

BANKING ON THE FUTURE OF ROCK OUTCROPS: ANALYSIS AND COMPARISON
OF SOIL SEEDBANKS AND EXTANT ABOVEGROUND VEGETATION COMPOSITION
OF TWO HIGH ELEVATION COMMUNITIES

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By

Amanda-Jean Blackburn

Director: Dr. Katherine Mathews
Professor of Biology
Biology Department

Committee Members: Dr. Beverly Collins, Biology
Dr. Gary Wein, Highlands-Cashiers Land Trust

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ABSTRACT

BANKING ON THE FUTURE OF ROCK OUTCROPS: ANALYSIS AND COMPARISON OF THE SOIL SEEDBANK AND EXTANT ABOVEGROUND VEGETATION OF TWO HIGH-ELEVATION COMMUNITIES

Amanda-Jean Blackburn, M.S.
Western Carolina University (April 2021)
Director: Dr. Katherine Matthews

The Southern Appalachian Mountains are an ancient region that supports an assortment of rare high-elevation communities. Rock outcrops are understudied communities that support a rich biodiversity of rare and endemic species and alpine relicts' representative of the Pleistocene. Rock outcrop communities can be classified into different types, but the two addressed in this study are montane red cedar outcrops (MRC, defined by the presence of *Juniperus virginiana*), and non-red cedar outcrops (NMRC, lacking *Juniperus virginiana*). These precariously balanced communities are strongly threatened by habitat destruction, air pollution, and global warming; increased knowledge of the biodiversity and biogeography of flora will aide in conservation and management efforts. Understanding the soil seed bank and its similarity to the aboveground vegetation is imperative to truly evaluate the differences in species diversity; processes affecting species composition such as environmental filtering, and seed dispersal and recruitment between outcrop types. Soil core samples were taken in Spring 2020 from 3 MRC and 3 NMRC rock outcrops and utilized in a combined direct seedling emergence and counting experiment to analyze species abundance and diversity within the soil seed bank. Herbaceous flora on the same sites was inventoried over two summer months to determine similarity between the soil seed bank and the extant aboveground vegetation on MRC and NMRC sites. Overall, both MRC and

NMRC sites displayed high soil seedbank and aboveground vegetation species diversity. The results indicate wide variation in the soil seedbank composition of both MRC and NMRC sites. The species compositions of the aboveground vegetation of MRC and NMRC sites was significantly different with strong clustering by site type, while the seedbank compositions was not significantly different. Further, broad separation between the soil seedbank composition and extant vegetation of both MRC and NMRC sites was demonstrated. These results indicate that MRC and NMRC sites are similarly affected by seed dispersal/recruitment. However, processes such as environmental filtering may influence the germination and establishment of seedlings and perpetuate differences in species composition of MRC and NMRC rock outcrop communities.

CHAPTER 1: INTRODUCTION

The Southern Appalachian Mountains region supports an assortment of high-elevation island communities including health balds, spruce-fir forests, grassy balds, and rock outcrops. Rock outcrops are isolated high-elevation communities characterized by expanses of open canopy over exposed bedrock (Wiser 1994, Wiser et al. 1996, Wiser & White 1999, Wiser et al 1996, Wiser et al. 1998, Culatta & Horton 2014, Mathews & Collins 2014 Horton & Culatta 2016) They are difficult to study due to their remote locations, distance from each other, and low economic importance. However, they support a rich biodiversity of rare and endemic plant species and serve as a model system for insular communities (Wiser 1994, Wiser et al. 1996, Wiser & White 1999, Wiser et al 1996, Wiser et al. 1998, Culatta & Horton 2014, Mathews & Collins 2014 Horton & Culatta 2016).

Flora present on rock outcrops is rare and distinctive. Forty species present on outcrops are considered rare in North Carolina and Tennessee, including five rare rock outcrop endemics (Wiser 1994, Wiser et al. 1998, Mathews & Collins 2014). Seven species found on these outcrops are also found in the alpine of New England and the arctic; it is hypothesized that these species are relicts of an alpine flora that once flourished in the Southern Appalachian Mountains during the previous ice age (Wiser 1994, Wiser et al. 1996, Wiser 1998, Wiser et al. 1998, Mathews & Collins 2014). Due to the current warm periods, most of these alpine relicts have retracted and populations have fragmented (Wiser et al. 1996, Mathews & Collins 2014). However, disjunct populations remain as a result of high light intensity and low competition driven by harsh environmental factors such as open canopies, shallow soil, exposed bedrock, and high amounts of runoff (Wiser 1994, Wiser et al. 1996, Wiser et al. 1998, Culatta & Horton

2014, Mathews & Collins 2014 Horton & Culatta 2016). The proportion of these species increases with latitude, elevation, and slope (Wiser et al. 1996, Wiser et al. 1998).

High elevation rock outcrops occur in the high peaks of western North Carolina and eastern Tennessee, and communities are often dominated by summer flowering, herbaceous perennials, shrubs, and stunted trees around the edge regions (Horton & Culatta 2016, Wiser 1994, Wiser et al. 1996). It has often been demonstrated that elevation is a dominant limiting factor within mountainous regions. Outcrops within the elevational range of 1200-2030m support over 80% of rare and endemic species populations, many of which are derivatives of past alpine tundra flora (Wiser & White 1999). These outcrops are mostly naturally occurring, but some were created more recently via severe soil erosion or debris avalanches (Wiser & White 1999). These high elevation regions are characterized by harsh environmental conditions such as shallow soil and high insolation which drives an increase in water stress (Culatta & Horton 2014, Horton & Culatta 2016, Small & Wentworth 1998). Intense light availability (though exposure varies widely from barren open granitic domes to partially shaded areas from surrounding forests), low temperatures in winter, and a short growing season limit growth on these rare sites (Horton & Culatta 2016). However, low competition allows resilient rock outcrop specialists to thrive in these harsh conditions (Horton & Culatta 2016). All rock outcrops can be characterized by these harsh conditions, but communities can occur over geological ranges and variations, producing several different types of rock outcrop communities (Wiser et al. 1996, Wiser et al. 1998).

Rock outcrops and their species composition and diversity can be influenced by elevation, geography, moisture, vegetation parental material, and bedrock type (Wiser et al. 1996, Wiser et al. 1998, Wiser 1998, Small & Wentworth 1998). Wiser et al. (1996) found that

site scale factors (elevation, slope, aspect, bedrock type), and microsite factors (soil depth, vegetation height, soil nutrients) were strongly correlated with species composition at the 1m² level. Moreover, outcrop communities are best classified by their elevation and bedrock type. High elevation rock outcrops can occur on a variety of bedrock compositions including felsic bedrock (granite, gneiss, schist) and mafic bedrock (gabbro and amphibolite) (Wiser et al. 1996, Wiser 1998, Wiser et al. 1998). Circumneutral soil refers to a nearly neutral pH (6.5-7.5), while mafic bedrock soil, more prominent in the northern regions, is richer in iron and magnesium and lower in aluminum compared to felsic bedrock soil (Wiser et al. 1996). Nevertheless, sampling bedrock type is laborious, and therefore presence of certain plant species can also be used to identify and classify types of rock outcrops.

High elevation rock outcrops can be divided into different categories: rocky summits (several subtypes), granitic domes, and red-cedar glades (Schafale 2012). Rocky summits are distinguished by a structure of sparse, herbaceous vegetation and extensive fractured rock (Schafale 2012). These are differentiated from granitic domes by presence of profusely fractured rock, as opposed to the smooth bedrock of domes, and can appear on both felsic and mafic bedrock (Schafale 2012). The extensively fractured rock allows for deeper soil pockets that allow more substantial plant coverage (Schafale 2012). Common plant types (though variations are possible depending on the subtype) include: *Kalmia latifolia*, *Carex misera*, *Micranthes petiolaris*, *Danthonia spicata*, *Solidago glomerata*, *Carex umbellata*, and *Dichanthelium acuminatum* (Schafale 2012). High elevation granitic domes, recognized by a smooth surface and absence of deep soil pockets, are dominated by bare rock lichens or shallow *Bryodesma tortipila* mats (Schafale 2012). Other common plant species include *Krigia montana* and *Houstonia longifolia* var. *glabra* (Schafale 2012). In this study, known felsic bedrock granitic domes and

rocky summits are combined into a single category entitled non-montane red cedar (NMRC) outcrops.

Montane red-cedar (MRC) woodlands, the other category considered in this study, are a rare open-canopy woodland with shallow soils, bare rock, and sparse vegetation that occur on steep, south-facing rock outcrops in the Southern Appalachian Blue Ridge Provinces (Small & Wentworth 1998, Schafale 2012). Vegetation surveys completed by Dellinger (1992) and Pittillo (1994) noted unusual vegetation, governed by *Juniperus virginiana*, indicative of high basic soils or mafic bedrock occurring on rock outcrops in the Southern Appalachians, and Small & Wentworth (1998) found that MRC plots supported many basophilic taxa associated with high base (mafic) substrates (Small & Wentworth 1998, Dellinger 1992, Pittillo 1994). Distinguishing features include an open canopy dominated by eastern red cedar (*Juniperus virginiana*), and lower strata herbaceous species requiring high light and circumneutral soil conditions such as: *Schizachyrium scoparium* var. *scoparium*, *Danthonia* spp., *Carex pensylvanica*, *Dichanthelium* spp., *Coreopsis pubescens*, *Solidago* spp., *Physocarpus opulifolius*, and *Philadelphus hirsutus* (Small & Wentworth 1998, Schafale 2012). Recorded basophilic taxa included: *Dodecatheon meadia*, *Lonicera flava*, *Rhus aromatica*, *Sedum glaucophyllum*, and *Symphoricarpos orbiculatus* (Small & Wentworth 1998). Eastern red cedar is a widely distributed conifer in the eastern United States and is highly adapted to the harsh environmental conditions associated with outcrops (Small & Wentworth 1998). Therefore, the presence of red cedars could be used as an indicator of bedrock type and soil pH.

The natural regeneration of plant life in these remote outcrop communities is dependent on seed dispersal and seedling recruitment (germination, establishment, and survival) (Du et al. 2007). Seed dispersal strongly influences the vegetation composition. Subjected to constant

erosion, soil saturation, and trampling, floral communities found on these sites are mostly components of primary succession (pioneer species). Therefore, the early stages of plant life (seed dispersal and seedlings) are the most important in natural regeneration (Du et al. 2007). (Du et al. 2007).

Soil seed banks represent all viable seeds present on or in the soil or associated litter (Allessio et al. 1989). Each seed bank has spatial and temporal dimension in relation to the vertical or horizontal dispersal throughout the soil (Allessio et al. 1989). These may also be classified as either transient, seeds that germinate within a year of dispersal, or persistent, seeds that lie dormant for many years before germination. Seed bank input is dependent on “seed rain” from parent plants and modes of dispersal may include fire, wind, water, animals, mechanical, or passive. Seed bank losses are controlled by factors such as light, temperature, water, pathogens, animal interactions, and burial depth. The inputs and outputs directly control the seed density of the soil and therefore the diversity and composition of the vegetation (Allessio et al. 1989).

Seed dispersal is necessary for maintaining populations on island communities (Mahanand & Behera 2019). Plants are specifically adapted to their dispersal type, and the success of their dispersal helps to define the survival rate of the species in a new habitat (Mahanand & Behera 2019). Abiotic dispersers such as wind/water are more efficient at long-distance dispersal, while biotic dispersers are better at overcoming obstacles that challenge dispersal ability (Mahanand & Behera 2019). Species that are adapted to both disperser types are typically more prolific throughout their habitat range than species that rely on one disperser type (Mahanand & Behera 2019).

Rock outcrops are terrestrial islands that likely rely on seed rain from chance dispersal by wind/water, and present parental vegetation input. The extant vegetation in the center outcrop

region and the edge forest region directly impacts the species composition and diversity on both MRC and NMRC outcrops. MRC outcrops are characterized by the presence of *Juniperus virginiana* (mostly) around the margins of the outcrop and species indicative of basic and circumneutral soil conditions (Small & Wentworth 1998, Schafale 2012). NMRC outcrops, consisting in this study of rocky summit and granitic dome subtypes, are distinguished by the presence of species that are absent in low elevation communities. They are also characterized by the presence of species not found in the high-peak subtype (Schafale 2012). Differences in soil parent material on MRC and NMRC rock outcrops could be driving differences in species composition/diversity on sites. Moreover, geographical isolation from other outcrops could limit dispersal ability of endemic outcrop species and influence species composition. The sites considered in this study are separated by a range of geographical distances (4.51–31.59 km). However, in insular communities, the dispersal network is often regulated more by the ecology of the receiving island rather than the proximity of the mainland seed source (Mahanand & Behera 2019). MRC rock outcrops are characterized by the presence of red cedar in an open-canopy woodland with sparse understory and exposed bedrock. NMRC rock outcrops are characterized by the absence of red cedar on either a granite-dome or rocky summit subtype. Both MRC/NMRC sites are affected by harsh environmental conditions such as shallow soil, high-light intensity, constant erosion, and edaphic drought that influence the extant vegetation (Culatta & Horton 2014, Horton & Culatta 2016, Small & Wentworth 1998). Therefore, differences in environmental filtering processes on MRC/NMRC rock outcrops may be impacting species composition.

Experimental studies have demonstrated that seed dispersal influences the spread and persistence of natural populations, but seed persistence (survival of seeds following maturity)

increases the chance of germination and establishment ((Levine & Murrell 2003, Long et al. 2015). The length of persistence, a period of time called dormancy, can impact community structure and composition because of its connotations for interspecific and intraspecific competition (Long et al. 2015). The breaking of seed dormancy is affected by a wide variety of environmental factors that determine if the seed exits the seed bank via germination. Seed rain is also influenced by other factors such as density/distribution, fecundity of parent plants, and physical site features (Levine & Murrell 2003). Therefore, though it is necessary for sustaining populations, seed dispersal may not be the primary driver of species composition on high-elevation rock outcrop communities. This is supported by evidence of long-term persistence of alpine disjuncts and Pleistocene relicts. Broad geographical distance and lack of habitat that meets the specific microsite conditions that these species need, suggests that seed dispersal is unlikely to occur between southern and northern populations, yet disjunct populations continue to survive. This is indicative of other factors influencing the species composition and diversity on rock outcrops such as environmental filtering processes that impact seed germination and establishment.

Seed germination is critical to the life cycle of plants and is restricted to locations that have a specific set of environmental conditions (Albrecht & Penagos 2012). In temperate habitats most seeds are dormant at maturity and cannot germinate immediately because conditions are unfavorable for seedling establishment (Albrecht & Penagos 2012). Temperature is regarded as the primary factor regulating seed dormancy but following the break of dormancy other environmental factors may influence the germination process of plant species (Albrecht & Penagos 2012). Though seed germination conditions are species specific, it is possible that soil pH could play a role in germination ability of outcrop species.

Soil pH is mainly influenced by parent material and organic/inorganic content (Basto et al. 2015). Solid matter constitutes the majority of the soil volume and therefore is the primary influencer of soil pH (Basto et al. 2015). The mineral and organic matter makeup of the soil affects the accumulation/production of H⁺ ions, decreasing soil pH (Ji et al. 2014, Basto et al. 2015). In contrast, carbonates “consume H⁺ ions” allowing the soil to maintain alkalinity (Ji et al. 2014, Basto et al. 2015). Climatic and anthropogenic factors may also influence the soil pH by disrupting the balance of H⁺ and OH⁻ ions and impacting seed persistence in the soil (Ji et al. 2014, Basto et al. 2015).

Though it tends to be considered a “constant species trait”, seed persistence (survival of seeds following maturity) in the soil may be impacted by environmental variation (Basto et al. 2015). Microenvironmental factors like temperature, moisture, and nutrient availability have been shown to affect seed persistence, but other factors such as soil acidity may also be influencing seed persistence (Basto et al. 2015). In grassland studies, soil pH has been demonstrated to be a main influencer of grassland diversity and composition (Basto et al. 2015, Kalusová et al. 2009). Because soil pH is often connected to other environmental factors, it is difficult to draw direct links between soil pH and seed persistence (Fernández-Calviño et al. 2011, Basto et al. 2015) Therefore, it is easier to observe the indirect effects of soil pH on seed persistence, specifically by examining the impact on seed germination (Basto et al. 2015).

Soil acidity has also been demonstrated to constrain root growth thereby negatively impacting germination in plant species (Turner et al. 1988, Haling et al. 2010). Constant root growth is crucial to nutrient and water uptake in plants, subsequently affecting plant resilience and productivity (Harling et al. 2010). This is especially important in habitats with high water stress; the establishment of a root system is crucial on rock outcrops where the soil is thin and

edaphic drought is increased (Small & Wentworth 1998, Haling et al. 2010, Culatta & Horton 2014, Horton & Culatta 2016). Haling et al. (2010) demonstrated that a decrease in soil strength and the presence of macropores can help to alleviate the effects of soil acidity in some habitats, however this is unlikely to occur on rock outcrops as the soil is thinly stretched over bare rock with no access to deep underground water reserves. Therefore, if soil pH differences are present on MRC/NMRC rock outcrops it may be acting as an environmental filter and perpetuating a difference between what is dispersing to these sites and what is forming a component of the flora.

A proportion of extant species found on rock outcrops are either relations of disjunct populations of alpine species that are separated by a wide geographical range or are relict species that flourished during the Pleistocene. The outcrops are geographically isolated from each other indicating that chance dispersal by wind or animal or directly from surrounding vegetation parental material are the main methods of seed input. Changes in environmental conditions could disrupt these patterns permanently. Species often respond to these environmental changes through migration to more suitable habitat or phenotypic plasticity (Culatta & Horton 2014). However, because of the high elevation, low genetic diversity as described by Culatta & Horton (2014), and highly specific required conditions for outcrop endemics, it may limit population's ability to shift their ranges or respond to environmental changes with genetic selection of phenotypic plasticity (Culatta & Horton 2014). The transitional zones between the southern disjunct populations and their northern counterparts are likely uninhabitable as they don't meet the specific environmental conditions needed for survival, therefore the migratory ability of these species is severely limited. Further, as the climate changes, the outcrop specialists may be unable to respond with phenotypic plasticity and will likely be outcompeted by more generalist species.

This would result in a loss of overall biodiversity in the Southern Appalachian Mountains with effects that could impact species-interactions and permanently alter the unique flora of the rare high-elevation rock outcrop communities. Vegetation surveys conducted by Mathews & Collins (2014) suggest that rock outcrops may already be losing sensitive and specialized species.

No work has been done to survey the soil seedbanks of the high-elevation rock outcrops in the southern Appalachian Mountains. Two techniques have been consistently used in the literature to establish seed bank densities following field collection: direct counting and seedling emergence (Gross 1990, Price et al. 2010, Erfanzadeh et al. 2013). Direct counting requires counting each individual seed found in the soil using techniques such as sieving or flotation for separation. This method accurately determines the total number of seeds within the soil but offers nothing on seed viability. A less tedious technique is seedling emergence, which allows for an estimation of the total number of viable seeds by germinating them under favorable conditions. This has been demonstrated to be a reliable estimate of the viable seeds in a sample (Page et al. 2006, Gross, 1990). A combination of these two techniques is encouraged for more precise results (Allessio et al. 1989).

Analyzing the soil seedbank of high-elevation rock outcrops is imperative to creating policies to preserve these rare habitats. Unprotected outcrops are strongly threatened by habitat destruction via development for resorts, private homes, and trampling/other damage by hikers and rock climbers (Wiser & White 1999, Mathews & Collins 2014). Air pollution is another major threat that may induce soil fertility changes and cation cycling as high-elevation outcrops are consistently immersed in fog and clouds that are often more acidic than precipitation (Wiser & White 1999, Horton & Culatta 2016). Global warming also poses a threat to these habitats, as shifts in temperature and cloud patterns may negatively impact rock outcrop flora (Culatta &

Horton 2014, Mathews & Collins 2014). Rock outcrop endemics have been demonstrated to rely heavily on frequent cloud immersion to alleviate water stress and shifts in global cloud trends threatens a greater possibility of drought (Culatta & Horton 2014, Horton & Culatta 2016). Moreover, increase in temperature and changes in precipitation may increase the length of growing seasons, decreasing plant carbon gain, productivity, and negatively impacting plant-animal and plant-pollinator interactions (Bemmels & Anderson 2019).

Due to low genetic diversity and migratory ability, it is unlikely that high-elevation species will be able to shift their distributions north (Culatta & Horton 2014, Bemmels & Anderson 2019). Island isolation has been demonstrated to decrease native species occurrence while the abundance of non-native, but efficient dispersers increase (Mahanad & Behera 2019). Anthropogenic impacts will have a prominent effect on the species composition and diversity of high-elevation rock outcrops. Examining the soil seedbank and extant aboveground vegetation will provide insights on the processes influencing species (seed dispersal/germination/establishment) composition and diversity on both MRC and NMRC rock outcrops. Moreover, increased knowledge on the differences in biodiversity and biogeography of flora on MRC and NMRC sites will aid in conservation/management by informing protective policy decisions specific to rock outcrop type. This knowledge can be obtained by exploring the following questions:

- 1.) Is there a disconnect between the soil seed bank and the present aboveground vegetation on MRC and NMRC outcrop communities?
- 2.) Is there a difference in species diversity and composition on MRC and NMRC sites?

To answer these questions, I completed vegetation surveys on three MRC and three NMRC outcrops in the Southern Appalachian Mountains. I took soil core samples and performed

a joint seedling emergence and direct counting experiment. I utilized field surveys and herbarium information to: (1) identify species and estimate species composition and diversity within the soil seed bank of both MRC and NMRC sites (2) analyze the differences between flora found on MRC and NMRC sites (3) determine how well the soil seed bank matches the above ground vegetation on MRC and NMRC sites. I expected to find differences in species diversity and composition on MRC and NMRC sites as germination on MRC sites is likely influenced by soil pH environmental filtering processes evidenced by the presence of basophilic taxa on sites. I also expect to see a difference in the soil seedbank and the extant aboveground vegetation of MRC sites due to environmental filtering. I expected to see similarities between the soil seedbank and extant aboveground vegetation of NMRC sites as there is no current evidence of environmental filtering occurring via the soil pH.

CHAPTER 2: METHODS

Field Site Descriptions

The study sites include six high-elevation rock outcrop communities within the Southern Appalachian Mountains of North Carolina. Within Jackson and Macon Counties, three montane red cedar (MRC; *Juniperus virginiana* present) and three non-montane red cedar (NMRC; *Juniperus virginiana* absent) sites were selected. Sites were chosen based on similarity in elevation, proximity, and walkability. ArcGIS was utilized to plot each outcrop and estimate outcrop area and distances between each site. A preliminary survey of each site was conducted to determine the walkable regions that may be sampled, and a matrix of geographical distances between sites was generated.

Cedar Knob (35°10'07"N, 83°15'26"W)

MRC woodland occurs on the slopes of Cedar Knob with a recorded elevation of 1234.3 m and an estimated total area of 126,000 m². Cedar Knob is located 6 miles east of Franklin in the Cowee Mountains of eastern Macon County. Access via Stiwinter Rd. was granted by Gary Wein and the Highlands-Cashiers Land Trust.

Cedar New (35°18'44"N, 83°06'29"W)

A private MRC rock outcrop located on the ridge line near Judaculla Rock in Cullowhee, Jackson County. Recorded elevation was 1000-1150 m with an estimated area of 73,600 m². Access was granted by land-owner Griffin Bell.

Doubletop (35°16'28"N, 83°05'28"W)

An MRC community located on a ridge in Tuckasegee, Jackson County, overlooking Cedar Cliff Lake. Max elevation was recorded at ~1300 m and estimated total area was ~55,000 m². Access was granted by Gary Wein and the Highlands-Cashiers Land Trust.

Laurel Knob (35°07'36"N, 83°07'59"W)

NMRC community located outside of Cashiers, Jackson County, near Lake Glenville, Laurel Knob sits at an elevation of ~1239.7 m, with an estimated area of 138,600 m². Access is granted via Gary Wein from the Highlands-Cashiers Land Trust.

Rock Mountain (35°06'34"N, 83°04'16"W)

A NMRC outcrop community located in Cashiers, Jackson County. Elevation was recorded at ~1300 m, and estimated total area was ~130,000 m². Access was granted by Gary Wein and the Highlands-Cashiers Land Trust.

Satulah (35°02'10"N, 83°11'31"W)

NMRC community located in Highlands, Macon County. Elevation was recorded at ~1383 m, and the estimated area was ~70,000 m². Access was granted via Gary Wein from the Highlands-Cashiers Land Trust.

Field Methods

Soil sample collection began in the spring of 2020 to allow for natural cold stratification of seeds (Erfanzadeh et. al 2013). An extant herbaceous species vegetation survey was conducted at the beginning of the collection period (late March/early April), and two additional herbaceous species surveys were conducted throughout the growing season: mid-summer (July/August), and early fall (September). Preliminary “checklists” formatted from herbarium data were compiled and utilized in the aboveground vegetation surveys of all six sites.

Soil Core Collection

On each site, a walkable sampling grid of 10 x10m quadrats was created using 50 m measuring tapes (Fig. 1). Each quadrat represents a 10m x10m² distance and was utilized to estimate the size of each outcrop. The first tape was run parallel to the slope of the outcrop, 5 m

from the edge of the walkable regions, until it reached the end of the walkable region or end of the tape. The second tape was placed perpendicular through the center of the first tape to create the edge outline of the grid. Five meters out from both ends of each tape marked the middle of the first 10x10m center quadrats (C3 on Fig. 1). The edge quadrats were identified by using the outside center quadrats (Fig. 1). Center and edge plots were assigned numbers and 30% of the center and 25% of the edge quadrats (with a minimum of 10 samples per site) were sampled using a random number generator. Samples were taken from the substrate within the selected quadrat as deep as possible, up to 10 cm, using a soil tin. Within each quadrat, 4 soil cores no closer than 1 m apart were pulled and combined into a Ziplock bag. Soil samples were transported back to Western Carolina University in Ziplock bags and stored in a refrigerator to prepare for the direct germination experiment.

[Fig. 1]

E1	E2	E3	E4	E5	E6	E7
E24	C1	C2	C3	C4	C5	E8
E23	C6	C7	C8	C9	C10	E9
E22	C11	C12	C13	C14	C15	E10
E21	C16	C17	C18	C19	C20	E11
E20	C21	C22	C23	C24	C25	E12
E19	E18	E17	E16	E15	E14	E13

Figure 1: Example visualization of grid sampling construction. The red cross hairs represent the 50m measuring tapes that were run perpendicular to each other to create the sampling grid of the walkable region of the outcrop. Each quadrat is 10m x 10m. C = center plots; E = edge plots

Vegetation Survey

A preliminary survey of the extant, aboveground herbaceous vegetation for each site was taken prior to soil sample collection. After soil core collection, two additional surveys of the aboveground vegetation were taken throughout the growing season: July/August, and September. On each outcrop, presence/absence data of herbaceous plant species was recorded in the center and edge regions, respectively. For 90 minutes, the center region and edge regions of each site were visually surveyed and presence/absence data of extant species was recorded. Starting at the top of the outcrop, herbaceous flora data was gathered in the center of the grid by walking longitudinal transects back and forth across the center region. The edge region was surveyed by walking along the perimeter of the outcrop. All present herbaceous vegetation identified to species using SERNEC herbarium references, iNaturalist, and seedling keys (Weakley 2005) in the combined 3-hour period was utilized in data analysis.

Greenhouse Methods

Soil core samples from each quadrat were transplanted all at once on May 10, 2020 into individual horticulture flats (with drainage holes) and stored in a greenhouse under advantageous conditions (21°C) for 114 days. Each flat was lined with a paper towel and a 1/8" layer of non-nutritious play sand for drainage. A layer of fine grain potting soil (Miracle Grow) was added, and large debris was removed from the soil samples. The soil samples were spread evenly across the top and tamped down. Flats were arranged haphazardly on a greenhouse bench, and the samples were watered with a fine mist hose daily to keep the soil moist. Trays were shifted around the bench weekly according to a predetermined, clockwise rotation schedule.

Emerging seedlings were marked with color-coded plastic toothpicks, and the number of individuals of each species were recorded regularly. Flats were monitored daily for newly

emerging seedlings to be sure no seedlings emerged but died before they could be recorded (Gross 1990). Living, photographed, or pressed seedlings were identified to the lowest taxonomic level possible using seedling keys (Weakley 2005), iNaturalist.org, SERNEC herbarium reference collections (sernecportal.org), and consultation with experts (K. Mathews, J.D. Pittillo, E. Schwartzmann, pers. comm.) Once individuals were identified, they were photographed/labeled, removed from the pot, and discarded. By September 1, any remaining unidentified seedlings were removed from the flats and transferred to individual pots, where they were grown to maturity for identification. The soil samples were continuously monitored for another two weeks, before a final census was taken (Gross 1990).

Data Analysis

Differences between MRC and NMRC species composition and diversity (species richness/evenness) were analyzed using the seedling emergence greenhouse experiment and extant vegetation survey data. Community differences between MRC/NMRC sites were identified by comparing the observed soil seedbank herbaceous species composition to the extant aboveground herbaceous species composition. Soil seedbank and vegetation survey data were subsampled via site type (MRC/NMRC) and plot type (center/edge) and tested to draw broader comparisons between the site types. Differences in the herbaceous plant communities between the six sites were tested for using presence/absence data for (1) total emergence (center + edge) (2) center emergence (3) edge emergence (4) total vegetation (center + edge) (5) center vegetation (6) edge vegetation data.

Diversity and Abundance of Seedbank and Extant Vegetation

Total herbaceous species abundance (species count) data for each site was calculated from the emergents in the soil seedbank samples. The most and least abundant species for both

MRC and NMRC sites were identified, and rank-abundance curves were constructed to visualize the distribution of species between site types. Rank-abundance curves calculated using R software (R Core Team 2016) were also used to visualize species evenness within the center and edge regions respectively of MRC and NMRC sites. Only presence/absence data were collected during aboveground vegetation surveys. Therefore, the Shannon Diversity Index was also utilized in estimating the species diversity within the soil seedbank and extant aboveground vegetation of MRC/NMRC sites.

The Shannon Diversity Index [$H = -\sum[(p_i) \times \ln(p_i)]$] was used to characterize species diversity in a community by calculating the proportion of a species relative to the total number of species (McCune & Mefford, 2016). Species diversity was estimated for subsamples of the soil seedbank emergence data and the aboveground vegetation survey data of all six sites including the (1) total emergence (2) center emergence (3) edge emergence (4) total vegetation (5) center vegetation (6) edge vegetation (R Core Team 2016).

Community Analysis of Seedbank and Extant Vegetation

Ordination methods using PCOrd v.7 (MJM software) were used to analyze the multivariate soil seedbank and aboveground vegetation data. Nonmetric Multidimensional Analysis (NMDS) was used to simplify and visualize the data by arranging sites in two-dimensional ordination space to best represent the similarity values calculated from the species presence/absence data. The closer the sites plot together, the more similar their species composition within the subsample. NMDS is a non-parametric approach well suited for accounts of species abundance utilizing the predetermined distance metric (McCune & Mefford 2016). Jaccard's distance was selected as the metric and was calculated using the soil seedbank and extant aboveground vegetation species data. NMDS plot ordinations were created for (1)

combined total emergence and vegetation (2) total emergence (center + edge) (3) total vegetation (center + edge) (4) total center (emergence + vegetation) (5) total edge (emergence + vegetation) (6) total MRC (emergence + vegetation) (7) total NMRC (emergence + vegetation) data.

Multi-response permutation procedure (MRPP) in PCOrd was used to test for differences in the herbaceous species composition of the (1) combined total emergence and vegetation (2) total emergence (3) total vegetation (4) total center (5) total edge data of all six sites. It was also used to test for differences between the species compositions of the soil seedbank and aboveground vegetation of MRC sites and NMRC sites, respectively. MRPP is a non-parametric procedure that tests for differences between the predetermined groups based on a chosen distance function (McCune & Mefford 2016); the predetermined groups were “type” (MRC/NMRC site) and “sample” (emergence/vegetation). Therefore, the null hypothesis is that observed separations are independent of site or sample type (McCune & Mefford 2016, Monar 2018). Jaccard’s distances were calculated from species data. Rows containing all zeroes were removed from the analysis. The alpha used to determine significance was 0.05.

Indicator species analyses were performed in PC Ord to identify species that indicate (1) soil seedbanks of MRC/NMRC sites (2) aboveground vegetation of MRC/NMRC sites (3) center plots of MRC/NMRC sites (4) edge plots of MRC/NMRC sites. Indicator species analysis is commonly used in ecology to describe the value of different species for indicating environmental conditions (McCune & Mefford 2016). It combines the concentration of species abundance data and the probability of the occurrence of a species in a particular group, producing indicator values for each species in predetermined groups (Type/Sample) (McCune & Mefford 2016). The alpha used to determine significance was 0.05 (McCune & Mefford 2016, Monar 2018). Dominance curves were also used to study the distribution of species in subsamples of

MRC/NMRC soil seedbanks and aboveground vegetation surveys. Dominance curves were constructed for the (1) combined total emergence and vegetation (2) total emergence (3) total vegetation (4) total center (5) total edge (6) total MRC (7) total NMRC data.

CHAPTER 3: RESULTS

Composition and Diversity of the Seedbank and Aboveground Vegetation

Seedbank

Samples germinated in the greenhouse were abundant and diverse; 34 species and 990 individuals were identified (Appendix A). Most species were not highly abundant and plot diversity was driven by 1 or 2 grass species. Emergents, seedlings germinated from the soil seedbank samples, were divided relatively evenly among site types: MRC (44%) and NMRC (56%) of total individuals. Of the MRC emergents, *Dichanthelium acuminatum* (19.3%), *Danthonia spicata* (15.2%), and *Phermeranthus teretifolium* (15%) were the most abundant species; *Verbena urticifolia* (0.001%) and *Brassica sp.* (0.001%) were the least abundant species. Of the NMRC emergents, *Dichanthelium acuminatum* (23.7%), *Danthonia spicata* (18.5%), and *Schizachyrium scoparium* (12.8%) were the most abundant species; *Hypericum stragulum* (0.002%), *Viburnum sp.* (0.002%), and *Danthonia sp.* (0.002%) were the least abundant species. Rank abundance curves were also used to visualize species richness/evenness in total, center, and edge regions of MRC and NMRC plots (Fig 2, 3, 4). The smooth and gradual slope of the rank abundance curves indicate similarity in species richness/evenness between the total, center, and edge of MRC/NMRC sites.

[Fig. 2]

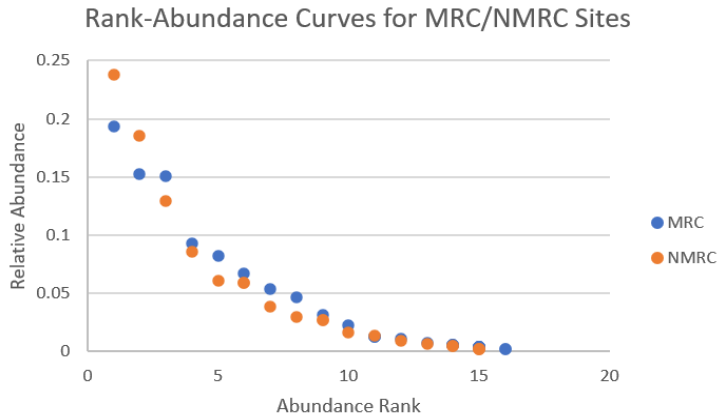


Figure 2: Rank abundance curve calculated from emergence data of MRC and NMRC sites.

[Fig. 3]

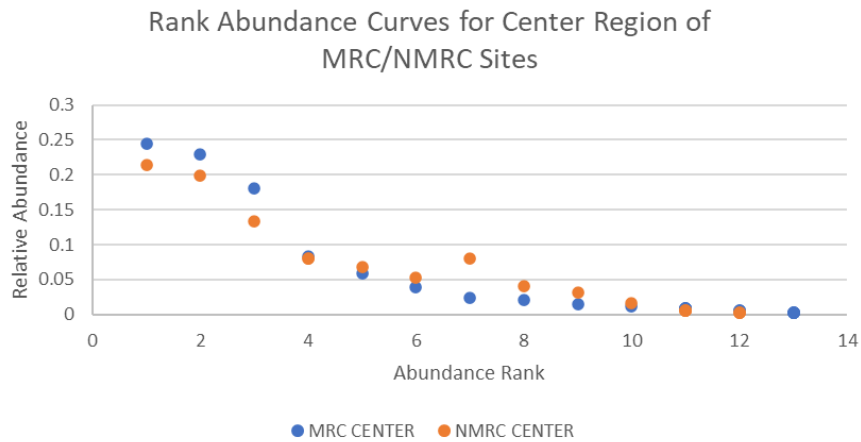


Figure 3: Rank abundance curve calculated from emergence data of center plots of MRC and NMRC sites.

[Fig. 4]

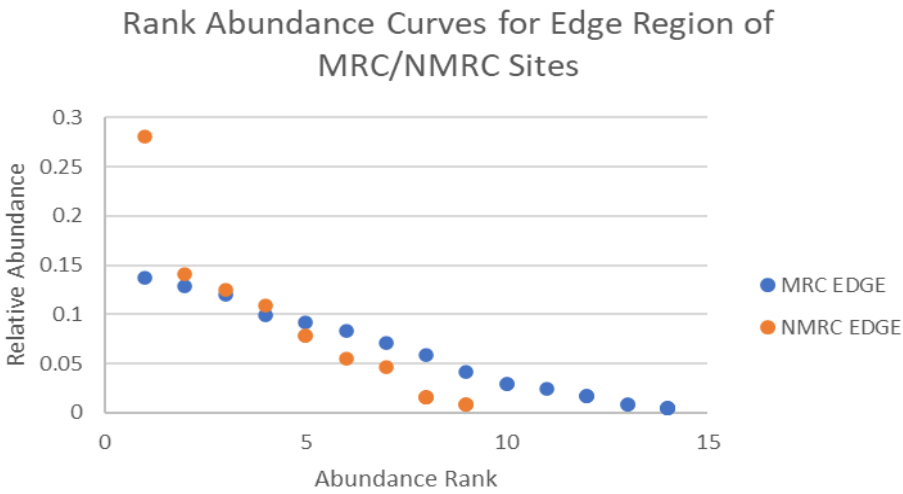


Figure 4: Rank abundance curve calculated from the emergence data of edge plots of MRC and NMRC sites.

Aboveground Vegetation

In the total (center + edge) aboveground vegetation presence/absence data of all 6 sites, 90 species were identified (Appendix B). Nine taxa were present in the soil seedbank and absent from the aboveground vegetation; 66 taxa were identified in the aboveground vegetation and absent from the soil seedbank; and 25 taxa were present in both. When classified by total (center + edge) site type, 42 taxa were present in MRC and absent from NMRC sites; 31 taxa were present in NMRC sites and absent from MRC sites; and 26 taxa were found in both. Within center and edge plots, 60 taxa were present in the center plots but absent from edge plots; 11 taxa were present in the edge plots but absent from center plots; and 26 taxa were present in both.

The Shannon Diversity Index [$H = -\sum[(p_i) \times \ln(p_i)]$] was used to evaluate species richness and diversity within the soil seedbank of both MRC/NMRC sites. The Shannon diversity analysis indicated high species richness in the total, center, and edge soil seedbanks of each site except

Laurel Knob. High species richness within the total, center, and edge vegetation data was also indicated by this analysis. Due to differences in size of each site, Shannon index numbers cannot be compared, rather they estimate species diversity within the sampling grid of each site.

Table 1. Shannon diversity index calculations for emergence and vegetation data of MRC/NMRC sites. Column headings are: TotEmerg = total (combined center and edge) emergence data; EmergC = center emergence plots; EmergE = edge emergence plots; TotVeg = total vegetation data; VegC = center vegetation data; VegE = edge vegetation data.

Site	Type	TotEmerg	EmergC	EmergE	TotVeg	VegC	VegE
Cedar Knob	MRC	2.3590888	1.8906315	1.377439	3.178054	3.367296	1.386294
Cedar New	MRC	1.9795880	1.8234377	2.243855	3.583519	2.944439	1.098612
Doubletop	MRC	2.1933951	1.9213363	1.418304	3.688879	3.295837	1.609438
Laurel Knob	NMRC	0.6495432	0.7683511	0.000000	3.637586	3.258097	1.386294
Rock Mountain	NMRC	1.9794868	1.9809465	1.565426	2.944439	3.044522	1.098612
Satulah	NMRC	1.9771614	1.0292816	1.588480	3.433987	3.401197	1.945910

Community Analysis Seedbank and Aboveground Vegetation

Mantel Test

Mantel test results for correlations between the matrix of the geographical distance (km) of all 6 sites and the matrix of ecological distance between subsamples of sites using the Jaccard's dissimilarity metric. All values were non-significant ($p > 0.05$), indicating little correlation between geographical distance of sites and differences in community species composition (Table 2).

Table 2. Statistical Mantel test for correlations between geographical and ecological distance of MRC/NMRC sites

Emergence/Vegetation	p-Value
Total Emergence	0.7935
Center Emergence	0.6999
Edge Emergence	0.4200
Total Vegetation	0.1763
Center Vegetation	0.1474
Edge Vegetation	0.2122

NMDS Ordination Analysis

NMDS ordination was used to visualize patterns of difference and overlap in herbaceous species composition via groupings by site (MRC/NMRC) and sample (emergence/vegetation) types. When grouped by site type, NMDS analysis indicated (1) wide variation in species composition between the total emergence (center + edge) data of both MRC and NMRC sites (Fig. 5) (2) similarity between the total aboveground (center + edge) vegetation species composition of MRC sites and NMRC sites respectively (Fig. 6).

[Fig. 5]

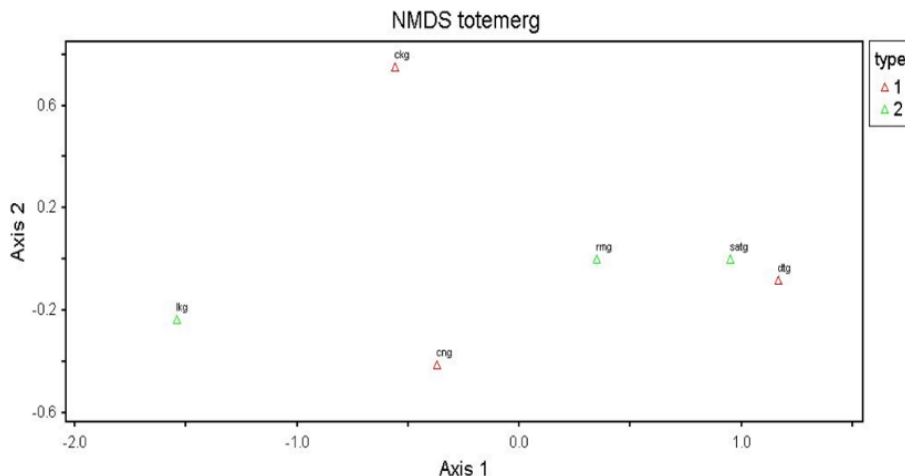


Figure 5: NMDS analysis of the total (center + edge) emergence data when grouped by site type: (1) MRC (2) NMRC

[Fig. 6]

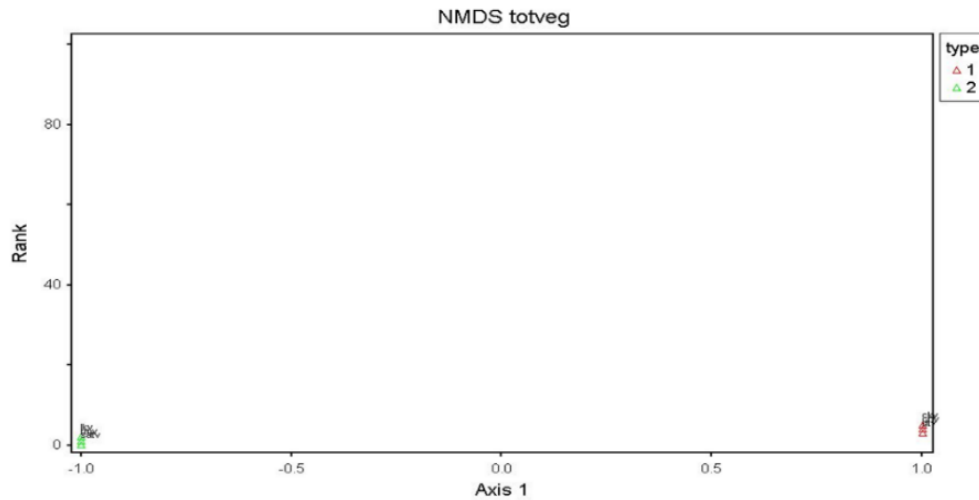


Figure 6: NMDS analysis for the total (center + edge) aboveground vegetation data grouped by site type: (1) vegetation (2) emergence

When the total MRC site data set was grouped by sample type, NMDS analysis indicated (1) similarity between the aboveground vegetation of Cedar Knob and Cedar New and separation from Doubletop (Fig. 7) and (2) wide variation in species composition of the soil seedbanks of each site (Fig. 7). When NMRC site data was grouped by sample type, analysis indicated (1) similarity between the aboveground vegetation of Laurel Knob, Rock Mountain, and Satulah (Fig. 8) and (2) similarity between the species composition of the soil seedbanks of Laurel Knob and Satulah and separation from Rock Mountain (Fig. 8).

[Fig. 7]

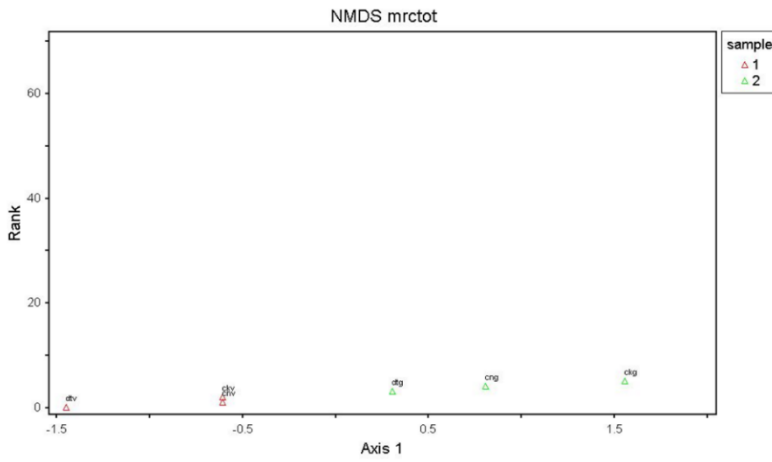


Figure 7: NMDS analysis of the total (center + edge) emergence and vegetation data of MRC sites grouped by sample type: (1) vegetation and (2) emergence

[Fig. 8]

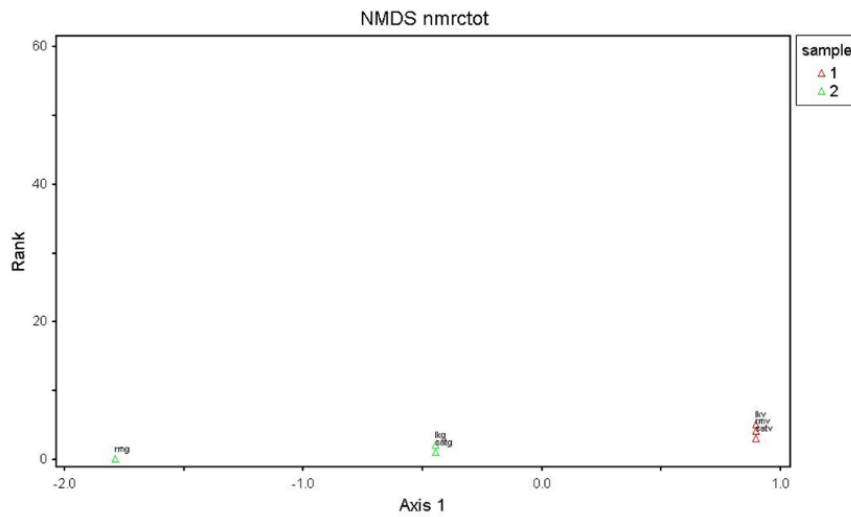


Figure 8: NMDS analysis of the total (center + edge) emergence and vegetation data of NMRC sites grouped by sample type: (1) vegetation and (2) emergence

NMDS analysis of the center and edge plots of both MRC and NMRC sites when grouped by site type indicated (1) wide variation in the center soil seedbank and aboveground vegetation of both MRC and NMRC sites (Fig. 9) (2) significant similarity in the edge soil seedbank and aboveground vegetation species composition of MRC and NMRC sites (excluding Laurel Knob) (Fig. 10)

[Fig. 9]

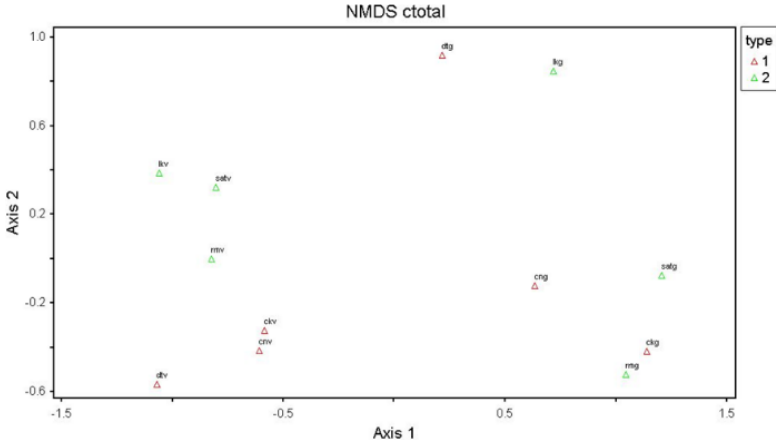


Figure 9: NMDS analysis of total center (emergence + vegetation) plots when grouped by site type: (1) MRC (2) NMRC

[Fig. 10]

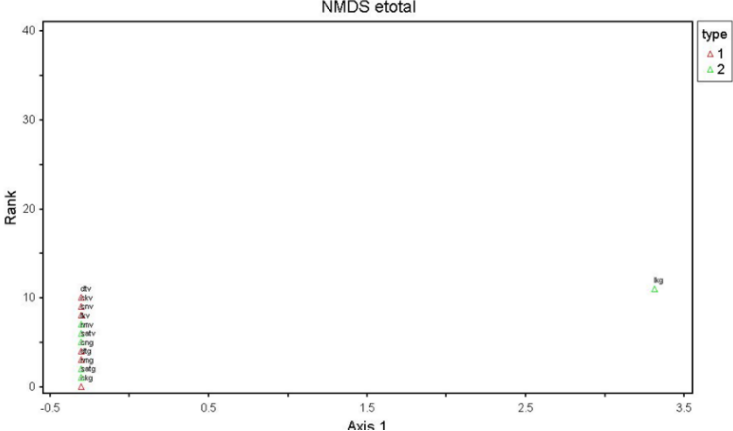


Figure 10: NMDS analysis of total edge (emergence + vegetation) plots when grouped by site type: (1) MRC (2) NMRC

When grouped by sample type, NMDS analysis of center and edge plots of MRC/NMRC sites indicated (1) wide variation of both soil seedbank and aboveground vegetation species composition of center MRC and NMRC plots (Fig. 11) (2) similarity between the soil seedbank and aboveground vegetation of edge MRC and NMRC plots (excluding Laurel Knob) (Fig. 12).

[Fig. 11]

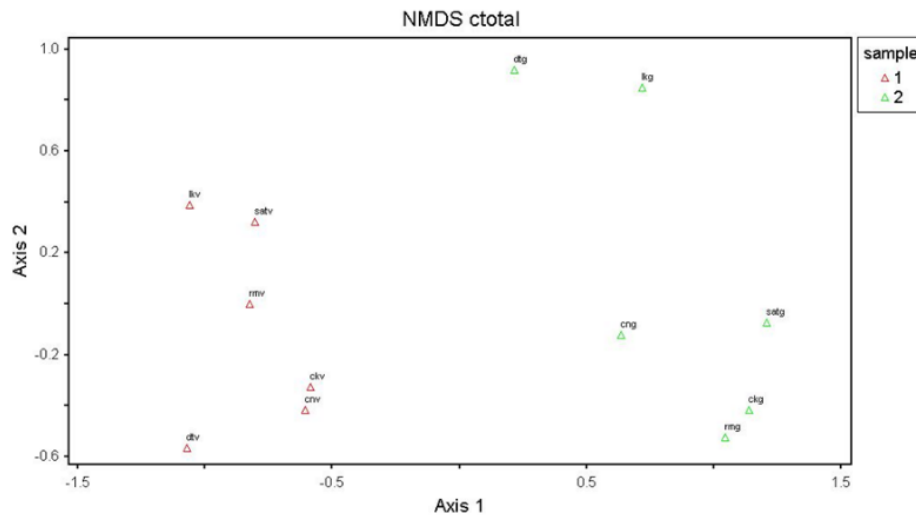


Figure 11: NMDS analysis of total center (emergence + vegetation) plots when grouped by sample type: (1) MRC (2) NMRC

[Fig. 12]

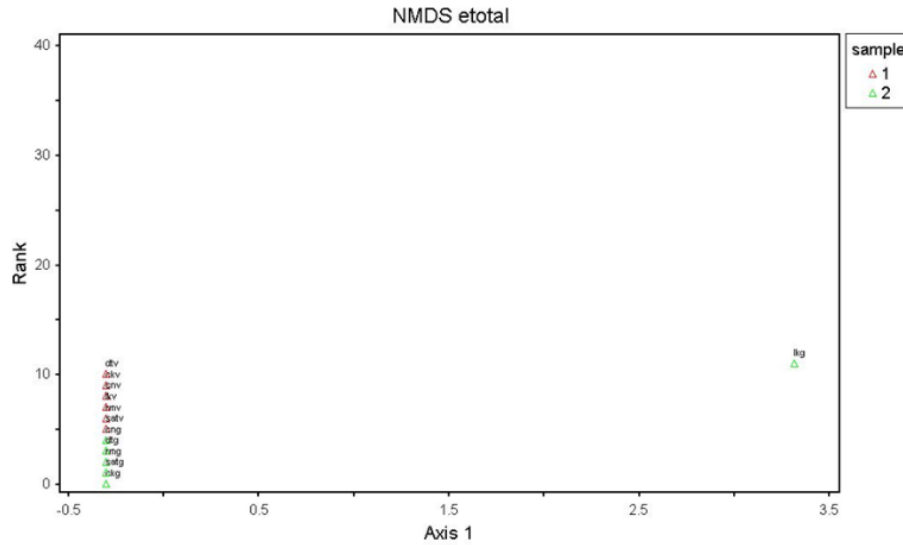


Figure 12: NMDS analysis of total edge (emergence + vegetation) plots when grouped by sample type: (1) MRC (2) NMRC

MRPP Analysis

Analysis of the greenhouse emergence data by MRPP indicated significant variation in the species composition between the total (center/edge of emergence and vegetation) soil seedbank and aboveground vegetation data ($p = 0.003$; Table 3). It also indicated significant variation in the species composition of the soil seedbank and aboveground vegetation of the (1) MRC ($p = 0.025$) and NMRC ($p = 0.023$) sites (2) center ($p = 0.0005$) plots of MRC and NMRC sites (3) edge ($p = 0.002$) plots of MRC and NMRC sites (Table 3). Further, significant variation in the species composition between the total (center + edge) aboveground vegetation of MRC and NMRC sites ($p = 0.024$) was demonstrated. The species composition of the soil seedbank and aboveground vegetation of both MRC and NMRC sites vary significantly (Table 3). Moreover, analysis indicated non-significant variation in the species composition of the soil

seedbanks of MRC and NMRC sites ($p = 0.739$) and in the species composition of the center ($p = 0.155$) and total edge ($p = 0.819$) plots of MRC and NMRC sites. Seedbank compositions were not significantly different between MRC and NMRC sites (Table 3).

Table 3. P-values from MRPP analysis of subsamples between sample types (emergence or vegetation data) (=Sample) and site types (MRC/NMRC) (=Type). Significance assessed at $p < 0.05$.

	Emergence vs Vegetation: p-value	MRC vs NMRC: p-value
Total	0.0029451	0.25233242
Total Emergence	-	0.73939395
Total Vegetation	-	0.02423766
MRC	0.02489015	-
NMRC	0.02252593	-
Total Center	0.00047819	0.15502734
Total Edge	0.00185428	0.81899401

Soil seedbank and vegetation species composition significantly differed in both MRC/NMRC, but there was broad overlap between them. Species composition of the soil seedbank MRC and NMRC sites largely varied, but differences in aboveground vegetation between site types were significant. Moreover, significant differences were detected between the soil seedbank and aboveground vegetation composition of the center and edge plots of MRC/NMRC.

Indicator Species Analysis

In the total (center + edge) MRC/NMRC dataset, *Dichanthelium. acuminatum* was a significant indicator of emergence ($p = 0.0032$) and *Micranthes petiolaris* was a significant indicator of aboveground vegetation ($p = 0.0150$) when grouped by sample type

(emergence/vegetation). No significant values were observed when grouped by site type (MRC/NMRC). In the total center (emergence/vegetation) dataset of MRC/NMRC sites, *Phermeranthus teretifolium* was a significant indicator of MRC ($p = 0.057$) and *Croton willdenowii* and *Capnoides sempervirens* were significant indicators of NMRC ($p = 0.058$; $p = 0.058$, respectively) when grouped by site type. Significant values were not observed when grouped by sample type. No other significant indicator species values were obtained in the subsamples.

Dominance Curves

The top three dominant species were identified in the (1) total emergence data (*D. acuminatum*, *Danthonia spicata*, and *Houstonia longifolia*) (Fig. 13) (2) total vegetation data (*Schizachyrium scorparium*, *H. longifolia*, and *M. petiolaris*) (Fig. 14) (3) total center data (*D. acuminatum*, *D. spicata*, *P. teretifolium*) (Fig 15) (4) total edge data (*D. acuminatum*, *Pycnanthemum montanum*, *H. longifolia*) (Fig. 16). When sampled by site type, MRC sites were dominated by *D. acuminatum*, *P. teretifolium*, and *D. spicata* and NMRC sites were dominated by *D. acuminatum*, *D. spicata*, and *S. scorparium* [Figs. 17, 18].

[Fig. 13]

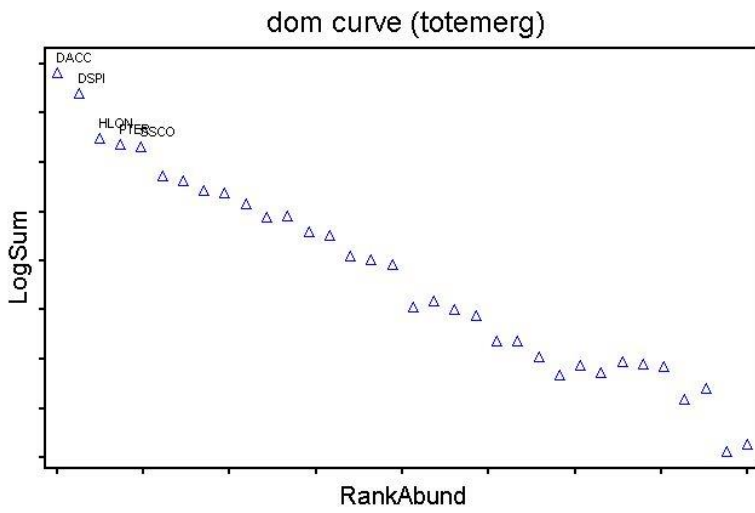


Figure 13: Dominance curve (dom curve) of the total emergence (totemerg) data. This shows the top 5 most dominant species in the greenhouse emergence data: *Dichantheium acuminatum* (DACC), *Danthonia spicata* (DSPI), *Houstonia longifolia* (HLON), *Phermeranthus teretifolium* (PTER), and *Schizachyrium scorparium* (SSCO).

[Fig. 14]

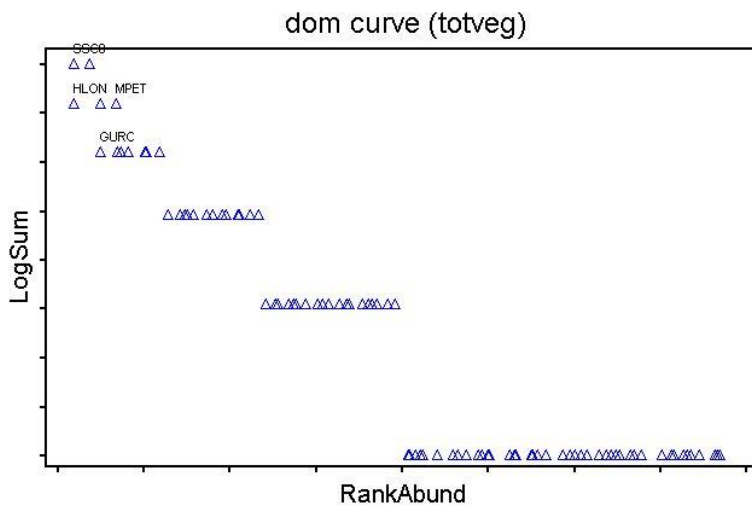


Figure 14: Dominance curve (dom curve) of the total vegetation (totveg) data. This shows the dominant species in the vegetation site survey data: *Schizachyrium scorparium* (SSCO), *Houstonia longifolia* (HLON), *Micranthes petiolaris* (MPET), and *Galax urticifolia* (GURC).

[Fig. 15]

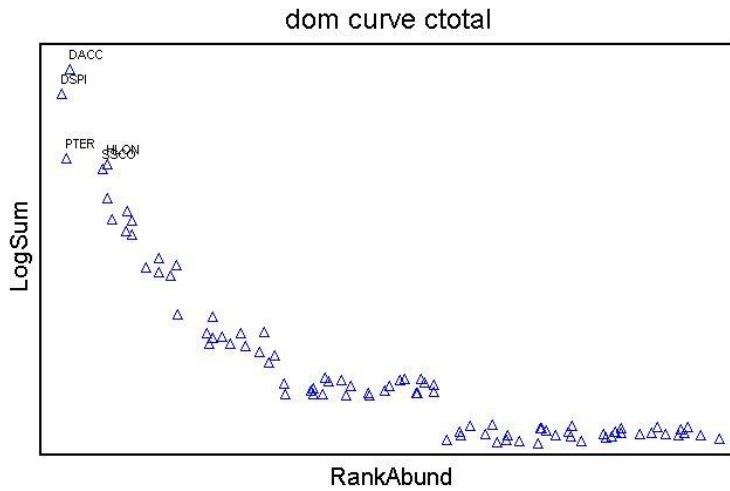


Figure 15: Dominance curve (dom curve) of the total center (ctotal) data. This shows the dominant species in the center emergence and vegetation data: *Dichantheium acuminatum* (DACC), *Danthonia spicata* (DSPI), *Phermeranthus teretifolium* (PTER), *Houstonia longifolia* (HLON), and *Schizachyrium scorparium* (SSCO).

[Fig. 16]

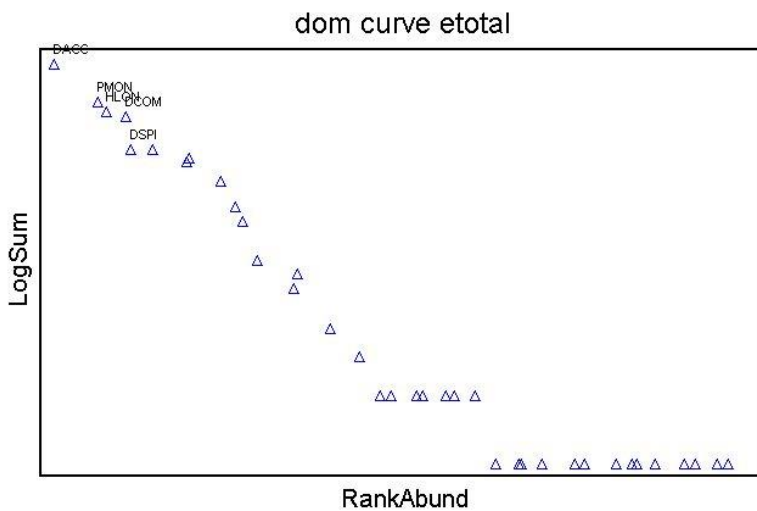


Figure 16: Dominance curve (dom curve) of the total edge (etotal) data. This shows the dominant species in the center emergence and vegetation data: *Dichanthelium acuminatum* (DACC), *Pycnanthemum montanum* (PMON), *Houstonia longifolia* (HLON), *Dichanthelium comutatum* (DCOM), and *Danthonia spicata* (DSPI).

[Fig. 17]

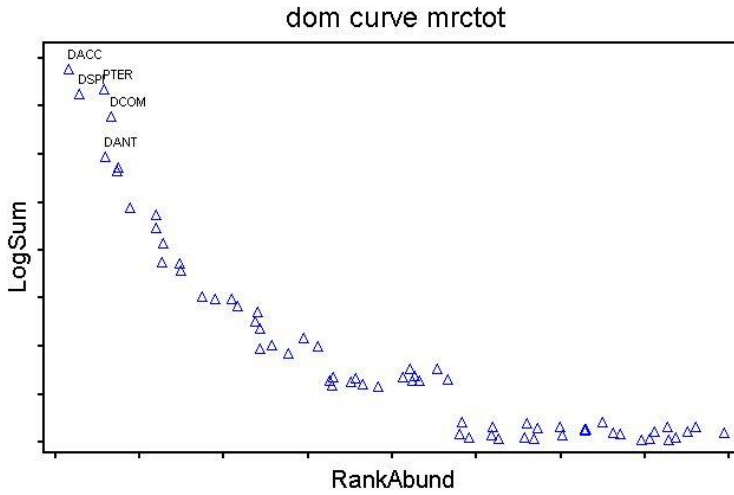


Figure 17: Dominance curve (dom curve) of the total MRC (mrctot) data. This shows the dominant species in the MRC emergence and vegetation data: *Dichanthelium acuminatum* (DACC), *Phermeranthus teretifolium* (PTER), *Danthonia spicata* (DSPI), *Dichanthelium comutatum* (DCOM), and *Danthonia* spp. (DANT).

[Fig. 18]

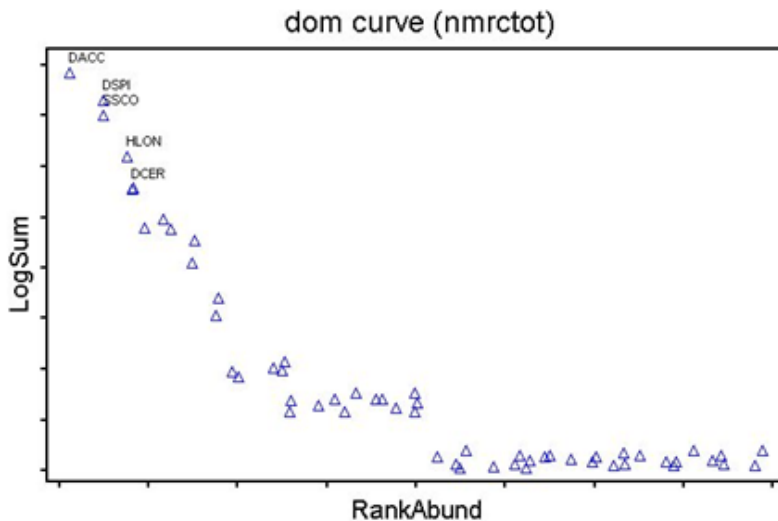


Figure 18: Dominance curve (dom curve) of the total NMRC (nmrctot) data. This shows the dominant species in the NMRC emergence and vegetation data: *Dichanthelium acuminatum* (DACC), *Danthonia spicata* (DSPI), *Schizachyrium scorparium* (SSC), *Houstonia longifolia* (HLON), *Danthonia cericea* (DCER).

Dichanthelium species dominated the total soil seedbank, while more traditional outcrop species dominated the total aboveground vegetation of all six sites. Moreover, when divided into center and edge plots *Dichanthelium* species continued to dominate. When *Dichanthelium* sp. were removed from the sample, characteristic outcrop herbaceous species dominated the center (*P. teretifolium*, *H. longifolia*, *M. petiolaris*) and edge (*P. montanum*, *H. longifolia*, *P. teretifolium*) of all six sites.

CHAPTER 5: DISCUSSION

This study investigated differences in species composition and diversity between MRC and NMRC sites as well as the difference between the soil seedbank and extant aboveground vegetation of both MRC and NMRC sites. The results from this community analysis indicate that there is a difference between the soil seedbank and the extant aboveground vegetation on both MRC and NMRC outcrop communities. Broadly speaking, herbaceous species richness and evenness of the soil seedbank and aboveground vegetation, calculated using rank-abundance curves and Shannon indices, were similar across MRC and NMRC rock outcrop types. Both MRC and NMRC outcrops demonstrated high species variability within the soil seedbank, but significant differences were observed between MRC/NMRC soil seedbanks and their aboveground vegetation and ordination analyses revealed significant differences between the aboveground vegetation of MRC and NMRC sites. However, no significant correlation was detected between the geographical distance of sites and the species composition of the soil seedbank and aboveground vegetation of sites. This indicates that differences in geographical distance of seed dispersal between rock outcrop types is not significantly influencing the soil seedbank or aboveground vegetation composition of both site types.

Similarities in herbaceous species abundance within the soil seedbank were observed in the greenhouse emergence data. Rank-abundance curves indicated that plot diversity in both MRC and NMRC soil seedbanks was dominated by grass species *Dichanthelium acuminatum* and *Danthonia spicata*, both of which are characteristic of high-elevation rock outcrops and montane red-cedar woodlands (Schafale 2012, Small & Wentworth 1980). This trend is also observed in the dominance curves; *D. acuminatum* dominated the total emergence, center, and edge plots of MRC and NMRC sites. *Dichanthelium acuminatum* is a tapered rosette grass with

a wide habitat distribution and is predominantly found in disturbed areas (USDA 2021). Upon removal of *D. acuminatum* from the data, more characteristic outcrop species dominated plots: *Ptermeranthus teretifolium*, *Houstonia longifolia*, *Micranthes petiolaris*, and *Schizachyrium scorparium*.

NMDS and MRPP ordination analyses highlighted patterns of similarity and dissimilarity of herbaceous species composition between site type (MRC/NMRC) and sample type (Emergence/Vegetation). NMDS visual ordinations and the calculated non-significant MRPP dissimilarity p-value (0.739) indicated high species variability within the soil seedbanks of all six sites (McCune & Mefford, 2016). This is supportive of congruent seed dispersal on all sites regardless of geographical distance. Additionally, NMDS ordination analyses indicated coupling of species composition by site type (MRC/NMRC) in the total aboveground herbaceous species composition; Therefore, it is unlikely that seed dispersal is the primary influencer of flora on rock outcrops, rather environmental filtering processes impacting germination and establishment have a more crucial role in influencing the species composition/diversity on both rock outcrop subtypes.

Site type groupings were still detectable within the center and edge aboveground vegetation NMDS plots but were not significant in the MRPP analyses: center (0.155), edge (0.818). This could be due to differences in site and sample sizes. Site grids were created based on their “walkability” leading to differences in total sample sizes for each site. Soil cores were taken from a percentage of the center and edge regions, leading to a difference in sample sizes. However, when grouped by sample type (Emergence/Vegetation), differences between the species composition of the soil seedbank and extant aboveground vegetation of the center, edge, MRC and NMRC plots were identified. This is additional evidence to support the hypothesis that

MRC/NMRC rock outcrop species composition is primarily impacted by environmental filtering processes that limit germination and establishment ability rather than seed dispersal ability.

When NMDS data was grouped by sample type, MRC sites demonstrated wide variability in the soil seedbank composition. However, strong similarities between the aboveground vegetation of Cedar Knob and Cedar New were identified, with some separation from Doubletop. This separation is likely due to differences in canopy structure; Doubletop is smaller, has less exposed bedrock, and greater canopy coverage than all of the other sites. Its structure is like that of a transition between rock outcrop and montane red-cedar woodland community (Wiser & White 1996, Small & Wentworth 1998, Schafale 2012). In contrast, the MRPP dissimilarity analysis between the MRC emergence and vegetation data calculated a significant p-value (0.0248).

In NMRC sites, similarities between the soil seedbank composition of Satulah and Laurel Knob were ascertained by ordination analyses, with both being separated from Rock Mountain. Rock Mountain is geographically closer to Laurel Knob (5.94 km) than Satulah is to Laurel Knob (11.43 km). Rock Mountain (1300 m) is also closer in elevation to Laurel Knob (1239.7 m) than Satulah (1383 m) is to Laurel Knob. Therefore, this separation could be due to difference in exposed bedrock influenced by human disturbance. Satulah is a popular hiking site that is open to the general public and is often trampled by hikers. Laurel Knob is owned by the Highlands-Cashiers Land Trust but is immediately adjacent to multiple private homes, and the homeowners and their pets may frequent the outcrops, which are essentially in their backyards. In contrast, Rock Mountain is surrounded by new residential developments that limit access by the general public. Restricted access by the general public limits trampling and results in dense vegetation mats and less exposed rock as compared to other sites. The presence of these mats

allows for greater seed trapping and likely driving the difference between the soil seedbank of Rock Mountain and Laurel Knob/Satulah. Overall, differences in human visitation to these sites could influence the amount of exposed bedrock and moss mats altering the soil seedbank composition (Mathews & Collins 2014).

Rock Mountain had many distinct herbaceous species germinate from soil core samples. Three species were present in Rock Mountain samples that were absent from Laurel Knob and Satulah; These include *Capnoides sempervirens*, *Croton wiildenowii*, and *Panicum virgatum*. Satulah and Laurel Knob each had only one unique species: *Danthonia cericea* (Laurel Knob) and *Solidago simulans* (Satulah). Differences in human disturbance levels could be driving soil seedbank compositions (Mathews & Collins 2014). Within the extant aboveground vegetation data, strong clustering of Laurel Knob, Satulah, and Rock Mountain was detected. This is supported by the significant p-value (0.0225) ascertained from the MRPP analysis of the emergence and vegetation data. This is evidence of site subtype specific environmental filtering processes impacting seed germination and establishment.

In center plots, NMDS visualization displayed wide variability in the soil seedbank and in the aboveground vegetation. In the emergence data, Rock Mountain and Cedar Knob plotted relatively close together, indicating similarity in soil seedbank composition. Rock Mountain and Cedar Knob are not geographically close (18.2 km) but are relatively close in elevation (RM: 1300 m, CK 1234.3 m). Rock outcrop vegetation is best classified by bedrock and elevation; therefore, this could account for the similarity in soil seedbank composition (Wiser & White 1999). Within the center aboveground vegetation data, there was some clustering of site types, and Cedar Knob and Cedar New plotted close to each other with some disconnect from Doubletop. This is unsurprising as Doubletop is structurally different from the other MRC sites.

There was no strong overlap in the vegetation data of NMRC sites. Emergence and vegetation data observably clustered by sample type, indicating differences in the center species composition of the soil seedbank and aboveground vegetation of MRC/NMRC sites. This is further supported by the significant p-value (0.0004) calculated from MRPP analysis.

In the edge plots, NMDS ordination displayed strong overlap between the soil seedbank of MRC/NMRC sites excluding Laurel Knob. This could be due to differences in sample size, as only 25% of the edge regions of sites were sampled, and sample grid sizes depended on the walkability of individual sites. The plot ordination also indicated strong overlap between the edge aboveground vegetation of MRC/NMRC sites. This could be attributed to similarity in parental input via seed dispersal in the surrounding woodlands, and deeper pockets for greater soil accumulation. However, the surrounding forest of MRC and NMRC sites appear relatively similar in their overstory with a dominant presence by oak and pine trees. However, NMRC edge regions typically had a thicker, shadier understory dominated by *Rhododendron catawbiense*, *Kalmia latifolia*, *Kalmia buxifolia*, *Amelanchier arborea*, and *Vaccinium spp.*, while MRC edge regions typically had a less dense and more scattered understory with scattered patches of *Vaccinium spp.*, *Ceanothus americanus*, and *Symphoricarpos orbiculatus*. There is also some separation between the soil seedbank and the aboveground vegetation of edge plots that is supported by the significant p-value (0.001). Thus, these results suggest that the process of environmental filtering may perpetuate a difference between species that are dispersing and species that are thriving on MRC and NMRC sites rather than seed dispersal.

As the global climate rapidly changes, high-elevation communities will be disproportionately impacted as they are often restricted by low local adaptive ability, genetic diversity, and migration ability (Mathews & Collins 2014, Bemmels & Anderson 2019). Due to

rising global temperatures and changes in weather patterns/cloud immersion coupled with the impact of anthropogenic disturbance, these disjunct, refuge populations are likely unable to compete with more generalist species (Mathews & Collins 2014). Rare and endemic rock outcrop specialists are adapted to the harsh environmental conditions associated with high elevations and exposed bedrock such as thin soil, morning cloud-immersion, high light intensity, and short growing seasons (Wiser et al. 1996, Wiser et al. 1998, Small & Wentworth 1998, Culatta & Horton 2014, Mathews & Collins 2014). Outcrop specialists likely do not have the genetic diversity needed to locally adapt to changing environmental conditions (Culatta & Horton 2014, Mathews & Collins 2014, Bemmels & Anderson 2019).

Mathews & Collins (2014) phylogenetic community analyses demonstrated that though the outcrop flora of the Southern Appalachians is taxonomically diverse, it is “phylogenetically shallow”, comprising of outcrop obligates and their close relatives. They further demonstrated that outcrop specialists are not ubiquitous across sites, suggesting outcrops have already randomly lost species over time (Mathews & Collins 2014). Though these habitats have been demonstrated to be resistant to invasion by nonnative species due to the harsh environmental conditions on sites, it is likely they will become more susceptible to invasion in the future (Wiser 1994, Mathews & Collins 2014). Horton & Culatta (2016) demonstrated that due to shallow soil and low water retainability, rock outcrop flora is likely to be especially sensitive to decreases in cloud immersion driven by the increase in global temperature. Climate change has also been demonstrated to impact growing seasons and disrupt plant-pollinator interactions on high-elevation communities in the Rocky Mountains (Bemmels & Anderson 2019).

Though climate change is influential, human visitation also poses an immediate threat to accessible rock outcrops. Hikers are likely to trample the moss mats that are essential to

germination of species and create bare rock patches. This decreases the overall herbaceous species diversity of the sites, and likely increases the abundance of trample-tolerant species, significantly influencing the species composition of sites (Mathews & Collins 2014). Mathews & Collins (2014) demonstrated the impact of human disturbance of lichen and vegetation mats on the species diversity and vegetation abundance of even low accessibility sites.

Studying the subtypes of high-elevation rock outcrop communities (MRC/NMRC) will assist in elucidating the natural processes influencing the unique species composition on these rare sites. These results suggest that rock outcrop vegetation is more influenced by environmental factors that impact seed germination rather than input into the seedbank by dispersal. This is validated by the observed differences between the species composition of the soil seedbank and extant aboveground vegetation on both MRC and NMRC sites. Further, observed differences between the aboveground vegetation of MRC and NMRC sites highlight the need for a comprehensive soil analysis of high-elevation rock outcrops subtypes to determine specific differences that could be affecting germination and establishment. This will further aid in future conservation and management strategies to preserve the rare and endemic species that rely on the distinct environmental conditions outcrops provide in the face of climate change. Land and natural resource managers may change policies to restrict visitation to bare rock regions or vegetation mats with disturbance-tolerant species only (Mathews & Collins 2014). This was demonstrated to be an effective strategy on Whiteside Mountain, which limits visitation with a predefined trail and barrier fence. Of the surveyed outcrops, Mathews & Collins (2014) observed the greatest flowering plant and pollinator species richness on Whiteside Mountain, indicating that visitation limitation is an effective preservation strategy. These results further

emphasize the importance of creating and maintaining policies specific to site type, to preserve the rare/endemic species assemblage on high-elevation outcrops.

Conclusions

High elevation rock outcrops are rare communities that support a rich biodiversity of rare and endemic species, and alpine relicts. Outcrops are also an excellent model for studying insular systems, and knowledge provided from this study will be applicable to other remote island studies. Though they all have similar underlying characteristics, the flora across different outcrop types is distinctive and therefore requires specific conservation and maintenance strategies. These communities are highly threatened by anthropogenic factors and climate change, and due to isolation, low genetic diversity, and lack of phenotypic plasticity it is unlikely that the highly adapted outcrop endemics will be able to survive these changes. Therefore, knowledge on distinct present flora and environmental processes occurring on distinct outcrop types (MRC/NMRC) from soil seed bank data and aboveground vegetation survey data is imperative to preserving these rare and delicate ecosystems.

Suggestions and Limitations

These results are limited by small and non-uniform sample sizes, as well as the limitation of sampling ability on sites. To obtain a more in-depth analysis of species composition across rock outcrop subtypes, a greater percent of the outcrop center and edge regions should be sampled and survey. Lack of abundance data from aboveground vegetation samples also limits the total vegetation analysis. Calculating the percent coverage of herbaceous species across the sites would result in a stronger community analysis. Future studies would also benefit from full soil and bedrock analyses to identify specific environmental differences in MRC and NMRC

sites that could influence species composition. Further, reciprocal transplant studies could be conducted to evaluate the germination of species on different rock outcrop subtypes.

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APPENDICES

APPENDIX: A

Appendix A: Complete identified herbaceous species list from emergence data; * = characteristic outcrop species as defined by Weakley (2005).

Taxa	Site	MRC/NMRC	Center	Edge
<i>Ageratina altissima</i> *	CN	MRC		X
<i>Antennaria plantaginifolia</i>	CN	MRC	X	
<i>Brassica sp.</i>	CN	MRC	X	X
<i>Capnoides sempervirens</i> *	RM	NMRC	X	
<i>Carex umbellata</i>	CK, DT, SAT	MRC/NMRC	X	
<i>Commelina communis</i>	CN	MRC	X	X
<i>Croton willdenowii</i> *	RM	NMRC	X	X
<i>Cyperus esculentus</i>	CK, CN, RM, SAT	MRC/NMRC	X	X
<i>Danthonia cericea</i>	LK	NMRC	X	X
<i>Danthonia spicata</i> *	CK, SAT	MRC/NMRC	X	X
<i>Danthonia sp.</i>	CN, RM	MRC/NMRC	X	X
<i>Dichantherium acuminatum</i>	CK, CN, DT, LK, RM, SAT	MRC/NMRC	X	X
<i>Dichantherium commutatum</i>	CK, CN, RM, SAT	MRC/NMRC	X	X

<i>Digitaria sanguinalis</i>	CN, DT, SAT	MRC/NMRC	X	
<i>Doch sp.</i>	DT	MRC	X	X
<i>Eleocharis acicularis</i>	CN, RM, SAT	MRC/NMRC	X	X
<i>Erechtites hieraciifolius</i>	CK, CN, DT, SAT	MRC/NMRC	X	
<i>Houstonia longifolia var. glabra *</i>	CK, CN, RM, LK, SAT	MRC/NMRC	X	X
<i>Hypericum stragulum</i>	CN, DT, SAT	MRC/NMRC	X	X
<i>Krigia montana *</i>	CK, RM	MRC/NMRC	X	
<i>Oxalis stricta</i>	CN	MRC		X
<i>Panicum virgatum</i>	RM	NMRC	X	
<i>Phermeranthus teretifolium</i>	CK, CN	MRC	X	X
<i>Plantago virginica</i>	DT	MRC	X	
<i>Polygonum sp.</i>	DT	MRC	X	
<i>Pycnanthemum montanum</i>	CN,SAT	MRC/NMRC	X	X
<i>Rubus alleghensis</i>	CK, CN, RM, SAT	MRC/NMRC	X	X

<i>Rubus occidentalis</i>	CK, CN, DT, RM, SAT	MRC	X	X
<i>Schizachyrium scorparium</i> *	CK, CN, LK, RM	MRC/NMRC	X	X
<i>Solanum nigrum</i>	DT	MRC	X	X
<i>Solidago sp.</i>	CN, DT	MRC	X	X
<i>Solidago simulans</i>	SAT	NMRC	X	X
<i>Verbascum thapsus</i>	CN	MRC	X	X
<i>Verbena urticifolia</i>	DT	MRC	X	

APPENDIX: B

Appendix B: Complete identified herbaceous species list from aboveground vegetation data; * = characteristic outcrop species as defined by Weakley (2005).

Taxa	Site	MRC/NMRC	Center	Edge
<i>Agalinis purpurea</i>	CK, DT	MRC	X	
<i>Ageratina altissima</i> *	CN, DT, SAT	MRC/NMRC	X	
<i>Agrostis perennans</i>	SAT	NMRC		X
<i>Allium sp.</i>	CN	MRC	X	
<i>Anaphalis margaritecea</i>	DT	MRC	X	
<i>Andropogon virginicus</i>	SAT	NMRC	X	
<i>Antennaria plantaginifolia</i>	CK, CN, DT	MRC	X	
<i>Artemisia sp.</i>	CK	MRC	X	

<i>Asclepias sp.*</i>	SAT	NMRC	X	
<i>Asplenium montanum</i>	SAT	NMRC	X	
<i>Bartonia virginica</i>	LK	NMRC	X	
<i>Calamagrotis coarctata</i>	LK, SAT	NMRC	X	
<i>Campanula rotundifolia*</i>	CK, DT, RM, SAT	MRC/NMRC	X	
<i>Capnoides sempervirens</i>	LK, RM, SAT	NMRC	X	
<i>Carex umbellata</i>	CK, DT, LK, SAT	MRC/NMRC	X	
<i>Chimaphila umbellata</i>	DT	MRC	X	
<i>Commelina communis</i>	CK, CN, DT	MRC	X	
<i>Coreopsis major</i>	DT, RM, SAT	MRC/NMRC	X	
<i>Croton willdenowii</i>	LK, RM, SAT	NMRC	X	
<i>Cyperus esculentes</i>	CK, CN, RM	MRC/NMRC	X	
<i>Cypripedium acaule</i>	RM	NMRC	X	
<i>Danthonia cericea</i>	LK	NMRC	X	
<i>Danthonia spicata*</i>	CK, SAT	MRC/NMRC	X	
<i>Dichanthelium acuminatum</i>	CK, CN, RM, SAT	MRC/NMRC	X	
<i>Dicanthelium commutatum</i>	CK, CN, RM	MRC/NMRC	X	
<i>Digitaria ischaemum</i>	LK	NMRC	X	
<i>Digitaria sanguinalis</i>	CN, SAT	MRC/NMRC	X	

<i>Erechtites hieraciifolius</i>	CK, CN, RM	MRC/NMRC	X	X
<i>Erigeron annuus</i>	CK, CN, DT	MRC	X	
<i>Eriogonum sp.</i>	DT	MRC	X	
<i>Euphorbia sp.</i>	DT	MRC	X	
<i>Eurybia sp.*</i>	CK	MRC	X	
<i>Eurybia surculose*</i>	LK, SAT	MRC	X	
<i>Galax urceolata*</i>	DT, LK, RM, SAT	MRC/NMRC		X
<i>Gaultheria procumbens</i>	LK, SAT	NMRC		X
<i>Helianthes sp.</i>	CK	MRC	X	
<i>Heuchera americana*</i>	CK, DT	MRC	X	
<i>Heuchera villosa*</i>	SAT	NMRC	X	
<i>Houstonia longifolia var. glabra*</i>	CK, CN, DT, LK, SAT	MRC/NMRC	X	
<i>Hypericum buckelyii*</i>	CK, CN	MRC	X	
<i>Hypericum gentianoides*</i>	LK, RM, SAT	NMRC	X	
<i>Hypericum punctatum</i>	DT	MRC	X	
<i>Hypericum stragulum</i>	CK	MRC	X	
<i>Krigia montana*</i>	CK, LK, RM, SAT	MRC/NMRC	X	
<i>Lechea sp.</i>	CK	MRC		X
<i>Leiophyllum buxifolium</i>	SAT	NMRC		X
<i>Lespedeza procumbens</i>	DT	MRC	X	

<i>Lobelia amoena</i>	LK	NMRC	X	
<i>Lobelia nuttallii</i>	LK	NMRC	X	
<i>Lobelia sp.</i>	DT	MRC	X	
<i>Luzula sp.</i>	DT	MRC		X
<i>Lysimachia quadrifolia</i>	RM	NMRC	X	
<i>Maianthemum racemosum</i>	DT, LK	MRC/NMRC		
<i>Melampyrum lineare*</i>	LK	NMRC	X	
<i>Micranthes petiolaris*</i>	CK, CN, DT, RM, SAT	MRC/NMRC	X	X
<i>Monarda sp.</i>	DT	MRC	X	
<i>Nabalus altissimus</i>	CK, DT	MRC	X	
<i>Opuntia humifusa var. humifusa*</i>	CN, DT	MRC	X	
<i>Oxalis stricta</i>	DT	MRC		X
<i>Panicum virgatum</i>	LK, SAT	NMRC	X	
<i>Penstemon sp.</i>	DT	MRC	X	
<i>Phermeranthus teretifolium</i>	CK, CN	MRC	X	
<i>Polygala curtissii</i>	SAT	NMRC	X	
<i>Polygala sanguinea</i>	LK, RM	NMRC	X	
<i>Polygala sp.</i>	CK	MRC	X	

<i>Polygonatum biflorum</i>	DT	MRC		X
<i>Polygonum sp.</i>	DT	MRC	X	
<i>Polytrichum juniperum</i>	SAT	NMRC	X	
<i>Potentilla sp. *</i>	CK, DT, SAT	MRC/NMRC	X	X
<i>Pteridium aquilinum</i>	LK	NMRC		X
<i>Pycnanthemum montanum</i>	CK, CN, DT, SAT	MRC/NMRC	X	X
<i>Rhus copallinum</i>	SAT	NMRC	X	
<i>Rubus allegheniensis</i>	CK, CN, LK	MRC		X
<i>Rubus occidentalis</i>	CK, CN, DT, LK, RM	MRC		X
<i>Rubus flagellaris</i>	CK	MRC	X	
<i>Rumex sp.</i>	DT	MRC		X
<i>Schizachyrium scorparium*</i>	CK, CN, DT, LK, RM, SAT	MRC/NMRC	X	
<i>Scleria triglomerata</i>	LK, SAT	NMRC	X	
<i>Sisyrinchium sp.</i>	RM	NMRC	X	
<i>Solidago sp.</i>	CK, CN, DT, RM, LK, SAT	MRC/NMRC	X	X
<i>Spiranthes cernua</i>	CK, CN	MRC	X	
<i>Stellaria media</i>	DT	MRC	X	
<i>Symphotrichum laeve</i>	LK	NMRC	X	
<i>Symphotrichum sp.</i>	CK, DT, RM	MRC/NMRC	X	

<i>Tradescantia sp.</i>	CN	MRC	X	
<i>Tradescantia subaspera</i>	CN, CK	MRC	X	
<i>Verbascum thapsus</i>	CN	MRC	X	
<i>Viola pedata</i>	CK, DT	MRC	X	
<i>Viola primulifolia</i>	LK, SAT	NMRC	X	
<i>Viola sagittata</i>	SAT	NMRC		X