetation, soil and community, and environment). The target species detected and undetected sites were combined in the third approach (environment) analysis to help alleviate small sample sizes.

Approach One: To identify if there were differences in the canopy, subcanopy, shrub and herb vegetation layers of our target species habitat compared to control sites.

We addressed this approach by taking the plant species percent cover data from the four vegetation layers and analyzing them in a non-metric multidimensional scaling (NMDS) model that compared the target species sites to the controls. An NMDS is an ordination technique that takes high-dimensional datasets and translates them into a lower dimension, more easily interpreted output (Pielou, 1984; Rabinowitz, 1975). It is ideal for community datasets due to this ability, its capacity to deal with zero abundances and small sample sizes and its general lack of statistical assumptions (Barbour, Michael, 1999; Rabinowitz, 1975). For each NMDS, we calculated a model normalized raw stress value and Tucker's coefficient of congruence (IBM Knowledge Centre, 2020a, 2020b).

Approach Two: To identify if there were differences in the soil texture, fertility, forest vegetation type and forest successional stage of our target species habitat compared to controls.

We addressed this approach by comparing the frequencies of control and target species sites in the different categories of soil texture, fertility, forest vegetation and successional stage with contingency table Chi-squared tests. A contingency table Chi-squared test is a hypothesis test used to determine whether there is a difference between the expected and observed frequencies between two nominal (categorical) variables (Everitt, 1992; McDonald, 2014). Its only statistical assumption was that the samples were independent (Anatolyev & Kosenok, 2009; McHugh, 2013). We specified the test to be a Fisher's exact test in SPSS to help alleviate problems associated with small sample sizes (<5) (Lipsitz et al., 2015).

Approach Three: To identify whether site aspect, slope, elevation, topographic position, soil drainage or soil moisture were influencing the target species populations.

We tackled this approach with two sequential methods. Initially, we used a principal component analysis (PCA) to gauge which of the six environmental variables were most associated with changes in the target species abundance. A PCA is another ordination technique that reduces the complexity of large datasets into simpler representations while retaining their relationships for easier interpretation (Abdi & Williams, 2010; Jolliffe, 2002). This ability and its limited statistical assumptions made it an ideal tool to analyze the environmental variables. A PCA assumes that there was a linear relationship between the variables and we checked this assumption with a Person's correlation matrix (Jolliffe, 2002) (Appendix 2.1- 2.3). We determined the number of principal components (PCs) to retain with scree plots and parallel analysis according to Horn (1965).

We then reviewed the environmental variables that our PCA showed to be associated with the target species and compared them to results from the Person's correlation matrix (Appendix 2.1- 2.3). With this approach, we aimed to maximize the types of environmental variables we could use in the following method while reducing redundancy from highly correlated variables. The selected environmental variables were analyzed with a generalized linear model (GLM) to gauge how they may be impacting the target species' abundances. A GLM is an extension of linear regression that is better adapted to handle response variables with non-normal distributions, such as datasets with species counts (Bolker et al., 2009; Hilbe, 2011; Iwasaki & Tsubaki, 2006). The assumptions of GLM are debated but there is general agreement that there must be homogeneity of the residuals (McCue et al., 2008). We tested this with a scatterplot of residuals (Appendix 3.1- 3.3) that revealed some clustering. We specified the predicated (dependent) variable to be the target species count and the covariates (independent) to be the remaining environmental variables from the PCA analysis. We defined the model to be a negative binomial with a log link function and made it estimate a calibrated dispersion factor. Our models were not able to run when datapoints had a target species abundance of zero, the log link function could not calculate a value of zero, so we added a value of one to each abundance in the datasets.

CHAPTER 3 RESULTS

Control Sites

We sampled 15 control sites in southwest Nova Scotia (Figure 1). Our control sites were mostly in coarse to medium textured soil with a poor to medium fertility (Figure 2A and B). They were in a range of forest vegetation groups, most notably spruce- hemlock, intolerant hardwood and mixedwood forests that were in a middle to late successional stage (Figure 2C and D).

During our analysis into the composition of the four vegetation layers we found that within the control canopy layer *Acer rubrum*, *Picea rubens* and *Betula alleghaniensis* occurred more frequently than other species (Appendix 4.1). In the subcanopy layer *Acer rubrum*, *Picea rubens* and *Abies balsamea* occurred most frequently (Appendix 4.2). For the shrub and herb layers *Abies balsamea*, *Acer rubrum* and *Betula alleghaniensis* as well as *Maianthemum canadense*, *Trientalis borealis* and *Cornus canadensis*, respectively, occurred most frequently (Appendix 4.3 and 4.4).

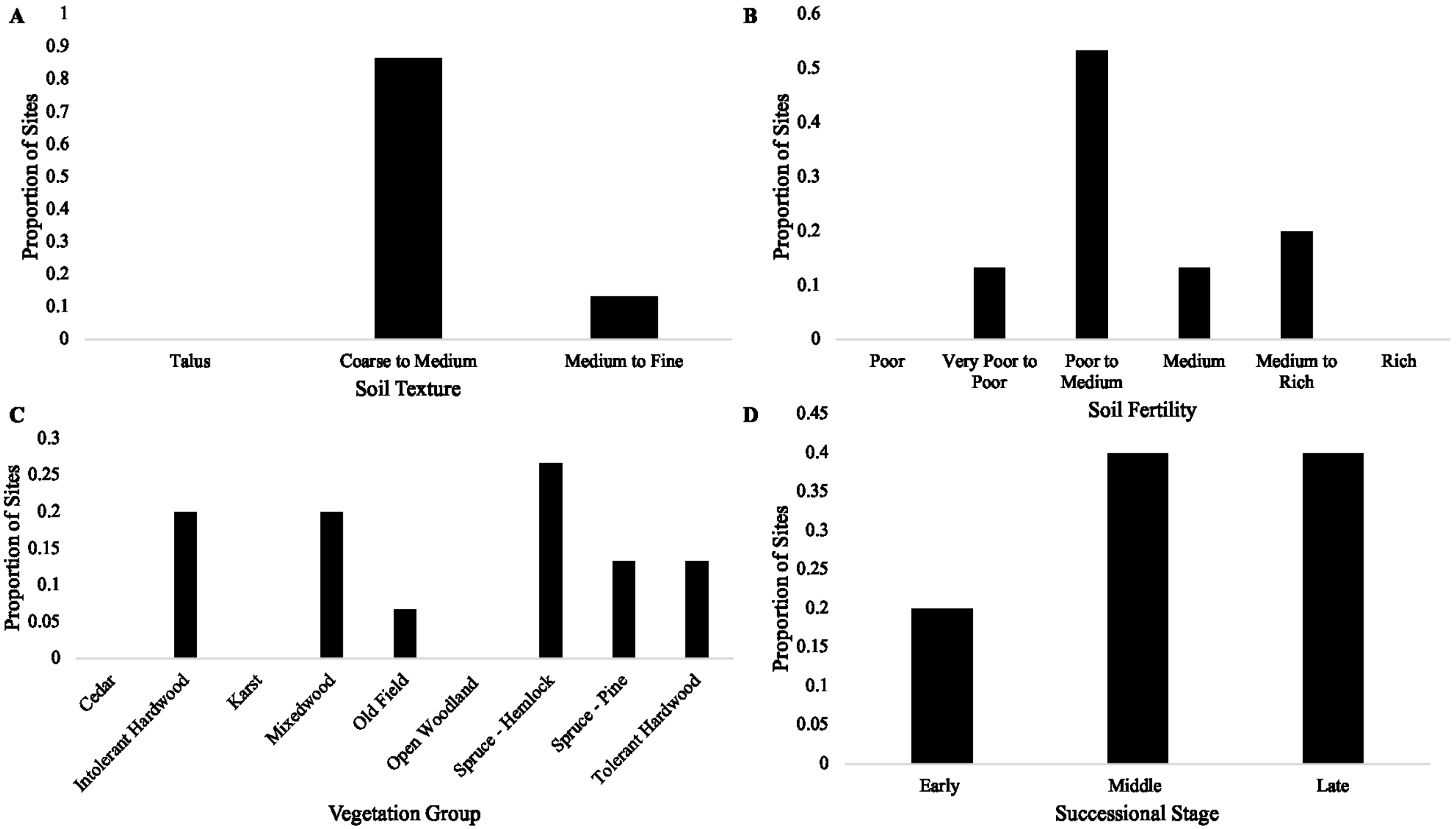

Figure 2 Proportion of control sites in categories of soil texture class (A), soil fertility class (B), forest vegetation group (C) and successional stage (D) (n=15). All control sites were in Southwest Nova Scotia.

Conopholis americana

We found *Conopholis americana* in 13 of the 15 sites we surveyed (Figure 1).

Non-Metric Multidimensional Scaling

All NMDS models for *Conopholis americana* had raw normalized stress values less than 0.05 and Tucker's coefficient of congruence values greater than 0.95, indicating the models created a strong repression of the dataset and a resilient fit (Figure 3). There was little separation between the control and *Conopholis americana* sites in the canopy and subcanopy plant communities (Figure 3A and B). However, there was clear separations between the site types in the shrub and herb level plant communities (Figure 3C and D).

Within the canopy layer at the *Conopholis americana* sites *Quercus rubra, Acer rubrum* and *Pinus strobus* occurred more frequently than other species (Appendix 4.1). In the subcanopy layer *Acer rubrum*, *Pinus strobus* and *Quercus rubra* occurred most frequently (Appendix 4.2). For the shrub and herb layers *Hamamelis virginiana*, *Pinus strobus* and *Fagus grandifolia* as well as Graminoids, *Trientalis borealis* and *Acer rubrum*, respectively, occurred most frequently (Appendix 4.3 and 4.4). In the canopy layer *Quercus rubra* and *Pinus strobus* differed from the controls. In the subcanopy *Pinus strobus* and *Quercus rubra* differed. In the shrub and herb layers all the dominant species differed except for *Trientalis borealis* in the herb layer.

Contingency Table Chi-squared

Conopholis americana sites were significantly different in soil texture, fertility, forest vegetation and successional stage compared to the control sites when analyzed with a contingency table Chi-squared test, p = 0.007, 0.013, 0.004 and 0.002 respectively (Figure 4A- D). *Conopholis americana* most often grew in medium to fine textured, medium fertility soils under a shade-intolerant early successional forest (Figure 4A-D). One *Conopholis americana* site could not be classified into a Nova Scotia FEC vegetation type because it had been partially harvested and the regenerating community did not fit well into a grouping.

Principal Component Analysis

One PC was retained based on the scree plot and parallel analysis for the *Conopholis americana* PCA (Figure 5A and B). The greatest amount of variation across site conditions was associated with soil moisture and drainage (Figure 6A and B). Topographic position, slope, elevation and south-facing index were not strongly correlated with PC1 (Figure 6A and B). Based on these results and review of the Pearson's correlation table (Appendix 2.1) we choose to include south-facing index, elevation and soil drainage in the GLM model.

Generalized Linear Model

The GLM built with the south facing index, elevation and soil drainage variables had a significant improvement in fit compared to a null model, P-value = 0.01 (Table 2). Changes in *Conopholis americana* populations were linked with soil drainage, P-value = 0.005, and to a lesser extent slope direction, P-value = 0.07 (Table 2). As sites became better drained and more south facing the population of *Conopholis americana* increased (Table 2). Site elevation was not associated with *Conopholis americana* populations (Table 2).

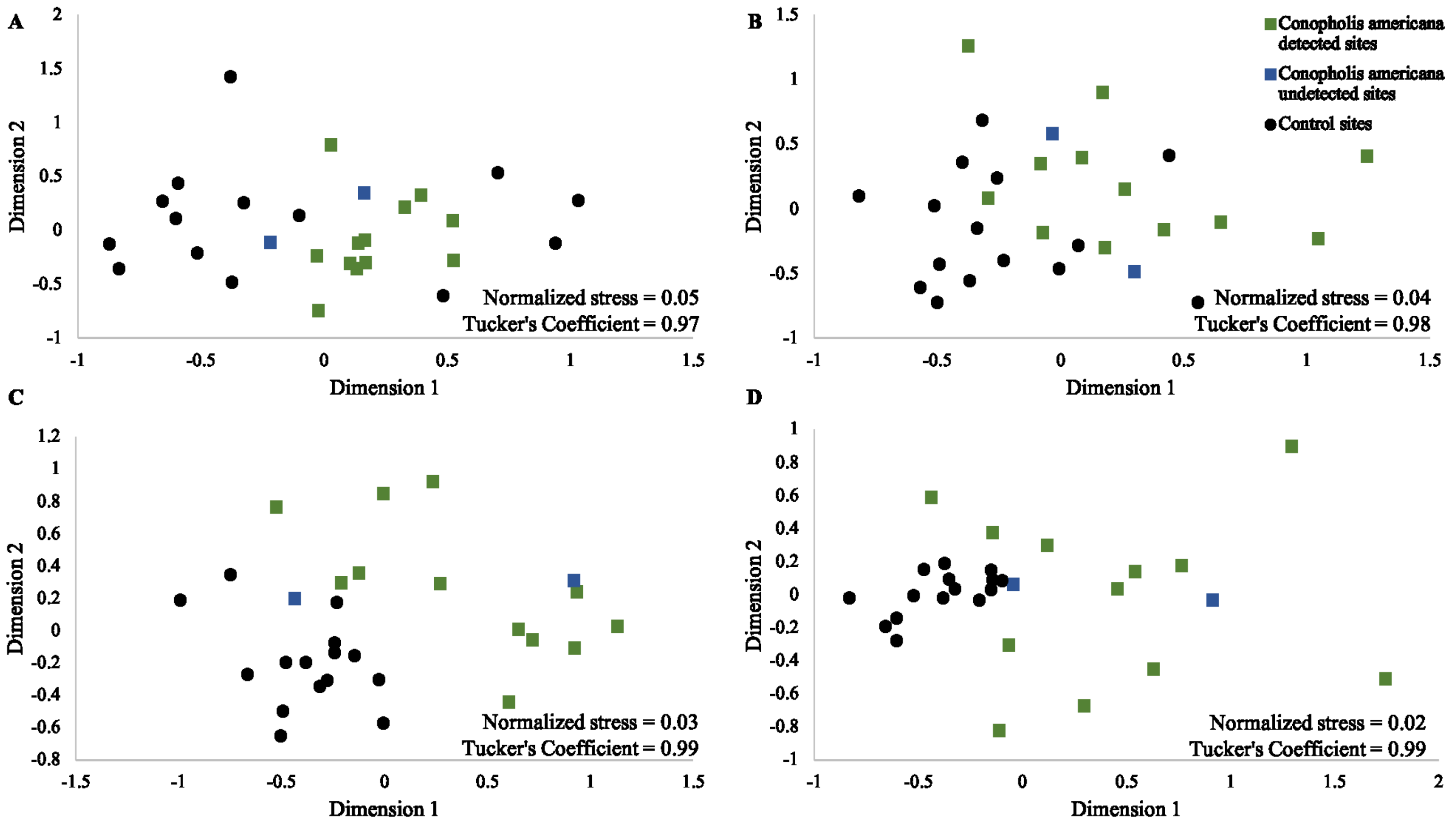

Figure 3 Non-metric multidimensional scaling (NMDS) biplot of control and *Conopholis americana* detected and undetected sites. An NMDS was performed on the canopy (A), subcanopy (B), shrub (C) and herb (D) level vegetation cover datasets with the control sites (n=15), *Conopholis americana* detected sites (n=13) and *Conopholis americana* undetected sites (n=2). Results shown with model normalized raw stress and tucker's coefficient listed.

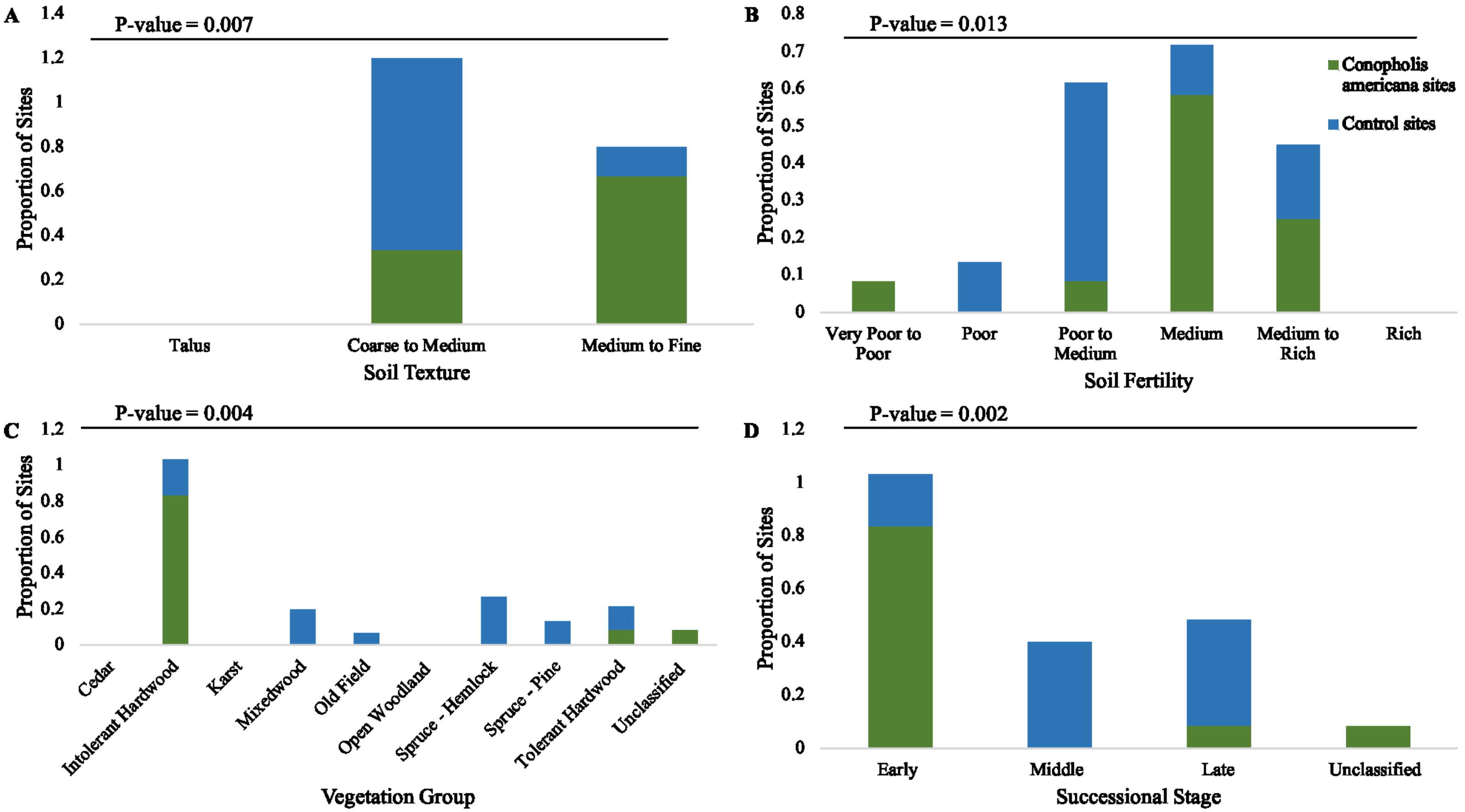

Figure 4 Contingency table Chi-squared analysis comparing control and *Conopholis americana* sites. A Chi-squared analysis was performed on soil texture class (A), soil fertility class (B), forest vegetation (C) and forest successional stage (D) datasets with control sites (n=15) and *Conopholis americana* sites (n=13). Results shown with Fisher's Exact Test (2-sided) p-values listed. Proportions are summed to a value of one separately for the control and target species sites.

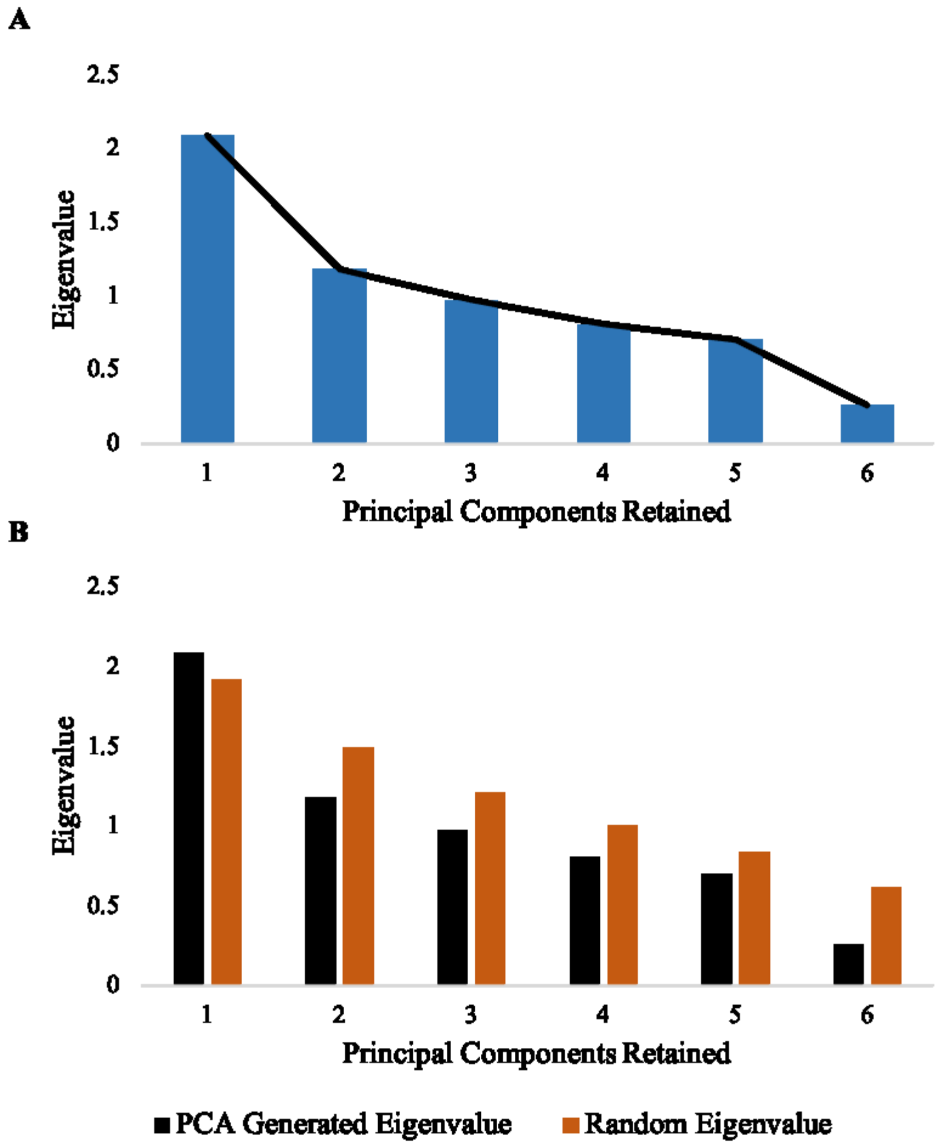

Figure 5 Principal component analysis (PCA) scree plot (A) and parallel analysis plot (B) generated from environmental variables of control sites (n=15) and *Conopholis americana* sites (n=14).

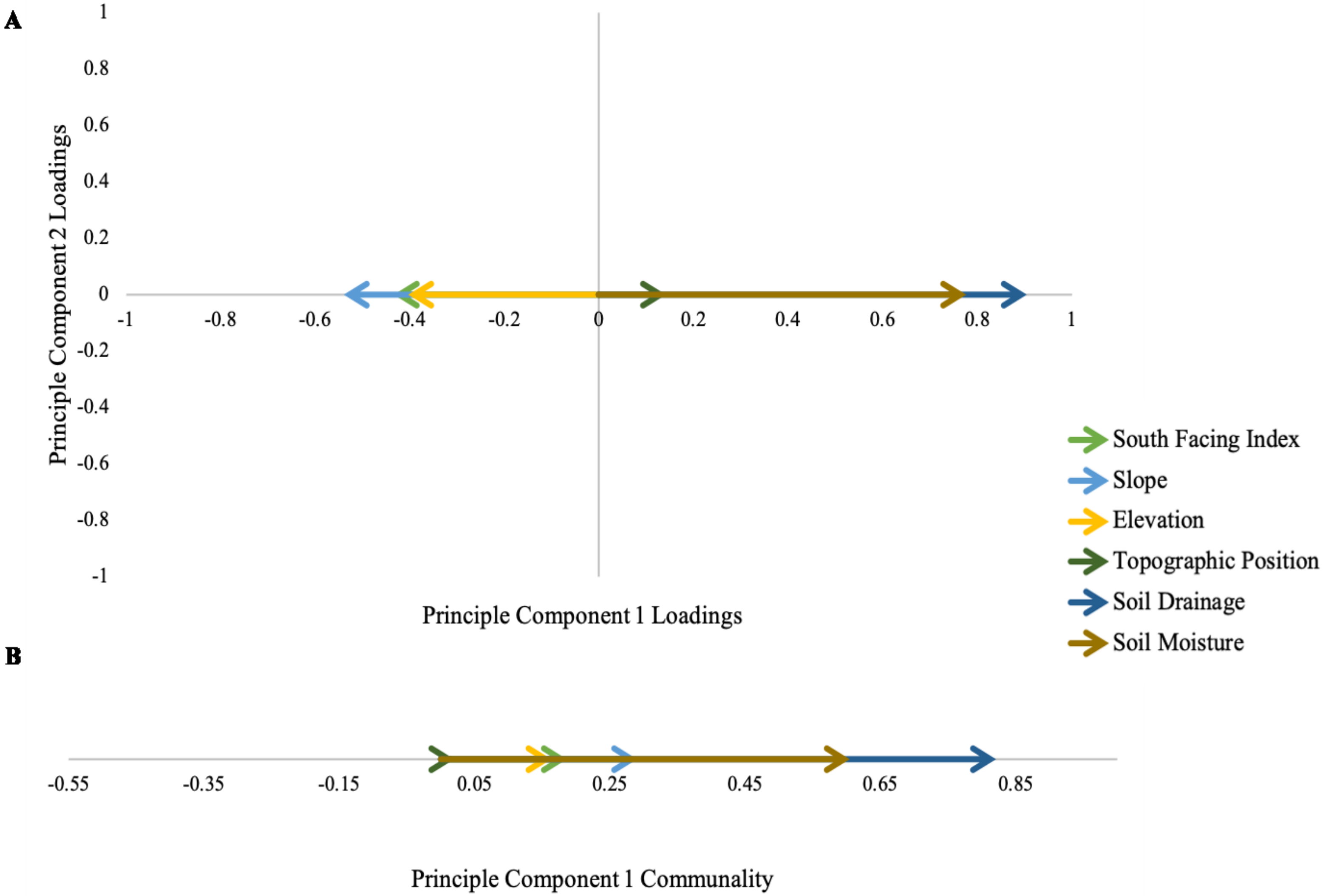

Figure 6 Principal component analysis loadings (A) and communality (B) values of environmental variables for control sites (n=15) and *Conopholis americana* sites (n=14).

Table 2 Generalized linear model (GLM) omnibus test results, test of model effects and parameter estimates of environmental variables from control sites (n=15) and *Conopholis americana* sites (n=14).

Environmental Variable	**Coefficient**	**Likelihood Ratio Chi-Squared Value**	**Degrees of Freedom**	**P-value**
Full Model		18.465	7	0.01
Factors and Their Levels		**Wald Chi-Squared Value**		
South Facing Index		8.655	4	0.07
North	-3.974	7.757	1	0.005
North West/ North East	-0.294	0.134	1	0.714
West/ East	-0.466	0.23	1	0.632
South West/ South East	-0.187	0.051	1	0.821
South	0*	N/A	N/A	N/A
Elevation	-0.008	1.777	1	0.182
Soil Drainage		10.56	2	0.005
Moderately Well	-3.316	6.727	1	0.009
Well	-0.004	0	1	0.996
Rapid	0*	N/A	N/A	N/A

0* indicates the variable was set to zero because this parameter is redundant (it was the baseline coefficient).

Goodyera pubescens

We found *Goodyera pubescens* in 14 of the 15 sites we surveyed (Figure 1).

Non-Metric Multidimensional scaling

All NMDS models for *Goodyera pubescens* had raw normalized stress values less than 0.05 and Tucker's coefficient of congruence values greater than 0.95, indicating the models created a strong repression of the dataset and a resilient fit (Figure 7). There was little separation between the control and *Goodyera pubescens* sites in the canopy and subcanopy plant communities (Figure 7A and B). However, there was clear separation between the site types in the shrub and herb level plant communities (Figure 7C and D).

Within the canopy layer at the *Goodyera pubescens* sites *Acer rubrum*, *Fagus grandifolia* and *Betula papyrifera* occurred more frequently than other species (Appendix 4.1). In the subcanopy layer *Fagus grandifolia*, *Abies balsamea* and *Tsuga canadensis* occurred most frequently (Appendix 4.2). For the shrub and herb layers *Abies balsamea*, *Fagus grandifolia* and *Picea rubens* as well as *Maianthemum canadense*, *Aralia nudicaulis* and *Trientalis borealis*, respectively, occurred most frequently (Appendix 4.3 and 4.4). In the canopy layer *Fagus grandifolia* differed from the controls. In the subcanopy *Fagus grandifolia* and *Tsuga canadensis* differed. In the shrub layer all the dominant species differed except for *Abies balsamea* and *Aralia nudicaulis* differed in the herb layer from the controls.

Contingency Table Chi-squared

Goodyera pubescens sites were significantly different in soil texture, fertility and successional stage compared to the control sites when analyzed with a contingency table Chi-squared test, $p = 0.027$, 0.026 and 0.033 respectively (Figure 8A, B and D). *Goodyera pubescens* most often grew in medium to fine textured, medium to rich fertility soil under a late successional forest (Figure 8A-D). There were no significant differences in forest vegetation, p = 0.105 (Figure 8C), however more *Goodyera pubescens* sites were found in tolerant hardwood forests.

Principal Component Analysis

Two PCs were retained based on the scree plot and parallel analysis for the *Goodyera pubescens* PCA (Figure 9A and B). The greatest amount of variation across site environmental conditions was associated with soil moisture, drainage and slope (Figure 10A and B). Elevation was more closely correlated to PC2. Topographic position and south facing index had roughly equal correlation to both PCs (Figure 10A and B). Based on these results and review of the Pearson's correlation table (Appendix 2.2) we chose to include south-facing index, slope and soil moisture in the GLM model.

Generalized Linear Model

The GLM built with the south-facing index, slope and soil moisture variables had a significant improvement in fit compared to a null model, P-value = 0.026 (Table 3). Changes in *Goodyera pubescens* populations were linked with south facing index and soil moisture, P-value = 0.0024 and 0.03 respectively (Table 3). As sites become more north facing and dryer the population of *Goodyera pubescens* increased, although the soil moisture pattern is less clear (Table 3). Slope was not associated with *Goodyera pubescens* populations (Table 3).

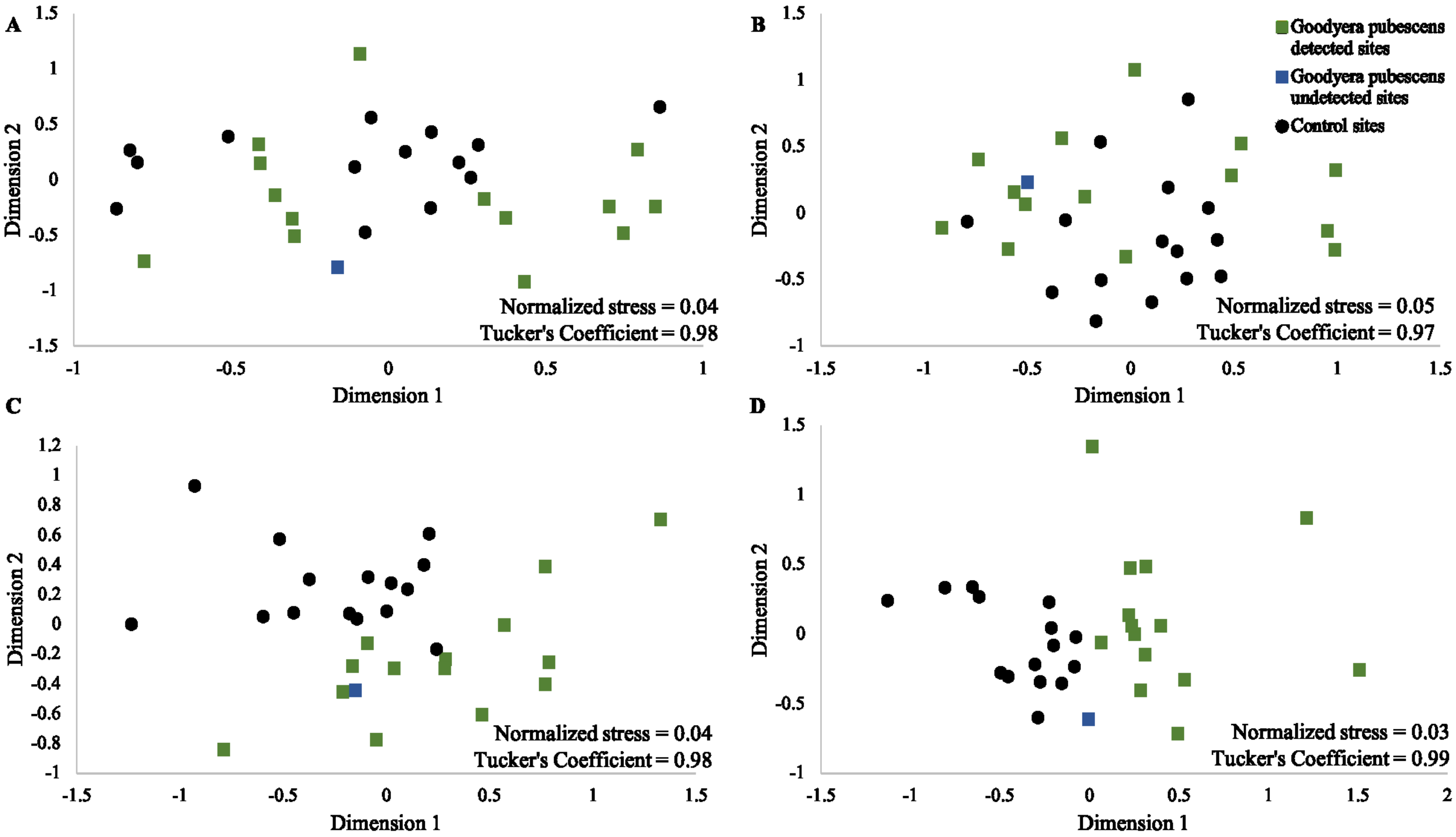

Figure 7 Non-metric multidimensional scaling (NMDS) biplot of control and *Goodyera pubescens* detected and undetected sites. An NMDS was performed on the canopy (A), subcanopy (B), shrub (C) and herb (D) level vegetation cover datasets with the control sites (n=15), *Goodyera pubescens* detected sites (n=14) and *Goodyera pubescens* undetected sites (n=1). Results shown with model normalized raw stress and tucker's coefficient listed.

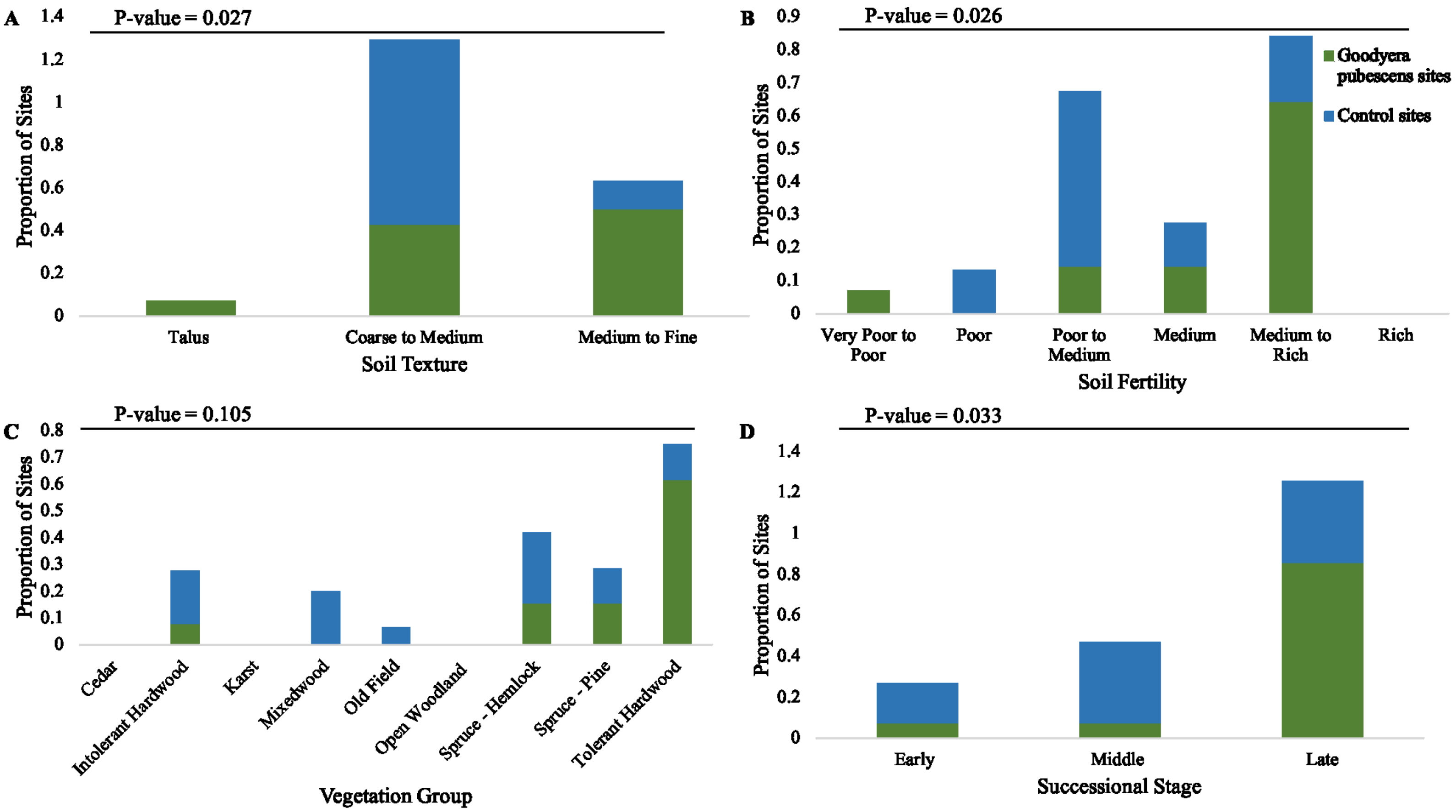

Figure 8 Contingency table Chi-squared analysis comparing control and *Goodyera pubescens* sites. A Chi-squared analysis was performed on soil texture class (A), soil fertility class (B), forest vegetation (C) and forest successional stage (D) datasets with control sites (n=15) and *Goodyera pubescens* sites (n=14). Results shown with Fisher's Exact Test (2-sided) p-values listed. Proportions are summed to a value of one separately for the control and target species sites.

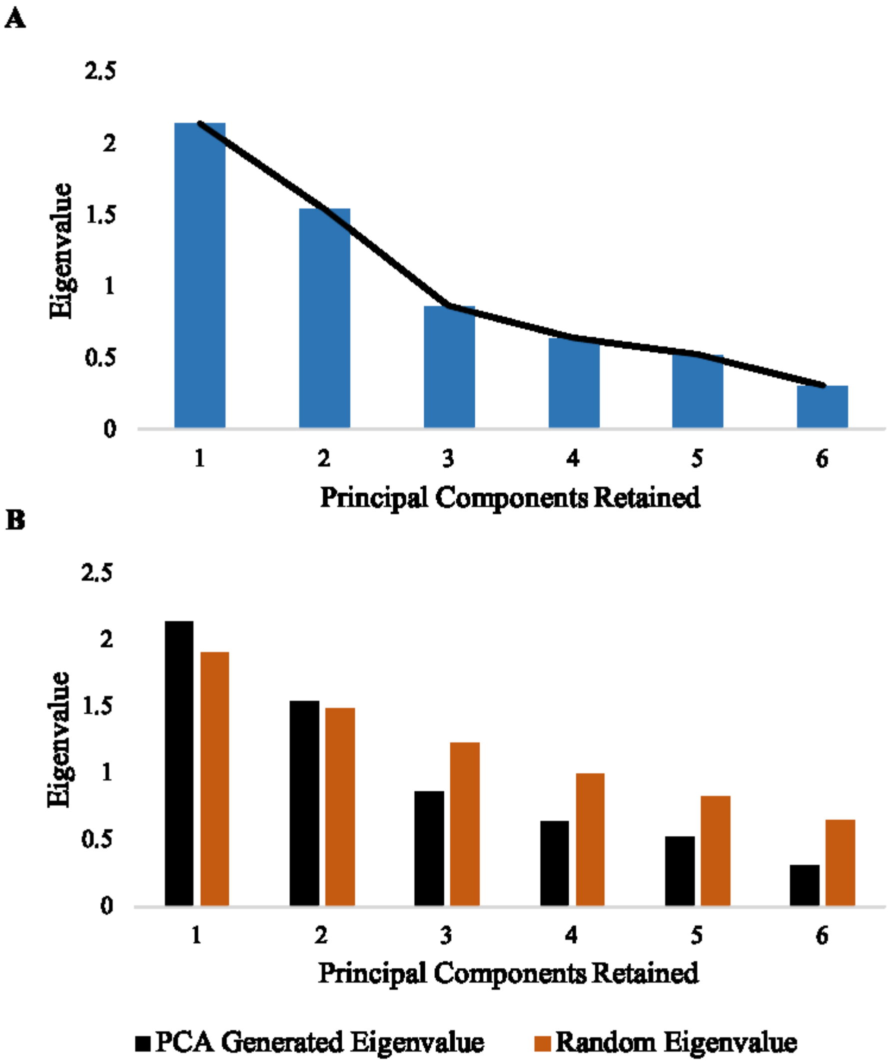

Figure 9 Principal component analysis (PCA) scree plot (A) and parallel analysis plot (B) generated from environmental variables of control sites (n=15) and *Goodyera pubescens* sites (n=15).

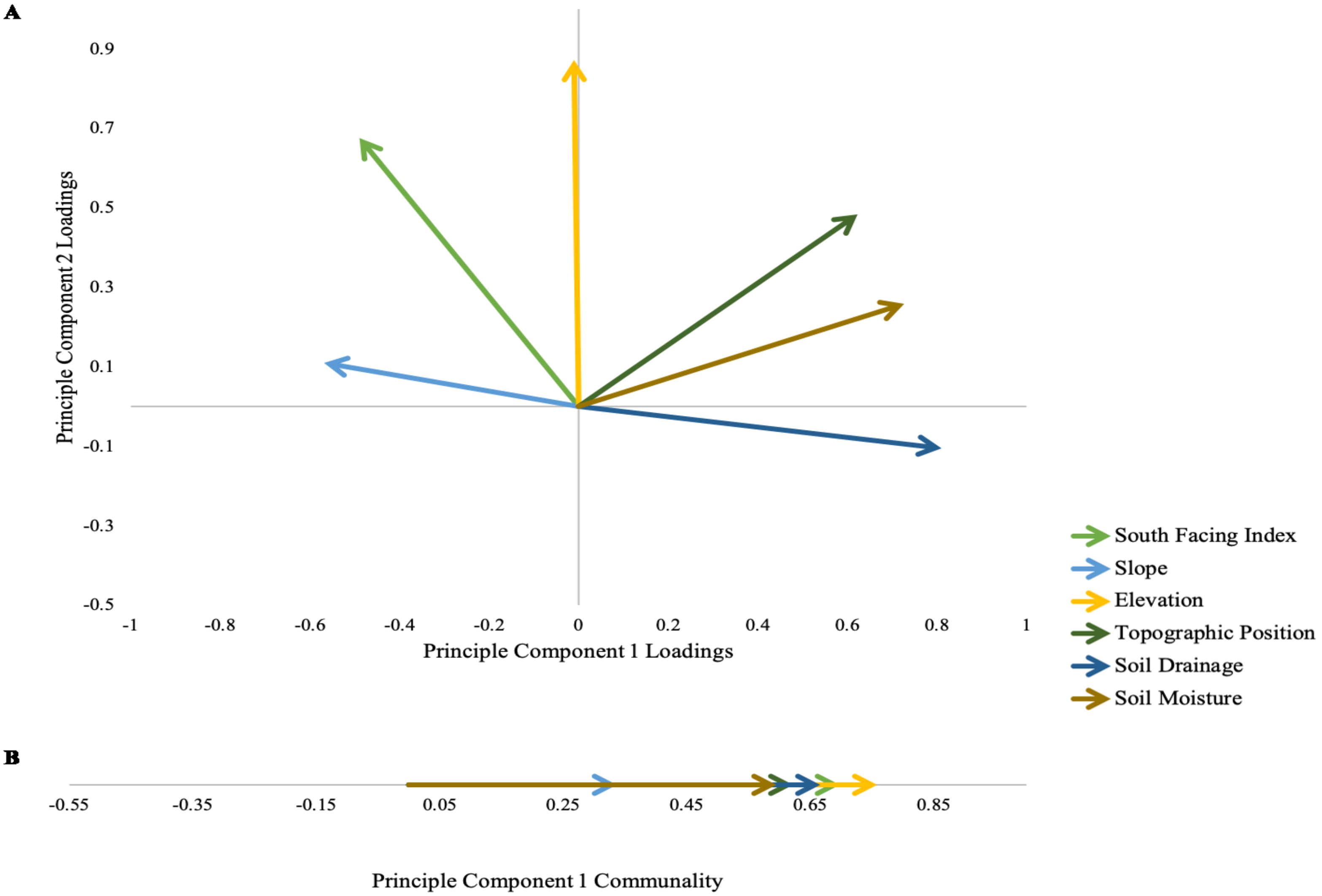

Figure 10 Principal component analysis loadings (A) and communality (B) values of environmental variables for control sites (n=15) and *Goodyera pubescens* sites (n=15).

Table 3 Generalized linear model (GLM) omnibus test results, test of model effects and parameter estimates of environmental variables from control sites (n=15) and *Goodyera pubescens* sites (n=15).

Environmental Variable	**Coefficient**	**Likelihood Ratio Chi-Squared Value**	**Degrees of Freedom**	**P-value**
Full Model		17.441	8	0.026
Factors and Their Levels		**Wald Chi-Squared Value**		
South-Facing Index		11.262	4	0.024
North	2.004	0.874	1	0.35
North West/ North East	1.431	0.452	1	0.501
West/ East	0.446	0.044	1	0.833
South West/ South East	-2.701	1.857	1	0.173
South	0*	N/A	N/A	N/A
Slope	-0.082	2.251	1	0.134
Soil Moisture		8.981	3	0.03
Fresh/Moist	-4.012	5.984	1	0.014
Fresh	-6.708	7.478	1	0.006
Dry	-6.426	5.019	1	0.025
Very Dry	0*	N/A	N/A	N/A

0* indicates the variable was set to zero because this parameter is redundant (it was the baseline coefficient).

Anemone americana

We found *Anemone americana* in 6 of the 15 sites we surveyed (Figure 1).

Non-Metric Multidimensional Scaling

All NMDS models for *Anemone americana* had raw normalized stress values less than 0.1 and Tucker's coefficient of congruence values greater than 0.95, indicating the models created a good repression of the dataset and a resilient fit (Figure 11). There was little separation between the control and *Anemone americana* sites in the canopy and subcanopy plant communities (Figure 11A and B). However, there was clear separation between the site types in the shrub and herb level plant communities (Figure 11C and D).

Within the canopy layer at the *Anemone americana* sites *Acer rubrum, Fraxinus americana* and *Picea rubens* occurred more frequently than other species (Appendix 4.1). In the subcanopy layer *Abies balsamea, Picea rubens* and *Acer rubrum* occurred most frequently (Appendix 4.2). For the shrub and herb layers *Abies balsamea, Acer rubrum* and *Picea rubens* as well as *Graminoids, Abies balsamea* and *Polystichum acrostichoides*, respectively, occurred most frequently (Appendix 4.3 and 4.4). In the canopy layer *Fraxinus americana* differed from the controls. The subcanopy and shrub did not differ from the controls, except for *Picea rubens* in the shrub layer. In the herb layer all the dominant species differed from the controls.

Contingency Table Chi-squared

Anemone americana sites were significantly different in soil texture and fertility compared to the control sites when analyzed with a contingency table Chi-squared test, p = 0.001 and 0.033 respectively (Figure 12A and B). *Anemone americana* most often grew in medium to fine textured, medium fertility soils (Figure 12A and B). There were no significant differences in forest vegetation and successional stage, p = 0.592 and 0.805 (Figure 12C and D). However, more *Anemone americana* sites were found in tolerant hardwood, late successional stage forests (Figure 12C and D). One site could not be classified into a Nova Scotia FEC vegetation type because it was partially harvested and the community did not fit well into a grouping.

Principal Component Analysis

Two PCs were retained based on the scree plot and parallel analysis for the *Anemone americana* PCA (Figure 13A and B). The greatest amount of variation across site environmental conditions was associated with soil moisture and drainage (Figure 14A and B). Slope and elevation were more closely correlated to PC2; topographic position and south facing index had roughly equal correlation to both PCs (Figure 14A and B).

Based on these results and review of the Pearson's correlation table (Appendix 2.3) we choose to include south facing index, slope and soil moisture in the GLM model.

Generalized Linear Model

An omnibus test could not be calculated for the *Anemone americana* GLM because the null model did not contain enough information to generate a dispersion parameter. Changes in *Anemone americana* populations were linked with south facing index and soil moisture, P-value = 0.008 and 0 respectively (Table 4). As sites become more south facing and drier the population of *Anemone americana* increased (Table 4). Slope was not associated with *Anemone americana* populations (Table 4.

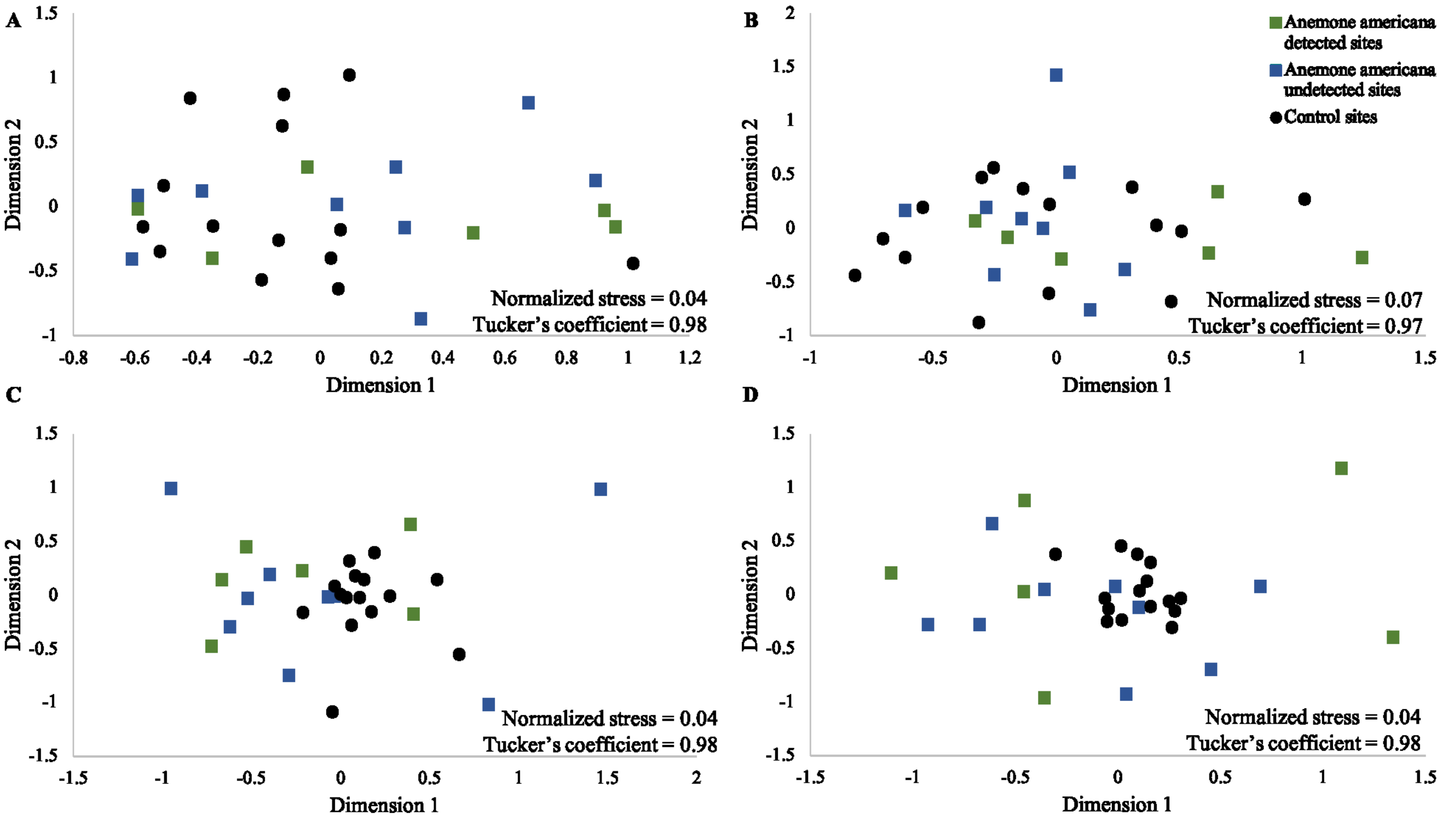

Figure 11 Non-metric multidimensional scaling (NMDS) biplot of control and *Anemone americana* detected and undetected sites. An NMDS was performed on the canopy (A), subcanopy (B), shrub (C) and herb (D) level vegetation cover datasets with the control sites (n=15), *Anemone americana* detected sites (n=6) and *Anemone americana* undetected sites (n=9). Results shown with model normalized raw stress and tucker's coefficient listed.

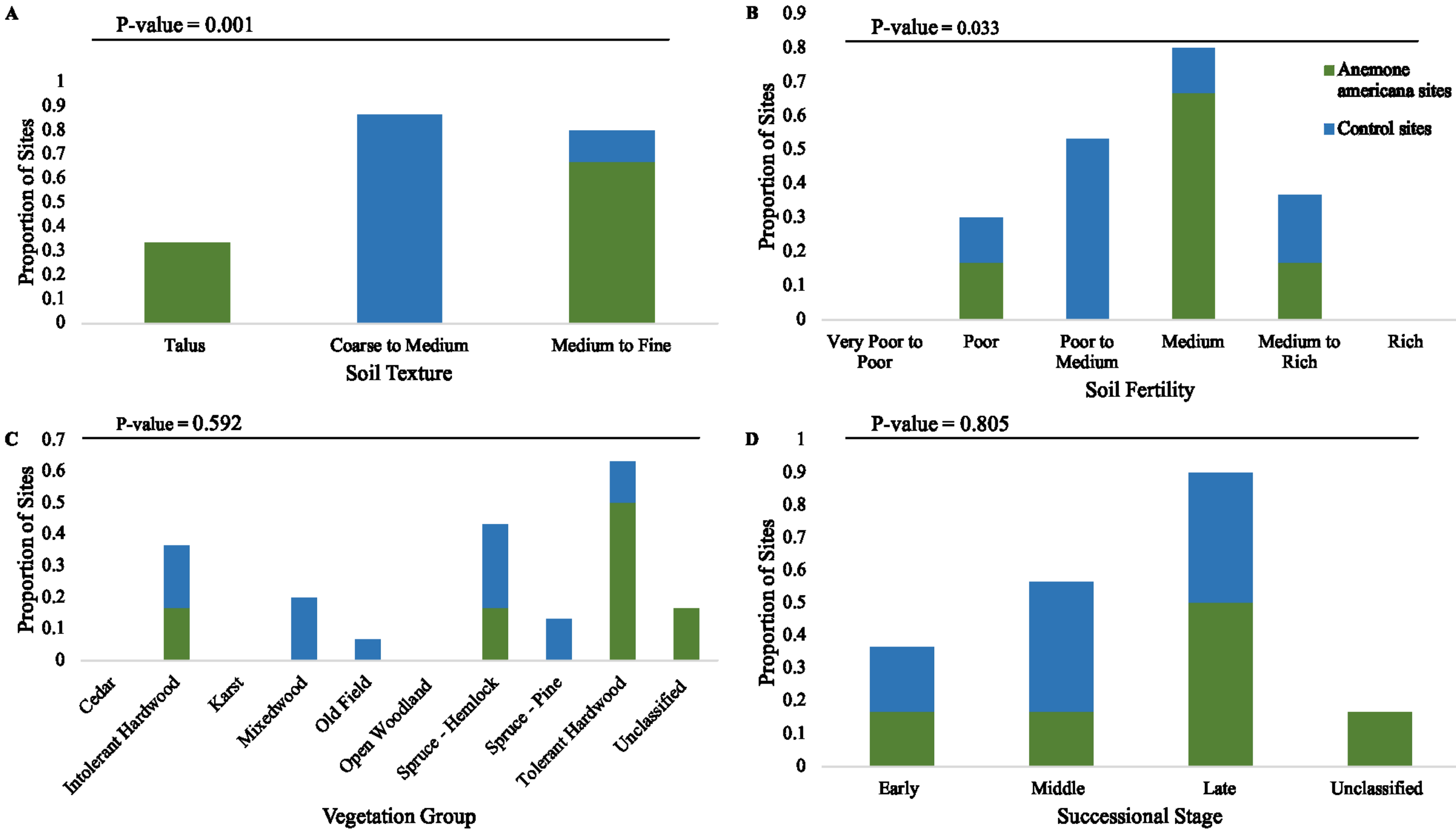

Figure 12 Contingency table Chi-squared analysis comparing control and *Anemone americana* sites. A Chi-squared analysis was performed on soil texture class (A), soil fertility class (B), forest vegetation (C) and forest successional stage (D) datasets with control sites (n=15) and *Anemone americana* sites (n=6). Results shown with Fisher's Exact Test (2-sided) p-values listed. Proportions are summed to a value of one separately for the control and target species sites.

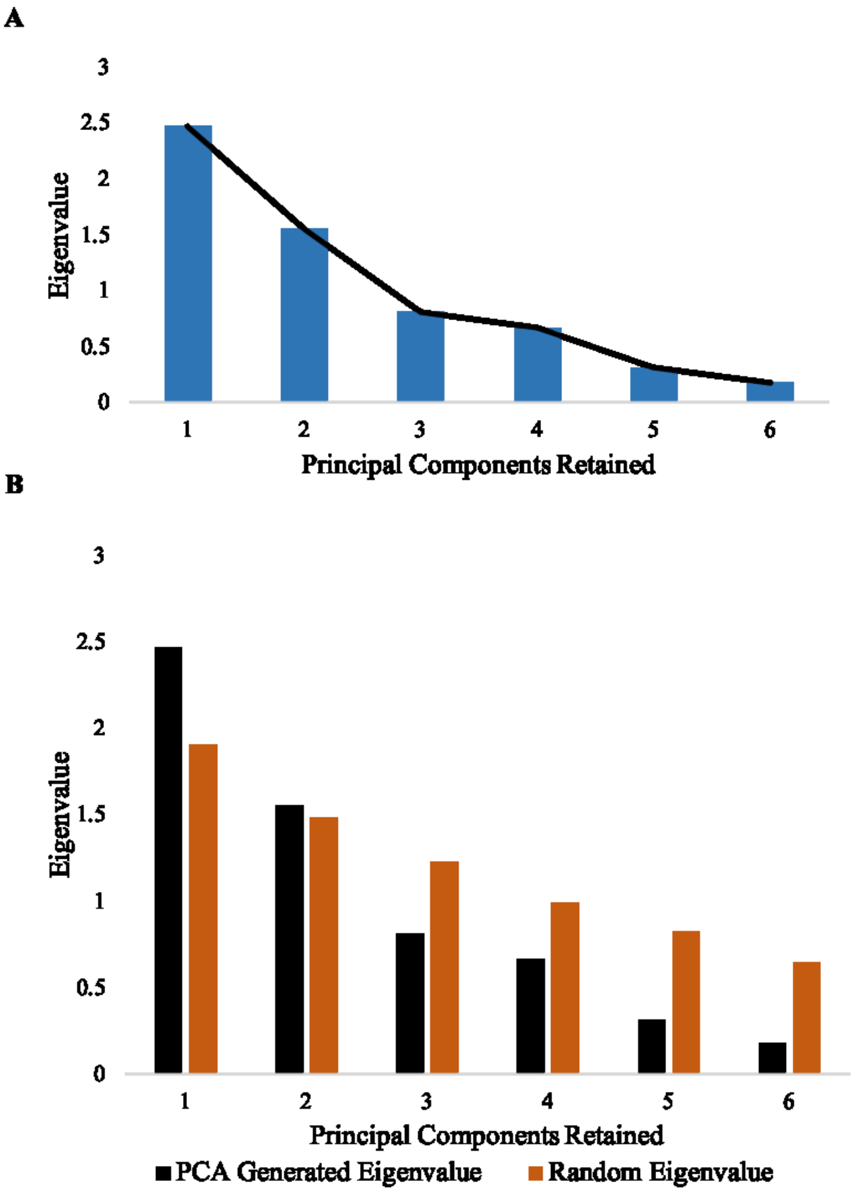

Figure 13 Principal component analysis (PCA) scree plot (A) and parallel analysis plot (B) generated from environmental variables of control sites (n=15) and *Anemone americana* sites (n=15).

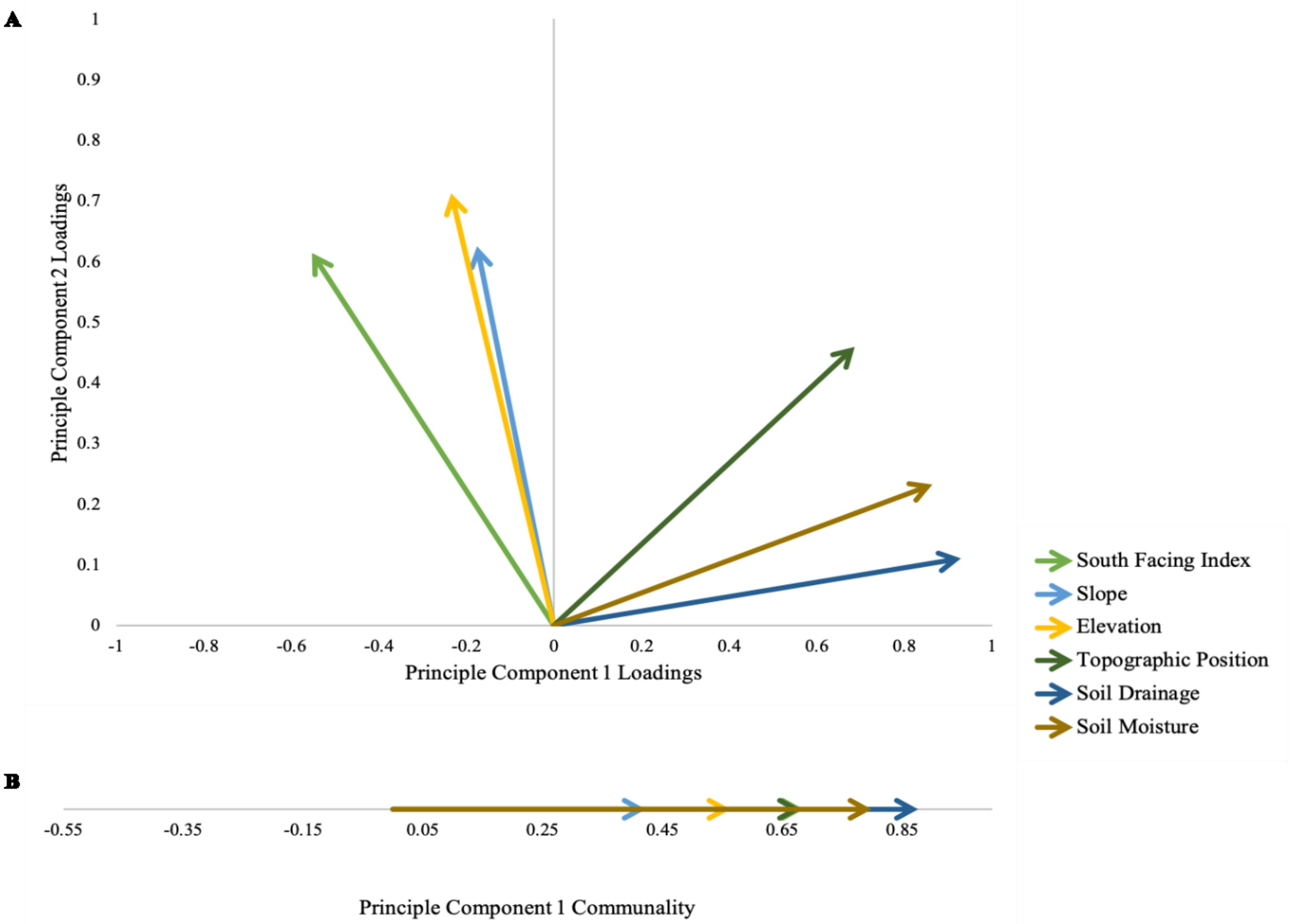

Figure 14 Principal component analysis loadings (A) and communality (B) values of environmental variables for control sites (n=15) and *Anemone americana* sites (n=15).

Table 4 Generalized linear model (GLM) omnibus test results, test of model effects and parameter estimates of environmental variables from control sites (n=15) and *Anemone americana* sites (n=15).

Environmental Variable	**Coefficient**	**Likelihood Ratio Chi-Squared Value**	**Degrees of Freedom**	**P-value**
Full Model		N/A	N/A	N/A
Factors and Their Levels		**Wald Chi-Squared Value**		
South Facing Index		6.955	1	0.008
North	-0.552	0.169	1	0.681
North West/ North East	-0.306	0.04	1	0.842
West/ East	1.571	1.381	1	0.24
South West/ South East	2.068	0.762	1	0.383
South	0*	N/A	N/A	N/A
Slope	0.039	0.889	1	0.346
Soil Moisture		25.376	5	0
Moist/ Wet	0.313	0.015	1	0.904
Moist	0.582	0.056	1	0.812
Fresh/ Moist	-1.338	0.252	1	0.615
Fresh	-2.516	3.055	1	0.08
Dry	3.433	3.409	1	0.065
Very Dry	0*	N/A	N/A	N/A

0* indicates the variable was set to zero because this parameter is redundant (it was the baseline coefficient).

CHAPTER 4 DISCUSSION

Our research set out to identify broad habitat characteristics of three rare forest plants: *Anemone americana*, *Conopholis americana* and *Goodyera pubescens*. Specifically, we focused on the vegetation, soil and community, and the environment of these species' habitats with three corresponding objectives.

Forest Vegetation

We found that the plant communities in the shrub and herb vegetation layers were different than control sites for all three target species and this may indicate that each target species is part of a distinct plant community. This is supported by our preliminary results showing that the control sites had mostly different dominant plant species in the four vegetation layers compared to the target species sites.

Conopholis americana

We found *Conopholis americana* most often growing in shade intolerant forests with medium to fine textured soils of medium fertility that were in an early successional stage. Cancer root populations were highest in better drained soils and when facing south. These findings are not surprising considering *Conopholis americana* is a parasitic plant to oak trees. Nova Scotia only has one native oak species, northern red oak (Munro et al., 2014), so we expected that cancer root would be associated with conditions favoured by red oak.

Red oak grows best in deep, rich loam or silt loam (medium to fine-textured) soils that are well-drained. They prefer middle and lower slopes with north, north-easterly aspects (Munro et al., 2014; Nesom & Kat, 2003). It is a versatile tree species and can grow in many forest types, sometimes even dominating the canopy but usually they are part of a mixed forest community. Red oak has an intermediate shade tolerance and is often found in early to middle successional communities (Munro et al., 2014; Nesom & Kat, 2003).

Cancer root is associated with mixed wood forests on well drained soils that have some component of northern red oak (Deshaye et al., 2019; Haynes, 1971; Inman & Pelton, 2002; Musselman, 1982; Percival, 1931; Rodrigues et al., 2016). Interestingly, it is associated with both north, northeast slopes, like red oak, (Huebner & McGill, 2018; Olivero & Hix, 1998) and south facing slopes (Deshaye et al., 2019).

Our results that cancer root was associated with south facing slopes matches the less often reported findings of Deshaye et al. (2019). Interestingly, their research was in Quebec, Canada while the other studies reporting cancer root in north-facing slopes were done in the United States. These apparently conflicting results may reveal a survival advantage for cancer root when facing south in the northern portion of its distribution. South facing slopes receive a higher duration and intensity of sunlight which create a warmer and drier forest soil (Desta et al., 2004; Searcy et al., 2003; Warren, 2008). These conditions may promote growth, or the increased light levels may benefit the host oak tree, which in turn could benefit cancer root. Cancer root has been reported to be sensitive to desiccation (Musselman, 1982) so further investigation is needed to clarify the relationship between cancer root and aspect. Another, less speculative explanation is that our sampling sites by chance captured an unrepresentative sample of cancer root and therefore did not accurately reflect its growth preferences, at least concerning aspect.

At first glance, it appears that our results of cancer root mostly growing in shade intolerant hardwoods go against most reports finding it in predominately mixed forests. However, this is not the case. The Nova Scotia FEC mixedwood forest group is defined by a canopy with deciduous and coniferous tree components between 25% and 75% (Neily et al., 2013). In the literature, mixed forest is used more freely and can include any forest that has some component of deciduous and coniferous trees. Therefore, some forests that would be considered mixed wood in the literature are not in the FEC. The shade intolerant and tolerant forest groups, not the mixedwood, are the forest groups most often associated with northern red oak in the Nova Scotia FEC. The mixedwood group is instead associated with birch and maple species and to a lesser extent balsam fir (Neily et al., 2013). When we consider the types of Nova Scotian forest communities with red oak and its general versatility, our results match the forest groups we would expect to find cancer root growing in.

Limitations with Research on Conopholis americana

A possible limitation of our cancer root research was that we relied on counting the flowers as a measurement for the number of individuals, which in turn was used as a metric for species success in an area. Although almost no work has investigated the connection between cancer root flowers and below ground biomass or abundance, Baird (1986) reported that the flowers may account for roughly 40% of the population. This estimate needs validation as it likely fluctuates with growing conditions. Future work should address this. Unfortunately, this limitation is unavoidable unless researchers harvest all the cancer root biomass in an area. This should be avoided considering cancer root is a species of conservation concern.

The high degree of clustering in our cancer root sites was likely another limitation to our results. Eight of the 13 cancer root sites were in Kejimkujik National Park and Historic Site. This clustering may have introduced unintentional pseudoreplication into our study and influenced our results. The minimum distance between our closest cancer roots sites was over 150 m, however all the sites within Kejimkujik likely share similar landscape conditions. We made efforts to capture as many sites outside of the park as possible but unavoidably, most known cancer root sites in Nova Scotia are within Kejimkujik.

Goodyera pubescens

We found *Goodyera pubescens* growing in several forest communities, mostly shade tolerant deciduous forests, in medium to fine textured soils of medium to rich fertility in a late successional stage. Downy plantain was most abundant in dryer upland soils and when facing north.

Our results matched reports that downy plantain grows under various canopy species, typically deciduous trees in a late successional staged forest (Brackley, 1985; Lamont & Stalter, 2007; McCormick et al., 2012, 2016; Reddoch & Reddoch, 2013; Sorrells & Warren, 2011; Warren, 2007, 2008; Whigham, 2004). Our findings that downy plantain grew best in dry soils that were facing north goes against reports that it prefers growing in moist, but not saturated, south facing forest soils (Diez, 2007; Olivero & Hix, 1998; Sorrells & Warren, 2011).

An orchid’s strong dependence on soil fungi for nutrients can help to explain a lot of our results. All orchids are obligate mycoheterotrophs; they get some or all their food from parasitizing soil fungi. This relationship can last for several months or the entire lifetime of an orchid and usually requires specific fungal hosts (McCormick et al., 2004, 2012; Rasmussen & Whighman, 1998; D. Taylor et al., 2002). Due to this reliance, an orchid's distribution is often limited by the distribution of its host (McCormick et al., 2012). Downy plantain follows this trend and is limited by environmental factors that influence its fungal host species, *Tulasnella* spp. (McCormick et al., 2004, 2012). This includes factors such as forest successional stage, which has both direct effects on downy plantain abundance by improving seed germination success and an indirect effect by supporting a higher abundance of its fungal host (McCormick et al., 2012, 2016; Olivero & Hix, 1998). *Tulasnella* spp. grow in earlier successional forests, but tends to have less diversity and lower abundances, even when supplemented into the soil (McCormick et al., 2012). *Tulasnella* spp. are saprotrophic, they breakdown nonliving organic matter for nutrients, and are reliant on decomposing deadwood (Leake et al., 2002; McCormick et al., 2004). Late successional forests tend to have higher proportions of deadwood (Bujoczek et al., 2018; Moroni & Ryan, 2010) and this is likely why these forests have higher abundances of *Tulasnella* spp.

The orchid- mycorrhizae connection may also help explain our results related to soil texture, fertility and site aspect. *Tulasnella* spp. is associated with moist and rich forest soils (Diez, 2007). Fine textured soils have higher fertility because of their ability to capture and hold more water and other soil nutrients (O'Geen, 2013). Fine soils also tend to have higher amounts of deadwood and organic matter because they support more productive communities which in turn increases soil nutrient and moisture levels (Prassad & Power, 1997; Rice, 2002). North facing soils also tend to be wetter because of reduced evaporation (Warren, 2008). Thus, richer, fine textured, north facing soils would have more moisture and nutrients available for *Tulasnella* spp., which in turn could support higher abundances of downy plantain. This can also help to explain why downy plantain is sensitive to drought, especially in thin soils (McCormick et al., 2004; Reddoch & Reddoch, 2013).

The orchid- mycorrhizae connection and previous findings call into question our results that downy plantain grows best in dry forest soils. Based on what we currently know about this species our results seem unlikely, but more research should be done to further investigate the relationship between downy plantain and soil moisture. Our results might reflect our limited sample sizes rather than an accurate description of the species.

Our results found that downy plantain grew in several forest groups, including forests dominated by deciduous and coniferous trees. This matches the limited reports that it grows in mixed wood, pine- oak and deciduous forests (Brackley, 1985; Lamont & Stalter, 2007; Sorrells & Warren, 2011). It is a fast colonizer species in deciduous forests (Sorrells & Warren, 2011), is associated with single burn sites (Huebner & McGill, 2018) and is shade tolerant (Warren, 2008). Together, these findings indicate downy plantain can grow in several different forest communities but strongly associates with late successional deciduous forests, as overviewed above.

Although we found downy plantain in a variety of forest communities, most of the sites were in shade tolerant deciduous forests. This is likely from several factors. First, shade tolerant trees form late successional staged forests because their seedlings can grow under the canopy of shade intolerant trees (Barbour, Michael, 1999). Deciduous forests also tend to grow in richer, finer textured, moist soils with high concentration of organic matter (Adams et al., 2019). Thus, these two factors likely benefit downy plantain through the mycorrhizae- orchid connection. Deciduous forests also have higher levels of annual available light for evergreen plants than coniferous forests. Evergreen plants grow and reproduce the most in the fall and spring before trees unfurl their leaves (Whigham, 2004). This needs to be verified for downy plantain but it does offer a reasonable explanation.

Anemone americana

We found *Anemone americana* most often growing in medium to fine textured, medium fertility soils under shade tolerant deciduous forests in a late successional stage. Round hepatica populations were highest in dryer upland soils and when facing south.

Our soil texture and fertility results match other reports that round hepatica prefers richer, fine textured soils (Angelo & Boufford, 2011; Pivorunas, 2013) but can also grow in shallow soils (Inghe & Tamm, 1985). Round hepatica’s preference for richer soils is expected when we consider its life history strategy. Hepatica is a spring ephemeral; it quickly grows leaves and flowers before or shortly after snowmelt to take advantage of the intense sunlight and reduced competition in the understory (Lapointe, 2001). To deal with this short growing window, spring ephemerals have evolved a high photosynthetic rate to quickly produce carbohydrates for growth, reproduction and storage in underground tissues (Lapointe, 2001; Risser & Cottam, 1968; Sparling, 1967; R. J. Taylor & Pearcy, 1976). To be able to support this high metabolism, spring ephemerals need higher concentrations of nutrients, especially nitrogen, compared to other forest herbs (Anderson & Eickmeier, 2011; Muller, 1978; Nault & Gagnon, 1988). In forests with nutrient poor mineral soil, spring ephemerals are rare (Rogers, 1982).The high nutrient demand of spring ephemerals explains why round hepatica was most often found in rich, fine textured soils. Only by growing in these conditions would hepatica be able to sustain its short, but intense, metabolism.

We also found that round hepatica most often grows in shade tolerant deciduous forests. Our findings mirror reports of round hepatica growing in deciduous dominated or mixed wood forests with a significant deciduous component (Bruce Trail Conservancy, 2017; Inghe & Tamm, 1985; Minnesota Wildflower Society, 2006; Motten, 1982; Podgórska, 2010; Tamm, 1956). These results can also be explained by round hepatica being a spring ephemeral. Round hepatica relies on the short growing window in early spring. In deciduous forests, around 60% of the above canopy light levels reach the forest floor in the early spring. However, when deciduous tree leaves are unfurled or in conifer forests, light levels can drop down to as low as 1% (Endler, 1993; Packham & Willis, 1982). Spring ephemerals are not able to grow in year-round shade (Eickmeier & Schussler, 1993) and are rare in conifer forests (Rogers, 1982).

To our knowledge, no work has investigated the connection between forest successional stage and round hepatica. However, our results that round hepatica grows mostly in late successional staged forests are expected, again considering it is a spring ephemeral. As discussed with *Goodyera pubescens*, late successional forests tend to have higher proportions of deadwood, which in turn increases soil nutrients (Bujoczek et al., 2018; Moroni & Ryan, 2010) and better supports its high nutrient needs.

Our result that round hepatica had higher populations in dryer soils matched some reports (Angelo & Boufford, 2011; Minnesota Wildflower Society, 2006) but most suggest it grows best in intermediate soil moistures (Inghe & Tamm, 1985; Sorrells & Warren, 2011; Warren, 2007) or even that it prefers moist soils (Kimberley, 2012). Our results are interesting considering spring ephemerals are less able to deal with environmental stressors than other herbs (Augspurger & Salk, 2017; Mahall & Bormann, 1978; Rothstein & Zak, 2001). The intense metabolic activity of spring ephemerals combined with their high transpiration rates, low root biomass and the cold spring soil temperatures creates a high-water demand (Lapointe, 2001; R. J. Taylor & Pearcy, 1976). Even in forests with rich soil and lots of available water, spring ephemerals are sensitive to soil water and nutrient limitations (Lapointe, 2001; Rogers, 1982). Our results could have been influenced by an outlier in our dataset that had a very high population of hepatica, roughly four times the second highest populous site, and had a dry, coarse textured soil. This may have inaccurately inflated our results, but this should not invalidate our findings considering the success of this population, it had close to 2000 hepatica plants. Another explanation comes from the methods we used to record a site's soil moisture. When we classified a site according to the Nova Scotia FEC it was done at the center of the plot to be the most representative of the overall community and it did not incorporate microsite conditions. Round hepatica could grow best in moister soil, as supported by the literature, at a microsite level within our sites but when we characterized them at a broader scale, we identified them as dry.

We found hepatica had the highest populations when facing south. This contrasted previous findings that hepatica had higher survival and was more frequently found on north facing slopes (Warren, 2007, 2008). Our results may be due to the limited sample size we had for hepatica, which could have misrepresented the species, discussed below. However, considering round hepatica is a spring ephemeral and can be restricted by low light levels (Eickmeier & Schussler, 1993) it is interesting that it may prefer north facing habitats that would have less sunlight and cooler temperatures (Galicia et al., 1999; Geiger, 1951; Radcliffe & Lefever, 1981; Searcy et al., 2003). These conflicting results may be related to south facing slopes being warmer and therefore having faster evaporation rates causing dryer conditions (Bolstad et al., 1998; Cantlon, 1953; Desta et al., 2004; Shanks & Norris, 1950; Werling & Tajchman, 1984), or some other factors might be at play. More work needs to be done in Nova Scotia and elsewhere to validate these results and clarify the situation.

Limitations with Research on Anemone americana

The major limitation for our research on round hepatica was that of the 16 sites we surveyed, we were only able to confirm it in six. The low sample size, especially compared to the other target species, reduced the power of our analysis and created some uncertainty around our findings. In the future, our sampling sites should be revisited to validate hepatica's absence in these areas and investigate reasons for its local extirpation.

Our control sites were selected in Southwest Nova Scotia because we wanted to limit the resources it would take to sample them. However, many of our *Anemone americana* sites were outside of Southwest Nova Scotia and this may have introduced spatial variation into our data. Considering there are differences in the geology, climate and forests of Colchester and Hants counties compared to Southwest Nova Scotia (*Natrual Landscapes of Nova Scotia: Summary Descriptions*, 2002; Neily et al., 2013) this could have contributed to the differences we found between the control and target species sites. Future work needs to either add controls sites in areas outside of Southwest Nova Scotia or develop a new sampling method.

Control Sites

Our control sites were mostly in coarse to medium textured soil with a poor to medium fertility under a range of forest vegetation groups in a middle to late successional stage.

It is somewhat unclear how well our controls represent typical forest conditions in Southwest Nova Scotia. The Nova Scotia FEC soil section (Neily et al., 2013) indicates that most forests in this part of the province have coarse to medium soil textures with poor to medium fertility. Most forests in the province are conifer dominated or are a mix of deciduous and conifer species, in early to middle successional stages (Basquill & Baldwin, 2020; Loo & Ives, 2003; Loucks, 1962; Munro et al., 2014; Nova Scotia Department of Lands and Forests, 2016; A. R. Taylor et al., 2020). Overall, our controls seem to have represented upland forests in Southwest Nova Scotia fairly well although they were in a later successional stage than typical.

Mechanisms of Plant Rarity and Connections to Target Species

If a factor interferes with a plant occupying any aspect of its niche, such as habitat, life-form, phenology and regeneration, it will impact survival and could lead to the species rarity (Gravel et al., 2011). Many factors can cause a species to become rare (Gravel et al., 2011; Poot & Lambers, 2003; Prendergast et al., 1993; Tittensor et al., 2010) but plant rarity is usually linked to intrinsic restraints, dispersal capabilities and establishment, herbivores, forest disturbance, abiotic factors and habitat (Bricker & Maron, 2012; Clark et al., 2007; DiTommaso et al., 2014; Maron et al., 2014; Mooney & McGraw, 2009; Olsen & Klanderud, 2014; Thomson, 2005; Turnbull et al., 2000; Wamelink et al., 2014). Many of the limitations on plant species are becoming increasingly connected to human activities (Brook et al., 2008; Corlett, 2016; Dávalos et al., 2014; Sala et al., 2000; Tylianakis et al., 2008) but for many rare plants, they are not well understood.

Our project did not set out to identify the mechanisms causing our target species to be rare. Instead, we examined the habitat ecology of these species with the hopes of clarifying if some of their habitat preferences might be limiting them. Our results that the habitat of *Conopholis americana* closely matched that of its red oak host and that *Anemone americana* relied on deciduous forests with rich soils did not provide a lot of insight into their rarity considering red oak and rich deciduous forests are common. However, our finding that *Goodyera pubescens* grew best in late successional deciduous forests did give us some indication of why it is rare. Forestry, agriculture and habitat fragmentation across North America has heavily impacted forest ecosystems and promoted younger, earlier successional forests (Pan et al., 2011), likely restricting downy plantain. Another factor that is likely contributing to the rarity of our target species in Nova Scotia is that all three plants are in the northern extent of their range and the colder climate may be limiting them.

More work needs to build upon our research to get a clearer understanding of why our target species, and other rare forest plants, are rare. Other mechanisms or other habitat features not covered in our research are likely limiting them.

We encountered the following topics during our research, but they are just some of the many possible mechanisms related to the rarity of our target species. Future research must investigate them, as well as others.

Cancer root's seed dispersal is unlikely to be a limiting factor to the species because it is mediated by two common North American mammals; white-tailed deer (*Odocoileus virginianus*) and American black bear (*Ursus americanus*) (Inman & Pelton, 2002; A. Johnson et al., 1995). Although cancer root seeds have low germination rates, each capsule contains hundreds of seeds and each inflorescence contains dozens of capsules, helping to offset the low germination rate (Baird & Riopel, 1986).

Cancer root may be limited because of an interaction with mycorrhizae. Sander et al. (1992) showed that the colonization of mycorrhizal fungi on a plant can influence if that plant becomes parasitized by another plant species. Considering the mycorrhizal community plays an extensive and powerful role in plant life (Bever et al., 2010; Clay & Packer, 2000; Mills & Bever, 1998; Saikkonen et al., 1998; Schneider et al., 2012; van der Heijden et al., 2008) this relationship offers possible mechanisms limiting parasitic plant species.

Anemone americana is a self-fertilizing herb so pollination is not a limiting factor (Native Plant Trust, 2021). However, it does rely on ants for seed dispersal and is not capable of reproducing through vegetative means (Warren & Lake, 2013). Ant based seed dispersal is a slow process that only advances about 1 m a year (Cain et al., 1998; Oberrath & Bohning-Gaese, 2002; Sorrells & Warren, 2011). If seed dispersal is limited it can prevent a plant from reaching new habitat, no matter its suitability (Ehrlen & Eriksson, 2000; Matlack, 1994; Mitchell et al., 2002).

Rare Plant Conservation

Plant species are facing growing threats to their survival because of human activities and rare plants maybe more at risk (Butchart et al., 2010; Díaz et al., 2006;

Reich et al., 2001; Tilman et al., 2006; Wamelink et al., 2014). The biggest threats to plants come from habitat loss, invasive species, unsustainable harvesting and climate change (Bohlen et al., 2004; Collard et al., 2010; Corlett, 2016; Côté et al., 2004; Fisichelli et al., 2013; Tanentzap et al., 2012; Thuiller et al., 2005). Many species are now facing several threats at once and these factors often interact in synergistic ways, exacerbating their overall impact (Dávalos et al., 2014; Didham et al., 2007).

Temperate forest ecosystems have been one of the most heavily impacted ecosystems in North America because of forestry, land development and fragmentation and very few areas are now left in pristine condition (Sharik et al., 2010). North American forests are much younger and in an earlier successional stage than would naturally occur (Pan et al., 2011), especially in Nova Scotia which has as little as 0.01% of the province remaining in old growth condition (Loo & Ives, 2003; Stewart et al., 2003). Past forest disturbances may also be having long term lingering effects that we have yet to see the full extent of (Honnay et al., 2005).

Forest ecosystems are facing enormous challenges to their ecosystem stability and many of the species that live within them are declining or disappearing. The need to have a comprehensive understanding of plant species habitats is critical, especially for rare plants, if we are to conserve them. We also need immediate and widescale changes in how we interact with forests and their species to prevent them from being further damaged.

Project Limitations and Future Work

The simplest limitation in our project to rectify is to further analyze the differences in the shrub and herb vegetation layers between the control and target species sites. Future work can begin by analyzing the biodiversity of the plant communities with species richness and evenness counts or by calculating a biodiversity metric like the Shannon-Wiener diversity index.

Our fieldwork was restrained by the difficulty we faced consistently finding our target species and this prevented us from recording more sampling sites. If we did not have access to previous sightings of our species this project would have been very limited. Developing resources to identify and monitor rare plant species in Nova Scotia, and elsewhere, would be beneficial to all future work.

Our data collection relied on one sampling season between May and November of 2019 and was only done once at each site, which could have introduced seasonal variation into our dataset. For instance, because we only started sampling in May, we could have missed spring ephemeral species that did not retain tissue above the soil surface later into the year. The differences in the vegetation between control and target species sites might have also been influenced by changes in plant abundances as the growing season progressed. However, because we knew the locations of our sampling sites before starting the field season, we were able to sample all sites in an area (regardless of site type) before moving onto a new area of the province. Often this meant sampling several site types within the same week. This did not completely remove seasonal variation from our data, but it does help to diminish it. Future work can further reduce seasonal influences by sampling each site several times throughout the growing season.

Although we found our control sites represented typical upland forests in Southwest Nova Scotia, a more efficient approach could involve designing paired control and experimental plots. First, the target species site could be identified, and its habitat analyzed, then a nearby area with similar characteristics but without the target species could be identified and sampled. This approach may reveal features of rare plant habitat not captured in our design, such as microsite conditions.

We converted site aspect into a new categorical variable, called the south facing index, to analyze it. However, our approach reduced the ability of our models to differentiate between east and west and may have introduced a bias because the categories were relative measures of direction. A more appropriate approach would make compass bearings relative to ±180°. For instance, 270° (W) becomes -90° and 90° (E) stays 90°.

A common limitation in ecological studies is the sample size, our work is no exception, especially with our analysis of *Anemone americana*. Future work to incorporate more samples into our dataset would greatly benefit the power of our analyses.

To help alleviate our small sample sizes we treated detected and undetected sites in the same way for our PCA and GLM analysis. We did this because the target species had been detected by other researchers as part of the Atlantic Canada Conservation Data Centre databases. We assumed the habitat conditions were unlikely to have changed substantially in the last 19 years (the maximum amount of time between our field sampling and the last time the target species were recorded). However, this might not be true and could have influenced our results.

Our work has provided critically needed details about broad scale habitat characteristics of our target species. There are many more aspects of a plant species habitat outside of our scope that needs to be addressed. This could include but is not limited to microhabitat conditions, soil nutrients, mycorrhizal associations, relationship with disturbance and impacts from harvesting. There also needs to be a better understanding of why our target species are rare. Current work is not sufficient to explain their scarcity. To get a reasonable understanding of their rarity more research needs to be done to address all aspects of their niche, including habitat, life-form, phenology and regeneration.

Research Importance

Anemone americana, *Conopholis americana* and *Goodyera pubescens* cover large ranges across eastern North America and are considered rare in a lot of their jurisdictions. Yet, very little work has comprehensively investigated their habitat even on a broad scale.

Our research was the first of its kind to give a detailed investigation into the abiotic and biotic conditions of these species' habitat in Nova Scotia, Canada. It has provided robust baseline information that can be refined with future projects to give a detailed understanding of these species' habitats.

Conclusion

The forest understory plant community is often marginalized in research, yet it plays an important role in many aspects of a forest ecosystem including in nutrient cycling, biodiversity and succession following disturbance. Forest ecosystems in North America have been heavily altered by human activities and the coming century poses increasing threats if they are not seriously addressed. This adds growing pressure on many rare forest plants, which have not been investigated sufficiently to understand the mechanisms behind their scarcity. Specific habitat requirements are a major driver of plant rarity and our work focused on this area by investigating the habitat characteristics of three rare forest plant species, *Anemone americana*, *Conopholis americana* and *Goodyera pubescens* in Nova Scotia, Canada. We have shown that *Conopholis americana* is associated with shade intolerant forests with medium to fine textured soils of medium fertility that were in an early successional stage and therefore closely matched the habitat preferences of its red oak host. *Goodyera pubescens* mostly grew in shade tolerant deciduous forests, with medium to fine soils of medium to rich fertility in late successional stages; its preferences likely influenced by its mycorrhizal host. Finally, we identified that *Anemone americana* predominately grew in shade tolerant deciduous forests with medium to fine textured, medium fertility soils in late successional stage. This habitat reflected its needs to sustain the high metabolic demands of a typical spring ephemeral. Our work has been the first to broadly investigate the habitat of our target species, especially in a Nova Scotian context. Previous research has only addressed specific aspects of their habitat and not an overall picture. More research needs to build upon our work to develop a detailed overview of these and other rare plant species so that they can be conserved across their distributions.

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APPENDIX 1 IMAGES OF *ANEMONE AMERICANA* (A), *CONOPHOLIS AMERICANA* (B) AND *GOODYERA PUBESCENS* (C)

APPENDIX 2.1 PEARSON'S CORRELATION MATRIX FOR *CONOPHOLIS AMERICANA* ENVIRONMENTAL VARAIBLES (N=29)

		South Facing Index	**Slope**	**Elevation**	**Topographic Position**	**Soil Drainage**	**Soil Moisture**
South Facing Index	Pearson Correlation	1					
	Sig. (2-tailed)						
Slope	Pearson Correlation	0.02	1				
	Sig. (2-tailed)	0.919					
Elevation	Pearson Correlation	0.047	0.078	1			
	Sig. (2-tailed)	0.81	0.688				
Topographic Position	Pearson Correlation	-0.107	-0.064	0.113	1		
	Sig. (2-tailed)	0.58	0.741	0.559			
Soil Drainage	Pearson Correlation	-0.36	-0.387*	-0.237	0.19	1	
	Sig. (2-tailed)	0.055	0.038	0.217	0.324		
Soil Moisture	Pearson Correlation	-0.127	-0.238	-0.228	-0.067	0.638**	1
	Sig. (2-tailed)	0.512	0.215	0.234	0.73	0	

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).

APPENDIX 2.2 PEARSON'S CORRELATION MATRIX FOR *GOODYERA PUBESCENS* ENVIRONMENTAL VARAIBLES (N=30)

		South Facing Index	**Slope**	**Elevation**	**Topographic Position**	**Soil Drainage**	**Soil Moisture**
South Facing Index	Pearson Correlation	1					
	Sig. (2-tailed)						
Slope	Pearson Correlation	0.296	1				
	Sig. (2-tailed)	0.112					
Elevation	Pearson Correlation	0.406*	-0.024	1			
	Sig. (2-tailed)	0.026	0.898				
Topographic Position	Pearson Correlation	-0.002	-0.231	0.266	1		
	Sig. (2-tailed)	0.99	0.219	0.155			
Soil Drainage	Pearson Correlation	-0.289	-0.301	-0.165	0.382*	1	
	Sig. (2-tailed)	0.121	0.106	0.385	0.037		
Soil Moisture	Pearson Correlation	-0.204	-0.155	0.194	0.370*	0.504**	1
	Sig. (2-tailed)	0.279	0.412	0.305	0.044	0.005	

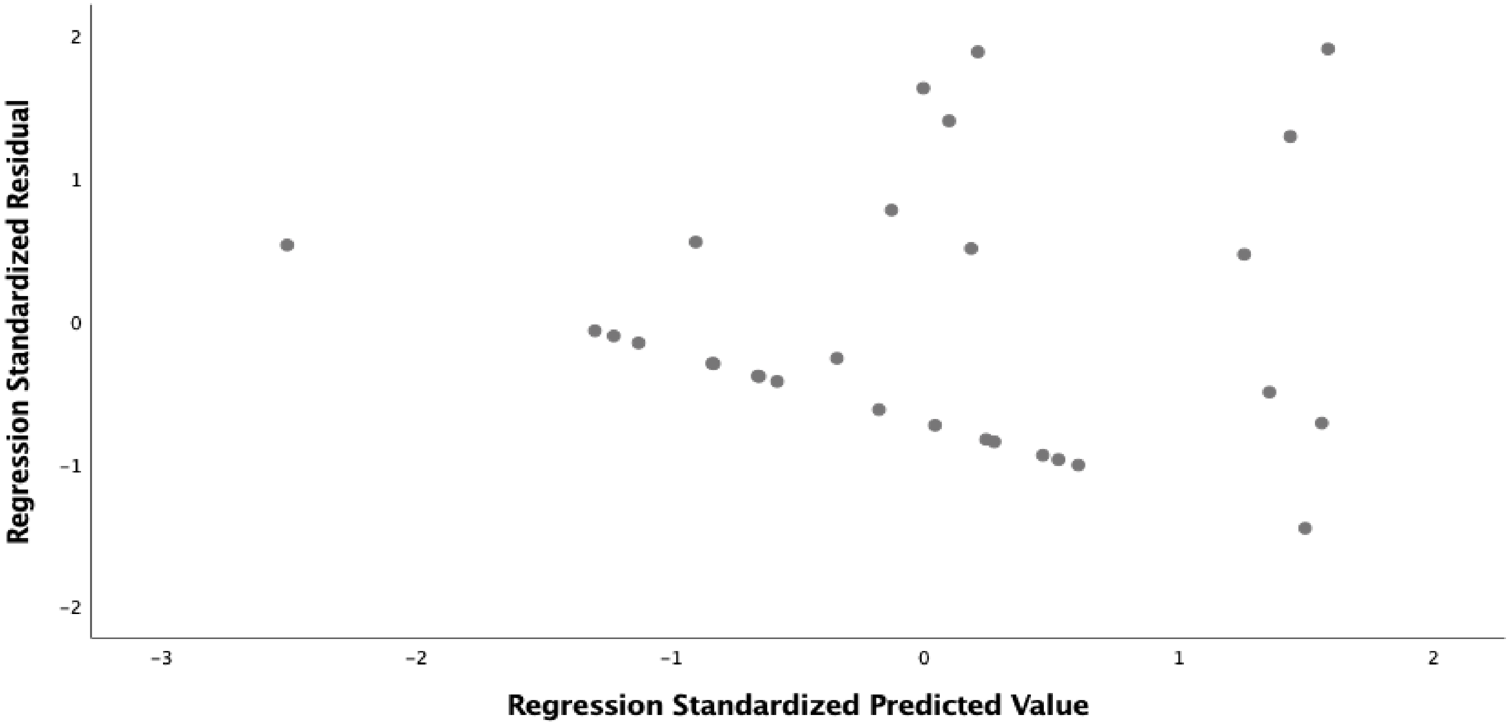
* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).

APPENDIX 2.3 PEARSON'S CORRELATION MATRIX FOR *ANEMONE AMERICANA* ENVIRONMENTAL VARAIBLES (N=30)

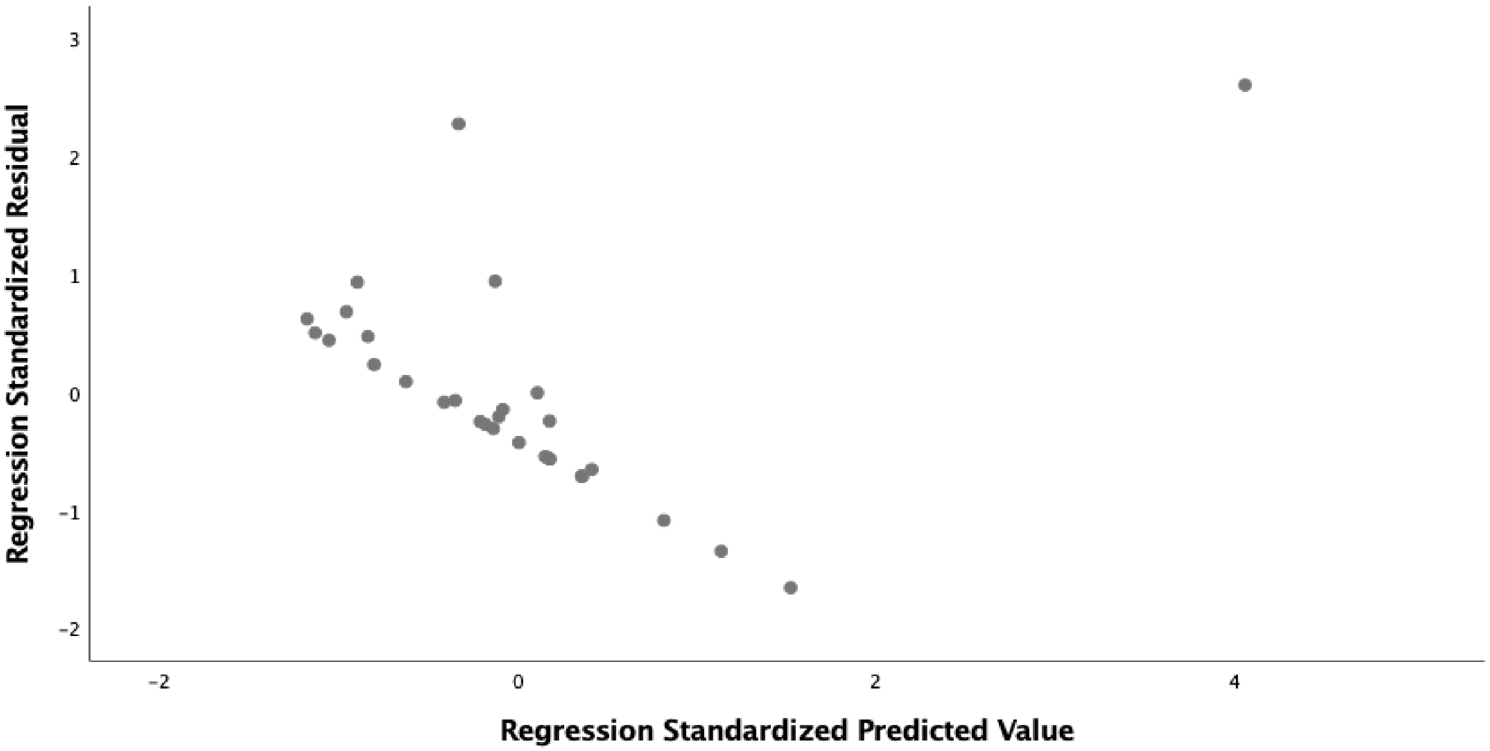
		South Facing Index	**Slope**	**Elevation**	**Topographic Position**	**Soil Drainage**	**Soil Moisture**
South Facing Index	Pearson Correlation	1					
	Sig. (2-tailed)						
Slope	Pearson Correlation	0.276	1				
	Sig. (2-tailed)	0.14					
Elevation	Pearson Correlation	0.410*	0.224	1			
	Sig. (2-tailed)	0.024	0.235				
Topographic Position	Pearson Correlation	0	0.085	0.015	1		
	Sig. (2-tailed)	1	0.655	0.937			
Soil Drainage	Pearson Correlation	-0.412*	-0.067	-0.149	0.613**	1	
	Sig. (2-tailed)	0.024	0.724	0.432	0		
Soil Moisture	Pearson Correlation	-0.334	-0.06	0.057	0.531**	0.779**	1
	Sig. (2-tailed)	0.071	0.754	0.763	0.003	0	

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).

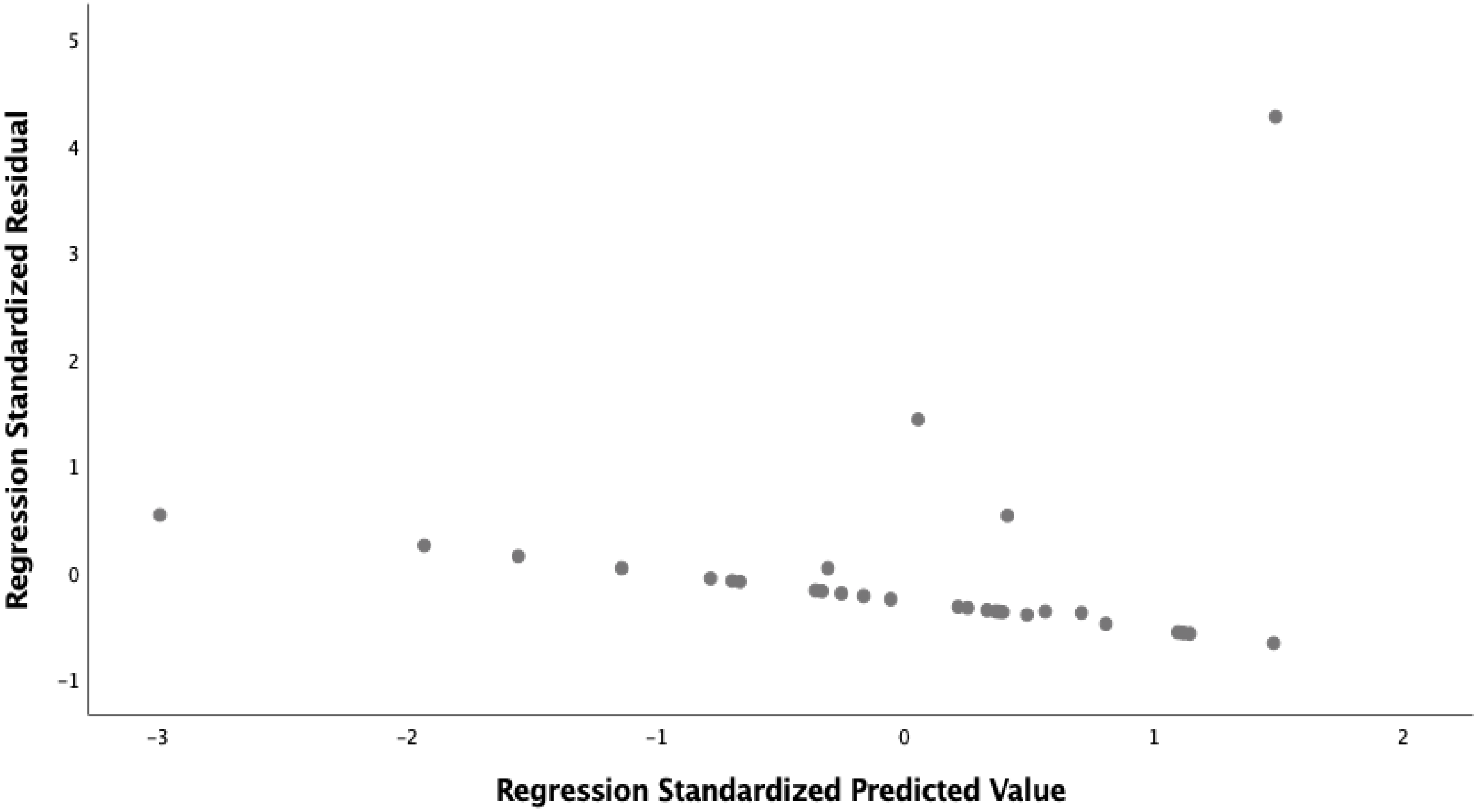
APPENDIX 3.1 PLOT OF RESIDUALS FOR *CONOPHOLIS AMERICANA* ENVIRONMENTAL VARAIBLES



APPENDIX 3.2 PLOT OF RESIDUALS FOR *GOODYERA PUBESCENS* ENVIRONMENTAL VARAIBLES



APPENDIX 3.3 PLOT OF RESIDUALS FOR *ANEMONE AMERICANA* ENVIRONMENTAL VARAIBLES



APPENDIX 4.1 CANOPY PLANT COMMUNITY COMPOSITION IN TARGET SPECIES AND CONTROL SITES

Plant Name		**Number of Sites with Plant Species**			
Common Name	**Botanical Name**	**Control**	***Anemone americana***	***Conopholis americana***	***Goodyera pubescens***
Balsam fir	*Abies balsamea*	5	5	0	1
Moose maple	*Acer pensylvanicum*	0	0	0	0
Red maple	*Acer rubrum*	12	11	11	13
Sugar maple	*Acer saccharum*	3	5	0	2
Mountain maple	*Acer spicatum*	0	0	1	0
Speckled Alder	*Alnus incana*	0	0	0	0
Yellow birch	*Betula alleghaniensis*	6	2	0	4
Paper birch	*Betula papyrifera*	2	4	8	8
Wire birch	*Betula populifolia*	0	0	1	0
American beech	*Fagus grandifolia*	1	0	1	9
White ash	*Fraxinus americana*	1	8	1	2
Witch hazel	*Hamamelis virginiana*	0	0	0	0
Ironwood	*Ostrya virginiana*	0	4	1	1
White spruce	*Picea glauca*	1	1	0	0
Black spruce	*Picea mariana*	4	0	0	0
Red spruce	*Picea rubens*	6	6	0	3
Red pine	*Pinus resinosa*	1	0	1	0
White pine	*Pinus strobus*	4	4	11	6
Large tooth aspen	*Populus grandidentata*	0	1	0	0
Trembling aspen	*Populus tremuloides*	0	3	3	0

Plant Name		**Number of Sites with Plant Species**			
Common Name	**Botanical Name**	**Control**	***Anemone americana***	***Conopholis americana***	***Goodyera pubescens***
Red oak	*Quercus rubra*	1	2	13	5
Eastern hemlock	*Tsuga canadensis*	2	2	0	6

APPENDIX 4.2 SUBCANOPY PLANT COMMUNITY COMPOSITION IN TARGET SPECIES AND CONTROL SITES

Plant Name		**Number of Sites with Plant Species**			
Common Name	**Botanical Name**	**Control**	***Anemone americana***	***Conopholis americana***	***Goodyera pubescens***
Balsam fir	*Abies balsamea*	7	11	2	8
Moose maple	*Acer pensylvanicum*	0	1	3	2
Red maple	*Acer rubrum*	12	6	10	5
Sugar maple	*Acer saccharum*	4	4	0	2
Mountain maple	*Acer spicatum*	0	0	0	0
Speckled alder	*Alnus incana*	0	0	0	0
Yellow birch	*Betula alleghaniensis*	5	0	0	0
Paper birch	*Betula papyrifera*	4	2	5	2
Wire birch	*Betula populifolia*	2	1	0	0
American beech	*Fagus grandifolia*	2	2	5	9
White ash	*Fraxinus americana*	1	3	2	2
Witch hazel	*Hamamelis virginiana*	0	0	0	0
Ironwood	*Ostrya virginiana*	0	4	3	1
White spruce	*Picea glauca*	1	1	0	0
Black spruce	*Picea mariana*	3	0	0	0
Red spruce	*Picea rubens*	8	7	0	5
Red pine	*Pinus resinosa*	1	0	0	0
White pine	*Pinus strobus*	5	2	7	2
Large tooth aspen	*Populus grandidentata*	0	0	0	0
Trembling aspen	*Populus tremuloides*	0	0	0	0
Pin cherry	Prunus pensylvanica	0	1	0	0

Plant Name		**Number of Sites with Plant Species**			
Common Name	**Botanical Name**	**Control**	***Anemone americana***	***Conopholis americana***	***Goodyera pubescens***
Red oak	*Quercus rubra*	1	1	7	2
Eastern hemlock	*Tsuga canadensis*	2	3	0	6

APPENDIX 4.3 SHRUB PLANT COMMUNITY COMPOSITION IN TARGET SPECIES AND CONTROL SITES

Plant Name		**Number of Sites with Plant Species**			
Common Name	**Botanical Name**	**Control**	***Anemone americana***	***Conopholis americana***	***Goodyera pubescens***
Balsam fir	*Abies balsamea*	13	13	3	15
Moose Maple	*Acer pensylvanicum*	5	2	2	3
Red maple	*Acer rubrum*	13	6	1	1
Sugar maple	*Acer saccharum*	2	2	0	0
Mountain maple	*Acer spicatum*	1	0	0	0
Red baneberry	*Actaea rubra*	0	1	1	0
Speckled Alder	*Alnus incana*	0	1	0	0
Serviceberry	*Amelanchier sp.*	2	0	0	0
Aster	*Aster sp.*	0	1	0	0
Yellow Birch	*Betula alleghaniensis*	6	0	0	0
Paper birch	*Betula papyrifera*	0	1	1	1
Wire birch	*Betula populifolia*	0	0	0	0
Sweetfern	Comptonia peregrina	0	0	1	0
Hazelnut	*Corylus cornuta*	0	3	3	0
Hayscentede fern	*Dennstaedtia punctilobula*	1	0	0	0
American beech	*Fagus grandifolia*	1	2	5	8
Glossy buckthorn	*Frangula alnus*	0	1	1	0
White ash	*Fraxinus americana*	2	4	1	1
Witch hazel	*Hamamelis virginiana*	0	0	10	0
Canada holly	Ilex verticillata	0	1	0	0

Plant Name		**Number of Sites with Plant Species**			
Common Name	**Botanical Name**	**Control**	***Anemone americana***	***Conopholis americana***	***Goodyera pubescens***
Fly Honeysuckle	*Lonicera canadensis*	0	1	0	0
Sensitive fern	*Onoclea sensibilis*	0	1	0	0
Cinnamon fern	*Osmunda cinnamomea*	1	0	0	0
Ironwood	*Ostrya virginiana*	0	4	2	1
White spruce	*Picea glauca*	0	1	0	1
Black spruce	*Picea mariana*	2	0	0	0
Red spruce	*Picea rubens*	5	6	1	7
Red pine	*Pinus resinosa*	0	0	0	1
White pine	*Pinus strobus*	3	1	9	1
Large Tooth Aspen	*Populus grandidentata*	0	0	0	0
Trembling aspen	*Populus tremuloides*	0	1	0	0
Pin Cherry	Prunus pensylvanica	0	0	0	0
Braken fern	*Pteridium aquilinum*	1	1	0	0
Red oak	*Quercus rubra*	2	1	0	1
New york fern	*Thelypteris noveboracensis*	2	0	0	0
Eastern hemlock	*Tsuga canadensis*	1	3	1	5
Wild raisin	Viburnum nudum	0	2	0	0

APPENDIX 4.4 HERB PLANT COMMUNITY COMPOSITION IN TARGET SPECIES AND CONTROL SITES

Plant Name		**Number of Sites with Plant Species**			
Common Name	**Botanical Name**	**Control**	***Anemone americana***	***Conopholis americana***	***Goodyera pubescens***
Balsam fir	*Abies balsamea*	2	12	2	9
Moose Maple	*Acer pensylvanicum*	0	1	3	7
Red maple	*Acer rubrum*	0	6	10	7
Sugar maple	*Acer saccharum*	0	4	0	2
Yarrow	*Achillea millefolium*	0	1	0	0
Red baneberry	*Actaea rubra*	0	1	0	0
Speckled Alder	*Alnus incana*	0	1	0	0
Bristly Sarsaparilla	Aralia hispida	0	1	0	0
Wild sarsaparilla	*Aralia nudicaulis*	9	5	1	13
Aster	*Aster sp.*	0	5	2	0
Lady fern	*Athyrium filix–femina*	1	0	0	0
Bluebead Lily	*Clintonia borealis*	4	0	1	2
Sweetfern	Comptonia peregrina	0	0	1	0
Gold thread	*Coptis trifolia*	8	2	0	5
Bunchberry	*Cornus canadensis*	9	2	6	2
Moccasin flower	*Cypripedium acaule*	0	1	0	0
Hay scented fern	*Dennstaedtia punctilobula*	5	3	3	0
Ground Cedar	*Diphasiastrum digitatum*	0	0	0	3
Evergreen wood fern	*Dryopteris intermedia*	6	0	0	0
Beech drop	*Epifagus virginiana*	0	0	2	0
May flower	*Epigaea repens*	3	1	1	0
Field horsetail	*Equisetum arvense*	0	1	0	0

Plant Name		**Number of Sites with Plant Species**			
Common Name	**Botanical Name**	**Control**	***Anemone americana***	***Conopholis americana***	***Goodyera pubescens***
American Beech	*Fagus grandifolia*	0	1	4	4
Glossy Buckthorn	*Frangula alnus*	0	1	1	0
White ash	*Fraxinus americana*	0	2	2	0
Rough bedstraw	*Galium asprellum*	0	1	0	0
Creeping snowberry	Gaultheria hispidula	0	1	1	0
Teaberry	*Gaultheria procumbens*	4	0	7	4
Huckleberry	*Gaylussacia baccata*	0	0	1	0
Graminoids	*Graminoids*	0	13	13	5
Oak fern	*Gymnocarpium dryopteris*	0	2	0	0
Witch hazel	*Hamamelis virginiana*	0	0	4	0
Golden lungwort	*Hieracium murorum*	0	0	0	0
Shining fir-moss	Huperzia lucidula	0	2	0	5
Pinesap	*Hypopitys monotropa*	0	0	4	0
Spotted touch me not	*Impatiens capensis*	0	1	0	0
Sheep laurel	*Kalmia angustifolia*	0	2	3	2
Twinflower	*Linnaea borealis*	1	0	0	0
Fly Honeysuckle	*Lonicera canadensis*	0	2	1	1
Wild lily of the valley	*Maianthemum canadense*	14	7	2	13
Cucumber root	*Medeola virginiana*	1	0	3	4
Partridgeberry	*Mitchella repens*	5	5	8	8
Ghost plant	*Monotropa uniflora*	3	0	9	0
Wood aster	*Oclemena acuminata*	4	2	0	0

Plant Name		**Number of Sites with Plant Species**			
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Sensitive fern	*Onoclea sensibilis*	0	2	0	1
Sidebells wintergreen	*Orthila secunda*	0	0	2	0
Cinnamon fern	*Osmundastrum cinnamomeum*	1	3	3	1
Ironwood	*Ostrya virginiana*	0	2	2	2
Wood sorrel	*Oxalis montana*	5	1	0	0
Northern beech fern	*Phegopteris connectilis*	1	0	0	0
Red spruce	*Picea rubens*	0	3	0	3
White pine	*Pinus strobus*	0	2	10	4
Common Plantain	*Plantago major*	0	1	0	0
Blunt-leaved Rein Orchid	Platanthera obtusata	0	1	0	0
Christmas fern	*Polystichum acrostichoides*	4	7	1	3
Braken fern	*Pteridium aquilinum*	5	2	8	1
Shinleaf	*Pyrola elliptica*	1	0	2	0
Red oak	*Quercus rubra*	0	1	1	1
Wood Buttercup	*Ranunculus abortivus*	0	2	0	0
Rose species	*Rosa sp.*	0	1	0	0
Dewberry	*Rubus hispidus*	0	0	0	0
Dwarf raspberry	*Rubus pubescens*	0	3	0	0
Goldenrod	*Solidago sp.*	0	3	0	1
Rosy twisted stalk	*Streptopus lanceolatus*	1	5	1	2
Dandelion	*Taraxacum officinale*	0	1	0	0
New york fern	*Thelypteris noveboracensis*	3	0	0	0
Bog fern	*Thelypteris simulata*	0	0	0	0
Poision Ivy	*Toxicodendron radicans*	0	1	0	0
Starflower	*Trientalis borealis*	13	6	12	12

Plant Name		**Number of Sites with Plant Species**			
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Trillium	*Trillium sp.*	2	0	0	4
Eastern Hemlock	*Tsuga canadensis*	0	0	1	2
Lowbush Blueberry	*Vaccinium angustifolium*	0	1	7	2
Velvet leaved blueberries	*Vaccinium myrtilloides*	0	0	4	0
Field Speedwell	*Veronica arvensis*	0	0	0	0
Birds-eye Speedwell	Veronica persica	0	1	0	0
Wild raisin	*Viburnum nudum*	0	0	0	1
Viola	*Viola sp.*	3	6	1	1