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Effect of Light Interception on Photosynthetic Capacity and Vegetative Reproduction of Running Buffalo Clover

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Citation

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Abstract

Running buffalo clover (*Trifolium stoloniferum*; RBC), is a recently delisted stoloniferous species that occurs in several eastern states of the United States. Once thought to be extinct, RBC was rediscovered in West Virginia in 1985 and it is generally accepted that it requires moderate disturbance and filtered sunlight to grow. We studied how light availability and photosynthetic parameters are related to stolon production and overall plant growth. We sampled six occurrences in the USDA Forest Service Fernow Experimental Forest (Parsons, WV) in September 2018. For each occurrence, we measured light, plant response, and environmental variables. There were no significant correlations among plant growth variables and light variables suggesting that light may not be the only driver in asexual reproduction of RBC. However, there were some correlations between light variables and environmental variables as well as environmental variables and plant growth variables which lead us to conclude that for management of the species, more than light variables may need to be taken into consideration. This information could be potentially useful in the conservation of the species.

KEY WORDS light response, *Trifolium,* stolons, asexual reproduction

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Cover Photo

Running buffalo clover (*Trifolium stoloniferum*). USDA photo by Melissa Thomas-Van Gundy.

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Introduction

Running buffalo clover (*Trifolium stoloniferum* Muhl. Ex Eaton) is a recently delisted stoloniferous species of clover that occurs in several eastern states (Madarish and Schuler 2002). Once thought to be extinct, running buffalo clover (RBC) was rediscovered in West Virginia in 1985 with additional rediscovery in Kentucky, Ohio, Indiana, and Missouri in the late 1980s. When this study began, RBC was considered federally endangered by the U.S. Fish and Wildlife Service (US FWS) (Madarish and Schuler 2002, US FWS 2007; see also FWS.gov); a proposal to delist the species was made in August of 2019 and formal delisting occurred in 2021.

Research has shown that RBC relies on small-scale disturbance over long periods of time for optimal survival (Madarish and Schuler 2002), and lack of disturbance has been a likely cause for near extinction (Watt 2011). Historically, RBC has been thought to thrive along streams and Native American trails due to the disturbance from walking and trampling by both humans and animals (Madarish and Schuler 2002). All known occurrences of RBC have been linked to moderate disturbance and the species distribution has been linked to gently sloping trails created by herbivores (Cusick 1989), although there is evidence of clovers growing on steeply sloped areas (Watt 2011). RBC occurs at the Fernow Experimental Forest (Fernow) in West Virginia along skid roads (narrow, unsurfaced, temporary roads used for logging) probably due to the disturbance that logging provides (Madarish and Schuler 2002, Thomas-Van Gundy 2022). To prevent extinction, existing occurrences are being maintained via manual disturbance, managing invasive species, and reducing habitat succession (US FWS 2007).

Disturbance has been connected to encouraging vegetative (asexual) reproduction and maintaining diversity (Ledo and Schnitzer 2014). For clovers, vegetative reproduction occurs when the stolons growing from the main crown produce plantlets that embed in the earth thus creating a new, genetically identical, central plant (Fig.1). This is a beneficial mode of reproduction because not only does it require less energy than seed production, but the offspring have a stronger vitality and higher survival rate (Fu et al. 2010). A study by Lopp and Sammul (2016) suggests that vegetative reproduction could potentially be influenced by habitat light availability for the clonal ramets. It is generally accepted that RBC thrives in shaded mesic habitats with filtered sunlight and does not grow well under direct light conditions for prolonged periods of time (Burkhart et al. 2013, Watt 2011). This is further supported by an observational study conducted by Madarish and Schuler (2002) that found that RBC sites had greater open sky and more canopy gaps than sites that did not support RBC. It has also been noted that there are no extant populations of RBC that grow in full sun or dense shade (Cusick 1989). Except for a few studies, there is a general paucity of research on the light requirements, photosynthetic capacity, and the drivers of stolon production of running buffalo clover (Watt 2011).

Our objective was to conduct a pilot study on the potential relationship between RBC asexual reproduction, as measured by stolon length and number, and leaf-level light response, light availability, and edaphic and topographical variables to provide a foundation for future research and conservation efforts.

Figure 1.—Running buffalo clover (RBC; *Trifolium stoloniferum*) image from the Fernow Experimental Forest, WV. USDA photo by Melissa Thomas-Van Gundy.

Materials and Methods

Site Description

Located near Parsons, WV, in Tucker County, the Fernow Experimental Forest (39.03°N, 76.67°W) is within the Monongahela National Forest and is administered by the USDA Forest Service's Northern Research Station.[1] The Fernow is known for long-term research on a variety of silvicultural and watershed topics including sustainable management of mixed mesophytic and oak-dominated forests. Common overstory tree species include yellow-poplar (*Liriodendron tulipifera*), sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), northern red oak (*Quercus rubra*), white oak (*Q. alba*), black cherry (*Prunus serotina*), and white ash (*Fraxinus americana*). The Fernow is approximately 1,860 ha and averages 450 m in elevation with steep slopes and shallow basins (Kochenderfer 2006).

This study took place in compartment 20A (Fig. 2) which has a northwest aspect and limestone rich soil (Burkhart et al. 2013). The known RBC occurrences are located along skid roads in natural sub-populations of 5 to 699 individuals.[2] These locations were established through annual or biannual monitoring surveys of the logging roads of the Fernow by Northern Research Station staff. Further description of the compartment can be found in Madarish and Schuler (2002).

[1] The Northern Research Station of the USDA Forest Service covers the area in the northeastern United States, from Minnesota to Maine, and from Missouri to West Virginia and Maryland. https://www.nrs.fs.fed.us/.

[2] Unpublished monitoring reports on file at the USDA Forest Service Northern Research Station office in Parsons, WV.

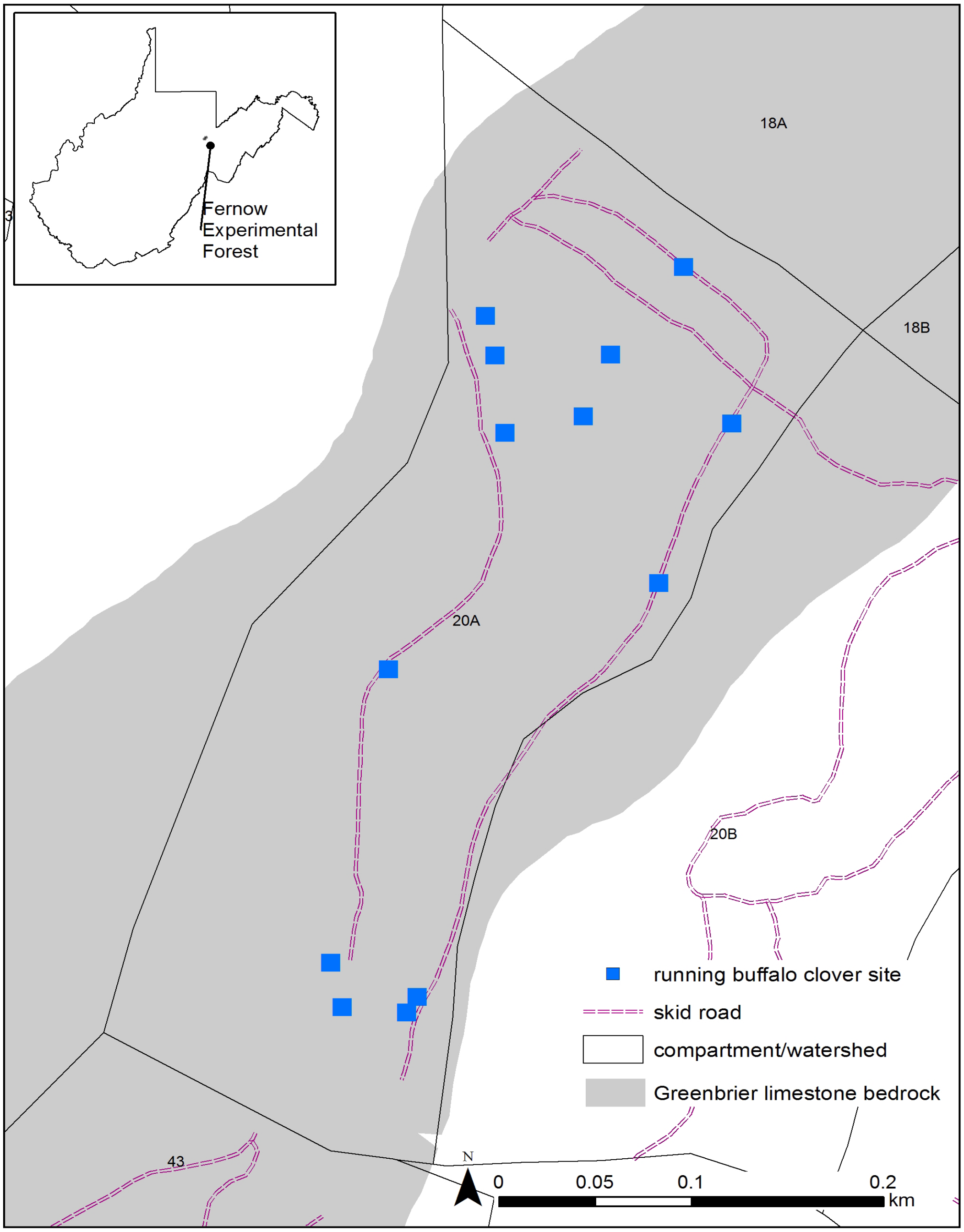

Figure 2.—Location of study area, running buffalo clover (RBC; *Trifolium stoloniferum*) monitoring sites in Compartment 20A, skid roads, and limestone bedrock on the Fernow Experimental Forest, Parsons, WV. The running buffalo clover sites depicted here are the long-term monitoring locations from which the specific study populations or sub-populations were chosen. There are 13 sites depicted and some were divided into sub-populations to yield 19 occurrences for study.

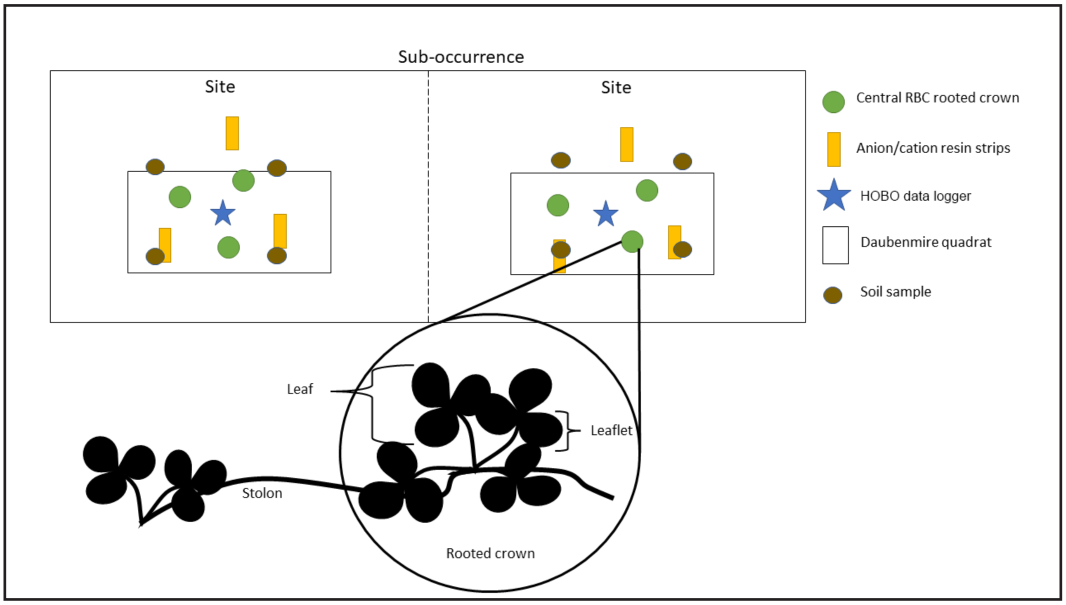

Figure 3.—Schematic illustration of running buffalo clover (RBC; *Trifolium stoloniferum*) morphology definitions and sub-occurrence and site sampling layout.

Design

We gathered preliminary measurements on all known RBC occurrences in compartment 20A over two 10-day periods during June 2018. We used results from this initial investigation to refine the study to focus on six sub-occurrences for closer analysis. The sub-occurrences were chosen based on key characteristics that distinguished them from the rest of the 19 original occurrences to ensure study of the range of environmental variables (e.g., high/low light, moist/dry soil, herbaceous competition). Because some sub-occurrences were spread out over a distance of several meters, we divided, roughly in half, each sub-occurrence into two sites to account for environmental variability (Fig. 3). The three rooted crowns nearest to the site's center were used for all sampling and yielded 12 sites (each of six sub-occurences divided into half) and 36 individuals for observation.

Photosynthesis and Morphology

Of the three rooted crowns being sampled, the one nearest the center of the site was used to obtain a light response curve using a LI-COR® Li-6800 portable photosynthesis meter (LI-COR BioSciences, Lincoln, NE) on the first fully expanded, eastern leaflet (parameters: air relative humidity: 75; soda lime: scrub auto; CO_2 sample: 400 μmol mol^{-1}; fan speed: 10,000 rpm; head light source: 1,500 μmol m^{-2} s^{-1}; temperature: 27.5 °C; flow setpoint: 500 μmol s^{-1}; valve: 0.1 KPa; gas exchange area: 3.00 cm^{2}) with 12 points taken from zero μmol m^{-2} s^{-1} to 1500 μmol m^{-2} s^{-1}. Photosynthetic measurements were taken September 20, 2018. We extracted light response curves from LI-COR light response data points according to Heberling and Fridley (2013) and Wavrek et al. (2017) using the nonlinear least squares (NLS) function in R (R Core Team 2019) to obtain compensation points, saturation points, and visual representations of the light response curves.

Placed next to the centermost crown, a HOBO Pendant® MX data logger (Onset, Bourne, MA) recorded illumination (lux) every minute from July 12 to September 4 (~8 weeks) and, at 15-minute intervals, calculations of mean, standard deviation (std. dev.), and maximum and minimum lux were recorded. To relate photosynthetically active light (PAR) to total incident light as lux, we calculated a linear regression curve based on the relationship between lux (as measured by the HOBO) and PAR using a LI-190R quantum sensor (LI-COR BioSciences, Lincoln, NE) according to Wagner and McGraw (2013) so PAR could be recorded as photosynthetic photon flux density (PPFD). We retrieved data recorded by the HOBO sensors and we calculated maxima and averages across the 8-week period using Microsoft® Excel.

We counted current-year stolons and measured stolon length to the nearest 0.1 cm for each of the three crowns at each site. All measurements used in data analysis are averages from the three central crowns of each site. From the crown used for photosynthetic data, we measured plant height (cm) from the ground to the top of the tallest shoot as well as the east leaflet width (cm).

Biotic and Edaphic Environment Sampling

Because RBC is light sensitive, we quantified ground cover (litter, bare-ground, rock) and local competition for light using the Daubenmire method and a 0.5 m^2 quadrat (Daubenmire 1959) at the center of each occurrence (Fig. 3). We categorized herbaceous cover as short (<1.5 m) or tall (1.5 to 3 m) and recorded tree sapling cover separately. We estimated overstory canopy closure using a spherical densiometer (Lemmon 1956) with the observer standing at the center of each site and facing the four cardinal directions. For both the skid road and true site terrain, we determined slope (degrees) and aspect (degrees) using the Dioptra™ app (Workshop 512, http://workshop512.com/) for Android. We calculated degrees distant from north to include aspect in the analysis such that the western half of the compass was negative, and the eastern half was positive, i.e., west = -90° and south = 180°.

Using a core sampler (2 cm), we sampled soil from four locations around the center (Fig. 3) of the site to a depth of 5 cm, or to the depth of a restricting rock layer. Soil samples were analyzed for pH following method S-2.2 and organic matter using method S-9.2 according to Gavlak et al. 2005. To determine whether soil nutrient availability differed among sites, we deployed 5 x 2.5 cm anion and cation resin strips (Membranes International, Inc., Ringwood, NJ) (Johnson et al. 2005, Skogley and Dobermann 1996) at the soil surface (to penetrate to 5 cm) in three locations within ~30 cm of the center of each site. The radius was approximate, as we avoided RBC roots and stolons during placement. Two cation and two anion strips were inserted at each of the three locations and left in place for 11 days at which time they were removed, rinsed, and extracted in bulk by site using two different extractions (KCl and NH_4Cl). We froze the resin extracts until N, Ca, and Mg could be determined colorimetrically and using ICP (Clesceri et al. 1995).

Data Analysis

We determined bivariate correlations among environmental (litter depth, average and maximum light, short and tall herbaceous cover, bare ground, rock cover, canopy cover, soil nutrients, organic matter, and pH) and RBC photosynthetic and morphological variables (light compensation point, saturation point, plant height, leaflet width, stolon length, stolon number, and shoot height) using Spearman's correlation coefficient (r_s) in R (R Core Team 2019). To further explore and visualize relationships among environmental and RBC response variables, we used nonmetric multidimensional scaling (NMDS). Using the "vegdist" function in the vegan package (Oksanen et al. 2007) of R (R Core Team 2019), we calculated dissimilarity (euclidean) among sites based on z-score standardized parameters and performed the NMDS ordination using the vegan package function "metaMDS" (Oksanen et al. 2007).

We included in the model the following variables: litter depth; aspect; slope; soil pH; percentage organic matter; extract nutrient concentrations for Ca, P, Mg, nitrate, and ammonium; and vegetative competition characteristics (cover by overhead canopy, saplings, tall and short herbaceous understory). We excluded two variables: 1) Count of light measurements exceeding the light saturation point because only two sites had non-zero values; and 2) Count of light measurements exceeding compensation point because they are strongly correlated with maximum and average light (r_s = 0.94, 0.82, respectively) and was redundant. "Stress" (0-1) and stressplots were used to verify goodness of fit. Finally, we fitted z-score standardized RBC characteristic vectors to the NMDS ordination using function "envfit" and plotted only vectors with a significant relationship with the ordination space (alpha =0.1) based on 999 permutations. To aid in interpretation of relationships, we overlaid vectors for the same environmental variables used to create the ordination but included only those with the strongest relationships ($r^2 \geq 0.5$, $p < 0.05$) with the ordination space to maintain legibility. Because this pilot study had a small sample size, we did not perform statistical hypotheses tests.

Results

For the three central rooted crowns measured, stolon count ranged from zero to seven stolons (mean=2, stolons, std. dev.=2.1 stolons) and total stolon length ranged from 0 to 16.6 cm (mean=7.2 cm, std.dev.= 6 cm) (Table 1). Shoot number, photosynthetic saturation point (PPFD), and quantum yield (AQY) were highly variable (Table 1). Canopy cover ranged from 62 percent to 88 percent (mean=78%, std.dev.=6.9%) and sapling cover was low for most sites (mean=2.5%, std. dev.= 4.1%; Table 2). Tall herbaceous cover varied little other than two sites with high cover (mean=21%, std. dev.= 36.3%), whereas short herbaceous cover ranged from 0 to 63 percent (mean=32%, std. dev.= 20.6%; Table 2). Maximum light intensities varied from 654 to 8185 μmol m-2 s-1 (mean=4316 μmol $m_2 s_1$, std. dev.= 2760.9 μmol m-2 s-1) and average light ranged between 42 and 249 μmol m-2 s-1 (mean=107 μmol $m_2 s_1$, std. dev.= 72.6 μmol m-2 s-1; Table 2). Aspect ranged from SE at two sites to NE at four sites; most sites had a NW aspect. With regard to topographical and soil variables (Table 3), litter depth and Mg concentrations varied little. Mean slope was 6.6 percent (std. dev.=3.6%) and varied little; however, there were two very shallow slope outliers. Soil pH ranged between 5.2 and 6.1 and organic matter ranged from 8 to 17.2 percent.

RBC stolon length and number had a moderately strong positive correlation with one another and with tall herbaceous cover (Fig. 4, Table 4). Stolon length was positively correlated with soil NH_4 concentration (r_s = 0.69) and negatively correlated with slope (r_s = -0.59; Fig. 4, Table 4). Neither stolon length nor stolon number correlated with any RBC photosynthetic capacity variables, overhead canopy cover, or any of the environmental light variables we measured (Table 4). RBC photosynthetic capacity variables were moderately to strongly correlated with the environmental variables aspect, soil pH, soil organic matter, maximum and average light measured, and number of measurements greater than light compensation point (Table 4). In particular, light saturation point, maximum carbon assimilation (Amax), and quantum efficiency (AQY) are all negatively correlated with aspect and soil pH (p >0.05 for pH and light saturation point), whereas light compensation point positively correlated with these variables (Fig. 5, Table 4). Amax is also positively correlated with average (r_s = 0.71) and maximum light (r_s = 0.72) measured and the number of light measurements exceeding compensation point (r_s = 0.68; Table 4).

Table 1.—Summary statistics for running buffalo clover (RBC; *Trifolium stoloniferum*) morphological and photosynthetic characteristics at sites in sub-occurrences in the Fernow Experimental Forest, Parsons, WV

	Height (cm)	Shoots (number)	Stolons (number)	Stolon length (cm)	Leaflet width (cm)	Amax (μmol m^{-2} s^{-1})	Saturation point (lux)	Compensation point (lux)	Quantum efficiency (AQY)
Min	0.7	2.7	0	0	2.1	0.7	69	3.7	0.03
Median	12.7	5	2	9.1	2.6	3.2	173.9	13	0.042
Mean	11.8	4.5	2	7.2	2.6	3.4	247.1	19.6	0.1
SD	5.9	0.9	2.1	6	0.3	2	195.1	14.2	0.1
Max	20.2	5.3	7	16.6	3.1	7.4	689.4	45.8	0.263

Table 2.—Summary statistics for light availability characteristics of running buffalo clover (RBC; *Trifolium stoloniferum*) sites in sub-occurrences in the Fernow Experimental Forest, Parsons, WV

	Max light (μmol m^{-2} s^{-1})	Avg light (μmol m^{-2} s^{-1})	Light measurement periods > saturation point (no.)	Light measurement periods > compensation point (no.)	Canopy cover (%)	Tall herbaceous cover (%)	Low herbaceous cover (%)	Sapling cover (%)
Min	655.0	41.8	0	0	61.8	0	0	0
Median	4884.1	78.7	0	4.5	80.1	2.5	37.5	2.5
Mean	4316.4	107.0	0.7	13.2	78.1	20.8	31.7	2.5
SD	2760.9	72.6	1.8	19.6	6.9	36.3	20.6	4.1
Max	8185.2	249.1	6	56	88.2	97.5	62.5	15

Table 3.—Summary statistics for edaphic characteristics of running buffalo clover (RBC; *Trifolium stoloniferum*) sites in sub-occurrences in the Fernow Experimental Forest, Parsons, WV

	Litter depth (cm)	pH	Ca (ppm)	P (ppm)	Mg (ppm)	NO_3 (ppm)	OM (%)	NH_4 (ppm)	Slope (°)
Min	0.1	5.2	14.58	0.00	2.57	0.7	8.0	0	0
Median	0.7	5.6	37.46	0.07	4.64	1.4	12.8	0.6	7.5
Mean	1	5.6	41.00	0.10	5.70	1.4	12.6	0.7	6.6
SD	1	0.3	23.30	0.00	3.40	0.6	3.8	0.5	3.6
Max	3.5	6.1	82.24	0.11	12.36	2.5	17.2	1.3	11

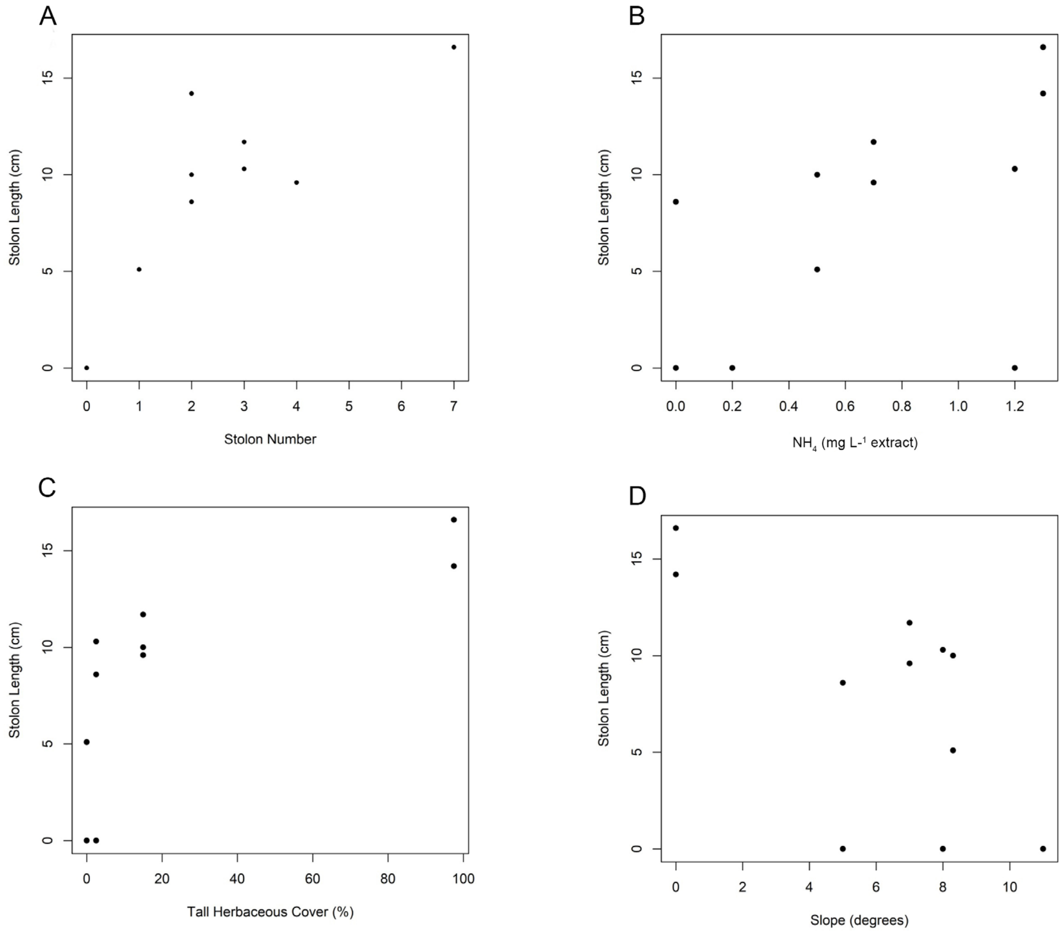

Figure 4.—Running buffalo clover (RBC; *Trifolium stoloniferum*) total stolon length correlates with A) total stolon number (r_s=0.87, p <0.001); B) available soil NH_4 (r_s=0.69, p <0.05); C) 1.5 to 3 m tall herbaceous cover (r_s=0.83, p <0.001); and D) skid road slope (r_s= -0.59, p <0.05). N=12.

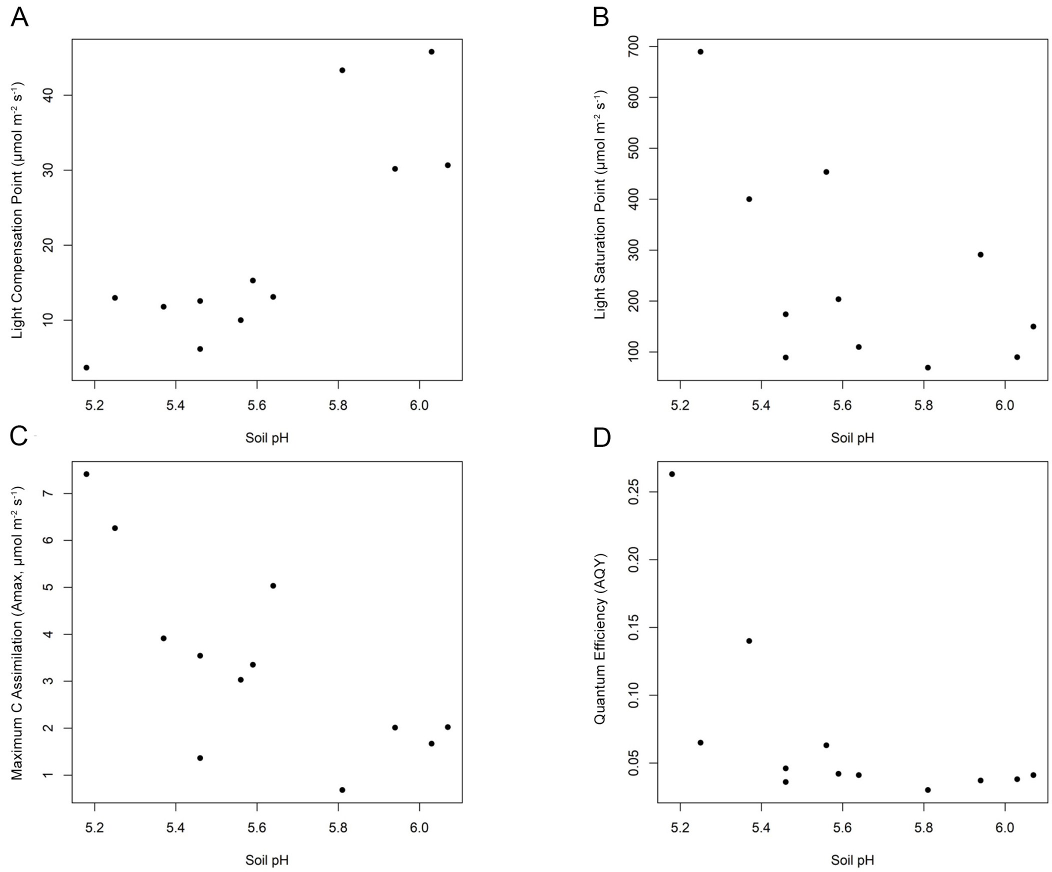

Figure 5.—Relationships among soil pH and running buffalo clover (RBC; *Trifolium stoloniferum*) A) light saturation point (r_s= -0.49, p >0.05); B) light compensation point (r_s=0.84, p <0.001); C) maximum carbon assimilation Amax (r_s= -0.66, p <0.05); and D) quantum efficiency AQY (r_s= -0.71, p <0.01). N = 12.

Table 4. —Spearman's correlation coefficients (r_s) among running buffalo clover (RBC; *Trifolium stoloniferum*) plant morphological and photosynthetic variables and environmental variables at sites in sub-occurrences of in the Fernow Experimental Forest, Parsons, WV

	Ht	N_shoot	N_Stol	StPt	CpPt	AQY	L_stol	Amax	W_lflt	Litr	Ca	Aspect	pH	P
Ht														
N_shoot	-0.04													
N_Stol	0.10	-0.55												
StPt	-0.64*	0.18	-0.24											
CpPt	0.45	0.20	-0.08	-0.49										
AQY	-0.61*	-0.08	-0.34	0.76**	-0.72**									
L_stol	0.45	-0.51	0.87***	-0.38	0.08	-0.45								
Amax	-0.40	-0.12	-0.33	0.62*	-0.63*	0.87***	-0.31							
W_lflt	0.54	-0.18	0.36	-0.49	0.54	-0.56	0.50	-0.23						
Litr	-0.15	0.41	-0.08	0.06	0.03	-0.16	-0.20	-0.48	-0.57					
CA	-0.11	0.39	-0.33	-0.03	0.14	-0.17	-0.47	-0.04	0.23	-0.27				
Aspct	0.16	0.14	0.09	-0.70*	0.61*	-0.78**	-0.02	-0.82**	0.23	0.34	0.34			
pH	0.33	0.22	0.11	-0.49	0.84***	-0.71**	0.20	-0.66*	0.62*	0.11	0.13	0.62*		
P	0.18	-0.53	0.13	-0.16	-0.03	0.15	0.35	0.41	0.23	-0.69*	-0.26	-0.34	-0.30	
Mg	-0.18	0.41	-0.52	0.00	0.20	-0.05	-0.58	0.07	0.06	-0.30	0.89***	0.28	0.01	0.03
NO_3	0.03	-0.31	0.53	0.05	-0.47	0.01	0.35	-0.09	-0.04	0.25	-0.26	-0.11	-0.15	-0.44
OM	-0.35	-0.38	0.09	0.10	-0.71*	0.36	-0.09	0.24	-0.64*	0.08	-0.09	-0.12	-0.79**	0.14
NH_4	0.24	-0.64*	0.56	-0.06	-0.25	0.13	0.69*	0.16	0.06	-0.08	-0.89***	-0.43	-0.20	0.43
Slope	-0.47	0.22	-0.52	0.20	-0.20	0.33	-0.59*	0.14	-0.78*	0.30	0.09	0.06	-0.47	0.09
Max_L	-0.08	-0.18	-0.24	0.08	-0.22	0.45	-0.14	0.72**	0.13	-0.75**	0.11	-0.41	-0.39	0.69*
Ave_L	-0.08	-0.01	-0.46	-0.01	-0.27	0.46	-0.33	0.71*	-0.01	-0.53	0.11	-0.31	-0.35	0.49
Num_StPt	0.23	-0.32	-0.06	-0.38	0.36	-0.22	0.23	0.04	0.36	-0.23	-0.27	0.09	0.37	0.41
Num_CpPt	-0.23	-0.20	-0.22	0.09	-0.16	0.41	-0.18	0.68*	0.18	-0.81**	0.27	-0.32	-0.30	0.69*
Cpy	0.26	0.09	-0.13	-0.28	-0.05	-0.27	-0.02	0.05	0.37	-0.29	0.55	0.14	0.12	-0.13
T_Herb	0.58*	-0.34	0.72**	-0.44	0.06	-0.40	0.83***	-0.21	0.60*	-0.23	-0.37	-0.01	0.19	0.23
L_Herb	-0.02	0.68*	-0.40	-0.03	0.24	-0.26	-0.49	-0.48	-0.18	0.59*	0.46	0.46	0.38	-0.82**
Sapling	-0.32	-0.08	-0.28	-0.12	0.13	-0.11	-0.48	-0.12	0	-0.11	0.71*	0.48	0.10	-0.19

	Mg	NO_3	OM	NH_4	Slope	Max_L	Ave_L	Num_ StPt	Num_ CpPt	Cpy	T_Herb	L_Herb	Sapling
Mg													
NO_3	-0.65												
OM	-0.03	0.29											
NH_4	-0.89***	0.44	0.14										
Slope	0.43	-0.50	0.54	-0.37									
Max_L	0.28	-0.34	0.18	0.08	0.11								
Ave_L	0.27	-0.30	0.20	0.00	0.20	0.90***							
Num_StPt	-0.15	-0.25	-0.27	0.29	-0.17	0.17	0.30						
Num_CpPt	0.46	-0.47	0.14	-0.08	0.17	0.94***	0.82**	0.22					
Cpy	0.32	0.18	-0.02	-0.30	-0.31	0.11	0.28	0.30	0.15				
T_Herb	-0.57	0.47	-0.16	0.64*	-0.75**	0.06	-0.07	0.09	-0.10	0.08			
L_Herb	0.33	-0.03	-0.27	-0.70*	0.15	-0.66*	-0.44	-0.31	-0.58*	0.17	-0.47		
Sapling	0.68*	-0.20	0.19	-0.68*	0.30	-0.05	0.02	0.09	0.18	0.41	-0.59*	0.35	

*, ** and *** indicate p <0.05, p <0.01 and p <0.001, respectively

Due to the likelihood of interactions among all the variables we measured, a multivariate approach to visualize relationships among RBC and environmental variables was necessary. We modeled environmental data using NMDS with both two and three dimensions. Stress was lower with three dimensions than with two dimensions (0.0439 and 0.0824, respectively), however, the actual interpretation of relationships between RBC and environmental variables did not differ between the two models. Thus, for ease of interpretation and explanation, we present the two-dimensional NMDS model (Fig. 5, Tables 5, 6). RBC site distance in the NMDS model was based on the environmental variables listed in the Figure 6 caption and Table 5. Those with the strongest relationship to the ordination were tall and short herbaceous cover; resin extract Ca, Mg, and NH_4 availability; pH; slope; and maximum and average light (Table 5). RBC variables that correlated with the NMDS ordination space ($p < 0.1$) were maximum photosynthetic assimilation (A_max), light compensation point, and RBC stolon number and length (Fig. 6, Table 6).

Table 5. — Nonmetric multidimensional scaling (NMDS) ordination of running buffalo clover (RBC; *Trifolium stoloniferum*) sites in sub-occurrences in the Fernow Experimental Forest based on soil, topographic, environmental light, and vegetative competition characteristics

	Coordinate 1	Coordinate 2	r2	p
Litter depth (cm)	-0.07	-0.55	0.31	0.195
Ca (ppm)	-0.67	0.51	0.72	0.005
pH	-0.37	-0.56	0.45	0.07
P (ppm)	0.58	0.45	0.55	0.025
Mg (ppm)	-0.26	0.85	0.80	0.002
NO_3 (ppm)	0.14	-0.25	0.08	0.705
OM (%)	-0.04	0.35	0.13	0.537
NH_4 (ppm)	0.76	-0.57	0.90	0.001
Slope (°)	-0.50	0.37	0.39	0.105
Max light (lux)	0.33	0.77	0.70	0.006
Avg light (lux)	0.25	0.86	0.81	0.001
Canopy cover (%)	-0.23	0.59	0.40	0.094
Tall herbaceous cover (%)	0.84	-0.10	0.71	0.009
Low herbaceous cover (%)	-0.86	-0.23	0.79	0.003
Sapling cover (%)	-0.49	-0.21	0.28	0.24
Aspect (° distant from N)	-0.54	-0.46	0.50	0.033

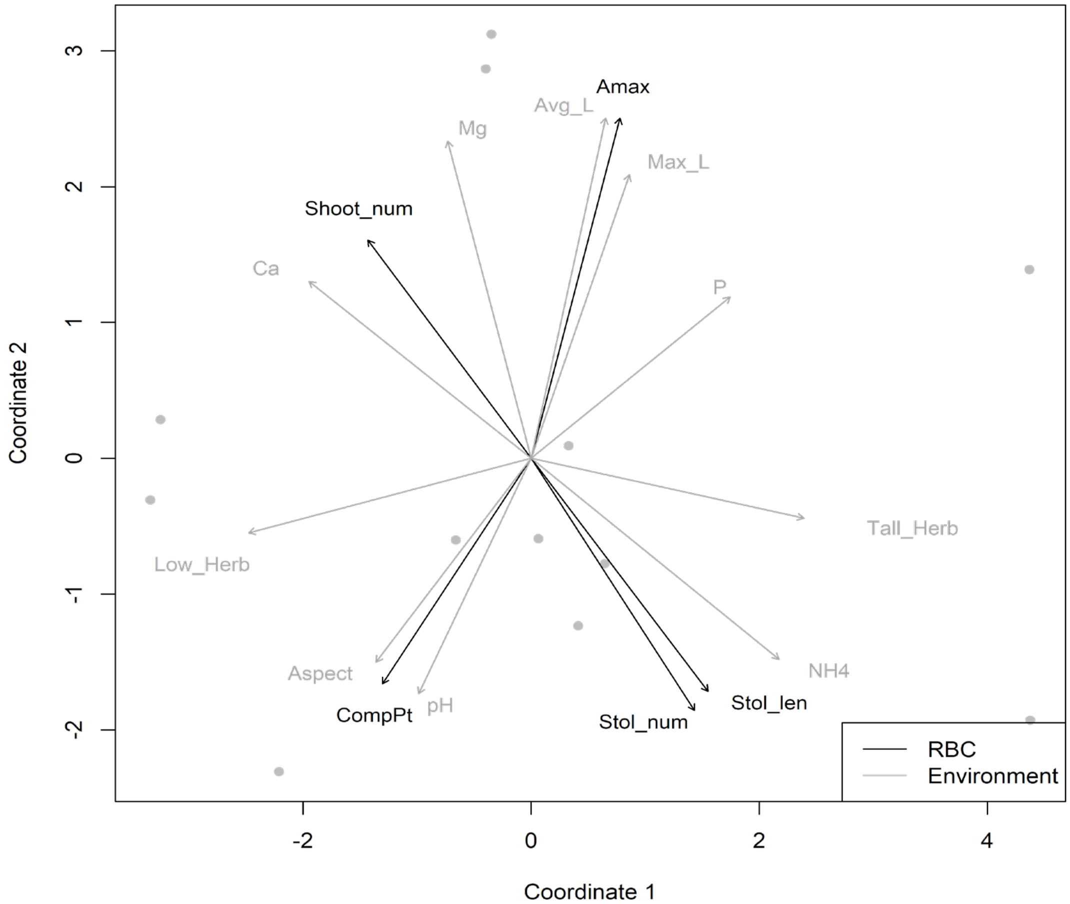

Figure 6.—NMDS ordination of running buffalo clover (RBC; *Trifolium stoloniferum*) sites in sub-occurrences in the Fernow Experimental Forest based on soil, topographic, environmental light, and vegetative competition characteristics: litter depth (cm), aspect (distant from north in degrees), skid road slope (degrees), soil pH, organic matter (%), resin extract nutrient concentrations for Ca, P, Mg, NO_3 and NH_4 (ppm), maximum Max_L, PPFD (μmol m^{-2} s^{-1}), and average (Avg_L, PPFD μmol m^{-2} s^{-1}) measured light and cover (%) by overhead canopy, saplings, tall (Tall_Herb) and low (Low_Herb) herbaceous understory (two dimensions, stress = 0.0824). RBC variables: plant height (cm), leaflet width (cm), number of shoots, number of stolons (Stol_num), saturation and compensation points (CompPt, PPFD, μmol m^{-2} s^{-1}) stolon length (Stol_Len, cm), maximum carbon assimilation (Amax, μmol C m^{-2} s^{-1}), and quantum efficiency (AQY) that are correlated ($p < 0.1$) with the environmental ordination are overlain as vectors (in black). The same environmental variables used to create the ordination are also added as vectors for interpretation purposes (gray, $p < 0.05$). Vector arrow direction indicates the direction of a gradient, and vector arrow length indicates the strength of the gradient. N = 12.

Table 6.—Running buffalo clover (RBC; *Trifolium stoloniferum*) variable relationship to nonmetric multidimensional scaling (NMDS) ordination of RBC sites in the Fernow Experimental Forest based on soil, topographic, environmental, light, and vegetative competition characteristics

	NMDS1	NMDS2	r_2	p
Height (cm)	0.11	-0.17	0.04	0.816
Shoots (number)	-0.45	0.36	0.33	0.149
Stolons (number)	0.48	-0.49	0.47	0.045
Stolon Length (cm)	0.50	-0.46	0.47	0.049
Leaflet Width (cm)	0.36	0.04	0.13	0.532
Amax (μmol C m^{-2} s^{-1})	0.28	0.79	0.71	0.006
Saturation point (μmol m^{-2} s^{-1})	0.00	0.33	0.11	0.569
Compensation point (μmol m^{-2} s^{-1})	-0.46	-0.52	0.48	0.056
Quantum efficiency	0.02	0.43	0.19	0.423

In the NMDS model, asexual reproduction, as stolon numbers and length, was positively related to topographic variables and edaphic variables (slope, base cations), and was nearly orthogonal to light conditions (maximum and average light; Fig. 6). Stolon length and number had an inverse relationship with slope of the skid road and a positive relationship with tall herbaceous cover and soil ammonium availability, confirming the bivariate correlations. Plant height, shoot number, and leaflet width did not have a strong relationship with the edaphic, topographic, or light variables included in the NMDS model. Regarding photosynthesis, maximum potential assimilation rate (Amax) was positively associated with light availability. The negative relationship between compensation point and available light might be due to potential RBC photosynthetic plasticity, however, photosynthetic measurements were highly variable, so these results should be interpreted with caution.

From the NMDS we can infer that light variables do not seem to be a large contributing factor to asexual reproduction of RBC compared to some other environmental variables such as soil pH, soil nitrogen, and competition from herbaceous understory plants. Overhead canopy was not strongly related to the NMDS model, nor any RBC characteristic, however, the presence of tall herbaceous competition is strongly related to stolon length and number at a given site. Light availability does correlate to RBC photosynthetic capacity, however, we did not detect direct relationships between photosynthesis and stolon production. Competition for resources other than light may be driving stolon production for nutrient foraging rather than light seeking.

Discussion and Conclusions

The objective of this pilot study was to elucidate the potential relationships among RBC asexual reproduction, leaf level light response, and edaphic and topographic variables. We concluded that while light has some effect on RBC populations, as seen by the relationship between tall herbaceous cover and stolon measurements, it may not be the main driving force in asexual reproduction. This conclusion is supported by the diverse light conditions of RBC's habitat. In the Fernow, we found RBC growing in low light areas as well as areas under direct sunlight. Madarish and Schuler (2002) found fully flowering RBC occurrences in open sunlight, which further refutes the hypothesis of necessary low-level light conditions for the survival of RBC. For example, one of our chosen sites of study appeared to be an outlier in all light-related measurements, as it always measured much higher than the other sites. There were also a few on the other end of the spectrum that measured lower than the others.

Further, Wang et al. (2012) make note that clonal plants under heterogeneous light supply will experience different levels of interspecific competition and clone performance, and growth could differ greatly. This observation could explicate the variability in our photosynthetic measurements. We chose to study sites that were dissimilar from each other to account for variability in RBC's habitat which may contribute to the lack of correlation between light availability and photosynthetic capacity. There have been several studies that make note of the genotypic plasticity within populations of RBC (Crawford et al. 1998, Franklin 1998, Hickey et al. 1991) that could potentially explain the variability in RBC photosynthetic and asexual reproductive relationships with light availability.

In general, clones growing under adverse light conditions show a great deal of plasticity in their specific leaf area and overall biomass (Slade and Hutchings 1987). A common behavior of stolon growth of ground ivy (*Glechoma hederacea* L.) under shaded conditions is to grow a singular long stolon to escape shading, whereas stolons would generally be shorter with many branch points under higher light conditions (Slade and Hutchings 1987). It has been observed that ground ivy stolons under adverse soil nutrient conditions behave similarly (Slade and Hutchings 1987), which could explain our observation of shorter stolons at sites where carbon assimilation was highest (Fig. 6). The RBC stolons may be exhibiting a stress response to limited nutrient availability rather than low light levels, thus explaining why we did not observe any strong relationships between photosynthetic capacity and asexual reproduction of RBC with light variables.

Thus, a variable more indicative of RBC's survival and reproduction may be soil nutrient availability rather than light levels or herbaceous competition. The relationship between pH and compensation point evidenced by the bivariate correlations was also apparent in the NMDS model and suggests that RuBisCO[3] availability and photosynthetic capacity may be related to soil pH or the underlying geology. We found that the compensation point increases as soil pH level increases (Fig. 5), which could be due to the level of nutrients in the soil needed for RuBisCO and other photosynthetic enzymes that drive the growth and reproduction of plants (Zhu and Jensen 1991). Although low compensation point can be due to adaptation to shading, based on Wang et al. (2012), we can assume clovers at sites with lower nutrient availability will have a lower compensation point because they are growing weakly due to limited resources, whereas plants at sites with higher soil pH and subsequently higher nutrient availability will have higher compensation points as they are growing vigorously and competing for resources. RBC populations are often associated with limestone substrate and soils with a more neutral pH relative to other soils in the region (US FWS 2007). Our findings support those of Wang et al. (2012), as we observed that there is a positive relationship between the soil nutrient NH_4 availability and stolon length (Fig. 4), providing further evidence of the effect of soil nutrients on asexual reproduction and survival. For species conservation, a possible tactic to increase species survival would be to amend soils with lime and carbonate to increase pH (Mulder and Van Veen 1960, Unkovich et al. 1996).

[3] Ribulose-1,5-bisphosphate carboxylase-oxygenase is commonly known by the abbreviations RuBisCo. It is an enzyme involved in the first major step of carbon fixation, a process by which atmospheric carbon dioxide is converted by plants and other photosynthetic organisms to energy-rich molecules such as glucose.

The relationships we found were based on a relatively small sample size of 36 individual rooted crowns within the 12 sub-occurrences. Compartment 20A was logged in the fall of 2018 so we are unable to obtain a larger sample size or conduct future studies using that location. Therefore, we intend for our findings to be used as reference to inform future studies that have access to larger sample sizes viable for statistical analyses.

It is possible that the relationship between photosynthetic and edaphic parameters are due to interactions we did not quantify. The forest understory is subject to dynamic light conditions, which leads to an area of interest in the presence of sunflecks. Sunflecks are categorized by high periods of photon flux density (PFD) that are unpredictable and intermittent with normal light conditions that have a positive effect on carbon assimilation (Pfitsch and Pearcy 1989). We took pilot measurements of sunflecks but were unable to collect substantial data for analysis due to time constraints. The number of sunflecks at an occurrence was determined by collecting data with the HOBO light loggers and counting the number of times average light values exceeded 50 μmol m^{-2} s^{-1} and were bound by light values less than 50 μmol m^{-2} s^{-1} of the average in 15-minute intervals (Pfitsch and Pearcy 1989). Our results were inconclusive, however studying sunflecks could provide understanding into RBC's light requirement as they have been identified as a vital resource for light-limited understory plants (Chazdon 1988).

Another avenue for future research is quantifying the presence of flowers under different kinds of environmental stress such as nutrient availability, light levels, and herbaceous competition. A study by Gonzales et al. (2017) elucidates the transgenerational effects of environmental stress in clonal plant asexual reproduction. Additional research on this topic could provide insights on the ratio of asexual to sexual reproduction for a different angle on conservation.

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