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Response of running buffalo clover (Trifolium stoloniferum Muhl ex. A. Eaton) to herbaceous competition and transplanting in Monongahela National Forest

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Response of running buffalo clover (*Trifolium stoloniferum* Muhl ex. A. Eaton) to herbaceous competition and transplanting in Monongahela National Forest

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Abstract

Response of running buffalo clover (Trifolium stoloniferum Muhl ex. A. Eaton) to herbaceous competition and transplanting in Monongahela National Forest

Ruben Sabella

Running buffalo clover (RBC) is a rare perennial plant that grows throughout the American Midwest and the Appalachian Mountains. It requires disturbed forests to establish and proliferate. It has been suggested that, in the past, these conditions were created by buffalo; now logging operations maintain RBC populations. However, forest managers have been looking for ways to create suitable habitat for RBC that do not involve harvesting practices. This could help create new populations in areas that cannot be logged. Once established, competing vegetation might influence RBC abundance and flowering. This study seeks to quantify this influence by measuring the vegetation in and around naturally occurring RBC patches. This study took place within five managed sites in the Cheat Wildlife Management Area within the Monongahela National Forest, WV. In these sites we completed an inventory of RBC patches, counting rosette numbers and flowers in each patch, and we collected data on neighboring trees. A year later, we returned to these patches and in the center 1 m^2 counted RBC rosettes and estimated RBC cover and RBC maximum height. We also measured the cover of various vegetation types, and the height of the tallest vegetation. We found that RBC patch size was positively related to tree canopy cover, and RBC flowering was negatively correlated with the presence of nearby trees. We also found that RBC rosette density, cover, and height were positively correlated with forb cover, and negatively correlated with shrub/vine cover. In a separate experiment, we established new RBC occurrences by transplanting five individuals into 1-m^2 experimental subplots with varying levels of prior ground disturbance. Treatments (n=15) comprised mowing vegetation, raking away litter, raking and mowing together, and undisturbed control. RBC survival rates in treated subplots one year after planting were increased by the disturbance treatments (83%) when compared to the control (57%). The number of rosettes that arose from each surviving transplant was also greater in the three disturbance treatments combined ($P = 0.009$) than the control, but there was no difference in the cover per surviving transplant ($P = 0.23$). Consequently, the total number of rosettes and their cover was greater after mowing and mowing+raking when compared with control. Raking alone resulted in values intermediate between the treatments involving raking and the control; values were not significantly different from the two raking treatments, but showed a statistical trend ($P \leq 0.1$) of having a greater number of rosettes (but not cover). The results of this study will help managers identify suitable canopy and herb-layer conditions for RBC to thrive, as well as create habitat for RBC without the use of logging disturbances.

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Introduction

1. Conservation Status and Range

Running buffalo clover (*Trifolium stoloniferum* Muhl ex. A. Eaton; RBC; Fabaceae) is a perennial plant indigenous to the eastern United States (Brooks 1983). Prior to 1983 it was thought to be extinct, with the last known specimen having been collected in 1940 (Cusick 1989). In 1983, populations were discovered in West Virginia. It has since been identified in other states that were part of its historic range. RBC was listed as federally endangered between 1987 and August 2021.

At present, RBC occurs in certain parts of Arkansas, Indiana, Kentucky (Campbell et al. 1988), Missouri (Taylor et al. 1994), Ohio (Cusick 1989), Indiana (USFWS 2007) and West Virginia (Brooks 1983), though historically it was also thought to be present in Illinois and Kansas (USFWS 2007). Currently, 175 populations are identified by the US Fish and Wildlife Service (USWFS 2021) in three geographical regions: the Appalachian (West Virginia, and southeastern Ohio), Bluegrass (southwestern Ohio, central Kentucky and Indiana), and Ozark (Missouri) regions. Most populations occur within the Appalachian and Bluegrass regions (USFWS 2007).

RBC's current range has decreased considerably from its historic range. This is thought to be based in the species' reliance on American bison (*Bison bison* L.) to maintain its habitat and for seed dispersal. The bison would graze in forest openings, thereby creating potential habitat for RBC. They also maintained large systems of trails simply from their migrations, and these trails are thought to have been potential habitat for RBC (Bartgis 1985). However, the last bison in West Virginia was reportedly killed in 1825 (Maxwell 1898). As the bison were removed from their forested range, the forest openings disappeared, greatly limiting suitable habitat where RBC could grow. Populations survived by growing in lightly shaded areas such as roadsides, along streams, and in cemeteries (USFWS 2007).

Because at present there is a relatively large number of populations and individuals, RBC was proposed for delisting in 2019, and officially delisted in August of 2021 (USFWS 2019, USFWS 2021). This decision came after it was determined that the threats to the species originally listed were no longer significant enough to deem RBC an endangered species. The primary threats to RBC, habitat destruction and succession, have been addressed by using logging practices to provide disturbance (USFWS 2021). However, invasive species like Japanese stiltgrass (*Microstegium vimineum* A. Camus) and Japanese honeysuckle (*Lonicera japonica* Thunb.) can outcompete RBC in the shaded areas that it inhabits (Jacobs and Bartgis 1987). Other invasive species historically utilized for reseeding purposes, such as white clover (*Trifolium repens* L.), also compete with RBC (Jacobs and Bartgis 1987). For these reasons, competition from invasive species is cited as a threat to RBC, especially following a disturbance event. Many management plans, including that of the Monongahela National Forest, specifically address invasive species by either removing them or by avoiding their use for reseeding purposes (USFS 2011). It is suggested that invasive species be manually removed from sites where RBC grows, as herbicides could harm RBC populations (USFWS 2007).

There is insufficient evidence to show that herbivory is causing a decrease in populations, though occurrences of herbivory by different animals, including slugs, rabbits and deer, has been reported (Pickering 1989, Ford et al. 2003). However, these RBC populations seem to return the next year mostly unaffected. Parasitism from other animals has been proposed as a threat, but a negative impact has not yet been observed. In fact, *Trifolium stoloniferum* has been found to be more resistant to root knot nematodes than non-native clover species (Quasenberry 1997). Some pathogens have been found on RBC planted in a greenhouse, notably viruses, and these have been found to decrease the survival rates of new plantings (Sehgal and Payne 1995). However, there is no evidence to show that RBC is impacted by these viruses in the wild (USFWS 2007).

2. Reproduction and Genetic Diversity

RBC reproduces clonally and sexually. Rooted crowns (rosettes) grow stolons or runners that root in the soil, producing new rooted crowns. Once established, these crowns can either remain attached to the parent plant or separate to create a new plant (Brooks 1983, USFWS 2007). While the growth patterns of RBC stolon growth are not fully understood, it can be compared to other stoloniferous plants. White clover (*Trifolium repens* L.) will have different stolon growth rates depending on various factors (Marriot et al. 1997). The extension rate of the stolons was 12 mm per week on the outside of the patch, whereas within the patch it was 7 mm per week. This may be caused by the adaptions white clover has to escape shade by growing stolons horizontally. Patch size may also affect growth rate of stolons and petioles, with smaller patches growing longer stolons and taller petioles. Smaller patches are more vulnerable to shading, having less leaf area, so they must grow more quickly to escape competition. This can be done by either growing horizontally through stolons or growing vertically through petioles (Marriot et al. 1997).

RBC flowers in May and early June, though RBC at higher elevations may flower as late as mid July. It has been observed that flowers can self-pollinate, and that resulting seeds can still develop into rigorous plants (Franklin 1998). Since the plant was once thought to be rare, it was inferred that inbreeding may have been prevalent between RBC populations (Hickey et al. 1991). Inbreeding can limit the genetic diversity of the species, making it susceptible to disease and other threats (Godt et al. 1996). Crawford et al. (1998) analyzed the DNA of RBC populations in

Ohio, Kentucky, West Virginia, Missouri, and Indiana. They found that the genetic diversity in RBC is low, and that much of the observed diversity occurred between different populations, rather than within them. They also found that small populations contribute as much to the species' diversity as large ones.

It is thought that seeds historically were consumed by large ungulates and spread through droppings (Ford et al. 2003). Therefore, seed dispersal may have decreased as bison and elk populations diminished, and this would explain the low levels of genetic diversity within populations of RBC (Crawford et al. 1998). Experimental studies showed that the seeds of RBC may benefit from scarification. Seeds scarified mechanically with sandpaper had a 90-100% germination rate, but unscarified seeds had little to no germination (Campbell et al. 1988). Chemical scarification, which simulated passage through a digestive tract, has been found to increase germination rates (Hattenbach 1996). However, RBC seeds that passed through the digestive tract of white-tailed deer (*Odocoileus virginianus* Zimmerman) could not produce evidence that seeds benefitted from this process (Ford et al. 2003).

RBC can also be propagated vegetatively using tissue culture. RBC shoot tips that were placed into culture medium were found to successfully root. Following this, there was a success rate of over 90% when transplanting the RBC grown in rooting medium into pots and growing them in a greenhouse (Singha et al. 1988).

3. Habitat Requirements and Habitat Creation

a. Habitat Requirements

There is no evidence that RBC supports rhizobial associations for nitrogen fixation (Campbell 1988, Morris et al. 2002). This is consistent with RBC's preference for rich limestone soils

(USFWS 2007), though it has been found to grow in other types of soils (Thomas-Van Gundy 2022). It has been suggested that, after being consumed by bison or other ungulates, the seeds would be left in excrement that would aid them in their growth. The lack of this nitrogen source may be part of the reason for RBC decline after the extirpation of the bison (Morris et al. 2002). In the apparent absence of bison droppings, stolon length and shoot number were positively correlated with NH_4 content in the soil (Labella et al. 2022). This suggests that sites with greater nutrient availability will have healthier RBC. Other abiotic factors such as slope have also been found to have an impact on RBC growth. A negative relationship was found between RBC stolon length and slope angle at the Fernow Experimental Forest, West Virginia. However, the number of RBC crowns were not correlated with slope angle (Labella et al. 2022).

Running buffalo clover grows successfully in partially filtered light. Plants are usually found growing in semi-shade (40-50% sunlight) (Cusick 1989). At the Fernow Experimental Forest, which contained primarily oak and maple tree species, leaf area index (LAI) 1 m above ground was found to be lower in sites that contained RBC (mean = 4.8) than at randomly selected points (mean = 5.3) (Madarish and Schuler 2002). The percentage of open sky was also found to be greater above RBC populations (mean = 4%) than the random points (mean = 1.9%). This suggests that light is a significant factor in the growth of RBC (Madarish and Schuler 2002). However, it has been found in cemeteries, where there is potentially more sunlight, and active disturbance from mowing regimes (USFWS 2007). Herb-layer competition has also been found to affect RBC growth. Specifically, the presence of tall herbaceous competition (1.5–3 m in height) was positively correlated with stolon number and length for RBC populations found in the Fernow Experimental Forest (Labella et al. 2022). Correlations have also been found between aspect and RBC populations size, with a study in West Virginia showing west-facing slopes having larger populations compared to other slopes (with slope angle of 20-30%) (Burkhart et al. 2013).

Extant populations of RBC grow in forests with periodically disturbed, mesic soils (Burkhart 2010). In a forested setting, this disturbance is usually caused by logging activities. Specifically, selective logging in uneven-aged stands (thinning cuts) is most beneficial, since a complete loss of forest canopy does not create suitable habitat (USFWS 2021). In addition, RBC is often found on skid roads after a forest harvest (Madarish and Schuler 2002). On the Fernow Experimental Forest, habitat is created and maintained by logging that recurs every 10-20 years. Madarish et al. (2002) concluded that these disturbances promoted growth of RBC populations that were otherwise declining. They noted that immediately after the harvest, rooted crown density (crowns/m^2) declined, but after 2 years, density began to increase, reaching a peak after 7 years (Madarish and Schuler 2002). At the same study area, RBC rooted crown density began to decrease 14 years after disturbance, and sites that had not been disturbed for 20 years had little or no likelihood of supporting RBC (Burkhart et al. 2013). In the absence of a disturbance regime, RBC decline can be predicted from the size of the individual populations. For example, on the Blue Grass Army Depot in Kentucky it was found that if an RBC patch contains 20 or less crowns, it would have about a 50% chance of persisting for the next 10 years. This would increase to about 95% if the patch had 75 or more crowns. (Dart-Padover 2016).

b. Habitat Creation

While it is known that RBC benefits from logging activities, little information exists on the effectiveness of other management techniques (USFWS 2007). A study conducted on lawn populations at an Ohio park (with scattered black walnut [*Juglans nigra* L.] employed a mowing regime that cut twice before RBC flowering took place, once after seeds had dropped, and afterwards continued mowing throughout the summer. This disturbance regime increased the population of about 100 individuals to 2271 individuals over the course of 13 years (Becus and Klein 2002).

Grazing by cattle has been attempted as a means of creating RBC habitat at the Bluegrass Army Depot in Kentucky. There, RBC had been found in forested riparian areas. Some of these areas were protected from grazing, while others continued to be grazed. Grass-specific herbicides were also applied to the patches both inside and outside of the grazing area. After years of monitoring, it appeared that RBC on sites with no grazing were more successful than those with grazing, implying that the grazing had been done at too high of an intensity (i.e., too many cows had been allowed in the enclosure) to promote RBC growth. In addition to this, sites were either mowed, had herbicide applied, or both. It was found that when both mowing and herbicides were applied together, RBC populations greatly benefitted. It was suggested that only mowing removes forbs while allowing grasses to become dominant, and the opposite was occurring when only grass-specific herbicides were applied. When both treatments were applied together, both types of competing vegetation were suppressed, allowing the RBC to thrive (Dart-Padover 2015).

Little work has been done to study the effectiveness of transplanting RBC to create new populations. In Missouri, experimental plantings were made to reintroduce RBC to more areas of the state. RBC from remnant populations of Missouri, West Virginia, Kentucky, Ohio, and Indiana had previously been brought to the Missouri Botanical Garden to be propagated. Between 1990 and 1993 the Missouri Department of Conservation (MDC) used these to establish 27 experimental plots on MDC lands in different parts of the state. These were chosen based on descriptions of historical sites, as well on conditions of known RBC habitat in other states. Into each of these, 48 RBC stem cuttings were planted (16 in each of three 1-m^2 plots). In 1993 and

1994 some of the sites were replanted to make up for losses, and new plots were established on some of the sites. No management was applied to the sites, but wire cages were placed around the 1-m^2 plots to prevent herbivory. However, these were later removed as vegetation grew on them and limited sunlight. Rooted crowns and flower heads were counted annually. For each year until 1998 many of the plots had decreasing populations. By 1998, 17 of the plots had 0 rooted crowns, and seven plots had less than 10. Reasons for this may have been inappropriate site conditions, or other factors including herbivory, disease, drought, and flooding. The remaining three plots contained between 14 and 21 rooted crowns by the end of the experiment. Further plantings were not established by the MDC after this experiment (Smith 1998).

In addition to this study, between 1991 and 1994 the U.S. Forest Service created separate experimental plots to reintroduce RBC on the Mark Twain National Forest in Missouri. Many of the RBC in these showed symptoms of disease a year after planting, and experienced significant dieback. Some of these sites also experienced significant flooding, so the planted RBC was lost (Hickey 1994).

Statement of Need

While RBC has been removed from the endangered species list, the task is now to manage populations so that they do not decline again. While studies have quantified how changes of the environment impact existing RCB, few have investigated how to create new populations of it. Of these few, none were successful in establishing new populations in the wild. Additionally, while there have been studies analyzing the habitat impact of forest harvests on RBC population sizes, the impact of competing vegetation on RBC growth between disturbances remains poorly understood. This research project will fill these knowledge gaps by studying a) conditions that result in the success or failure of transplanted RBC and, thus, provide insights into non-logging methods for establishing new populations of RBC, and b) how RBC abundance and flowering are affected by competing vegetation.

Objectives

The first objective of this study was to quantify the effects of competing vegetation on RBC abundance and flowering characteristics in naturally occurring RBC patches. The second objective was to quantify and compare the success rates of RBC when transplanted into different habitat conditions. Specifically, this project compared short-term (one-year) survival rates, crown number, and cover of RBC transplants in areas that were disturbed (mowed, raked, mowed and raked) prior to transplanting and an undisturbed control.

Hypotheses

Objective 1

H_o: Higher cover of competing vegetation will not have an impact on RBC abundance and flowering characteristics in naturally occurring RBC patches.

H_a: Higher cover of competing vegetation will decrease RBC abundance and negatively impact flowering characteristics in naturally occurring RBC patches.

Objective 2

H_o: There is no difference in survival rate, crown number, and cover between RBC transplanted into undisturbed areas (control) and areas with prior ground disturbance.

H_a: Survival rate, crown number, and cover of RBC transplanted into undisturbed areas (control) will be lower than RBC planted into areas with prior ground disturbance.

Methods

a. Study Sites

This study was conducted within a wildlife management area in the Monongahela National Forest, on Cheat Mountain (38°39'09.9" N, 79°55'17.4" W). It can be accessed via FR 1560 off WV state highway 250. This study was conducted in five sites (labeled A through E, Figure 1) along this road. The sites were similar with regards to soil composition, topography, and disturbance history. Average annual precipitation is 47 inches, and average summer temperatures range from highs of 79°F to lows of 57.7°F. Three series of soils have been mapped in these sites: the Calvin and Dekalb soils are acidic soils derived from sandstone, and the Belmont series is less acidic and derived from limestone parent material (USDA 2023). Sites were located on spur ridges with a northwest aspect (Figure 1). Previous management on these sites includes timber harvests that took place from the late 1800's to the early 1900's, then again within the last 40 years. The West Virginia Department of Natural Resources has also maintained conifer species and apple orchards on the sites (USFS 2016).

The forest within the sites is primarily a mixed hardwood forest type. Trees within the sites are in multiple different age classes, but most common is the 80 to 120-year age class (62% of trees) (USFS 2016). Prominent tree species on these sites include black cherry (*Prunus serotina* Ehrh.), sugar maple (*Acer saccharum* Marshall), and hickory (*Carya* spp.). Sites D and E also contain many hawthorn (*Crataegus* spp.) and apple trees (*Malus domestica* Borkh.). Common understory species include wood nettle (*Laportea canadensis* (L.) Wedd.), avense (*Geum* spp.), and bedstraw (*Galium* spp.).

In the summer of 2017, habitat improvement areas (HIA) were created within flat areas of the study area via a bulldozer pushing aside litter and downed trees. This treatment was intended to promote the growth of RBC, as well as provide habitat for wildlife. In the summer of 2022, a thinning timber harvest commenced on these sites that would fully encompass HIAs on sites A, B and E but only partially affect HIAs on sites C and D.

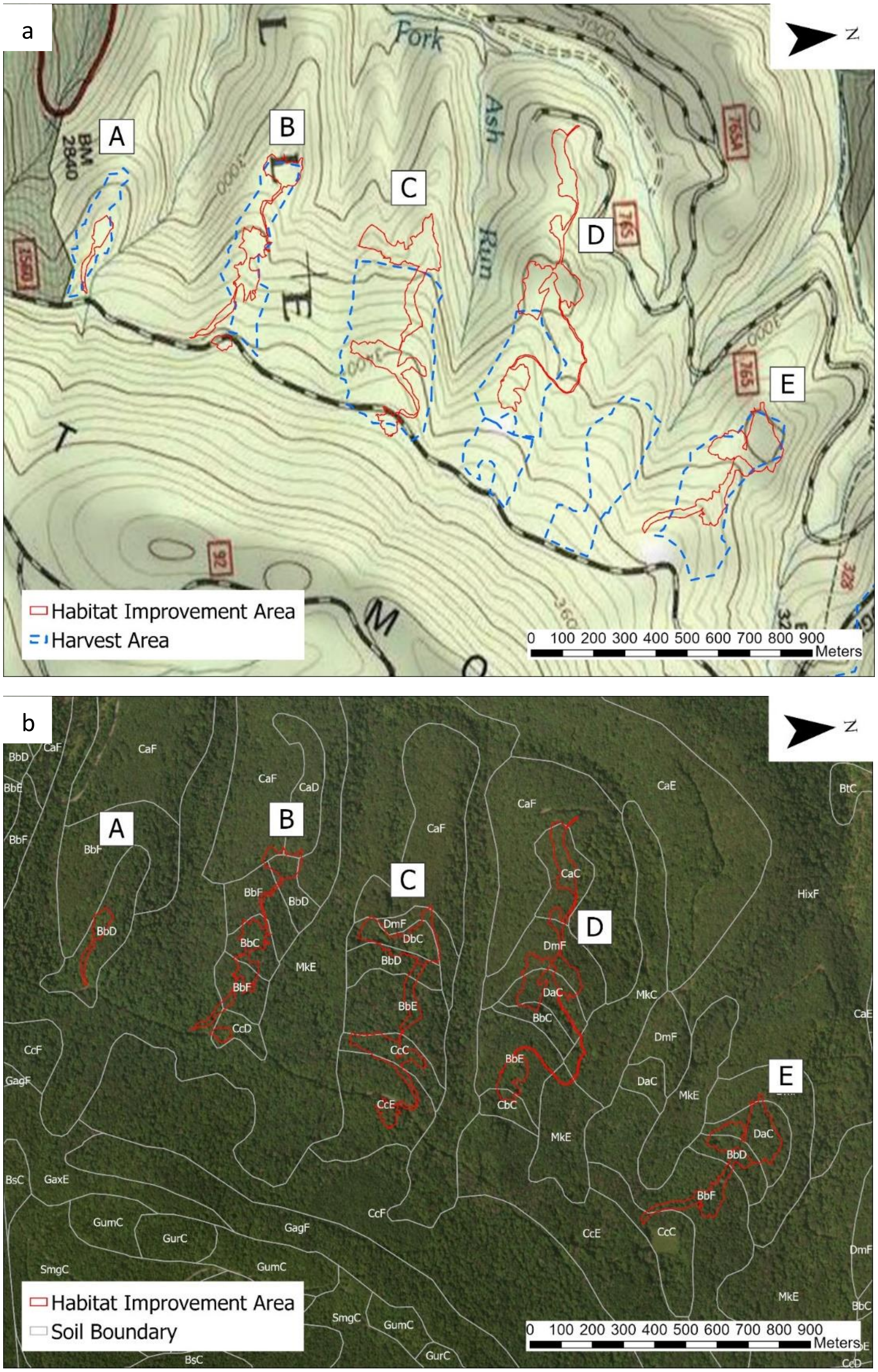


Figure 1. Map of study sites (a) and soils (b) on Cheat Mountain in the Monongahela National Forest, West Virginia, USA. Areas outlined in red denote habitat improvement areas created in 2017 in which mechanical treatment (via bulldozing) removed litter and downed trees. Areas in blue show a 2022 thinning harvest. Soils that begin with D, C, and B represent Dekalb, Calvin, and Belmont series soils, respectively.

b. Data Collection

i. RBC inventory

An inventory of RBC on the sites was conducted in the summer of 2021 (June 28 to July 6). Research personnel searched for RBC patches while walking back and forth across the site at a consistent pace, following a meandering line with lines spaced about 4 m apart. An RBC patch was defined as comprising at least one rooted crown (rooted rosettes that stay in the ground when gently pulled) and lacking physical connections to other patches via stolons (Figure 2). Search time varied with the area of the site and depending on how many personnel were involved. This search was only conducted within the HIAs; cursory searches for the species within full-canopy forest outside HIAs yielded no RBC patches. Any RBC patches found were marked with a stake flag. Location data for each patch was recorded using a Garmin GPS unit (Figure 3). In addition, a tree was identified that would function as a marker for each patch. In the areas with scheduled timber harvest, the selected tree was known to not be intended for harvest (i.e., it was not marked for removal during the timber inventory). The distance from this tree to the patch stake flag was recorded using a tape measure; the direction from the tree to the stake flag was determined using a compass. The tree was marked with flagging tape and its GPS location was recorded. This way, a patch could still be found even if the flag was lost.

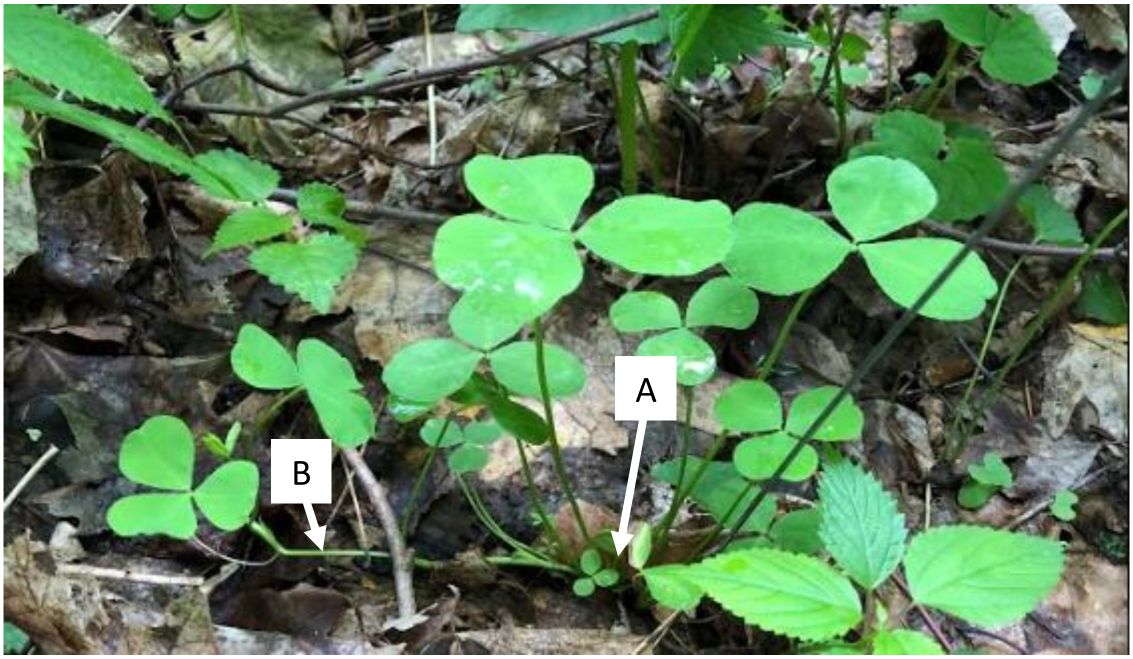


Figure 2. Running buffalo clover rooted crown (A) with stolon (B) at the study site.

Each “patch” stake flag had a number ID that was written directly on the stake flag. Within each of these patches, rooted crowns were counted. This was aided by placing a “counting” stake flag next to every rooted crown identified; these flags would be tallied as they were pulled up to avoid double-counting or missing rooted crowns (Thomas-Van Gundy 2022). If flowers were present, they were also counted.

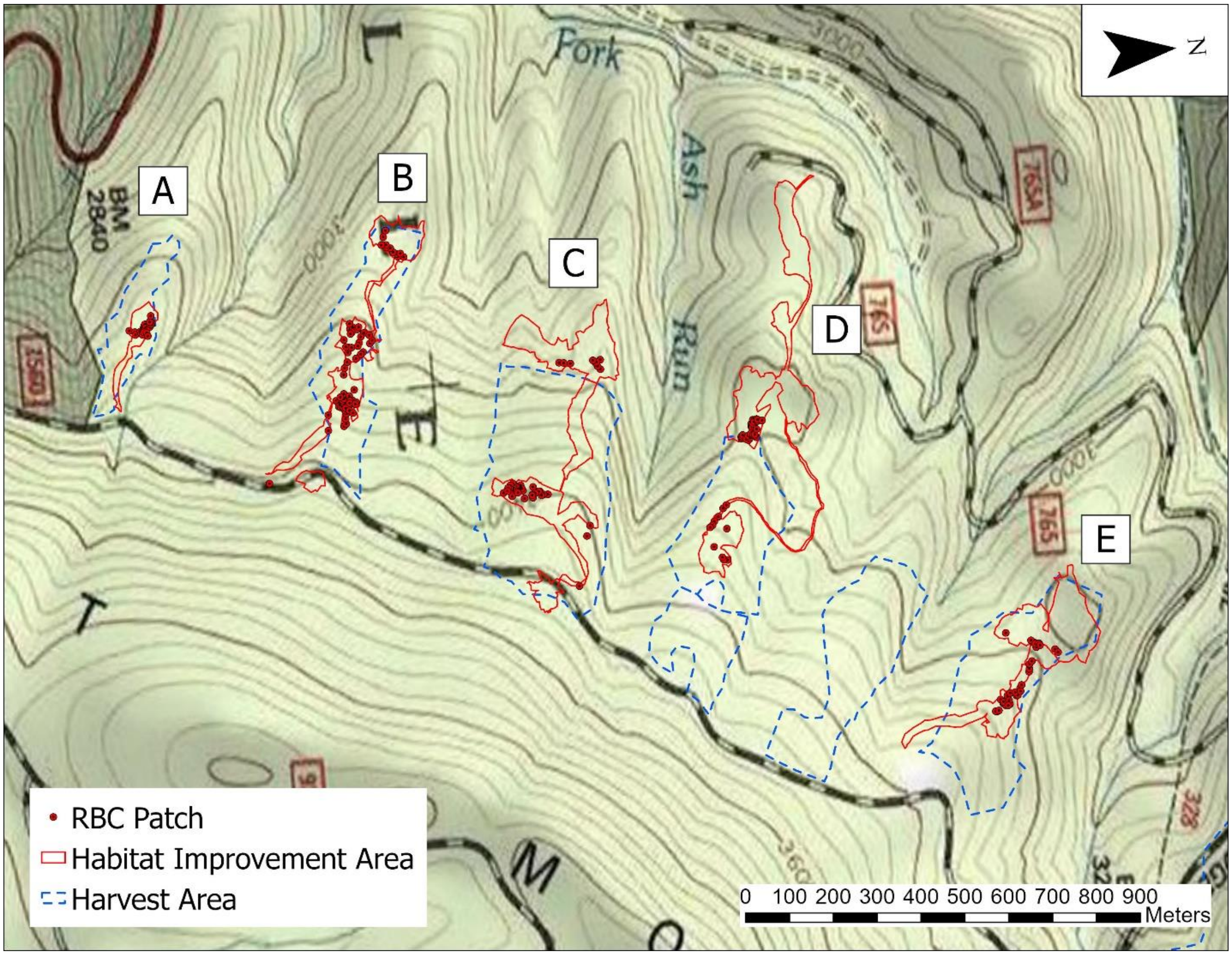


Figure 3. GPS locations of RBC patches found during the inventory.

ii. Competing vegetation (Objective 1)

In early summer of 2022 (28 June - 6 July), research personal recorded cover of vegetation in the known RBC patches found during the 2021 inventory. For each RBC patch, a 1m × 1m PVC sampling frame was placed centered on the patch's stake flag. This flag had been placed to denote the patch center during the 2021 inventory.

Within the sampling frame, RBC rooted crowns were counted, and RBC cover was estimated as a percentage of this area when viewed as a projection from above. Cover was measured to the nearest 10 cm^2, or 0.1% of the sampling frame. Cover of the other vegetation types (fern, grass, forbs, shrubs/vines, tree seedlings) were recorded in the same manner (Coulloudon 1999).

Maximum height was determined for the tallest herb-layer plant and RBC in each sampling quadrat. The tallest herb-layer plant was identified to species level; the maximum height of the RBC represented the length of the longest petiole.

For each patch, the three nearest trees had been located during the inventory; their diameter at breast height (DBH), distance from patch, and species were recorded. These data were used to estimate stem area (ha^{-1}) and tree stem density (ha^{-1}) in the immediate vicinity of each patch by using the farthest tree to delineate a circular area of reference.

iii. Transplant Experiment (Objective 2)

In August of 2021, five and ten 2.5m × 2.5m transplant plots were established within the HIAs outside the scheduled harvest area of sites C and D, respectively (Figure 4). These transplant plots were located about 5 m from existing RBC patches to facilitate growing conditions similar to areas that obviously supported the growth of the species. Within each plot, there were four square 1-m^2 subplots, each being 0.5 m from the neighboring subplot. All plots were oriented in north-south direction (Figure 4, Figure 5).

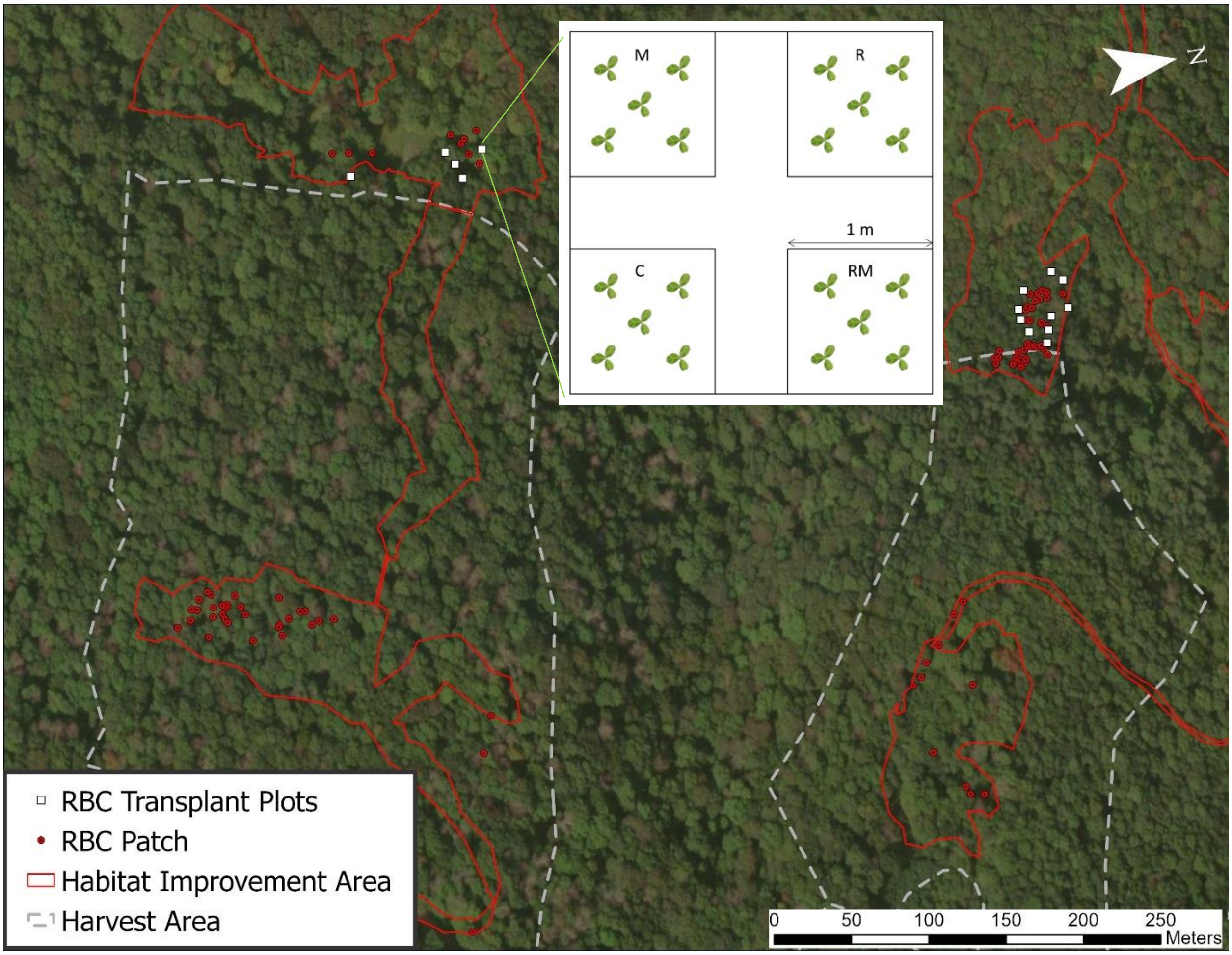


Figure 4. Transplant plot locations on sites C and D and plot layout (inset). Each of the 15 transplant plots comprised four different treatments in respective 1m x 1m subplots (C – control, M – vegetation was mowed to ground level, R – litter was raked from subplot with fire rake, MR – mowed and subsequently raked). Each leaf symbol (inset) represents one transplanted RBC individual.

At each subplot, delineated by placing a 1m × 1m PVC frame, cover of the herb-layer vegetation (≤1 m height) was recorded before treatments were applied. Herb-layer cover was measured for plant groups (fern, grass, forbs, shrubs/vines, tree seedlings) using the hand-area (HA) method (Walter et al. 2015). This method involves using a hand, palm side down, to represent 1% of a 1-m^2 area. The number of "hands", representing the total leaf area of each plant group, was recorded. Tree canopy cover was recorded in the center of each plot using a densiometer. This was done by measuring canopy cover facing each cardinal direction and taking the average of them.

The four subplots in each transplant plot were randomly assigned a treatment. One served as the control (C) and received no treatment. One subplot was raked (R) using a fire rake, creating surface soil disturbance, damaging some leaf tissue, and removing most of the litter (simulating disturbance by bison hooves). In the third subplot, all vegetation was mowed (M) using shears to simulate herbivory; all herb-layer plants within these plots were cut down to the ground. The cut biomass was removed from the plot. The fourth subplot was raked after the vegetation had already been clipped (MR).

After applying the treatments, five rooted crowns of RBC were transplanted into each of the subplots. These rooted crowns were sourced from HIAs within the harvest areas (Table A1). Using a hand trowel, 300 rooted crowns were collected and placed into a cooler containing a moist paper towel. Each transplant was a section cut from a stolon and had at least 1 root and 1 leaf. On average the roots were about 3 cm in length and 2 mm in width. They were immediately transported to the transplant plots. RBC cuttings were planted evenly spaced apart, with one in the center and the other four in the centers of the 0.5×0.5m quarters of the subplot (Figure 4, inset). Cuttings were transplanted on August 17, 2021, before a precipitation event was forecasted. Twelve days after transplanting, the sites were revisited, and any plants that had died (34 of the 300) were replaced.

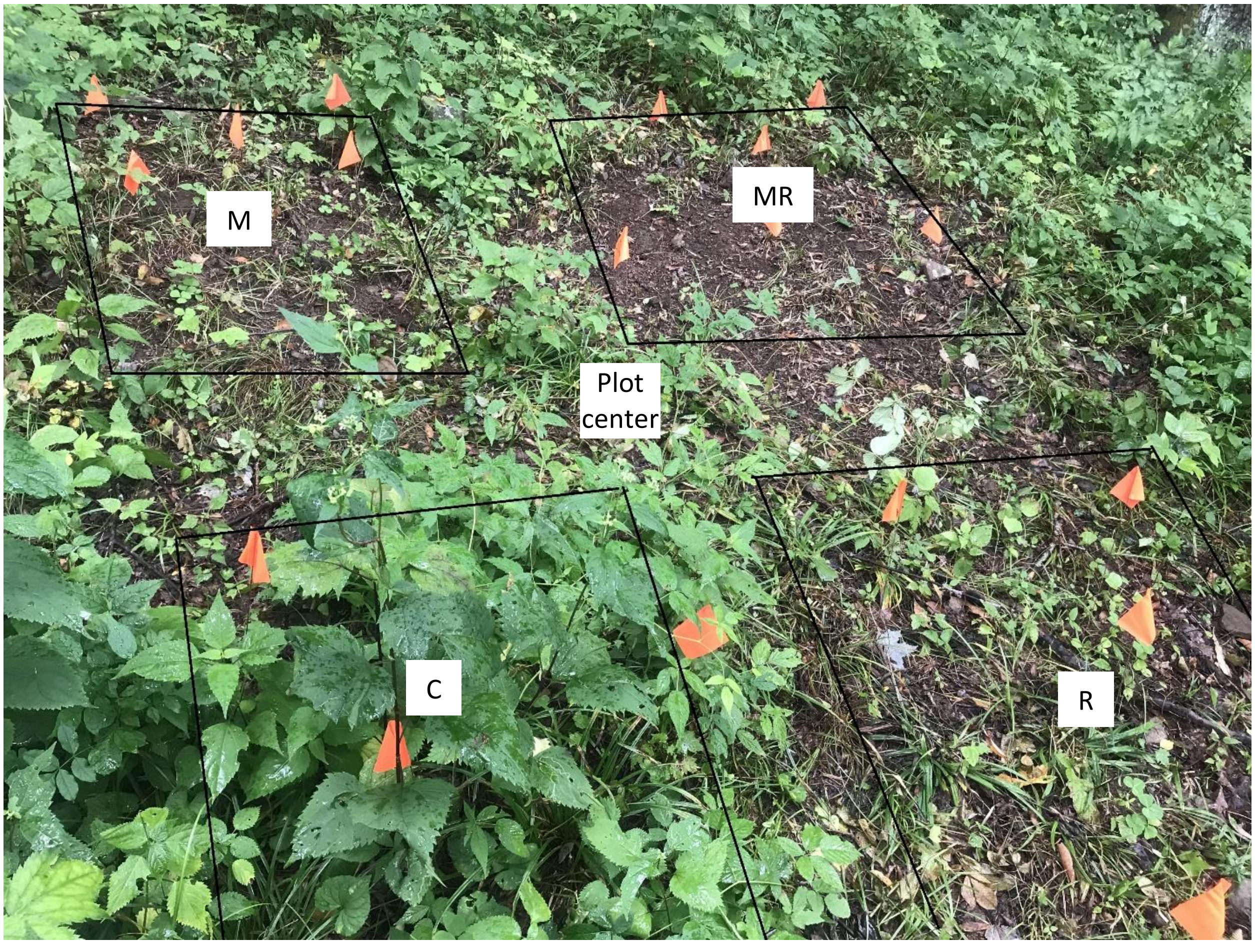


Figure 5. A transplant plot showing the four ground disturbance treatments. Each stake flag denotes the location of a RBC rooted crown transplanted after the ground disturbance. C – control, M – vegetation was mowed to ground level, R – litter was raked from subplot with fire rake, MR – mowed and raked.

In the summer of 2022 (July 4-5), the sites were revisited. Survival rates were measured based on how many of the five transplant locations per subplot still contained at least one RBC individual. In addition, RBC rooted crowns were counted in each subplot and RBC cover was estimated as percent ground cover when foliage was vertically projected from above. Measuring cover this way does not consider layering of foliage (Coulloudon 1995). Within each subplot, total herb-layer cover and the cover of ferns, vines and shrubs, forbs, grasses, and tree seedlings was also recorded.

iv. Data analysis

Data were analyzed using JMP and SAS software (JMP®, Version Pro 16.0.0, SAS Institute Inc., Cary, NC, Copyright ©2021; SAS®, Version 9.4, SAS Institute Inc., Cary, NC, Copyright ©2002-2012). The significance criterion alpha for all tests was 0.05.

In the analysis of effects of competing vegetation on naturally occurring patches of RBC (Objective 1), Spearman's rank correlation coefficients were determined for two datasets. One dataset (A) comprised the number of RBC crowns and flowers counted per patch, tree canopy cover, and data collected from neighboring, mature trees such as density and stem area. The other dataset (B) included data from within 1-m^2 sampling quadrats at the patch center: the number of RBC crowns and RBC maximum height; percent cover of RBC, ferns, vines/shrubs, forbs, grass, and tree seedlings; tree canopy cover; and maximum height of competing vegetation.

The data collected from the transplant plots (Objective 2) were analyzed using a generalized linear mixed model (GLMM) to assess the effect of the different treatments on success of transplanted RBC, utilizing PROC GLIMMIX of SAS. Analyses included the fixed effects of disturbance treatment on RBC a) survival, b) number of rooted crowns, and c) cover. Additional models were explored; they included treatment, cover of herb-layer vegetation types, tree canopy cover, and their interactions as predictor variables. In all models, site was included as a random effect to account for the differing conditions between the sites. Prior to analyses, a Shapiro-Wilk test was conducted to assess normality of the response variables. For survival and rooted crown data, a constant of 0.01 was added to all observations. Survival data were analyzed using Poisson distribution, with log link, and Kenward-Roger degrees of freedom method. Rooted crown data were square root transformed to achieve normal distribution. RBC cover was normally distributed and did not require transformation prior to analysis. Treatment means were compared by constructing contrasts (three disturbance treatments vs. control, or treatments involving mowing vs. raking/control) or by conducting multiple comparisons with Tukey adjustment.

Results

i. RBC Inventory

During the inventory, 233 RBC patches, comprising 5186 rooted crowns with 521 flower heads, were found in the 12.7 ha of habitat improvement areas across all five sites (Table 1). RBC patch size ranged from 1 to 156 (mean = 22.3, SD = 25.9) rooted crowns per patch. The number of flowers in a patch ranged from 0 to 47 (mean = 2.2, SD = 5.3). The ratio between the number of flowers and rooted crowns per patch was calculated, and this value ranged from 0 to 1.67 (mean = 0.11, SD = 0.22). Site B contained the most RBC, with 1309 individuals in 75 patches; Site D had the largest mean patch size (36 rooted crowns per patch). There was a positive correlation between the number of rooted crowns and area of HIA (R^2 = 0.38), although the distribution of RBC patches was uneven within HIAs (Figure 3) and RBC was almost always encountered on the limestone derived soils of the Belmont series (Figure 1,3, A1).

Table 1. Patch size and flowering information on running buffalo clover (RBC) found within habitat improvement areas (HIA) on each of the five sites within the study site, collected during RBC inventory in summer of 2021.

	Site				
Site/ RBC Characteristics	A	B	C	D	E
Size of HIA (ha)	0.49	1.95	3.65	3.49	3.12
Number of RBC patches found	25	75	43	50	40
Total number of rooted crowns	241	1309	751	1786	1099
Mean number of rooted crowns per patch	10	17	17	36	27
Min–max number of rooted crowns per patch	1–37	1–80	1–96	1–156	2–148
Total number of flower heads	61	211	70	37	142
Mean number of flowers heads per patch	2.44	2.81	1.63	0.74	3.26
Min–max number of flowers heads per patch	0–20	0–47	0–15	0–15	0–34

ii. Competing Vegetation

In the 222 patches inventoried in 2021 (and from which no crowns were taken for the transplanting experiment, Table A1), RBC cover measured in 2022 within a 1-m^2 sampling quadrat around the patch was variable (0% to 70%), but most plots only contained moderate RBC cover (mean = 4.8%) (Table 2, Figure 6). Twelve of the original patches contained 0% RBC in 2022. These patches had mostly been small, containing between 1 and 12 rooted crowns the year prior, with only one of them containing more (23 rooted crowns). The maximum height of RBC was 26 cm (mean = 15). Mean RBC density was 18 crowns per 1 m^2, though it ranged from 0 to 165. Herb-layer cover (measured as vertical projection onto the ground) was abundant, with mean total cover at 74%. Most frequent and abundant were the forbs which occurred in every quadrat and, in some cases, had a cover of 100% (mean = 45) (Table 2). Ferns were least frequent, only occurring in 16 of the quadrats. In these 16 quadrats, fern cover ranged from 1% to 30%, with a mean of 9.5%. Shrub and vine cover (mostly comprising blackberry (*Rubus* spp.) and greenbrier (*Smilax* spp.)) was low to moderate in most quadrats (mean = 11.89), but high on some, particularly on site A, where sometimes cover was 80% (Figure 6). Grass cover varied greatly, ranging from 0% to 70% (mean = 12.8). Tree seedlings were often small and therefore had low cover (mean = 2.15) (Figure 6, Table 2).

Maximum height of herb-layer vegetation within the sampling area ranged from 1 to 145 cm (mean = 64). False wood nettle (*Boehmerica cylindrica* (L.) Swartz) was the dominant (tallest) plant species in 107 of the quadrats. Other dominant vegetation included *Rubus* spp. (41 quadrats), wood nettle (29 quadrats), various grasses (ten quadrats), and trees seedlings (eight quadrats). Multiflora rose (*Rosa multiflora* Thunb.) was found in two of the patches. This was the only invasive species that was encountered within the quadrats that were sampled.

Tree canopy cover over the RBC patches ranged from 35% to 97.5% (mean = 85, SD = 9.9) (Table 3). Canopy trees near the RBC patches comprised 20 different tree species, with common species being sugar maple, black cherry, and white ash (*Fraxinus americana* L.). Among the three trees closest to RBC patches, tree diameter at breast height (DBH) ranged from 9 cm to 175 cm (mean = 45.4, SD = 25.6). Based on the measurements of these trees, extrapolated tree density ranged from 17 to 9549 trees per hectare (mean = 507, SD = 843). Stem area ranged from 2 to 1400 m^2 per hectare (mean = 80, SD = 127) in the immediate vicinity of RBC patches.

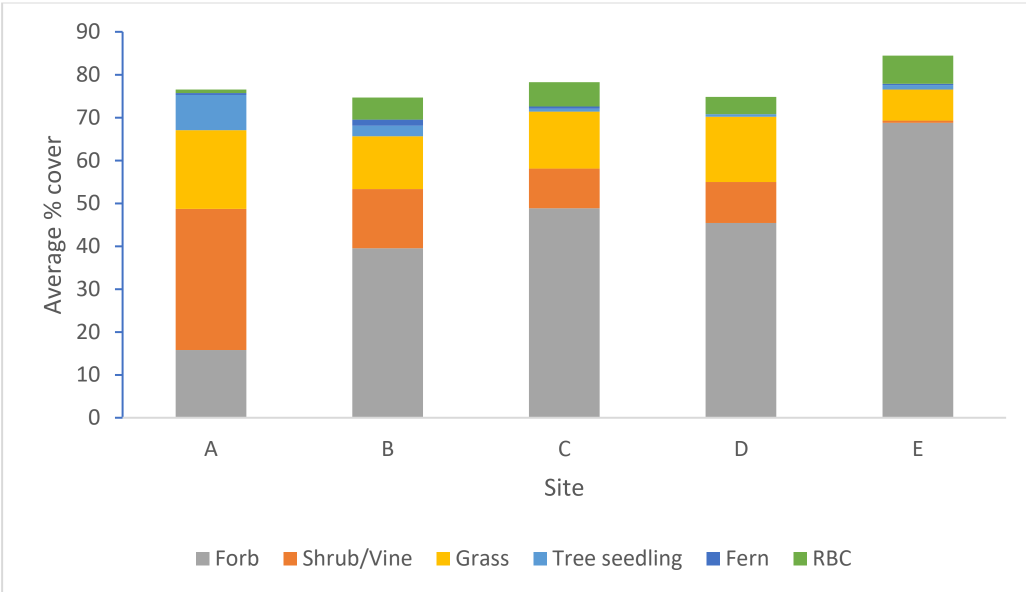


Figure 6 – Mean cover of vegetation found within sampling quadrats, separated by site. Cover (measured as vertical projection onto the ground) is expressed as % of a 1-m^2 area around the center of the patch (see Figure 3).

Table 2 – Summary statistics of RBC data and herb layer cover (as leaf area projected onto the ground; % of 1-m^2 quadrat) in clover patches. “Total” refers to the cover of the herb-layer (including RBC). “Crowns” is the number of RBC rooted crowns, “hplant” and “hclover” show the height (cm) of the tallest competing vegetation and tallest RBC petiole, respectively. “Frequency” represents the % of plots that contain a certain cover type.

	Running Buffalo Clover			Herb-layer plants (<1 m height)						
	Crowns	Cover	hclover	Total	Fern	Shrub/ Vine	Forb	Grass	Tree Seedl.	hplant
Min	0	0	0	20	0	0	1.3	0	0	1
Max	165	70	26	100	30	80	100	70	40	145
Mean	17.85	4.82	15.26	73.98	0.68	11.89	45.04	12.77	2.15	64.18
SD	19.17	8.21	5.31	16.55	3.46	17.43	25.31	13.57	4.93	19.86
Frequency					7.21	58.56	100.00	93.69	48.65	

Table 3 – Data on trees found near RBC patches on the five sites of this study area. Date were collected during 2021 RBC inventory.

Ridge	All	A	B	C	D	E
Tree canopy cover (%)	85	84	79.4	89	92.5	84.4
Mean DBH of three neighboring trees (cm)	45	50	54	42	38	40
Mean tree density in patch vicinity (Tpha)	507	176	207	305	1368	422
Mean tree stem area in patch vicinity (m^{-2} ha^{-1})	80	69	50	41	184	58

ii. A. Competing Vegetation: Trees

RBC patch size was not significantly (P≤0.05) correlated with any of the variables relating to nearby trees. These included trees per hectare (P=0.14) and stem area per ground area (P=0.87) (Table 4). Tree canopy cover was not significantly correlated with patch size, but there was a positive trend between the two (P=0.07). The number of flowers in a patch was negatively correlated with each of these variables. The ratio of the number of RBC flowers and crowns was also calculated, and this, too, was negatively correlated with the three tree variables (Figure 7).

Table 4 – Spearman's correlation values from RBC inventory conducted in summer 2021. Data collected includes RBC patch size (based on rooted crown #), RBC flower #, and the ratio between flower number and crown number. Data on nearby trees were also collected, including the tree canopy cover above each patch. Trees per hectare, and stem area were calculated using size and distance to nearby trees. Negative ρ values show a negative correlation. Significant P-values are specified using an asterisk ($^{\#}$≤ 0.1, * ≤ 0.05). The complete correlation matrix is shown in the appendix (Table A5).

	Patch Size		Number of Flowers		Flower: Crown Ratio	
	ρ	P-value	ρ	P-value	ρ	P-value
Tree Canopy Cover	0.1209	$0.0659^{\#}$	-0.2882	<.0001*	-0.3326	<.0001*
Trees/ha	0.0963	0.1435	-0.2686	<.0001*	-0.2799	<.0001*
Stem Area (m^2/ha)	-0.0108	0.8706	-0.1909	0.0035*	-0.1766	0.007**

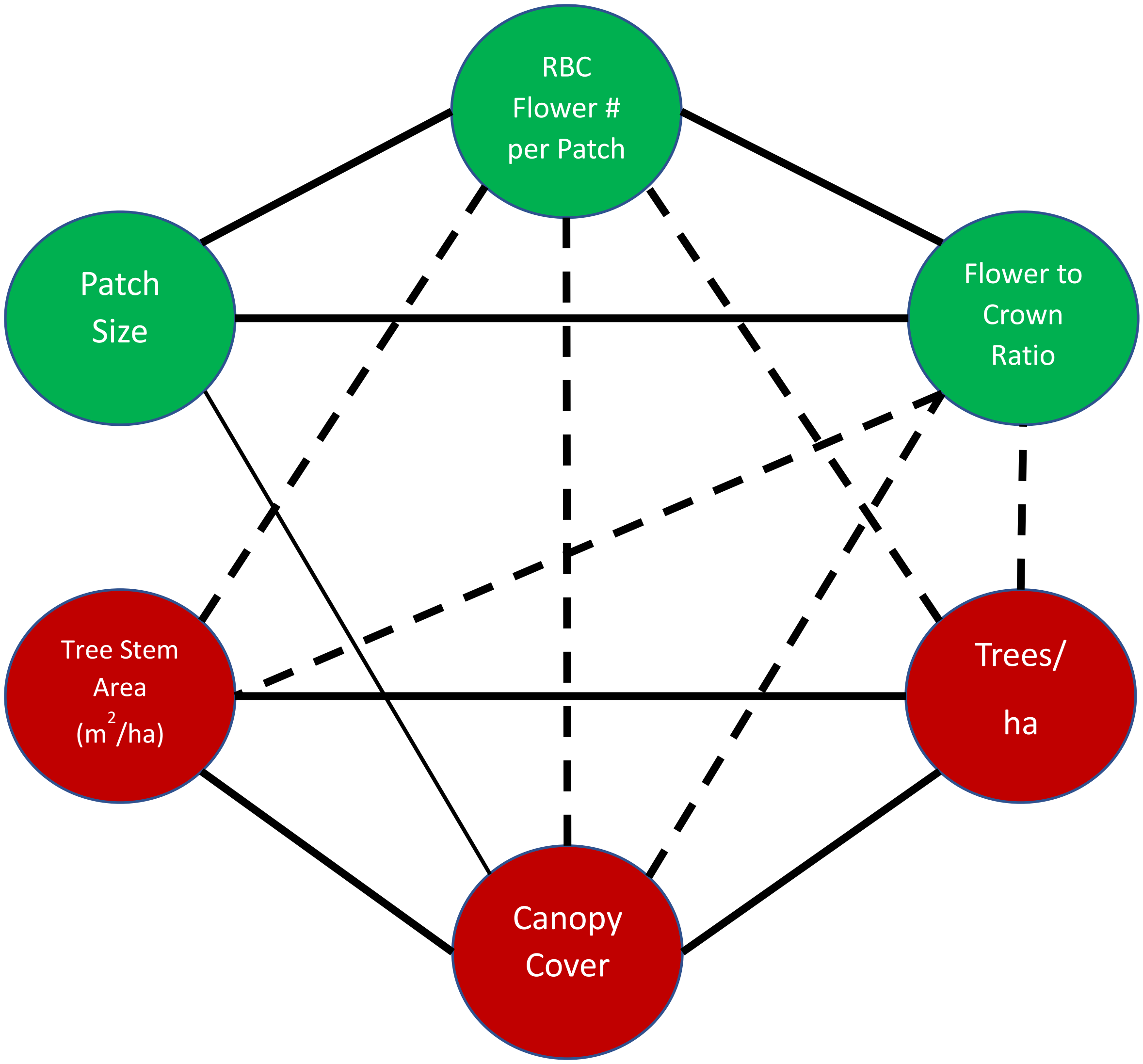


Figure 7 – Correlation wheel showing which data from the 2021 RBC inventory are significantly correlated. Data collected includes RBC patch size (total number of rooted crowns per patch), RBC flower number, and the ratio between flower number and crown number. Data on nearby trees were also collected, including the tree canopy cover above each patch. Trees per hectare, and stem area were calculated using the three nearest trees. Thick lines show a P-value of <0.05, while thin lines show a P-value of ≤ 0.1. Solid lines show positive correlations; dashed lines represent negative correlations.

ii. B. Competing Vegetation: Herb layer

In a separate correlation analysis utilizing data collected with the 1-m^2 sampling quadrats at the RBC patch center, tree canopy cover was positively correlated with both RBC cover and crowns (P=0.04 and P=0.0004, respectively). Tree canopy cover was not significantly correlated to RBC height (P=0.662). Cover of shrubs/vines was negatively correlated with RBC cover, crowns and height, while forb cover had positive correlations with these RBC variables. Grass cover was negatively correlated with RBC height, while maximum herb-layer height was positively correlated with RBC height (Table 5, Figure 8).

Table 5 – Spearman's correlation values from RBC cover, crowns, height, and characteristics of competing vegetation. Negative ρ values show a negative correlation. P-values ≤ 0.05 are specified using an asterisk. The complete correlation matrix is shown in the appendix (Table A4)

	RBC Cover		RBC Crowns		RBC Height	
	ρ	P-value	ρ	P-value	ρ	P-value
Tree Canopy Cover	0.138	0.040*	0.232	<0.001*	0.029	0.662
Total_Herb	0.108	0.110	0.184	0.006*	0.172	0.010*
Fern	-0.044	0.514	-0.055	0.414	0.003	0.963
Vine/Shrubs	-0.338	<0.001*	-0.344	<0.001*	-0.177	0.008*
Forb	0.220	0.001*	0.274	<0.001*	0.266	<0.001*
Grass	-0.077	0.253	-0.059	0.382	-0.226	0.007*
Tree seedlings	-0.228	0.001*	-0.214	0.001*	-0.043	0.527
Max herb-layer height	-0.085	0.207	0.000	0.995	0.153	0.023*

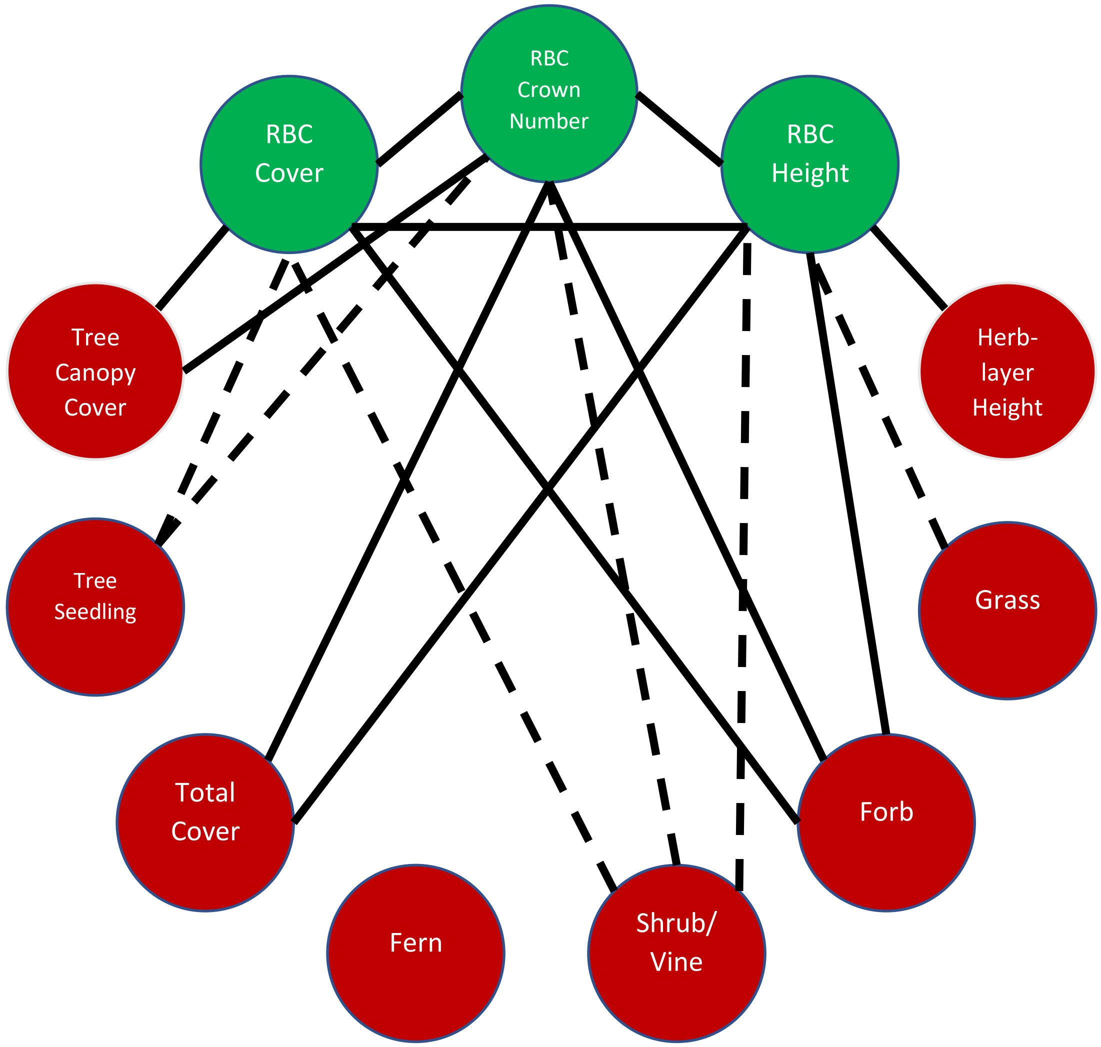


Figure 8 – Correlation wheel showing which data from the 2022 RBC collection are significantly correlated. Data includes RBC crowns, maximum height of RBC in each patch, tree canopy cover, and % cover of RBC, forbs, shrubs/vines, grasses, ferns, and tree seedlings within a 1-m^2 quadrat. Lines show a P-value of ≤ 0.05. Solid lines show positive correlations; dashed lines represent negative correlations.

iii. Transplanting

In the transplant subplots, similarly to the centers of naturally occurring patches, the herb-layer was dominated by forbs before treatment and one year after treatment (Figure 9). Before treatment, forb cover on the transplant subplots ranged from 6–75%, with an average of 30.5%.

Grass cover ranged from 0–30%, with an average of 10.3%. Most subplots had at least some grass, with only 3 of the 60 subplots containing none. Similarly, cover of shrubs and vines ranged from 0-23%, with an average of 4.9%. There were nine subplots that contained no shrubs or vines. In contrast, ferns were absent from 50 of the 60 subplots, with a mean cover of 0.8% across all subplots (min–max 1–19% if present). Tree seedlings were absent from 39 of the 60 subplots, with a mean cover of 0.65% (min–max 1–7% if present) (Figure 9a). Before the implementation of treatments, subplots of different treatments did not have different levels of cover in respective plant types (e.g., forbs $P = 0.24$), although for two plant types there was a trend for pre-treatment differences (Tree Seedlings $P = 0.07$, Total Cover $P = 0.08$) (Figure 9a). Tree canopy cover of mature trees measured at the plot level ranged from 80–97%.

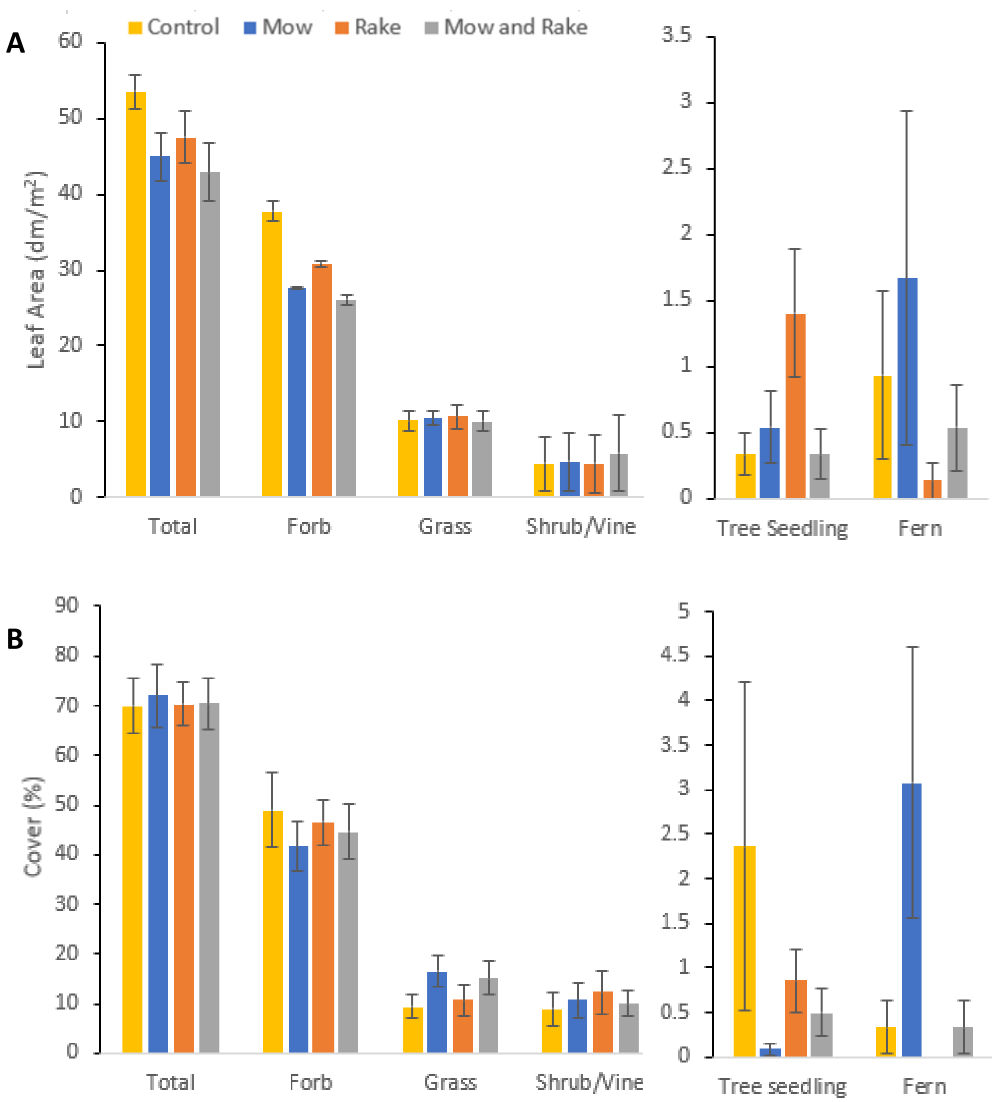


Figure 9. - Transplant plot characteristics regarding a) pretreatment herb-layer cover (measured in dm of leaf area over 1 m^2 of ground), and b) cover 1 year post treatment (measured as percent of vertically projected cover). Error bars are 1 SE, n = 15.

One year after planting, the RBC in the Control experienced increased mortality compared to the disturbance treatments, with little difference in survival between the three disturbance types (Figure 10a). Survival rate was 57% in the control versus 83% in the disturbance treatments. Considering only the surviving transplants, transplants in the disturbed subplots grew better than in the control (P = 0.009). Within the Control subplots each surviving transplant had multiplied to an average of 1.7 crowns after one year; in the three disturbance treatments, each surviving transplant had multiplied to between 2.5 and 3.25 crowns (Figure 11a). When tallying the number of rooted crowns in each subplot, RBC rooted crowns in the disturbance treatments had doubled or tripled compared to the five planted one year prior. In the Control, only about half as many rooted crowns were present than in disturbed plots (Figure 10b). When expressing RBC abundance as cover, a very similar pattern was seen compared to the rooted crowns, with cover being lower in the Control than in the three treated plots (Figure 10c). RBC cover per surviving transplant (indicating plant vigor) was similar when contrasting the control to the disturbance treatments combined ($P = 0.023$). Plant vigor was similar between the two treatments involving mowing, and between the two non-mowed treatments (Control, Raking) (Figure 11b). There was a statistical trend in the comparison between mowed and non-mowed treatments ($P= 0.08$), with mowing before transplanting benefitting plant vigor one year later.

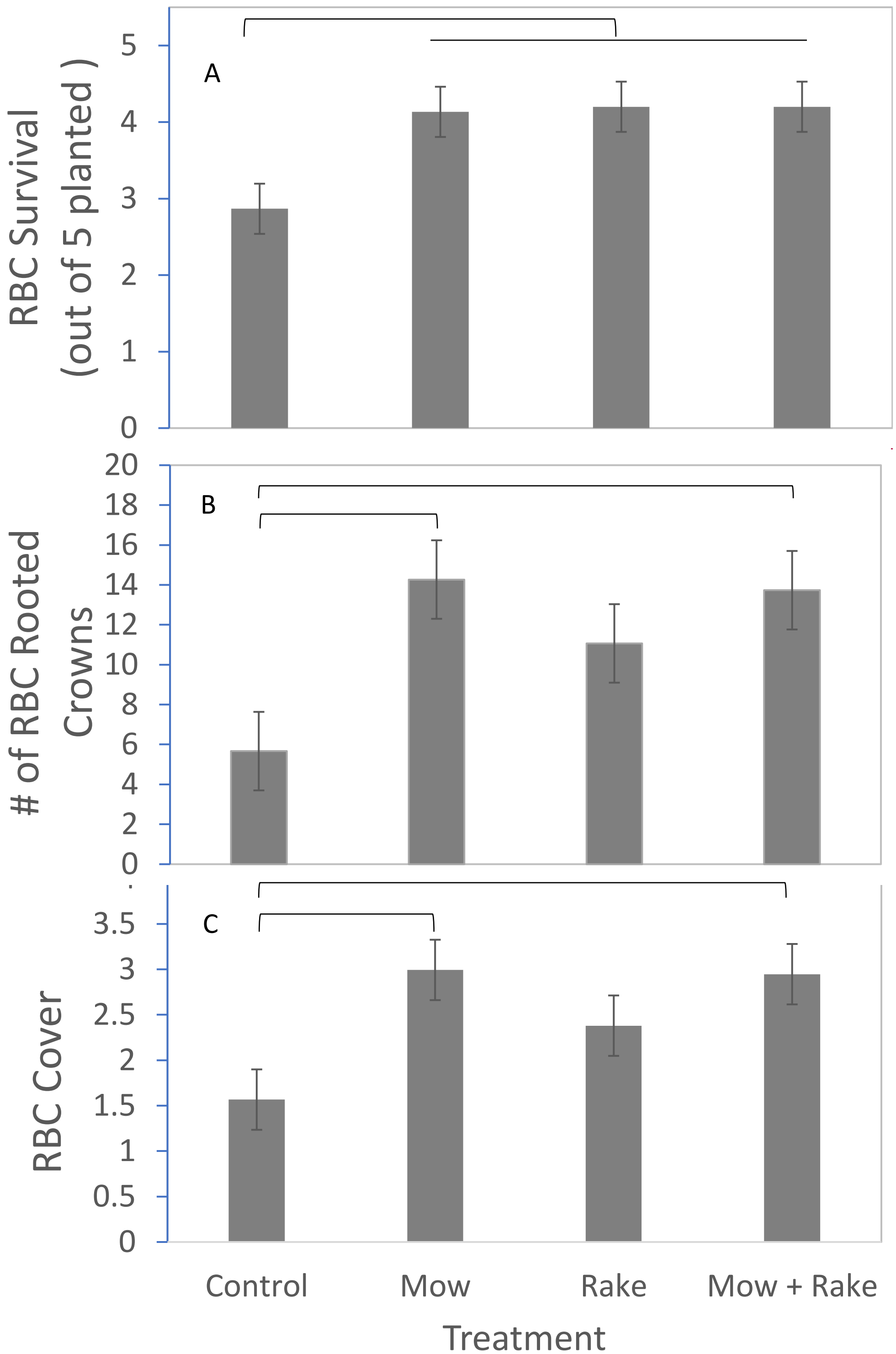


Figure 10 – RBC survival (out of five planted) (A), the average number of RBC crowns (B), and cover % (C) for each of the disturbance types one year after the disturbance. Error bar shows 1 SE. Brackets show significant differences based contrasts (A) or multiple comparisons (B,C). Corresponding violin plots are shown in the appendix (Figure A2).

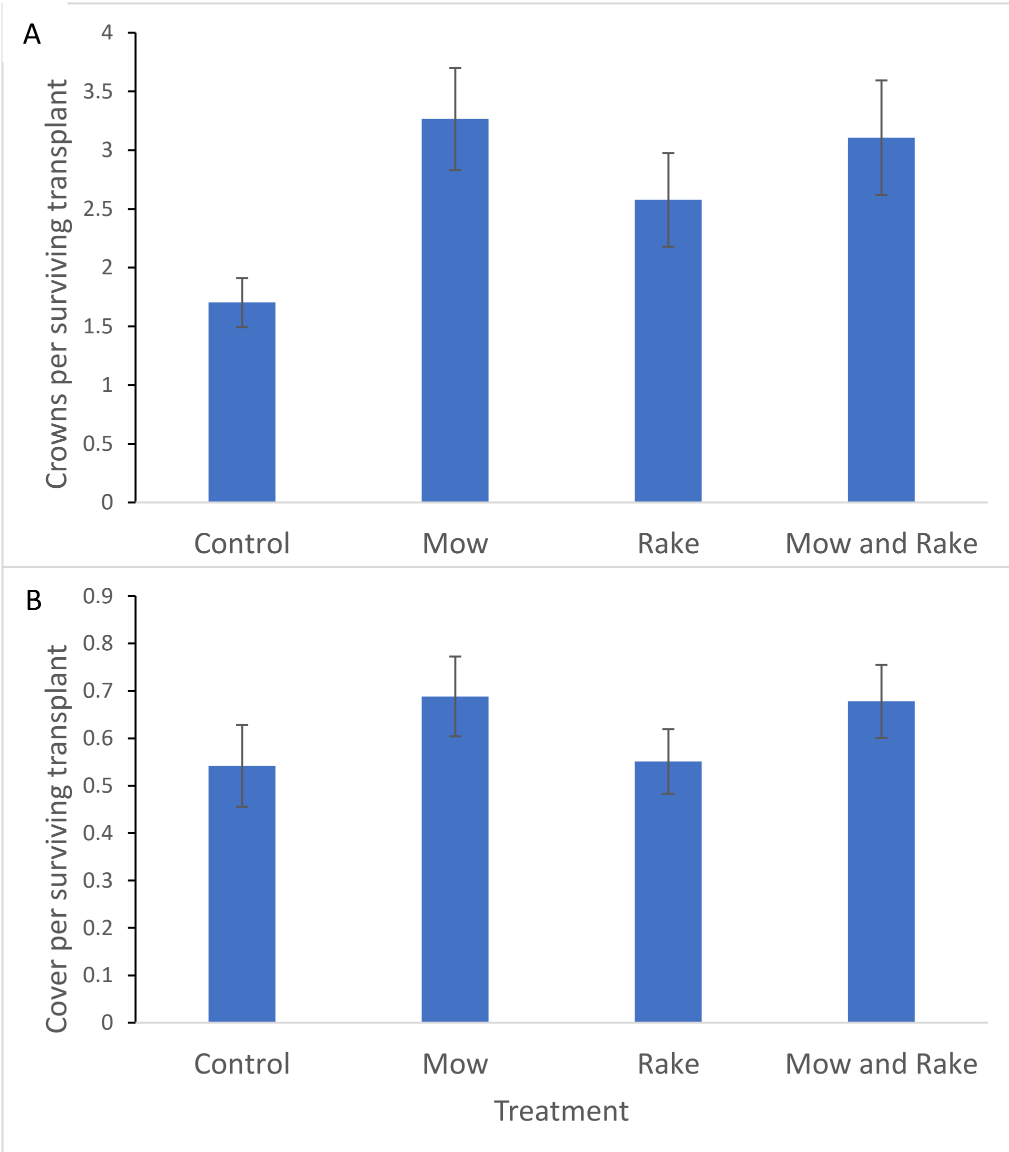


Figure 11 – Rooted crowns grown from surviving transplants (a) and cover (projected onto the ground in a 1-m^2 area) per surviving transplant (b) one year after planting.

Statistical analyses showed that the impact of the disturbance treatments on RBC survival was not statistically significant when Treatment was the sole predictor variable (Table 6, Model 1). Other models were explored that included a range of predictor variables, including tree canopy cover (Table 6, Models 2,3), cover of different types of herb layer vegetation before subplots were treated (Table 6, Models 3–9), and different types of herb-layer cover one year after transplanting (Table 6, Models 10–16). In model 7, including pretreatment forb cover, the Treatment effect was statistically significant for RBC survival. While pretreatment forb cover was not a significant predictor in the overall model 7 (Table 6), forbs were the most prominent ground cover on the subplots (Figure 9), and their abundance before the treatments appeared to have a negative impact on RBC survival in Mowed subplots (Figure 12). In none of the other models with RBC survival was Treatment or any other predictor variable significant (Table 6).

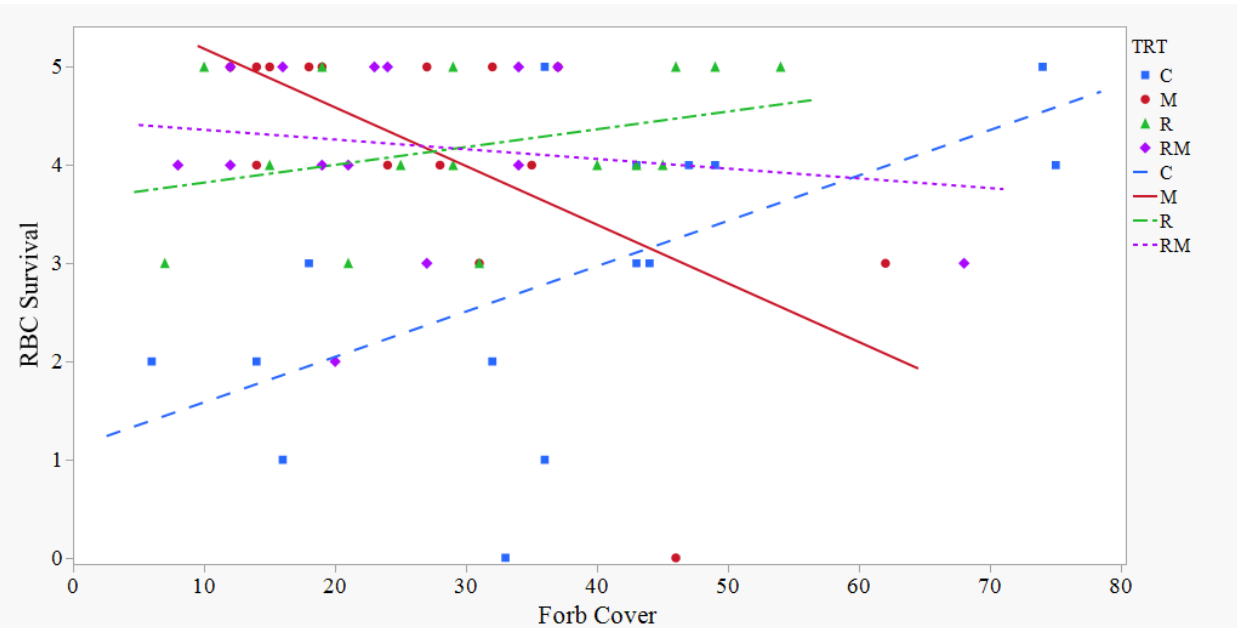


Figure 12 – Interaction of treatment and forb cover (as leaf area in dm^2 m^{-2} or %) before treatment on RBC survival rates (out of the 5 planted).

Table 6 – Results of GLM tests run on transplant variables. P-values represent effects of response variables on RBC survival, number of RBC crowns, and RBC cover. TRT is the disturbance treatment applied, "TreeCanopyCover" represents the percentage of sky view obscured by trees around the transplant plots. Variables written with "Pre" represent cover values in 2021 prior to applying disturbance treatments, while "Post" represents 2022 cover data. Significant P-values are specified using an asterisk (* ≤ 0.1, ** <0.05).

Model	Effect	Survival	Crowns	Cover
1	TRT	0.188	0.006**	0.019**
2	TRT	0.723	0.230	0.250
	TreeCanopyCover	0.710	0.360	0.531
	TRT*TreeCanopyCover	0.990	0.461	0.449
3	TRT	0.202	0.004**	0.011
	TreeCanopyCover	0.667	0.383	0.398
	Total_Others_Pre	0.691	0.128	0.300
4	TRT	0.133	0.022**	0.004**
	Total_Others_Pre	0.869	0.463	0.999
	TRT*Total_OthersPre	0.286	0.131	0.013**
5	TRT	0.280	0.025**	0.069*
	FernPre	0.610	0.596	0.666
	FernPre*TRT	0.889	0.724	0.806
6	TRT	0.653	0.159	0.029**
	ShrubVinePre	0.484	0.885	0.998
	TRT*ShrubVinePre	0.429	0.498	0.182
7	TRT	0.033**	0.0003**	0.006**
	ForbPre	0.950	0.702	0.631
	TRT*ForbPre	0.116	0.011**	0.036**
8	TRT	0.712	0.895	0.273
	GrassPre	0.898	0.070*	0.013**
	TRT*GrassPre	0.457	0.190	0.643
9	TRT	0.343	0.032**	0.100*
	TreeSeedlingPre	0.778	0.487	0.168
	TRT*TreeSeedlingPre	0.887	0.661	0.769
10	TRT	0.175	0.002**	0.008**
	Fern	0.749	0.096*	0.542
	ShrubVinePre	0.661	0.998	0.255
	ForbPre	0.680	0.017**	0.469
	GrassPre	0.762	0.016**	0.005**
	TreeSeedlingPre	0.934	0.523	0.519
11	TRT	0.899	0.334	0.710
	Total_OtherHerbsPost	0.912	0.619	0.428
	TRT*Total_OtherHerbsPost	0.716	0.136	0.401
12	TRT	0.319	0.114	0.077*
	FernPost	0.658	0.083*	0.028**
	TRT*FernPost	0.868	0.525	0.067*
13	TRT	0.889	0.790	0.116
	ShrubVinePost	0.765	0.279	0.146
	TRT*ShrubVinePost	0.503	0.215	0.346
14	TRT	0.627	0.102	0.252
	ForbPost	0.906	0.313	0.518
	TRT*ForbPost	0.907	0.305	0.348
15	TRT	0.891	0.896	0.220
	GrassPost	0.785	0.157	0.954
	TRT*GrassPost	0.726	0.314	0.976
16	TRT	0.813	0.213	0.162
	TreeSeedlingPost	0.509	0.881	0.227
	TRT*TreeSeedlingPost	0.756	0.532	0.469

The disturbance treatments had a statistically significant effect on RBC crown number and cover (collectively termed abundance). This effect was consistent between the model including only Treatment (Table 6, Model 1) and most models including pretreatment cover of the herb layer (Table 6, Models 3-10). Pretreatment forb cover was found to have a significant impact on RBC crowns and cover (Model 7, Table 6) (Figure 13a,b), as did the presence of grasses before treatment (Model 8 and 10, Table 6) (Figure 13c,d). Among models considering posttreatment herb-layer cover, only fern cover was a significant predictor of RBC number of crowns and cover (Table 6, Model 12) (Figure A2). However, ferns were infrequent and any relationship with RBC abundance might have been driven by one subplot with very high fern cover (Figure A2). Tree canopy cover was not a significant predictor of RBC abundance (Model 2-3, Table 6).

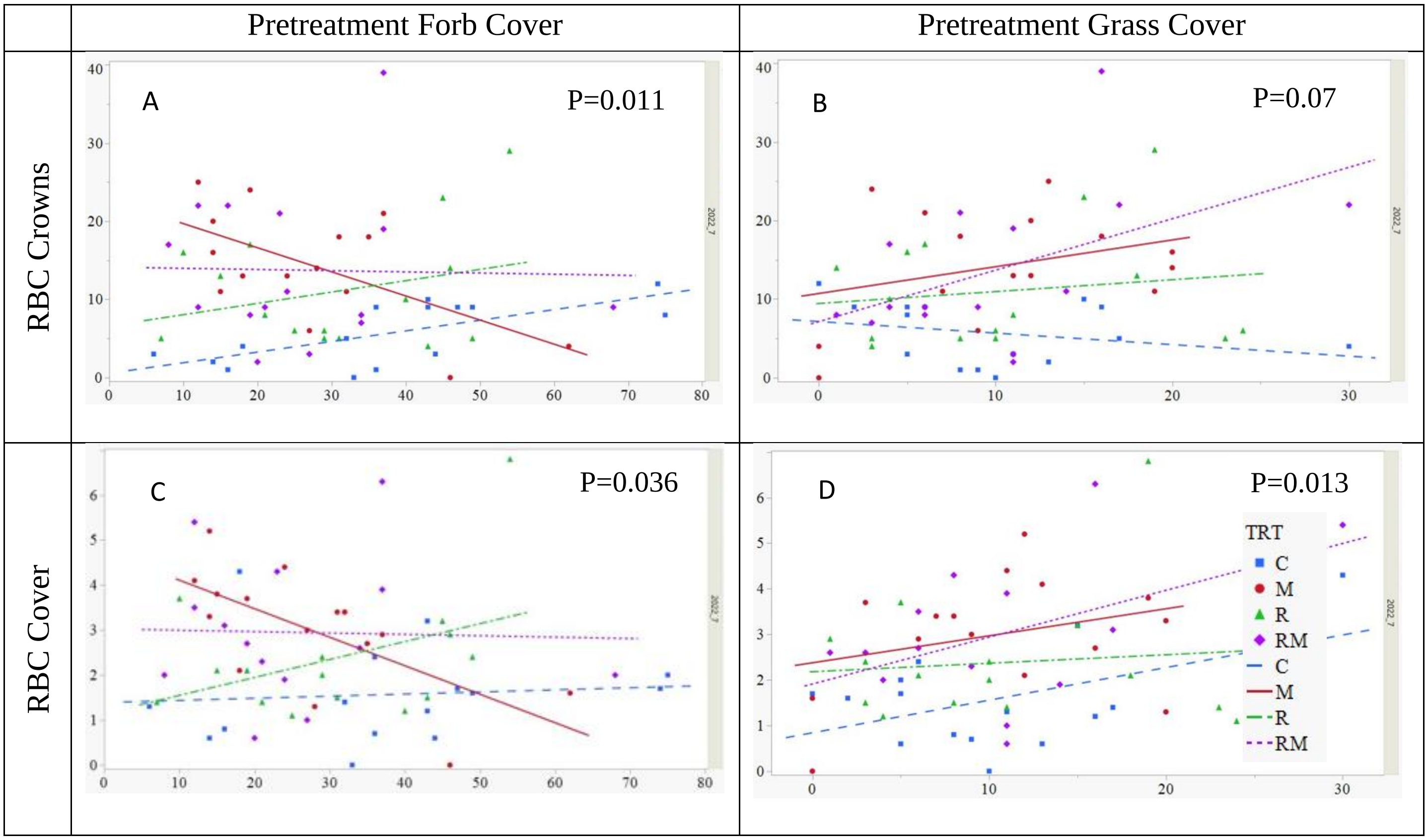


Figure 13 – The interaction of treatment and cover before treatment on the number of RBC crowns and cover one year after transplanting: forb cover and RCB crowns (A), forb cover and RBC cover (B) (Model 7 of Table 6), grass cover and RBC crowns (C) and grass cover and RBC cover, separated by treatment (Model 8 of Table 6). P-vales are for interactions of Treatment and Forb or Grass cover, respectively (Table 6).

Of the models with Treatment as significant predictor variable ($P \leq 0.05$), Akaike Information Criterion (AIC) values were used to determine which of the models to use for further investigation (Table 7). Model 7 (including pretreatment forb cover) was selected for RBC survival, and Model 1 (only including Treatment as a predictor variable) was chosen for RBC crown number and cover.

Table 7 – Akaike Information Criterion (AIC) for each of the models shown in Table 6. AIC values for models including Treatment as significant (P≤0.05) predictor variable are highlighted in light gray; models in which predictors other than treatment were statistically significant (P≤0.1) have AIC values highlighted in darker gray. The lowest AIC value of models with Treatment as significant predictor (P≤0.05) are specified with an asterisk.

	Model	Survival	Crowns	Cover
1	TRT	224.27	187.89*	208.16*
2	TRT + TreeCanopyCover + TRT*TreeCanopyCover	231.96	201.09	220.32
3	TRT + TreeCanopyCover + Total_OthersPre	227.91	195.19	218.38
4	TRT + Total_OthersPre+TRT*Total_OthersPre	228.22	201.76	215.15
5	TRT + FernPre + TRT*FernPre	231.52	191.7	209.74
6	TRT + VinePre + TRT*VinePre	228.98	202.01	216.09
7	TRT + ForbPre + TRT*ForbPre	225.55*	200.67	220.24
8	TRT + GrassPre + TRT*GrassPre	229.55	200.29	216.78
9	TRT + TreePre + TRT*TreePre	231.64	187.99	206.31
10	TRT+FernPre+VinePre+ForbPre+GrassPre+TreePre	233.59	204.54	220.28
11	TRT+Total_OtherHerbsPost+TRT*Total_OtherHerbsPost	188.81	181.35	185.45
12	TRT+FernPost+TRT*FernPost	187.67	159.69	156.32
13	TRT+ShrubVinePost+TRT*ShrubVinePost	187.61	176.56	180.83
14	TRT+ForbPost+TRT*ForbPost	189.57	181.64	182.61
15	TRT+GrassPost+TRT*GrassPost	188.81	176.13	183.81
16	TRT+TreeSeedlingsPost+TRT*TreeSeedlingsPost	182.29	157.49	161.97

When using multiple comparisons, RBC survival in individual ground disturbance treatments was not significantly (P>0.05) different than the Control; however, when constructing contrasts between the Control and the combination of the three disturbance treatments, RBC survival in the disturbance treatments was significantly higher than in the Control (P=0.0077) (Figure 7a, Figure A2a). When considering RBC abundance, multiple comparisons showed that mowing plots increased the number of rooted crowns (P=0.009), as did the combination of mowing and raking (P=0.015), when compared to the control (Figure 10b, Table A2, Figure A2b). Mowing also increased RBC cover (P=0.028), as did mowing and raking (P=0.036) (Figure 10c, Table A3, Figure A2c). Raking alone did not significantly impact the number of RBC crowns (P=0.1) or cover (P=0.36) relative to the Control (Figure 7b,c, Figure A2b,c).

When testing the impact of treatments on herb-layer cover, we found that overall, Treatment had no effect on any of the herb-layer components(Table 8, Figure 9b). When assessing contrasts between the disturbed subplots combined and the control, there was no significant difference (Table 8). However, when contrasting the plots that were mowed (M and RM) with the ones that were not mowed (C and R), cover of grasses and ferns showed a trend of being positively impacted by the mowing treatments (P=0.093 and P=0.094 respectively); tree seedling cover was found to be significantly negatively impacted by treatment (P=0.0171 (Table 8, Figure 9b).

Table 8 – P-values of effect of treatment (TRT) on the cover for different vegetation types one year after treatment. Results of GLM tests run on transplant variables.

Cover Type	TRT P-values	C vs M/R/MR P-values	C/R vs. M/MR P-values
Total_OtherHerb	0.9953	0.8845	0.8544
Forb	0.8772	0.537	0.4775
Grass	0.3877	0.2332	0.0925
Fern	0.2067	0.7535	0.094
Shrub_Vine	0.9639	0.6918	0.9781
Tree_Seedling	0.107	0.0544	0.0171

Discussion

i. RBC Inventory

Based on inventory results, we found that the treatments applied to create the HIAs support RBC populations. The removal of litter and small vegetation in the HIAs had evidently created favorable conditions for RBC, and five years later they were still present and thriving. These populations were not confined to skid roads as RBC often is in West Virginia (Madarish and Schuler 2002) but spread unevenly throughout the HIA interior. A forest harvest was scheduled to take place on the HIAs at the beginning of this study. However, it was delayed until after data collection. Therefore, this study could not determine if HIAs or skid roads provide better RBC habitat. The HIAs had a lesser slope relative to the surrounding areas, and this might have contributed to the success of RBC. There is limited information on the effect of slope on growth of RBC. RBC stolon length has been shown to be negatively correlated to slope, but no relationship was found between slope and stolon number or number of rooted crowns (Labella et al. 2022). Many of the RBC patches contained flowers, showing that sexual reproduction is taking place, thereby increasing genetic diversity.

ii. Competing Vegetation

Tree canopy cover was positively correlated with RBC patch size (as a trend, Figure 7) and with RBC crown number and cover in the 1-m^2 patch center (Figure 8). However, both the number of RBC flowers per patch and the flower:crown ratio were negatively correlated with nearby tree density, tree canopy cover, and nearby tree size. This suggests that flowering in RBC may decrease when there are more trees nearby. This supports our alternate hypothesis, which stated that competing vegetation would decrease flowering characteristics of RBC. This finding is of interest because increased flowering could lead to increases in seeding, which would promote genetic diversity in this species. As tree canopy cover is easily measured, it can be a helful tool to managers for promoting vegetative or sexual reproduction of RBC.

In this study we found that RBC in the 1 m^2 area at the patch center with higher cover had more rooted crowns and had longer petioles, but not higher cover (Figure 8). Previous research on stoloniferous plants suggests that stolon growth is an adaptation developed to escape herb-layer competition by growing horizontally (Van Kleunen and Fischer 2001). The findings of this study would support this idea, since RBC facing more herb-layer competition appeared to have more rooted crowns but without a proportional increase in leaves (i.e., cover), so that it can move away from its competition. It may also be growing taller petioles to escape its competition vertically.

However, RBC cover, number of crowns, and maximum height were all positively correlated with forb cover. The opposite was true for the shrubs/vines; their presence was negatively correlated with RBC height and abundance. This could be due to the vines and shrubs outcompeting RBC for light and nutrients. However, light seems unlikely as a cause, since mean forb cover surpassed shrub/vine cover (except on site A; Figure 9). Only 45 of the patches had vines or shrubs as the dominant vegetation, compared to the 169 which had a forb species (mostly false wood nettle) as the dominant cover. This suggests that, instead of competition, the habitat conditions preferred by RBC align more closely with those of other forbs than those of the shrubs or vines in this study area. It is therefore possible that another variable not measured in this study could be causing the positive and negative correlations observed with forbs and shrubs/vines, respectively. These findings only partially support our alternative hypothesis, which stated that competing vegetation will decrease RBC abundance.

iii. Transplant Experiment

One objective of this study was to measure the success rate of RBC after transplanting and determine which site preparation treatments can be applied to create the most favorable conditions for RBC. This study found that treating the plots with any combination of raking and mowing before planting improved the chances of RBC surviving for at least one year. It also increased the number of rooted crowns and cover of RBC one year after planting compared to the control with an intact herb-layer and undisturbed ground. This is most likely due to the removal of its primary competition, which in this case are forbs (Figure 9). These findings support our alternative hypothesis for this objective.

Among the treatment types, raking alone appeared somewhat less effective in promoting RBC than both mowing and the combined raking and mowing treatment (Figure 10). This suggests that in the short term, mowing the plots may be more effective at removing the forbs present on the sites than raking. However, it was possible that the sample size was too small to detect a significant effect raking. The effect of treatment on forb cover was tested, but it was not statistically significant. However, the method used for measuring cover data one year post treatment may not accurately reflect forb biomass, since it would not account for forb height or layering. Thus, there may have been reduced forb biomass on treated plots, but it was not detected with measuring cover as leaf area projected to the ground. If forb biomass was impacted by mowing, it would likely be due to forbs growing from an apical meristem (Fynn et al. 2004). This stem is lost when the site is mowed, halting forb growth. While forbs may grow back the following year, the RBC will have had a portion of the growing season with increased sunlight, and therefore a better chance at establishing. It should be noted that the transplanting occurred in early August, which would not have given the forbs enough time in the growing season to fully regrow from their roots. The results of this study may have been different if the transplanting had occurred earlier in the growing season. Thus, future work is needed on the optimal timing for transplanting.

Grasses are less affected by the mowing, having basal meristems that can easily grow back after they are cut (Fynn et al. 2004). In fact, grass cover benefitted from treatments including mowing compared to the control and raking. This is consistent with a previous study that applied non-logging disturbance to existing RBC patches. At the Blue Grass Army Depot in Kentucky, a combination of mowing and grass-specific herbicide was used to suppress competition in and around RBC patches. Researchers found both mowing and herbicide application together were necessary to promote RBC growth. When mowing was done alone, the grasses became much more prevalent and suppressed RBC, and when only the herbicide was applied, the forbs became dominant, outcompeting RBC (Dart-Padover 2015). A similar effect may be taking place on the plots of this current study, i.e., mowing is suppressing forb growth. However, there was relatively low grass cover on the subplots pretreatment (Figure 9a), so grasses would not have had the chance to become dominant one year post-treatment.

Raking may not be an effective method for removing ground cover compared to mowing. The process of raking removes litter and damages some plant tissues. However, it was observed that the apical meristems of the forbs often remained mostly intact, even if they were knocked over. This would enable them to recover more quickly after raking than mowing, and allow them to compete with RBC. Raking was also more effective than mowing in reducing tree seedling cover (Table 8). While tree seedlings found in the subplots were often small, they were unlikely to compete with RBC. However, mowing would maintain tree canopy cover in the long term at the level conducive to RBC at present. It should be noted that it is difficult to draw conclusions regarding the efficacy of raking, given the low sample size of this study.

Canopy tree cover was not a significant predictor of RBC transplanting success, rooted crowns, or cover. This is of note, as it is known that RBC has specific light requirements for it to survive and persist. It is possible that the light levels were too similar throughout the HIAs, and, in order to detect a tree canopy cover effect, additional data is needed within HIA sites lacking RBC and outside of the HIA. It should be noted, however, that previous studies have made this comparison and found that light is a limiting factor for RBC viability (Madarish and Schuler 2002). It has also been suggested that factors other than light have a significant impact on RBC. These factors may include soil properties like ammonium content, or other environmental factors like slope (Labella et al. 2022).

Previous attempts at planting RBC have not been successful. They resulted in the RBC becoming infected with diseases or damaged by flooding (Smith 1998). It appears that site conditions in these cases were not favorable to RBC survival. Based on this present study, favorable conditions for success of transplanted RBC appear to be sites with about 85% tree canopy cover. This is lower than the 40-50% sunlight estimate given by Cusick (1998) but is similar to light conditions found on the Fernow Experimental Forest where RBC thrives (Madarish and Schuler 2002, Labella et al. 2022). In the present day, the herb layer was dominated by forbs such as wood nettle and false wood nettle. These conspicuous forbs may serve as an indicator species for RBC, since studies have shown that many plants in the family Urticaceae (nettles) also require limestone-based soils to grow (Rief et al. 1985).

While about 60% transplants survived in the Control, survival >80% was achieved with disturbing the herbaceous layer prior to transplanting. The number of crowns produced per surviving transplant in disturbed plots was also larger than in the control, supporting improved growing conditions for surviving transplants.

Thus, when establishing plantings of RBC, prior management of forbs and potentially grasses might be needed. If grass cover is high, a different management strategy than for forbs will be needed to suppress these, such as the application of grass-specific herbicide. However, in a forested setting, grasses are often limited by light resources, so they are unlikely to be abundant (Cole and Weltzin 2005).

Conclusions

This project contributed new knowledge about RBC interactions with other herb-layer vegetation and on managing running buffalo clover by habitat creation without logging.

RBC likely competes with other plants of the herb layer for resources. However, sites with higher RBC abundance often contained higher forb cover, while sites with lower RBC growth contained higher cover of shrubs and vines. Thus, forbs that share similar habitat requirements, like wood nettle and false wood nettle, could be used as indicators of optimal RBC microhabitat, though further research will be required to confirm this. Managers that wish to promote flowering in RBC populations will also need to be conscious of nearby tree density and size, as it can significantly impact flowering rates.

Prior to this study, attempts at transplanting RBC had been unsuccessful. This study shows that new populations of RBC can be created through transplanting, and that this will be most successful if the transplant sites are treated by removing smaller trees (creating habitat via suitable canopy tree cover) and then disturbing the herb-layer prior to planting. Specifically, mowing vegetation will increase 1-year post-transplant survival and promote growth after establishment compared to simply planting RBC under existing vegetation. We hope that managers can use this to establish or increase RBC populations in areas where logging may not be possible.

The habitat requirements for RBC can be compared to those of American ginseng (*Panax quinquefolius* L.), as they both require well drained, calcium rich soils and grow well in shaded environments (Anderson et al. 2001). These favorable conditions can be created by limiting disturbance and keeping the tree canopy intact. Previous studies have found that disturbance from forest harvests can decrease existing populations (Chandler and McGraw 2015). Since this species grows slowly, they are less likely to recover quickly after the harvest. For these reasons, management for RBC may not be compatible with those of other species of concern.

This study only quantified RBC abundance at one point in time. Further studies will be needed to measure how both the planted and naturally occurring RBC will fare into the future. Previous studies have shown that RBC will begin to decline after about 7 years if disturbance does not consistently maintain its habitat (Madarish and Schuler 2002). The HIAs in this study have not been disturbed in 5 years, and the RBC population is large (>5000). Many of the patches (67%) found in the HIAs were small, containing 20 or less crowns. According to previous research, patches containing such few rooted crowns had only a 50% chance of persisting for the next ten years. Two of the patches did exceed 75 crowns, and these patches are 95% likely to persist for the next 10 years if habitat requirements continue to be favorable (Dart-Padover 2016). Other recent studies have found that after about 20 years, RBC will begin to decline in the absence of continued management (Thomas-Van Gundy 2022). Of note is, that, even if long-term establishment through transplanting may initially fail, seeds produced during RBC site occupancy could remain viable in the seed bank for 8 years. These seeds may be able to germinate after a disturbance in the area and reestablish RBC (Mills 2009).

The management activities required to establish and maintain RBC could increase the risk of introducing invasive species. While invasive species were rare at the study site at the time of this study, managers should be conscious of this when applying treatments prior to transplanting RBC and repeat disturbance to maintain existing populations.

Literature Cited:

Anderson, R. C., Anderson, M. R., & Houseman, G. (2002). Wild American ginseng. Native plants journal, 3(2), 93-105.

Bartgis, R. (1985). Rediscovery of *Trifolium stoloniferum* Muhl. Ex A. Eaton. Rhodora, 87(851), 425–429.

Becus, M. S., & Klein, J. B. (2002). Mowing schedule improves reproduction and growth of endangered running buffalo clover (Ohio). Ecological Restoration, 20(4), 295–296.

Brooks, R. (1983). *Trifolium stoloniferum*, running buffalo clover: Description, distribution, and current status. Rhodora, 85(843), 343–354.

Burkhart, J. (2010). Ecology of *Trifolium stoloniferum* (Muhl. Ex A. Eaton), a federally endangered vascular plant, at the Fernow Experimental Forest in West Virginia. West Virginia University.

Burkhart, J., Rentch, J. R., & Schuler, T. M. (2013). Effects of forest management on running buffalo clover (*Trifolium stoloniferum* Muhl. Ex A. Eaton) distribution and abundance in the Fernow Experimental Forest. Natural Areas Journal, 33(2), 156–162.

Campbell, J., Evans, M., Medley, M., & Taylor, N. (1988). Buffalo clovers in Kentucky (*Trifolium stoloniferum* and T. reflexum): Historical records, presettlement environment, rediscovery, endangered status, cultivation and chromosome number. Rhodora, 90(864), 399–418.

Chandler, J.L. and McGraw., J.B. (2015). Variable effects of timber harvest on the survival, growth, and reproduction of American ginseng (*Panax quinquefolius* L.), Forest Ecology and Management, Volume 344, Pages 1-9.

Cole, P. G., & Weltzin, J. F. (2005). Light limitation creates patchy distribution of an invasive grass in eastern deciduous forests. Biological invasions, 7, 477-488.

Coulloudon, B., (1999). Sampling vegetation attributes: interagency technical reference. U.S. Department of the Interior

Crawford, D., Esselman, E., Windus, J., & Pabin, C. (1998). Genetic variation in running buffalo clover (*Trifolium stoloniferum*: Fabaceae) using random amplified polymorphic DNA markers (RAPDs). Annals of the Missouri Botanical Garden, 85(1), 81–89.

Cusick, A. (1989). *Trifolium stoloniferum* (Facabeae) in Ohio: History, habitats, decline and rediscovery. SIDA, Contributions to Botany, 13(4), 467–480.

Dart-Padover, A. (2015). A comparison of management strategies for the federally endangered running buffalo clover (*Trifolium stoloniferum*) on the Blue Grass Army Depot, KY. Eastern Kentucky University.

Dart-Padover, A., Koslow, A., & Brown, D. (2016). Population trends of the federally endangered running buffalo clover (*Trifolium stoloniferum*) at the Blue Grass Army Depot, Kentucky. Natural Areas Journal, 36(2), 137–145.

Fynn, R.W.S., Morris, C.D. and Edwards, T.J. (2004), Effect of burning and mowing on grass and forb diversity in a long-term grassland experiment. Applied Vegetation Science, 7: 1-10.

Ford, W., Madarish, D., & Schuler, T. (2003). Influence of white-tailed deer digestion on running buffalo clover (*Trifolium stoloniferum*: Fabaceae Muhl. Ex A. Eaton) germination. The American Midland Naturalist, 149(2), 425–428.

Franklin, C. (1998). Self-compatibility and variation in seed production among Ohio populations of federally endangered *Trifolium stoloniferum* (Fabaceae). Ohio State University.

Godt, M., Johnson, B., & Hamrick, J. (1996). Genetic diversity and population size in four rare southern appalachian plant species. Conservation Biology, 10, 796–805.

Hattenbach, M. (1996). Edaphic relations of an endangered plant, *Trifolium stoloniferum* Muhl. Ex A. Eaton. Ohio State University.

Hickey, R. J., Vincent, M. A., & Guttman, S. I. (1991). Genetic variation in running buffalo clover (*Trifolium stoloniferum*, Fabaceae). Conservation Biology, 5, 309–316.

Hickey, E.E. (1994). Establishment of experimental populations of running buffalo clover Trifolium stoloniferum on the Mark Twain National Forest in Missouri. A report to Region 3, U.S. Fish and Wildlife Service.

Jacobs, J. F., & Bartgis, R. L. (1987). The running buffalo clover. In Audubon Wildlife Report 1987 (pp. 438-445). Academic Press.

LaBella, J., LaFantasie, J., Thomas-Van Gundy, M., & McDonald, L. M. (2022). Effect of light interception on photosynthetic capacity and vegetative reproduction of running buffalo clover. Research Note NRS-310. Madison, WI: US Department of Agriculture, Forest Service, Northern Research Station.

Madarish, D., & Schuler, T. (2002). Effects of forest management practices on the federally endangered running buffalo clover (*Trifolium stoloniferum* Muhl Ex A Eaton). Natural Areas Journal, 22(2), 120–128.

Marriott, C. A., Bolton, G. R., & Duff, E. I. (1997). Factors affecting the stolon growth of white clover in ryegrass/clover patches. Grass and Forage Science, 52(2), 147-155.

Mills, L. (2009). Gone But Not Forgotten? US Forest Service, Retrieved from https://www.fs.usda.gov/wildflowers/success/storydac5.shtml

Morris, D., Schuler, T., Harmon, P., & Baligar, V. (2002). Biological nitrogen fixation and habitat of running buffalo clover. Journal of Plant Nutrition, 25(4), 735–746.

Maxwell, H. (1898). The history of Randolph County, West Virginia, from its earliest settlement to the present. The Acme Publishing Company.

Pickering, J. (1989). Conservation efforts boost hopes for rare clover. The Center for Plant Conservation, 4(2), 3.

Quesenberry, K.H., N.M. Call, D.E. Moon, and R.A. Dunn. (1997). Response of native eastern North American Trifolium spp. to root-knot nematodes. Crop Science 37:270-274.

Reif, A., Teckelmann M., Schulze E. (1985) The Habitat Distribution of Urtica dioica L. — a Reevaluation of Phytosociological Site Descriptions Based on Ellenberg's Indicator Values. *Flora*, 176(5-6), 365-382.

Sehgal, O. P., & Payne, L. (1995). Viruses affecting running buffalo clover, Trifolium stoloniferum. Plant Disease, 79(3), 320.

Singha, S., Baker, B., & Bhatia, S. (1988). Tissue culture propagation of running buffalo clover (*Trifolium stoloniferum* Muhl. Ex A. Eaton). Plant Cell, Tissue and Organ Culture, 15, 79–84.

Smith, T. (1998) The reintroduction of running buffalo clover (*Trifolium stoloniferum*) in Missouri. Missouriensis, 19, 12-21

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Official Soil Series Descriptions. Available online. Accessed 04/25/2023.

Taylor, N. L., J. M. Gillett, J. J. N. Campbell, and S. Berger. 1994. Crossing and morphological relationships among native clovers of eastern North America. Crop Science 34(4): 1097-1100

Thomas-Van Gundy, M. (2022) Twenty-year trends in running buffalo clover (*Trifolium stoloniferum* Muhl. Ex A. Eaton; Fabaceae) on a managed forest in northeastern West Virginia. Natural Areas Journal 42, 1: 79-88

U.S. Fish and Wildlife Service. (2007). Running buffalo clover (*Trifolium stoloniferum*) recovery plan: First revision. U.S. Fish and Wildlife Service.

U.S. Fish and Wildlife Service. (2015). Threatened, endangered and sensitive plants element occurrence protocol and field guide.

U.S. Fish and Wildlife Service. (2019). Endangered and threatened wildlife and plants; removing *Trifolium stoloniferum* (running buffalo clover) from the federal list of endangered and threatened plants.

U.S. Fish and Wildlife Service. (2021). Endangered and threatened wildlife and plants; removing *Trifolium stoloniferum* (running buffalo clover) from the federal list of endangered and threatened plants.

U.S. Forest Service. (2011). Monongahela National Forest Land and Resource Management Plan

U.S. Forest Service. (2016). Tygart-Chestnut Ridge Wildlife and Watershed Project: Final Environmental Assessment

U.S. Forest Service. (2016). Upper Tygart – Chestnut Ridge Wildlife and Watershed Project: Environmental Assessment

Van Kleunen, M., & Fischer, M. (2001). Adaptive evolution of plastic foraging responses in a clonal plant. Ecology, 82(12), 3309-3319.

Walter, C. A., Burnham, M. B., & Gilliam, F. S. (2015). A reference-based approach for estimating leaf area and cover in the forest herbaceous layer. Environmental Monitoring Assessment, 187, 657.

Appendices

Appendix I – RBC data

Table A1 – Number of RBC crowns removed from their original patch for use in transplanting experiment. CrownsPre refers to the RBC rooted crowns present on the site before being taken. These patches were omitted from analyses involving RBC measurements after crowns had been removed for transplanting. Other patches omitted were 116, which no longer had crowns near plot center; 187, which had been destroyed by logging equipment; and 205, which had a tree fall on it.

Patch ID	Site	CrownsPre	CrownsTaken
120	C	56	54
122	C	96	36
127	C	68	24
184	D	83	9
191	D	133	66
192	D	119	112
193	D	156	20
224	D	36	13

Table A2 – Multiple comparisons between different treatment types on their effects on the number RBC Crowns one year after transplanting. Results are based on Model 1 (Table 6) comprising treatment as the only predictor variable. Statistically significant comparisons (P≤0.05) are after Tukey adjustment are underlined.

Differences of TRT Least Squares Means
Adjustment for Multiple Comparisons: Tukey

TRT	TRT	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P
M	**RM**	0.07422	0.4217	56	0.18	0.8609	0.9980
M	**R**	0.4055	0.4217	56	0.96	0.3404	0.7717
M	**C**	1.3926	0.4217	56	3.30	0.0017	<u>0.0088</u>
RM	**R**	0.3313	0.4217	56	0.79	0.4355	0.8607
RM	**C**	1.3184	0.4217	56	3.13	0.0028	<u>0.0145</u>
R	**C**	0.9871	0.4217	56	2.34	0.0228	0.1011

Table A3 – Multiple comparisons between different treatment types on their effects on the amount of RBC Cover one year after transplanting. Results are based on Model 1 (Table 6) comprising treatment as the only predictor variable. Statistically significant comparisons (P≤0.05) are after Tukey adjustment are underlined.

Differences of TRT Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

TRT	TRT	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P
C	**M**	-1.4267	0.4961	55	-2.88	0.0057	<u>0.0284</u>
C	**R**	-0.8133	0.4961	55	-1.64	0.1068	0.3655
C	**RM**	-1.3800	0.4961	55	-2.78	0.0074	<u>0.0361</u>
M	**R**	0.6133	0.4961	55	1.24	0.2216	0.6068
M	**RM**	0.04667	0.4961	55	0.09	0.9254	0.9997
R	**RM**	-0.5667	0.4961	55	-1.14	0.2583	0.6652

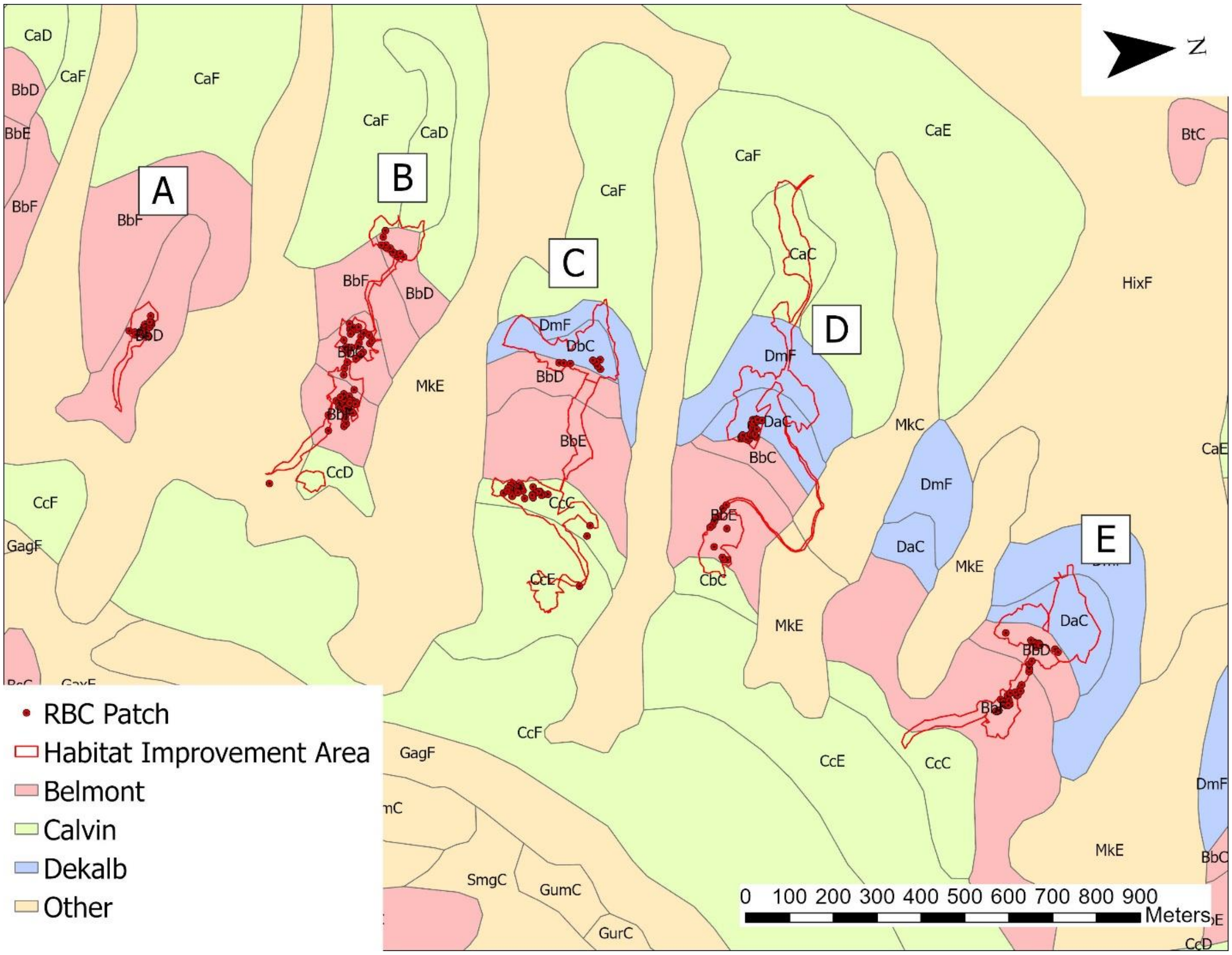


Figure A1 – RBC patch locations in relation to soil types.

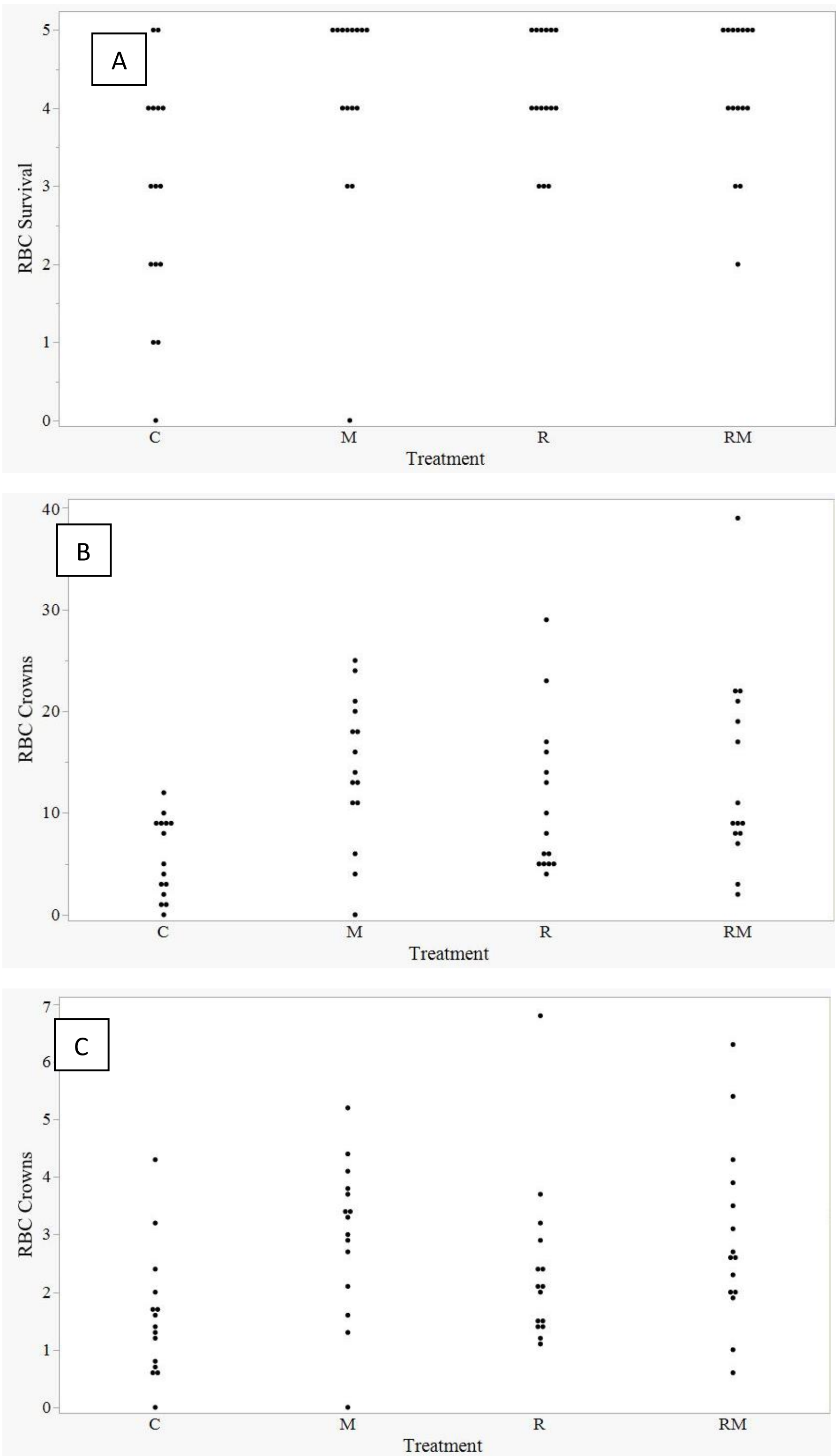
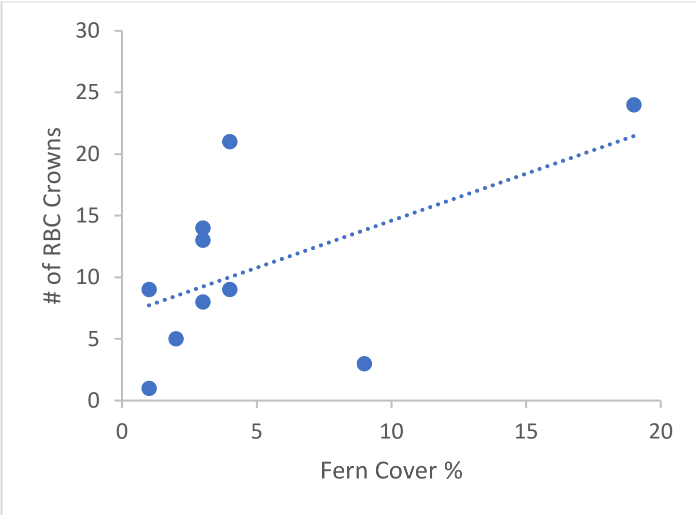


Figure A2 – The number of surviving RBC (A) number of crowns (B) and % cover (C) for each treatment type one year after planting five crowns. C – control, M – vegetation was mowed to ground level, R – litter was raked from subplot with fire rake, MR – mowed and raked.



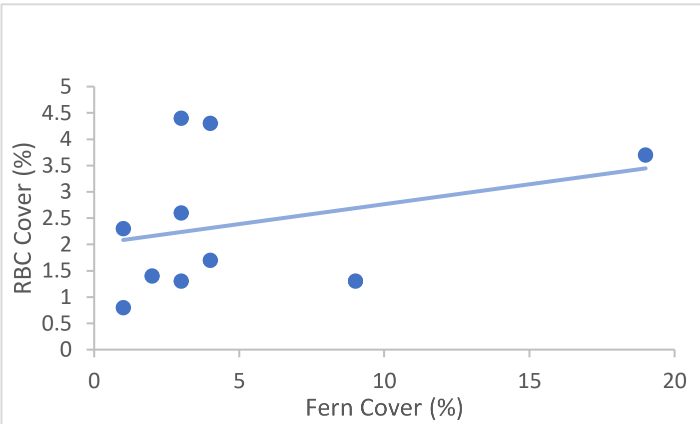


Figure A3 – RBC crowns and cover against fern cover on transplant plots that contained fern cover

Table A4 – Spearman's correlation coefficients for data collected during 2022 RBC inventory. Data includes RBC crowns, maximum height of RBC in each patch, tree canopy cover, and % cover of RBC, forbs, shrubs/vines, grasses, ferns, and tree seedlings within a 1 m^2 quadrat.

	RBC Cover	RBC Crowns	RBC height	Canopy	Total Herb	Fern	Vine	Forb	Grass	Tree
RBC Cover										
RBC Crowns	≤0.01									
RBC height	≤0.01	≤0.01								
Canopy	0.04	≤0.01	0.66							
Total_Herb	0.11	0.01	0.01	0.17						
Fern	0.51	0.41	0.96	0.02	0.64					
Vine	≤0.01	0.00	0.01	0.04	0.69	0.01				
Forb	≤0.01	≤0.01	≤0.01	0.36	≤0.01	≤0.01	≤0.01			
Grass	0.25	0.38	0.01	0.67	0.65	0.64	0.46	≤0.01		
Tree	≤0.01	≤0.01	0.53	0.01	0.86	≤0.01	≤0.01	≤0.01	0.82	
Herb_ht	0.21	0.99	0.02	0.20	≤0.01	0.04	0.22	≤0.01	0.03	0.99

Table A5 – Spearman's correlation coefficients for data collected during 2021 RBC inventory. Data collected includes RBC patch size (based on rooted crown #), RBC flower #, and the ratio between flower number and crown number. Data on nearby trees were also collected, including the % of open canopy above each patch. Trees per hectare, and stem area were calculated using size and distance to nearby trees.

	Patch Size	RBC flowers	RBC Flower Ratio	Canopy Cover	Trees/ha
Patch Size					
RBC flowers	≤0.001				
RBC Flower Ratio	0.009	≤0.001			
Canopy Cover	0.066	≤0.001	≤0.001		
Trees/ha	0.144	≤0.001	≤0.001	≤0.001	
Stem area	0.87	0.004	0.007	0.007	≤0.001