

**The effect of extensive green roof substrate composition
on native low-bush blueberry (*Vaccinium angustifolium*)
and crowberry (*Empetrum nigrum*) growth and health**



Vaccinium angustifolium



Empetrum nigrum

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Authors Contributions

C.B. and J.L. designed the experiment; C.B. collected, assembled and analyzed the data with the advice of J.L.; C.B. wrote the manuscript; J.L. and S.S. gave assistance and edited the manuscript.

Abstract

The establishment of native vegetation on green roofs may reduce maintenance requirements and increase local biodiversity and ecosystem services. In Nova Scotia, native species from coastal barrens have been selected for green roof use due to their adaptations to harsh climates; yet the persistence of dominant native species (*Vaccinium angustifolium* and *Empetrum nigrum*) on green roof systems is unexplainably low. To test whether commercial green roof substrate is limiting shrub growth, shrub health and survivorship were monitored in an experimental study using four different substrate treatments. Under greenhouse conditions, survival, growth (height and number of leaves) and a categorical ranking of health were compared between plants grown in four substrate compositions: (1) control, or 100% commercial green roof growing medium; (2) control+peat moss, (3) control+native soil inoculum; and (4) control+peat moss+native soil inoculum. After 12 weeks, the control treatment for both species had significantly higher height, number of leaves and health scores than the other treatment, with the exception of *V. angustifolium* in the peat moss treatment. The mixture treatment had the lowest pH and highest organic matter content of all treatments, making it the closest treatment to resemble two of the three characteristic factors of coastal barren soils: high organic matter content; low pH; and low nutrient levels. However, the mixture treatment had the lowest height, number of leaves and health scores apart from *V. angustifolium*'s number of leaves in the inoculum treatment. Plant growth and health was lower in mixture substrates due to increased nutrient levels, high organic matter levels in soils with a relatively basic pH to coastal barrens, or water-logged roots due to increased decomposition of added organic matter. The results indicate that substrate composition may not be limiting dominant native shrub growth on green roofs. However, before substrate composition is omitted as a potential explanation for shrub deficiency in green roof systems, this study must be replicated: (1) with a mixture treatment that has a lower pH, as pH is a leading factor in determining nutrient levels in soil; and (2) on an outdoor green roof system due to potential effects environmental factors have on shrub growth and health.

Key Words: Green Roof, Commercial green roof substrate, Native plant communities, Low-bush blueberry, Crowberry, Inoculum, Peat moss, Organic matter, pH.

1.0 Introduction

1.1 Motivation

The potential benefits of establishing native ecosystems on green roof systems are difficult to examine due to the inability to successfully reproduce native community structures on green roof systems. However, reproducing native ecosystems on green roof systems has the potential to reduce maintenance requirements, increase local biodiversity and enhance ecosystem services. In Nova Scotia, native species from coastal barrens have been selected for green roof use due to their adaptations to harsh climates; yet the persistence of dominant native species, specifically low-bush blueberry (*Vaccinium angustifolium*) and crowberry (*Empetrum nigrum*), on green roof systems is unexplainably low. This study will explore one of the potential explanations for dominant native species' deficiency in these systems in hopes to discover a clear limiting factor and propose mitigation, or to better understand barriers.

1.2 Overview on Green Roofs

Successful green roofs are capable of providing ecosystem services such as storm-water management, cooling buildings and pollutant removal from air and water (Oberndorfer et al., 2007). However, the efficiency of these services is directly tied to the type of green roof, namely extensive, containing up to 20 cm substrate, or intensive, containing greater than 20 cm of substrate, and its supported vegetation.

The environment on a green roof however is very harsh and the number and types of species that can establish themselves on these systems is limited. Conditions on rooftops vary due to climatic differences in geographic regions, but in general green roof species are subject to drought, extreme temperatures, high winds and high light intensities (Oberndorfer et al., 2007). These stressors are heightened on extensive green roofs due to their shallow substrate, making plants more susceptible to freezing in the winter and desiccating in the summer. Consequently, the majority of extensive green roofs support a small number of non-native species, typically in the *Sedum* genus, due to their drought-tolerance and ability to survive in these harsh conditions.

The traditional consideration of green roof functionality, which focuses on balancing economic benefits with engineering and architectural challenges (Dunnett & Kingsbury, 2007), typically ignores the role vegetation plays in regulating the efficiency of green roof services. As a result, since green roof origination in Europe, there has been little new or localized research on which species would be better suited to flourish on green roofs in North America (Oberndorfer et al. 2007).

Though it is inconclusive whether North American native species are better adapted to green roofs within North America than non-native species (Butler, Butler & Orians, 2012); it has been determined that a habitat template approach, or using species from ecosystems within a particular geographic region that have characteristics similar to green roof can be successful (Lundholm, 2006). Thus, the introduction of certain native species could aid the advancement of green roof systems as well as increase the native biodiversity in urban environments.

1.3 Why Encourage Natives¹?

The addition of native species to green roof systems is preferable to non-native species because they can increase various ecosystem services, such as roof temperature reduction and storm water capture (Lundholm, MacIvor, MacDougall & Ranalli, 2010), and do not pose risks of biological invasion. Despite these potential benefits, the use of native species in current landscape design is lacking. The limited use of native species is due to certain limitations, such as the absence of native species commercially available in nurseries and a deficiency in understanding the dynamics of native plant communities (Dunster, 2010). Nonetheless, it is likely that there is a native species suitable for any landscape situation; they just have not been identified (Simmons, Venhaus, & Windhager, 2007).

If appropriate native species are discovered, the ecological services of green roofs can be amplified while the whole ecological health of urban landscapes is enhanced. Use of native species on green roofs can increase the ecological function and diversity of a fragmented landscape by introducing corridors or islands of habitat (Simmons, Venhaus, & Windhager, 2007). Corridors or islands would increase pollination, food and habitat resources for native insects, birds and small mammals. In fact, recent research indicates that current *Sedum*-carpeted roofs have little biodiversity value compared to roofs dominated with other vegetation types (Dunnet & Kingsbury, 2007). Increased diversity has been shown to increase the

¹ Native being defined as a plant that arrived and evolved over thousands of years in a particular region without human agency (Kendle & Rose, 2000).

thermal efficiency and rainwater absorption services of green roofs (Dunnet & Kingsbury, 2007). Thus, usage of diverse native species on green roofs not only increases native biodiversity in an area but also augments their ecosystem services.

1.3 A Habitat Template Approach in Nova Scotia: the Coastal Barrens

The coastal barrens of Nova Scotia are open areas within a forest matrix that are dominated by dwarf shrubs (Burley & Lundholm, 2010). Coastal barren habitation and persistence is influenced by high elevation, shallow soils, extreme temperature, and wind exposure (Burley & Lundholm, 2010; Oberndorfer et al., 2007). Consequently, coastal barren communities exhibit many parallels with green roof environments; using the theory of the habitat template approach, it is presumed that coastal barren communities will perform well and persist on green roofs.

Coastal barren communities are desirable on green roof systems because they would increase the diversity in these systems. Coastal barren communities have been recorded to support 173 different species and a variety of different life-forms despite the harsh growing conditions (Oberndorfer, 2009). This diversity is much greater than the current *Sedum* monocultures found on many green roofs.

1.4 Knowledge Gaps

Though theoretically justifiable, it is unknown how coastal barren communities actually perform on green roof systems. A prevailing problem in

establishing these communities on green roofs is that native shrubs are being outcompeted by grass species before their services can be quantified (Lundholm, MacIvor & Ranalli, 2009). An additional discontinuity in determining coastal barren performance on green roof systems is the cause of this trend of grassy plant dominance is unknown. Based on theories within the literature, current commercial green roof substrate seems to be the most probable cause of native shrubs inability to thrive on green roofs; however, this has not yet been studied.

1.5 Research Problem

This study will focus on investigating the role of current commercial green roof substrate on native shrub growth, health and survival in Nova Scotia. It will seek to determine if green roof substrate is limiting shrub growth and health, or, if not, then eliminate one of the possible causes of shrub deficiency in these systems. The study will be focused on answering the following research questions:

1. Can we modify the organic matter content, pH and nutrient levels in green roof substrates to more closely resemble coastal barren soils?
2. Will *V. angustifolium* and *E. nigrum* have greater growth² in green roof substrates that more closely resemble coastal barren soils?
3. Will *V. angustifolium* and *E. nigrum* have improved health and survivorship in green roof substrates that more closely resemble coastal barren soils?

² Growth will be measured through plant height and number of leaves

1.6 Study Approach

Under greenhouse conditions, this study will explore and quantify the success of the two major native shrubs on coastal barrens, low-bush blueberry (*Vaccinium angustifolium*) and crowberry (*Empetrum nigrum*), in four different substrates: a control of just commercial green roof substrate; green roof substrate with peat moss added; green roof substrate with an inoculum of native soil added; and green roof substrate with both peat moss and an inoculum added. It will test the hypotheses that the current commercial green roof substrate is limiting plant success and that both *V. angustifolium* and *E. nigrum* will be more successful in the mixture substrate (65% commercial green roof substrate, 25% humus from coastal barrens and 10% peat moss) compared to the others because it will have higher organic matter and lower pH.

2. Literature Review

2.1 Introduction

The replacement of typical *Sedum* species with native species on green roof systems has been debated within current green roof research. In an analysis of 410 green roof papers, Butler, Butler and Orians (2012) found that 26% supported the use of native species on green roofs; the other 74% were either against the use of natives or did not recognize the importance of natives in their paper. In addition, through a thorough examination of the literature, it was found that scholarly

literature on reconciliation ecology, through Biological Abstracts, Science Direct and Web of Science databases, shows that there is a growing body of literature on incorporating native species into green roof systems (Francis & Lorimer, 2011).

This review will explain the importance of green roofs and native plant usage in reconciliation ecology. It will then examine the current studies involving native species on green roofs and will address the theory and practicality behind native success on these systems in general and then focus to green roofs within Nova Scotia. Finally, it will examine the significance of substrate type on plant success and parallel this to the potential success of coastal barren communities on green roofs.

2.2. Reconciliation Ecology and Green Roofs

Reconciliation ecology aims to modify urban environments to increase the diversity, number, and range of species, without compromising the functional aspect of these urban environments (Francis & Lorimer, 2011). Green roofs are an important step towards reconciliation ecology as they improve urban biodiversity and can be created directly by urban citizens (Francis & Lorimer, 2011). Green roofs help achieve five of the goals of reconciliation ecology and urban biodiversity conservation identified by Dearborn and Kark (2009), green roofs: (1) provide ecosystem services (Dearborn & Kark, 2009; Oberndorfer et al., 2007); (2) improve human well-being through the improvement of water and air quality (Yang, Yu & Gong, 2008; Monterusso, Rowe, Rugh & Russel, 2002); (3) tentatively create corridors for natural populations that increase pollination, food and habitat resources for native insects, birds and small mammals (Simmons, Venhaus, &

Windhager, 2007); (4) connect people with nature while providing environmental education, as the public's attitudes towards green roofs are generally positive (Jungles, Rakow, Allred, & Skelly, 2013), and (5) provide new opportunities for increased education about nature and green roof systems.

Some of these goals include using native species and increasing biodiversity conservation while others place importance on functionality and cost-effectiveness (Dearborn & Kark, 2010). Nevertheless, reconciliation ecology can incorporate ecological restoration by using native species in urban landscape design; further improving ecological value and reducing ecological damage to urban landscapes (Simmons, Venhaus, & Windhager, 2007). However, there are complications with the establishment and performance of native species on green roof systems.

2.3 Native Species on Green Roofs

There has not been conclusive evidence on how native species will thrive on green roofs. Much of the theory supporting native species on green roofs is based on assumptions that natives are better adapted and associated with enhanced environmental benefits (Butler, Butler, & Orians, 2012). People assume that because natives are favorable on ground-level environments, they will perform the same on rooftops (Butler, Butler, & Orians, 2012). However, this cannot just be assumed; the performance of natives on green roof systems is directly tied to the methods used to establish natives on green roofs (Sutton, 2008), their ability to adapt to harsh environments (Simmons, Venhaus, & Windhager, 2007), and the natural environment the native plant originated from (Bousselot, Klett, & Koski, 2009).

Some past studies assessing native success on green roofs have found sedums had greater survival than native species. Native species were found to be unsuitable for the drought-induced harsh environmental conditions on green roofs (Monterusso, Rowe, & Rugh, 2005). The factor that seems to have the greatest effect on plant survival is the water limitations on roofs (Monterusso, Rowe, & Rugh, 2005; Martin & Hinkley, 2007). However, subsequent studies have determined that study design and plant selection are paramount. Premature discontinuance of supplemented water before roots structures establish masks the tolerance properties of a plant by promoting plant failure (Sutton, 2008). Additionally, selecting plants that whose natural habitats involve shallow soil structures and low annual precipitation lead to native success on green roof systems (Bousselot, Klett, & Koski, 2010). Many studies do not take into consideration the physiological traits of the native species they test on roofs, which augments their failure. Phenotypic plasticity of plants proves more predictive of plant performance than the type (native or non-native) or life-form (monocots, dicots, shrubs, herbs, etc.) of the plant (Farrell, Szota, Williams, & Arndt, 2013). The extent of plasticity in plants can be determined by looking at their natural environments and its variable conditions, which would demand quick morphological, behavioral or physiological changes in its supported vegetation.

One approach to examine potential plant plasticity and to increase the amount of native biodiversity on green roofs is to use a habitat template approach when choosing species (Lundholm, 2006). This approach uses the ideology that many urban environments have natural analogs. Thus, by matching environmental

conditions in the natural and urban environments it can then be determined which plant communities would be most successful (Lundholm, 2006). In terms of green roofs, it would be ideal to find a natural environment that has shallow substrates as well as extreme temperature and moisture variations to determine which plant communities would be most successful in green roofs' harsh environment.

2.4 Green Roofs in Nova Scotia and the Coastal Barrens

In Nova Scotia, the use of native species from coastal barren habitats on green roofs has been recommended by Licht & Lundholm (2006) due to parallels between the two extreme environments. Both green roof and coastal barren species are subject to drought, extreme temperatures and high winds (Licht & Lundholm, 2006; Oberndorfer et. al., 2007). Thus, based on the theory behind the habitat template approach, coastal barren species would thrive on green roofs within Nova Scotia.

Plant communities characteristic of coastal barrens are dominated by ericaceous vegetation, typically of the dwarf shrub life-form (Oberndorfer & Lundholm, 2009). Further, the most dominant species on the coastal barren is *E. nigrum*. However, another species characteristic of coastal barrens in Nova Scotia, but less abundant, is *V. angustifolium*. The dense shrub cover on Nova Scotia coastal barrens results in little grassland invasion, as the few grass species found within the barrens have low abundances (Oberndorfer & Lundholm, 2009).

Green roof research in Nova Scotia has successfully cultivated coastal barren species on green roofs. However, there has been difficulty sustaining the intended

shrub dominated communities on these systems (Lundholm, MacIvor, & Ranalli, 2009). Within a year the plant communities are taken over by grassy natives, which is not characteristic of natural coastal barrens (Oberndorfer & Lundholm, 2009). On green roof systems, shrubs such as *E. nigrum* and *V. angustifolium* demonstrated slow growth rates compared to grasses such as red fescue (*Festuca rubra*) (MacIvor & Lundholm, 2011). Grassy species are outcompeting shrubs before shrubs' establishment on green roofs. This makes the assessment of shrubs on green roofs difficult to study unless shrubs are grown in monocultures.

Current studies looking at shrub performance on green roofs are inconsistent. Shrubs have been found to either perform similarly to or underperform other life-forms. A study by Nardini, Andri and Crasso (2011) found that there was no significant difference between grass-covered and shrub-covered roofs when it came to storm water runoff capture, both storing more than 90% of rainfall. Conversely, MacIvor & Lundholm (2011) found that native grass species performed better than shrubs for most ecosystem services. However, in both studies the experimental units were grown in monocultures, which are not characteristic of native environments. Only being able to examine shrub performance through monocultures makes the assessment of shrub dominated, but not exclusive, native plant communities on green roofs difficult. The lack of assessment of polycultures of shrub and grasses withholds information on how these species will perform together.

2.5 Plant success and Growth Medium

The performance of coastal barren communities on green roofs cannot be effectively studied until dominant coastal barren shrubs can be successfully established on these systems. It has been hypothesized that green roof substrate can limit plant growth due to its differences to natural soil composition (Dunster, 2010). Green roof substrate needs to be lightweight and porous to meet the needs of the plants without compromising the structural integrity of the building (Nagase & Dunnett, 2011). The main factors that determine green roof substrate composition involve trade-offs between weight, water-holding capacity, oxygen-holding capacity and long-term stability of the substrate (Emilsson, 2008). Consequently, green roof substrate lacks natural soil horizons (Dunster, 2010), is highly mineral based (Oberndorfer et al, 2007), contains low volumes of organic matter (Lundholm et al. 2010), and is deficient in essential microbiota communities (Dunster, 2010). These composition characteristics are inconsistent with natural soil configurations (Dunster, 2010) and can be limiting plant persistence.

In a study by MacIvor and Lundholm (2011), shrubs had the lowest growth rate of all the 15 coastal barren species tested. In particular, *V. angustifolium* and *E. nigrum* had very low growth rates (MacIvor & Lundholm, 2011) despite their characteristic and dominant nature on natural coastal barrens (Oberndorfer & Lundholm, 2009). However, the green roof substrate utilized in this study was a common green roof substrate, Sopraflor X (MacIvor & Lundholm, 2011), which has a pH around 6.0-7.0 and a dry organic matter content of 5-10% (Lundholm et al. 2010; MacIvor, Ranalli & Lundholm, 2011). Unlike common green roof substrates,

soil in the coastal barrens is acidic, having an average pH of 4.3 (Oberndorfer, 2006), and is composed of thick organic layers, around 55-60% organic matter by volume (Oberndorfer, 2006; Burley, Harper, & Lundholm, 2010). These differences in soil composition could explain why these shrubs did not perform well in a green roof system when in theory they should thrive.

The lack of microbiota (Dunster, 2010) and organic matter in green roof substrate can limit the health of green roof ecosystems and plant growth. Microbiota can aid plant growth and health in harsh environments by regulating the decomposition of organic matter, nutrient cycling, and activity rates within the soil to levels that promote plant health (Rumble & Gange, 2012; Schloter, Dilly & Munch, 2003). Organic matter increases soil characteristics beneficial for plant growth, such as increased water retention (Tisdall & Oades, 1982) and decreased element leaching through increased cation exchange capacity (Nagase & Dunnet, 2011).

Fortunately, changing the composition green roof substrate can have beneficial effects on the growth of plants. It has been found that the addition of native microbes into green roof substrate can aid the growth of native grasses sedges and forbs within the Midwestern United States (Sutton, 2008). Additionally, a study by Nagase and Dunnet (2011) found the addition of 10% organic matter to green roof substrate was optimal for all species tested.

There have been examples of successful manipulations of green roof substrate to enhance plant growth on green roofs. Thus, there is reason to believe if current green roof soils were manipulated to represent coastal barren soils through inoculation with native soil or organic matter additives, then success of native

coastal barren community structures, including the persistence of shrub species, on roofs could be examined.

2.6 Conclusion

The use of native species that already thrive in areas with shallow soils and little annual precipitation has proven successful on green roof systems. Within Nova Scotia the plants found on the coastal barrens are analogous to plants that survive in these harsh environments. However, the discrepancy between soil composition between green roof and coastal barrens is immense. There have been examples in other regions that reflect the importance of manipulation of green roof soils to help aid native plant growth. Therefore, modifying green roof soils to resemble coastal barren soils could promote enhanced plant growth, especially in shrubs, which would facilitate the establishment of natural community systems on green roofs.

3.0 Materials and Methods

3.1 Experimental Design

The experiment ran for 12 weeks, starting on the 22nd of November 2013 and finishing on the 14th of February 2014. 15 plants of each species were assigned to four treatments:

1. Control, which contained, by volume, 100% commercial green roof substrate.
2. Peat moss, which contained, by volume, 90% commercial green roof

- substrate and 10% peat moss.
3. Inoculum, which contained, by volume, 75% commercial green roof substrate and 25% humus from coastal barrens.
 4. Mixture, which contained, by volume, 65% commercial green roof substrate, 25% humus from coastal barrens, and 10% peat moss.

Pots were arranged in a complete randomized design (Figure 3.1). Over the study period, all plants were watered to field capacity (~17.3 mL) twice a week (Monday and Friday). The commercial green roof substrate used for this experiment was Sopraflor X (From Soprema Inc. Drummondville, QC, Canada), which is composed of expanded shale, sand, vegetable compost, perlite and blond peat and has a total porosity of 50-69% and bulk density of 1100-1200 kg/m³. A part of the four substrates were reserved for pH, levels of organic matter, P, K, and N analysis at the Harlow Institute on the Dalhousie Agricultural Campus (for complete analysis see Appendix I).



Figure 3.1. Completely Randomized design in a 12x10 pot layout for control, peat moss, inoculum and mixture treatments located in a greenhouse at Saint Mary's University, Halifax, NS.

3.1.1 Rationale of Additives in Substrate Treatments

Peat moss was used to increase the amount of organic matter and lower the pH of the sample. The volume of organic matter added to the sample (10%) has been determined by Nagase & Dunnett (2010) as optimal amount for plant growth. Only 10% organic matter was added rather than 58%, which is typical of coastal barren soils, due to the consequences of increased organic matter on rooftops.

Organic matter lacks stability due to decomposition, which can lead to the reduction and compaction of green roof substrate (Nagase & Dunnett, 2011). The reduction in volume of green roof substrate would require supplementary maintenance to place additional material on the roof. The compaction of green roof substrate can impede water drainage, which leads to plant stress (Friedrich, 2005). Additionally, increased organic matter could be a water quality issue, by increasing nutrients in output water if the plants cannot absorb them all, and a fire safety issue,

by increasing the volume of flammable material. It was expected that a 10% increase would be the least amount of organic matter needed to see a response in plant growth and health without making the large-scale implementation of a potential successful substrate impractical.

An inoculum was used to increase the organic matter and microbial activity in the sample (Dunster, 2010). The inoculum used was coastal barren humus collected from Chebucto head in October of 2013. Collected humus was taken from various areas within Chebucto head with *E. nigrum* or *V. angustifolium* cover. The top two inches of soil directly under these shrubs were removed, mixed together, and stored in a refrigerator (4°C) back at Saint Mary's University until the soil treatments were prepared in November 2013. In the inoculum and mixture treatment treatment, 25% native soil inoculum was added by volume based on methods from Andonian et al. (2012) for successfully inoculating soil.

3.2 Site and Species Selection

The experiment was conducted in the greenhouse on the green roof testing facility at Saint Mary's University, Halifax, NS to investigate the relationship between plant growth and substrate type as well as the relationship between plant health and substrate type. A greenhouse was be used to enable the study to be performed during the winter months of the year as well as to accurately control other environmental factors to ensure variance in plant growth and health is due to substrate type.

The two species used in this study, *V. angustifolium* and *E. nigrum*, are found on the coastal barrens of Nova Scotia, Canada and are of a dwarf shrub life-form. They were germinated from berries collected from Chebucto Head, Nova Scotia during the summer months of 2013.

3.2.1 Plant Germination and Transplantation

Collected berries were stored in a refrigerator (4°C) for up to three months to simulate a dormancy period before germination. The berries of each species were then blended separately with ~250 mL of water for approximately 10 seconds to separate the seeds from the fruit. After about two minutes, the majority of the seeds settled to the bottom and the fruit floated to the top and was removed. The water-seed mixture was then poured into a sieve (0.42mm). The remaining seed sludge was spread over a tray full of moistened peat moss substrate and was then covered with ~1 cm of moistened peat moss. This process was repeated for each species. The trays were then placed in a growth chamber set to optimal conditions for *V. angustifolium* germination (12 hours of dark at 15°C and 57% humidity and 12 hours of light at 20°C and 57% humidity) until their second set of true leaves formed.

After 3 months, 60 plants of each species, 120 plants in total, were transplanted into 200 mL plastic pots containing 150 mL (added by volume) of the assigned treatment soil described in Section 3.2. During transplantation, a mix of relatively large and small plants were used in each treatment to control for size differences. One week after initial transplantation, 31 plants were replaced due to

death via transplantation shock and an additional week was given to guarantee their true establishment before measurements began.

3.3 Plant Growth and Health Measurements

Plant growth was measured using height and number of leaves. Height (from the soil line to the highest leaf apex) and number of leaves of each individual plant was measured every two weeks during the study period, six times in total. Plant health was determined using a standardized categorical scale and survivorship. The relative appearance of each plant was evaluated using a categorical scale from 0 to 5: 0=dead, 1= stressed plant showing visible wilting or browning, 2= a plant that shows little change since planting, 3= slow growth, 4= healthy plant exhibiting a large amount of growth, and 5= exceptional growth and fullness (Monterusso, Rowe, & Rugh, 2005) during each measurement period. Final growth measurements and survival data were recorded on February 14th, 2014, the last day of the study.

3.4 Statistical Analysis

Differences in soil composition were analyzed within treatments using a one-way ANOVA for each soil parameter: Nitrate, Phosphate, pH, Organic Matter, CEC, and Na. Tukey's Honest Significant Difference test was used to determine the pairwise comparisons between each treatment for each soil parameter.

Differences in plant height and number of leaves were analyzed within species using one-way ANOVA. Differences in relative appearance were analyzed within species with a Kruskal-Wallis one-way ANOVA. Significance level was

determined at $\alpha=0.05$ for all statistical analyses. All analyses were performed using the statistical software, R.

4. Results

4.1 Soil Analysis

4.1.1 Similarity with Coastal Barren Soil

Some of the amendments to the soil resulted in substrate similar to coastal barren soil, though no one treatment shared significantly similar quantities to all parameters of the coastal barren soils. The inoculum treatment most resembled coastal barren soils, only having significant differences to coastal barren soils in four of the six soil parameters, compared to the mixture and peat moss treatment, which had five significant differences, and the control, which had six significant differences (Fig 4.1). The inoculum treatment was not significantly different from coastal barren soils in nitrate quantities (Tukey's adjusted p-value= 0.678) and cation exchange capacity (CEC) (Tukey's adjusted p-value=0.518).

The parameters that had the greatest difference between coastal barren soils and all treatments were phosphate (differences ranging from 449 to 930 kg/ha), pH (differences ranging from 1.46 to 3.36), and organic matter (differences ranging from -47.5 to -52.34%) (Fig 4.1). The inoculum treatment had the lowest phosphate quantities (Mean \pm SE: 492.7 \pm 9.2). The mixture treatment had the lowest pH (Mean \pm SE: 5.76 \pm 0.04) and highest organic matter content (Mean \pm SE: 11.07 \pm 0.78). The treatment that had the greatest difference for all parameters was the control

treatment, except the mixture treatment had a greater difference with coastal barren soil for nitrate quantity (Fig 4.1).

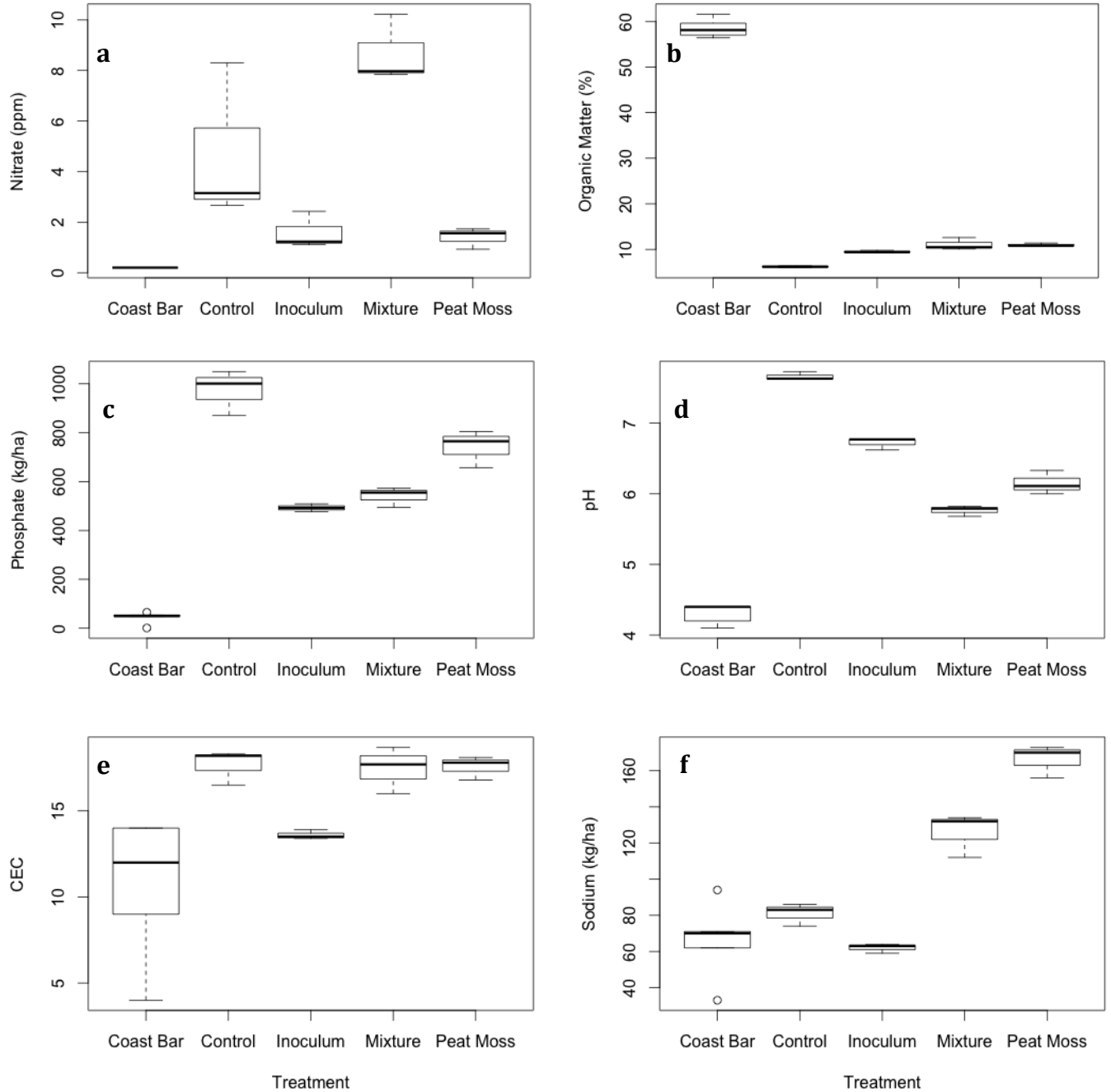


Figure 4.1 Soil Parameters for substrate treatments and coastal barren (coast bar) soils. Data from coastal barren from Oberndorfer 2006. Data from substrate treatments from soil analysis by Harlow Institute on Dalhousie Agricultural Campus. n=3 for substrate treatments. n=5 for coastal barren soils.

4.2 Plant Growth

Treatments showed variable growth responses in both species. After 12 weeks, there was a significant difference between treatments in *V. angustifolium* height ($F_{3,50}=4.15$, $p=0.016$) and number of leaves ($F_{3,50}=3.23$, $p=0.015$). Height and number of leaves were significantly greater in the control (Mean \pm SE: 58.18 \pm 2.32 mm and 20.80 \pm 2.45 leaves) and peat moss (Mean \pm SE: 55.78 \pm 3.70 mm and 18.3 \pm 4.18 leaves) compared to the inoculum (Mean \pm SE: 43.82 \pm 2.15 mm and 10.47 \pm 1.65 leaves) and mixture (Mean \pm SE: 44.16 \pm 2.58 mm and 13.23 \pm 1.77 leaves) treatments (Figure 4.2 & Figure 4.3). Throughout the 12 week period, the inoculum and mixture treatments had a moderately constant height around 45 mm, while the control and peat moss treatments had variable heights, peaking on December 20th, 2013 and February 14th, 2014 (Figure 4.2).

E. nigrum showed similar patterns to *V. angustifolium*; demonstrating significant differences between treatments in height ($F_{3,50}=6.90$, $p=0.0007$) and number of needles ($F_{3,50}=17.40$, $p=2.50e-7$), though *E. nigrum* heights were significantly lower than *V. angustifolium*. Unlike *V. angustifolium*, only the control treatment had significantly greater height (Mean \pm SE: 37.68 \pm 1.59 mm and 71 \pm 8.5 needles) than the other treatments, whose heights and number of leaves were approximately 28 \pm 2 mm and 30 \pm 3 needles, respectively (Figure 4.2 & Figure 4.3). Over the 12 weeks, the control's number of needles varied greatly compared to the other treatments, peaking on December 20th, 2013 and February 14th, 2014 (Figure 4.3). The control's height showed a slightly higher fluctuation compared to the other treatments, peaking again on December 20th, 2013 and February 14th, 2014 (Figure

4.2). However, this fluctuation was not as substantial as the variability in *V. angustifolium* heights.

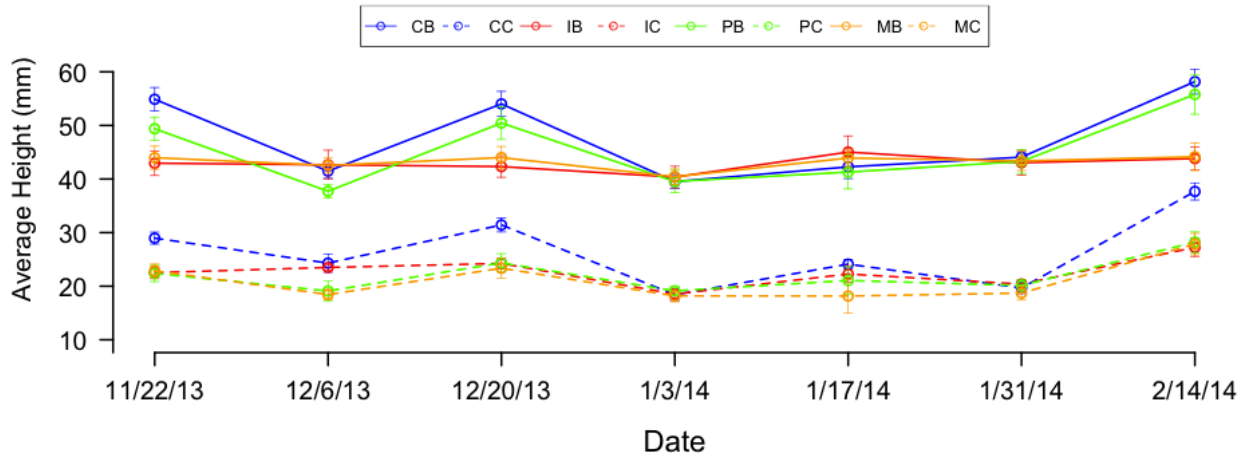


Figure 4.2. Average height (in mm) of low-bush blueberry (*Vaccinium angustifolium*) and crowberry (*Empetrum nigrum*) grown in various substrate treatments over 12 weeks under greenhouse conditions at Saint Mary’s University, Halifax, NS. CB= *V. angustifolium* in control treatment, CC= *E. nigrum* in control treatment, IB= *V. angustifolium* in inoculum treatment, IC=*E. nigrum* in inoculum treatment, PB= *V. angustifolium* peat moss treatment, PC=*E. nigrum* in peat moss treatment, MB= *V. angustifolium* in mixture treatment, MC= *E. nigrum* in mixture treatment. n=3-15

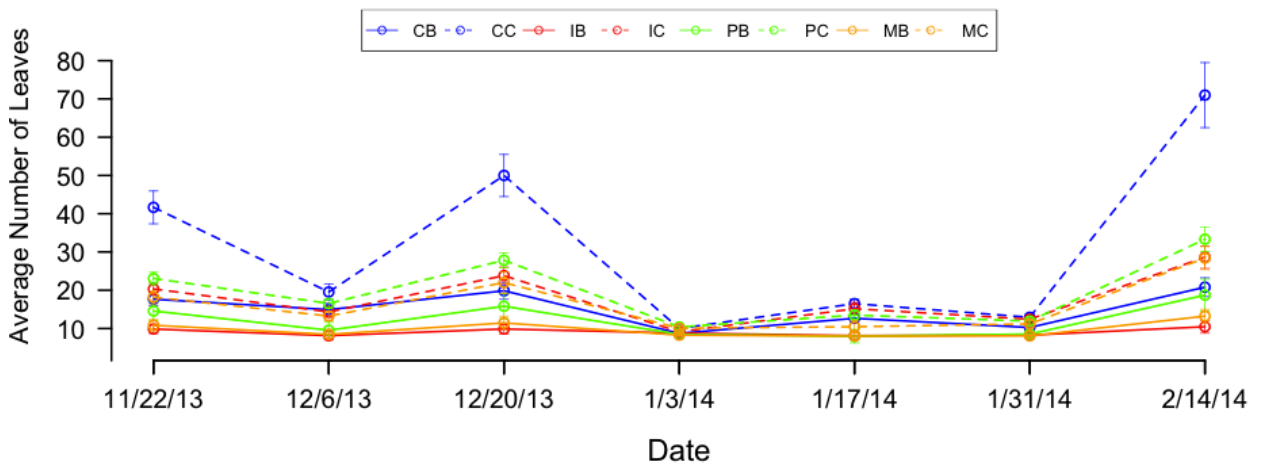


Figure 4.3. Average number of leaves of low-bush blueberry (*Vaccinium angustifolium*) and crowberry (*Empetrum nigrum*) grown in various substrate treatments over 12 weeks under greenhouse conditions at Saint Mary’s University, Halifax, NS. CB= *V. angustifolium* in control treatment, CC= *E. nigrum* in control treatment, IB= *V. angustifolium* in inoculum treatment, IC=*E. nigrum* in inoculum treatment, PB= *V. angustifolium* peat moss treatment, PC=*E. nigrum* in peat moss treatment, MB= *V. angustifolium* in mixture treatment, MC= *E. nigrum* in mixture treatment. n=3-15

4.3 Plant Health

Variability in plant health was only seen in *V. angustifolium*, not *E. nigrum*. After 12 weeks, only *V. angustifolium* had a significant difference in health scores ($\chi^2_3=11.99$, $p=0.007$). The control treatment for *V. angustifolium* had a significantly higher health score (Mean \pm SE: 3.47 ± 0.26) than the other *V. angustifolium* and *E. nigrum* treatments (Figure 3). Overall there was a parallel between how both species were affected by the treatments, as the same treatment for both species resulted in the highest, medium and lowest health scores. The control treatments had the highest health scores, followed by peat moss and inoculum treatments, then the mixture treatments (Figure 3). However, this relationship was not seen with survivorship.

Both species acted differently in the various treatments in terms of survival. In *V. angustifolium*, both the control and inoculum treatment had the highest survivorship (100%), while the peat moss treatment had the lowest survivorship (73.3%). The *E. nigrum* showed the opposite, the peat moss treatment had the highest survivorship (80%), while the control and mixture treatments had the lowest survivorship (66.7%).

Most species decreased in survivorship within the first three weeks of the study period; however, the control treatment for *E. nigrum*, inoculum treatment for *E. nigrum*, and mixture treatment for *E. nigrum* showed significant declines in the later weeks (Figure 4). Overall, *V. angustifolium* showed higher survivorship over than the *E. nigrum* during the study period.

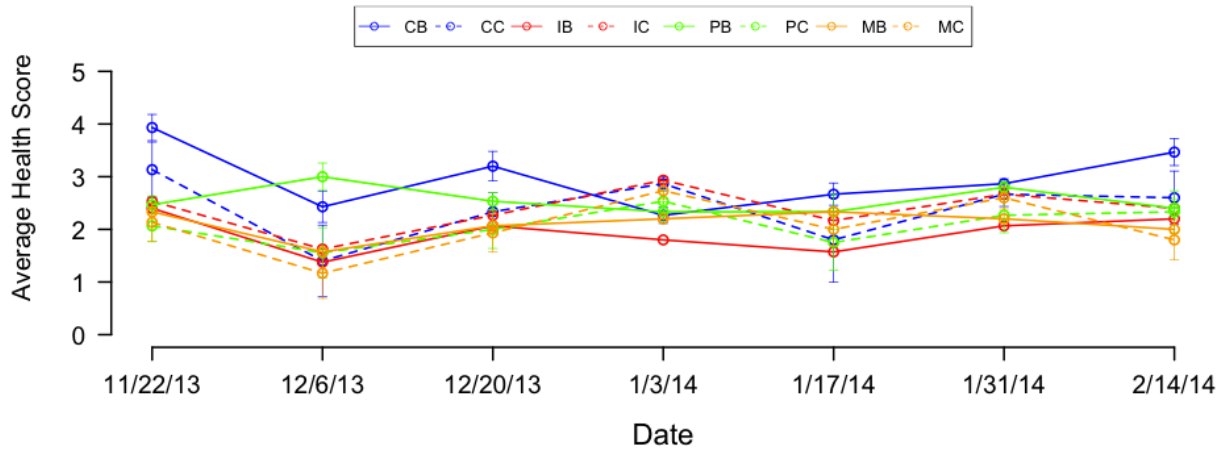


Figure 4.4. Average Health Score of low-bush blueberry (*Vaccinium angustifolium*) and crowberry (*Empetrum nigrum*) grown in various substrate treatments over 12 weeks under greenhouse conditions at Saint Mary’s University, Halifax, NS. 0=dead, 1= stressed plant showing visible wilting or browning, 2= a plant that shows little change since planting, 3= slow growth, 4= healthy plant exhibiting a large amount of growth, and 5= exceptional growth and fullness (Monterusso, Rowe, & Rugh, 2005). CB= *V. angustifolium* in control treatment, CC= *E. nigrum* in control treatment, IB= *V. angustifolium* in inoculum treatment, IC=*E. nigrum* in inoculum treatment, PB= *V. angustifolium* peat moss treatment, PC=*E. nigrum* in peat moss treatment, MB= *V. angustifolium* in mixture treatment, MC= *E. nigrum* in mixture treatment. n=3-15

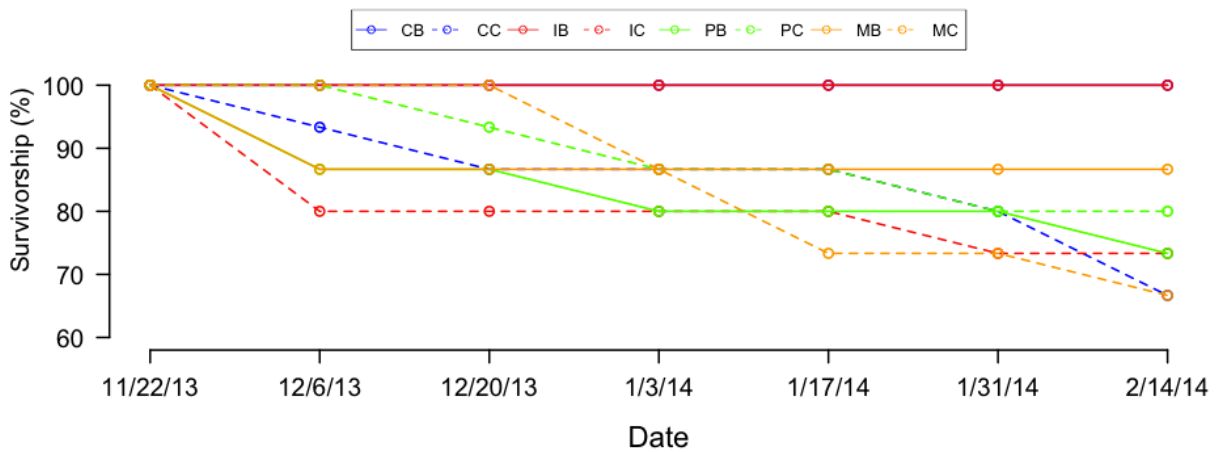


Figure 4.5. Survivorship (%) of low-bush blueberry (*Vaccinium angustifolium*) and crowberry (*Empetrum nigrum*) grown in various substrate treatments over 12 weeks under greenhouse conditions at Saint Mary’s University, Halifax, NS. CB= *V. angustifolium* in control treatment, CC= *E. nigrum* in control treatment, IB= *V. angustifolium* in inoculum treatment, IC=*E. nigrum* in inoculum treatment, PB= *V. angustifolium* peat moss treatment, PC=*E. nigrum* in peat moss treatment, MB= *V. angustifolium* in mixture treatment, MC= *E. nigrum* in mixture treatment. n=15

5.0 Discussion

5.1 Modifying Green Roof Substrate to Resemble Coastal Barren Soil

It is possible to modify green roof substrate to resemble coastal barren soils; however, a greater quantity of peat moss, inoculum, or another substance with high organic matter and low pH must be added. The inoculum treatment was found to have more soil parameters similar to coastal barren soils than the other treatments; however, it was missing two characteristics of coastal barren soils (i.e. low pH and high organic matter). The mixture treatment had the lowest pH and highest amount of organic matter, but had higher CEC and nitrate than the inoculum treatment and coastal barren soils (Fig 4.1. a & Fig 4.1. e).

The mixture treatment had two out of the three characteristics of coastal barren soils; however, it was missing the low nutrient characteristic. A higher CEC means the soil has more storage capability for nutrients, such as calcium, magnesium, potassium and ammonium, increasing the pool of nutrients available for plant uptake (Mengel, 1993). Nitrate is a form of nitrogen that can be easily taken up by plants. Typically, nitrogen is a limiting nutrient in ecosystems and regulates the construction and maintenance of plant cells (Forde & Clarkson, 1999). It is likely that the mixture treatment exhibited high nutrient availability compared to the inoculum treatment and coastal barren soil due to organic matter and pH relationships.

Major determinants of soil CEC are organic matter (Hunt, 1981), soil moisture, and pH (Barton & Karathanasis, 1997). As organic matter increases, the

CEC of soil also increases (Kweon, Lund & Maxton, 2013; Mengel, 1993). However, as the pH of soil decreases, the CEC of soil decreases (Carter & Gregorich, 2008). It is likely that the high variability of CEC seen in coastal barren soils (Figure 4.1 e) is due to varying pH and organic matter levels in different locations on the barrens. The mixture treatment had a significantly higher pH ($F_{3,8}=30.65$, Tukey's adjusted p-value=0.0e-7) than the coastal barren soils (Figure 4.1 c) and higher organic matter levels than the inoculum treatment (Figure 4.1 d); it is likely that the mixture treatments' CEC was representative of its higher organic matter and was not at a low enough pH to see the relationship between CEC and pH. It is possible that if the mixture treatment's pH was lower, it would have been at a CEC level closer to coastal barren soils.

The significantly higher pH level of the mixture treatment also explains the large amount of nitrate available in the treatment. Nitrate is a product of the nitrogen cycle, which is controlled by soil temperature (Toosi, Schmidt, & Castellano, 2014), pH, and microbial activity (Kemmit, Wright, Keith, & Goulding, 2006). Nitrate, in particular, is oxidized from ammonium by nitrifying bacteria in neutral or basic soils (Forde & Clarkson, 1999). However, in acidic soils, many of these bacteria cannot survive and the majority of nitrogen is typically in the form of ammonium (Forde & Clarkson, 1999). Further, increased nitrate production is a result of high organic matter, as there is more organic nitrogen to be broken down into ammonium and oxidized to nitrate. This is consistent with the soils analysis, which shows that the mixture treatment had the highest organic matter as well as the highest nitrate concentration (Figure 4.1 A & Figure 4.1 D). The high amount of

nitrate in the mixture substrate is a result of a too basic soil as well as its high organic matter content.

The low pH of the coastal barrens appears to have the greatest influence on the nutrient availability of the soil by retarding organic matter decomposition (Kemmit, Wright, Keith, & Goulding, 2006), reducing the CEC of the soil, and limiting the oxidation of ammonium into nitrate. Thus, there is reason to believe that decreasing the pH of the mixture substrate would be the most successful step in replicating coastal barren soils in green roof substrates, as the mixture treatment is already the most similar treatment to the two other defining characteristics of coastal barren soils.

5.2 Plant Response to Substrate Treatments

Overall, the control treatments had greater growth and health scores than the amended substrates, demonstrating that commercial green roof substrate may not be limiting shrub growth. Other than blueberries in the peat moss treatment, control treatments for both species resulted in significantly better growth in terms of height and number of leaves (Figure 4.1 and Figure 4.2). Further, the control treatments were the only treatment to possess significantly higher health scores than the other treatments (Figure 4.3). Survival however showed different trends. The inoculum treatment and control treatment resulted in the highest survival for *V. angustifolium*. Additionally, the peat moss treatment resulted in the highest survival for *E. nigrum*, which was 13.3% greater survival than the control treatment. Growth and health score results are inconsistent with the initial assumption that modifying

green roof substrate to mimic coastal barren soils would enhance shrub growth and health due to the immense differences between coastal barren soil and commercial green roof substrates. However, it was shown that modifying green roof substrate can increase survival in shrub species.

Soil analysis results confirmed the addition of peat moss and a native soil inoculum to green roof substrate significantly decreased the pH ($F_{3,8}=68.23$, Tukey's adjusted p-value= $6.98e-8$) and increased the organic matter content ($F_{3,8}=11.49$, Tukey's adjusted p-value= $1.6e-4$) of the substrate. Nonetheless, crowberries and blueberries in the mixture treatment exhibited some of the lowest plant heights, number of leaves, health scores and survival, with the exception of blueberry survival in this treatment. However, although the mixture treatment was closest to the coastal barren soils in terms of pH and organic matter concentrations, its nutrient levels were significantly higher than in coastal barren soils (for more detail see section 5.1).

Other studies, not on green roofs, have found that nutrient addition does not stimulate growth in *E. nigrum* (Chapin & Shaver, 1989). Further, *E. nigrum* has greater survival in areas with low concentrations of nitrate and phosphate (Bell & Tallis, 1973). The mixture treatment had the highest nitrate concentration (Figure 4.1a); this explains why *E. nigrum* had lower growth, health and survival; however, the control had the highest phosphate concentrations (Figure 4.1b), so it is unknown why *E. nigrum* had higher growth and health in this treatment.

Unlike *E. nigrum*, *V. angustifolium* has been observed to exhibit improved growth and health in response to increased nitrogen (Smagula & Hepler, 1980).

However, this response is only seen when *V. angustifolium* is transplanted correctly (Smagula & Hepler, 1980). Additionally, *V. angustifolium* more readily utilizes the ammonium form of nitrogen rather than nitrate (Hall, Forsyth, Aalders, & Jackson, 1972), thus the increased nitrate concentration in the mixture treatment would not show the same results even if *V. angustifolium* was transplanted correctly. These factors explain why *V. angustifolium* did not exhibit greater growth or health in the mixture treatment, but does not explain why growth in the mixture treatment was inferior to the control treatment.

It is possible that the levels of nitrogen were too high for *E. nigrum* and *V. angustifolium* growth. Due to low nutrient availability in their natural environment, both species have adapted to establish relationships with mycorrhizae fungi, which allows them to utilize all forms of organic nitrogen (Jonasson & Shaver, 1999; Hodge, Cambell & Fitter, 2001). The mixture treatment had significantly higher nitrate and total nitrogen concentrations than all other treatments (Fig. 4.1a & Appendix I). Assuming the added inoculum transferred mycorrhizae fungi to the substrates, both species would be able to uptake both inorganic and organic forms of nitrogen. These high concentrations of nitrogen available for *V. angustifolium* and *E. nigrum* uptake are antithetical to nitrogen availability in coastal barren soils; thus species growth and persistence would decline in these conditions.

Survivorship of *E. nigrum* was increased due to additives in the soil. The peat moss treatment resulted in 80% survivorship compared to 66.7% in the control treatment. Survivorship could have been higher due to the peat moss treatment having the lowest nitrate levels and *E. nigrum* survival is higher in low nitrate

conditions (Bell & Tallis, 1973). However, when compared to other studies, *E. nigrum* had survival of 99.4% when grown in commercial green roof substrate (MacIvor & Lundholm, 2009; Lundholm et. al, 2010). The soil amendments did not raise *E. nigrum* survivorship to its potential levels. It is possible that survivorship of *E. nigrum* was lower in this experiment due to differences in environmental conditions between greenhouse conditions and the natural environment.

Greenhouse conditions lack wind activity, have high moisture concentrations and have fairly stable day to night temperatures, which are all opposite of the extreme environments typical of coastal barrens (Oberndorfer & Lundholm, 2009) and green roofs (Nagase & Dunnet, 2011). One possible way environmental conditions can affect plant growth, health and survival is through changed decomposition rates. Substrate composition dictates the water storage capacity of a substrate (Nagase and Thuring, 2006) and the moisture level controls plant success (Monterusso, Rowe & Rugh, 2005); however, moisture level also plays a crucial role in determining decomposition rates (Friedrich, 2005). Thus, plant growth and survival is dependent on the most favorable substrate composition for moisture levels (Thuring, Berghange, Beattie, 2010).

Friedrich (2005) has demonstrated that organic matter acts differently depending on climate and moisture levels. When moisture and temperature levels are high, there is increased decomposition of organic matter. This leads to a production of fine particles creating a low-permeability mat, which can impede drainage, causing water build up and stress in plants. Dissimilarly to other forms of organic matter, peat moss has a high water storage capacity (Friedrich, 2005),

which allows decomposition to occur at slower rates (Ise, Dunn, Wofsy & Moorcroft, 2008). *E. nigrum*, in particular, is vulnerable to death via water logging and cannot survive prolonged periods of inundation (Bell & Tallis, 1973). This explains why *E. nigrum* survivorship was higher in the peat moss treatment than treatments with added organic matter that was not peat moss. However, when peat moss is exposed to higher temperatures and is inundated, this can increase the decomposition rates of peat moss (Ise, Dunn, Wofsy & Moorcroft, 2008). This could explain why *E. nigrum* survival was lower in greenhouses than previous studies on green roofs and do to *E. nigrum*'s water logging sensitivity, why *V. angustifolium* had greater survival than *E. nigrum* in all treatments (Figure 4.5). It is possible that moisture levels in the greenhouse were higher than what is typical of the outside environment, encouraging plant stress, especially in *E. nigrum*, and decreased survival in treatments with added organic matter.

5.3 Research Limitations and Future Research

This study was limited to a greenhouse experiment, which allowed for the control of other environmental factors; however, those environmental factors could be crucial to the growth and health of coastal barren shrubs. Another potential limitation is that the high nitrate concentration in the mixture treatment could be a result of a biased subsample. The control treatment had high variation (standard deviation=3.12 ppm) in nitrate concentration. It is possible that the mixture treatment was produced using a subsample of the control substrate with uniformly high nitrate concentrations, which resulted in significantly higher nitrate concentrations in the mixture treatment compared to the others. Future studies are

needed to compare the differences in environmental factors to determine if they are indeed significant as well as to replicate the experiment to assess any subsample biases.

5.3.1 Additional Future Research: Other Factors Promoting Shrub Failure on Green Roof Systems

Opportunities for future research to determine what other factors could be limiting shrub persistence on green roofs include examining the effects of salt spray, transplantation timing, and shading on shrub growth and health. Burley and Lundholm (2010) have determined salt spray to be a deterministic factor in coastal barren community structure. Based on the soil analysis in this study, sodium levels were not significantly higher in coastal barren soils compared to the control treatments. This suggests that sodium was not a limiting nutrient when previous studies failed to sustain *E. nigrum* and *V. angustifolium* on green roofs systems; however, this could be further studied. Time of transplantation has the potential to play a huge role in the success of shrubs, especially *V. angustifolium*, on green roof systems. It has been found that *V. angustifolium* is more successful when transplanted in the early spring during its dormancy than directly before the growing season (Lafond, n.d.). Lastly, *E. nigrum* and *V. angustifolium* are both shade-intolerant species (Burley, Harper, & Lundholm, 2010); a comparative study between sunlight intensity and duration between green roofs and the coastal barrens could provide insight into the shrubs' deficiency on green roof systems.

6.0 Conclusion

This experimental study was performed to determine if substrate composition was inhibiting Nova Scotian shrub persistence on green roof systems. The study was designed to verify or refute a potential explanation for the reoccurring issue of dominant shrubs being outcompeted by grass species when trying to establish natural coastal barren communities on green roof systems. Theoretically, coastal barren communities would increase diversity on green roofs, consequently increasing many of its' ecosystem services. However, without the successful establishment of the coastal barrens' dominant species, shrubs, on green roofs, it is unknown how these community structures will actually perform on green roof systems.

Based on theories within the literature and the incongruity between coastal barren soils and green roof substrate compositions, current commercial green roof substrate seemed to be the most probable cause of native shrubs inability to thrive on green roof systems. Thus, this study sought to answer to following questions:

1. Can we modify the organic matter content, pH and nutrient levels in green roof substrates to more closely resemble coastal barren soils?
2. Will *V. angustifolium* and *E. nigrum* have greater growth in green roof substrates that more closely resemble coastal barren soils?
3. Will *V. angustifolium* and *E. nigrum* have improved health and survivorship in green roof substrates that more closely resemble coastal barren soils?

Based on soil analysis, it was determined that green roof substrates can be modified to resemble coastal barren soils, but the addition of a substance with low pH and high organic matter is necessary. The mixture treatment was most characteristic of coastal barren soils as it had the lowest pH and highest organic matter. Analysis suggests that a low pH is crucial in determining the low nutrient concentrations in soil as it slows the decomposition of organic matter, reduces the cation exchange capacity, and sustains higher levels of organic nitrogen necessary for mycorrhizal functioning. If this study were to be replicated, it would be recommended that a substance with low pH be added to the mixture treatment in order to obtain a substrate representative of coastal barren soil.

The control treatments for both species had the greatest growth and health of all treatments. The mixture treatment, which most closely resembled coastal barren soils, had some of the lowest growth and health of all treatments. The failure of species in the mixture treatment could have been due to: high nutrient concentrations when plants prefer low nutrient conditions; unavailable concentrations of ammonium because the oxidation of ammonium to nitrate occurred too quickly; water-logged roots due to insufficient water drainage as a result of organic matter decomposition; or pathogens present in the native soil inoculum.

The results suggest that green roof substrate is not limiting *E. nigrum* and *V. angustifolium* growth and health. Further, modifying green roof substrates to resemble coastal barren soils decreases, rather than increases, *E. nigrum* and *V. angustifolium* growth and health. However, the study being limited to a greenhouse

experiment may have impacted the results by removing essential environmental conditions for species growth. Consequently, substrate composition cannot be removed as a potential factor until a green roof study is performed. Future studies should repeat this study on a green roof to see if the results stay consistent as well as examine other potential explanations for shrub deficiency in these systems such as: salt sprays' influence on coastal barren community structure; transplantation methodology; and the effects of shading on *E. nigrum* and *V. angustifolium* growth and health.

Despite the previous studies that found soil modification can enhance plant persistence and the variation between coastal barren soils and green roof substrate, green roof substrate does not seem to be limiting shrub growth and health in these systems. However, before substrate composition is omitted as a potential explanation for shrub deficiency in green roof systems, this study must be performed on a green roof.

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Appendix I

Table 1. Nutrient and pH in four different substrate treatments and respective ANOVA analysis, significance value at $\alpha=0.05$.

Parameter	Treatments				Statistical Results
	Mixture	Inoculum	Peat Moss	Control	
Nitrogen (%)	0.42 ± 0.01	0.39 ± 0.02	0.47 ± 0.03	0.28 ± 0.02	F _{3,8} =9.047 p-value =0.006
pH (pH Units)	5.76 ± 0.04	6.72 ± 0.05	6.15 ± 0.10	7.66 ± 0.04	F _{3,8} =181.9 p-value =1.07e-7
Buffer pH (pH Units)	7.65 ± 0.01	7.90 ± 0.01	7.74 ± 0.03	8.05 ± 0.01	F _{3,8} =89.2 p-value =1.72e-6
Organic Matter (%)	11.07 ± 0.78	9.5 ± 0.15	11.00 ± 0.20	6.20 ± 0.12	F _{3,8} =30.65 p-value =9.77e-5
Phosphate (kg/ha)	541 ± 23.58	492.67 ± 9.24	742.33 ± 44.20	974.00 ± 53.41	F _{3,8} =35.37 p-value =5.78e-5
K ₂ O (kg/ha)	346.33 ± 13.31	320.33 ± 4.96	405.67 ± 12.84	522.00 ± 31.05	F _{3,8} =24.18 p-value =2.3e-4
Nitrate-N (ppm)	8.68 ± 0.77	1.59 ± 0.42	1.41 ± 0.24	4.70 ± 1.80	F _{3,8} =11.38 p-value =2.9e-3
Base sat. K (%)	2.2 ± 0.06	2.5 ± 0.00	2.47 ± 0.03	3.13 ± 0.07	F _{3,8} =70.46 p-value =4.29e-6
Required Nutrient (kg/ha)	N, P2O ₅ , K ₂ O	N, P2O ₅ , K ₂ O	N, P2O ₅ , K ₂ O	N, P2O ₅ , K ₂ O	