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Evaluating Endangered Pitcher Plant (*Sarracenia jonesii*) Leaf Morphology And Flowering As Related To Soil Nutrient Status

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Abstract

As a federally endangered and critically imperiled carnivorous plant, there is limited information on *Sarracenia jonesii*. This study provides a greater understanding of how soil nutrients affect this pitcher plant's ability to grow carnivorous leaves and catch its own prey, as well as how it affects the plant's ability to flower and spread its dwindling genetic diversity. *S. jonesii* plants were found to have more flowers as the number of carnivorous leaves increased. The soil nutrient assessment of this population demonstrated that these carnivorous plants occupy soil that is high in nutrient content potential but low in plant-available nutrients, specifically nitrogen. Flowering and the presence of soil ammonium (NH_4), did not exhibit a relationship. The growth of non-carnivorous leaves, phyllodia, and the presence of soil NH_4 did not show a relationship. A historical assessment of one of the few remaining populations of *S. jonesii* showed a decline in clumps but showed increased leaf and flower production. This information can be used to inform future conservation strategies to ensure plants are propagated and planted in appropriate soil conditions for ideal growth, carnivory, and reproduction.

1. Introduction

Known globally for their animal-like adaptations, pitcher plants are carnivorous herbaceous plants that have evolved to trap, kill, and digest prey for their nutritional benefit. These hydrophytic plants live in extreme environments with high humidity, high light, and consistently saturated soil. Occupying soil that is commonly referred to as "nutrient poor", these plants are left with minimal nutrients to flower and reproduce (Butler et al. 2007). Their harsh and stressful soil conditions led to their uncommon adaptations to acquire the necessary nutrients through unconventional methods (carnivory). This study is separated into four parts: leaf morphological analyses, soil nutrient and plant relationships, a flowering examination, and historical assessments of an extant population.

Sarracenia is a genus of North American carnivorous plants, commonly referred to as pitcher plants, with about 10 species, five of which are federally endangered. *Sarracenia jonesii* (W.) Case and Case (Sarraceniaceae) is a federally and state endangered critically imperiled North and South Carolina evergreen species known for its tall stature, slightly bulging mouth, and long tapered down-facing hood (Murdock 1990; Wichmann 2021; Ainsworth and Ainsworth 1998; McPherson and Schnell 2012, Case and Case 1976). It is also known by its common names the Mountain Sweet Pitcher Plant and the Red Pitcher Plant (United States Fish & Wildlife 2019, Slack 1984). It has been extirpated from 43% of its known locations and currently exists in 12 different populations across five counties (seven populations in North Carolina, five populations in South Carolina), and at least three of these populations are introduced. Only a fourth of the current populations have not shown declining numbers in the past few years (United States Fish & Wildlife 2019). Being one of the rarest pitcher plants in North America, it is considered the species most at-risk for extinction in *Sarracenia* for its low genetic diversity and small number of clumps per population (Furches et al. 2013, Godt and Hamrick 1996).

1.1. Leaf morphology

Sarracenia grow modified leaves, often referred to as pitchers, that capture, kill, and digest their prey. The carnivorous leaves of *Sarracenia*, unlike other pitcher plant genera such as *Nepenthes*, have to photosynthesize and trap prey in the same structure leading to a decreased overall photosynthetic rate. To combat this decreased rate, one solution the North American genus has evolved is to grow more photosynthetically efficient leaves called phyllodia (Pavlovič et al. 2007; Karagatzides and Ellison 2009). Phyllodia have elongated keels, are more efficient at photosynthesizing, and are more nutritionally expensive to make than their normal pitchers (Beaulac et al. 2002; Ellison and Gotelli 2002; Pavlovič et al. 2007; Karagatzides and Ellison 2009). The Givnish cost/benefit model for botanical carnivory anticipates that carnivorous plants will only remain carnivorous when certain conditions (high light and lack of mineral nutrition) are met (Givnish et al. 1984). If the plant's current environment is not ideal for carnivory, however, it can grow more non-carnivorous leaves (phyllodia) that acquire carbon through photosynthesis. (Beaulac et al. 2002; Ellison and Gotelli 2002; Pavlovič et al. 2007; Karagatzides and Ellison 2009).

Another species in this genus, *Sarracenia purpurea*, has been shown to grow more phyllodia when given more plant-available nitrogen. Carnivorous plants *Sarracenia purpurea* and *Drosera rotundifolia* have been experimentally found to decrease their carnivorous abilities through the reduction of digestive organs (pitchers and sticky exudate, respectively) when plant-available nutrients are added into their growing medium (Ellison and Gotelli 2002, Thorén et al. 2003). Since the plants grow pitchers to obtain nutrients through carnivory, it is hypothesized that those leaves need not be grown if enough nutrients can safely and effectively be taken in through the roots.

Therefore, these non-carnivorous leaves with small to no "mouth"/carnivorous parts and a large keel can act as indicators for soil nutrient status (Ellison and Gotelli 2002). We hypothesize that more phyllodia will be found in the *Sarracenia jonesii* clumps with the most plant-available nutrients in their soil.

1.2. Soil nutrients

Soil fertility, or lack thereof, is integral to the identity of carnivorous plants as their soil conditions created the plant's evolutionary need to find another source of nutrients. Nearly all literature describes carnivorous plant soil as "nutrient poor" or "lacking in nutrients'' (Adamec 2002, Palfalvi et al. 2020, Butler et al. 2007). Extremely low plant-available amounts of nitrogen (N, primarily nitrate (NO_3) and ammonium (NH_4)) have been found in other carnivorous plant bogs and fens suggesting a large discrepancy compared to the total amount of nutrients (total nitrogen) in the soil (Adamec 1997; Roberts and Oosting 1958). *Sarracenia* plants are able to uptake some nutrients through their roots, but their carnivorous abilities appear to be their main source of nitrogen (Butler et al. 2007, Adamec 1997).

As *Sarracenia* will often live in waterlogged habitats, their soils generally have lower levels of oxygen leading to slower decomposition by anaerobic bacteria when compared to non-waterlogged soils with more active aerobic decomposing microorganisms. The high acidity of carnivorous plant soils can also reduce the amount and rate of decomposition leading to fewer plant-available nutrients released (McClaugherty 2001). The soil they inhabit will still have organic matter, but the limited decomposition from the saturated soil leads to lesser release of plant available nutrients. We hypothesize that soil in which *Sarracenia jonesii* grows is not nutrient poor, but rather nutrient-rich (high total N); however, these nutrients are not in plant-available forms. Gaining a better understanding of the soil this species grows in may aid in the explanation of why this plant has such a limited and diminishing habitat range.

1.3. Flowering

Carnivorous plants can generally live without the added benefits of carnivory but may not thrive or even flower without prey (Adamec 1997). There is limited research on *Sarracenia* flowering and its relationships to soil and prey-based nutrients and no known relevant literature for *S. jonesii*. Multiple studies suggest that when carnivorous plants (mainly *Drosera*, *Pinguicula*, *Sarracenia*, and *Dionaea*) are fed with insects either through human intervention or not, they flower more and create more seeds (Karlsson and Pate 1992, Thum 1998, Karlsson et al. 1991, Gibson 1983). However, experimental studies have found that when carnivorous plants are fertilized with plant-available nutrients via soil, the plants either do not change, or suffer in terms of reduced flowering (Thorén 2003, Roberts and Oosting 1958, Stewart and Nilsen 1992). We hypothesize that the higher the amount of plant-available nutrients (especially N in the forms of nitrate [NO_3] and ammonium [NH_4]) in the soil, the fewer flowers the *S. jonesii* clumps will produce.

1.4. Historical analysis

The metapopulation of *Sarracenia jonesii* at the Robbert's Nature Preserve (RNP) fen was observed in this study. The population is found in the Southern Appalachians and was first reportedly written about in the 1930s, but data collection in the form of population censuses started in 1979 (North Carolina Natural Heritage Program 2022, Casebeer and Caldwell n.d.). As one of the few extant sites, we hypothesize that the *S. jonesii* metapopulation at the RNP will show declining trends in population growth from 1979 to 2022.

2. Methodology

As a metapopulation, the RNP is classified as fen in North Carolina with two small remaining subpopulations of *Sarracenia jonesii* and one individual clump by itself across the property; an additional smaller historical subpopulation cannot be found and is likely extirpated from its last recorded sighting in 2018 (North Carolina Natural Heritage Program 2022). Pitcher plant clumps were defined as a group of *S. jonesii* individuals (potentially connected by the same rhizomes or not) that were distinctly separated from other individual clumps, usually separated by about half a meter. Individual seedlings were not counted as clumps and only incorporated in the counts if they were at least about 10 centimeters away from a larger clump. For each subpopulation, which were labeled "footpath" and "lakeside", ten plant clumps at each site were identified for analysis (n=20). A single, lone clump was found in a different location that was labeled "trailside". The footpath site had 10 clumps total, lakeside had 14 clumps total, and trailside had one. With each clump assigned a number, a random number generator was used to identify the 10 clumps at each subpopulation for testing at the lakeside site.

2.1. Leaf morphology

For the leaf morphology analysis, the number of carnivorous leaves, as well as the number of phyllodia, or non-carnivorous leaves, were counted and recorded for each clump for which soil was collected. The clumps contained multiple years worth of leaves and only leaves that had a recognizable still-attached lip and hood were counted. Clumps were thoroughly examined to count all leaves found in clumps, even those diminutive and hidden. The circumferences of the clumps were measured by wrapping a measuring tape around the clumps and carefully tightening it to reduce excessive gaps between the foliage.

2.2. Soil nutrients

Using a 2.5 cm diameter soil probe, six 10-cm depth soil samples were collected per clump and homogenized. Efforts were taken to remove leaf litter from the soil surface, and any remaining large roots or other out-of-place organic matter were also removed. From each homogenized soil sample, two soil analyses were conducted.

First, all solid soil samples were analyzed for total nitrogen (%) and carbon (%) with a Carlo Erba NC 2500 Elemental Analyzer conducted at the Central Appalachians Stable Isotope Facility (CASIF) in Frostburg, Maryland. Second, plant-available nitrogen (NO_3 and NH_4) in ppm of the soil was measured of 21 clumps across the RNP. First, 150 grams of KCl (potassium chloride) was dissolved into 1 Liter of deionized water to make 2M KCl. 10.0±1.0 grams of field moist soil was added into 21 labeled test tubes (10 from the footpath subpopulation, 10 from the lakeside subpopulation, 1 from the trailside site) (Tully n.d.). Then, 35 mL of the 2M KCl were poured into each test tube and placed on a shaking plate for 1 hour. After being thoroughly mixed, the test tubes were placed upright to separate the soil. The soils were filtered from the liquid in the test tubes using a vacuum and Whatman filters. The filter funnel and Büchner flasks were rinsed with deionized water before and after each test tube. The post-filter liquid products were poured into new labeled test tubes, frozen, and sent to North Carolina State University for analysis (Maynard et al. 1993).

To measure the gravimetric soil moisture content (GWC%) of the soil samples, an aluminum tin was tared on a balance and 10.0±1.0 grams of field wet soil was added and the exact weight was noted. The soils were then air-dried and re-weighed. Excessively large roots or leaf litter (approximately larger than 1 cm in length) were removed from soil samples before weighing. Equation (1) calculated the dry weight of the air-dried soil, and equation (2) was used to calculate the GWC%.

$$\text{Dry weight} = (\text{tin weight} + \text{air-dried soil weight}) - \text{tin weight} \quad (1)$$

$$\text{Gravimetric soil content (\%)} = [(\text{wet weight} - \text{dry weight}) / \text{wet weight}] \times 100 \quad (2)$$

2.3. Flowering

The number of inflorescences, with or without existing flowers, were counted for each clump for which soil was collected. The inflorescences did not need to be fresh and green to be counted.

2.4. Historical analysis

The data used were aggregated by the North Carolina Natural Heritage Program, and the information was collected by at least 22 different individuals all likely with varying methods of counting clumps, leaves, and flowers (North Carolina Natural Heritage Program 2022). Starting in 2013, the censuses changed their leaf count wording to “pitchers”, whereas from 1979 to 1999, the word “leaves” was used. The authors use the assumption that only pitchers were counted from 1979 to 1999. No phyllodia census is found for any year before 2022.

2.5. Statistics

Statistical analyses were performed in the R program using an alpha of 0.05. All input variable data and model residuals were assessed for normality and homoscedasticity. For most variables, the data were right-skewed and required a log transformation to conduct parametric statistics. When appropriate, specific tests assuming unequal variance were applied. The quantitative results (p-values, slope, r2 values) of linear regressions and ANOVA using the log-transformed data were reported in the writing and figure captions, however the graphical figures show axes with untransformed data for the purpose of reader interpretability and transparency of actual data counts/ranges.

3. Results

3.1. Leaf morphology

Leaf morphology (the growth of pitchers and phyllodia) did not have a significant relationship with the amount of plant-available nutrients in the soil (Figure 1). The data were presented per square foot to reduce variance introduced by clump size.

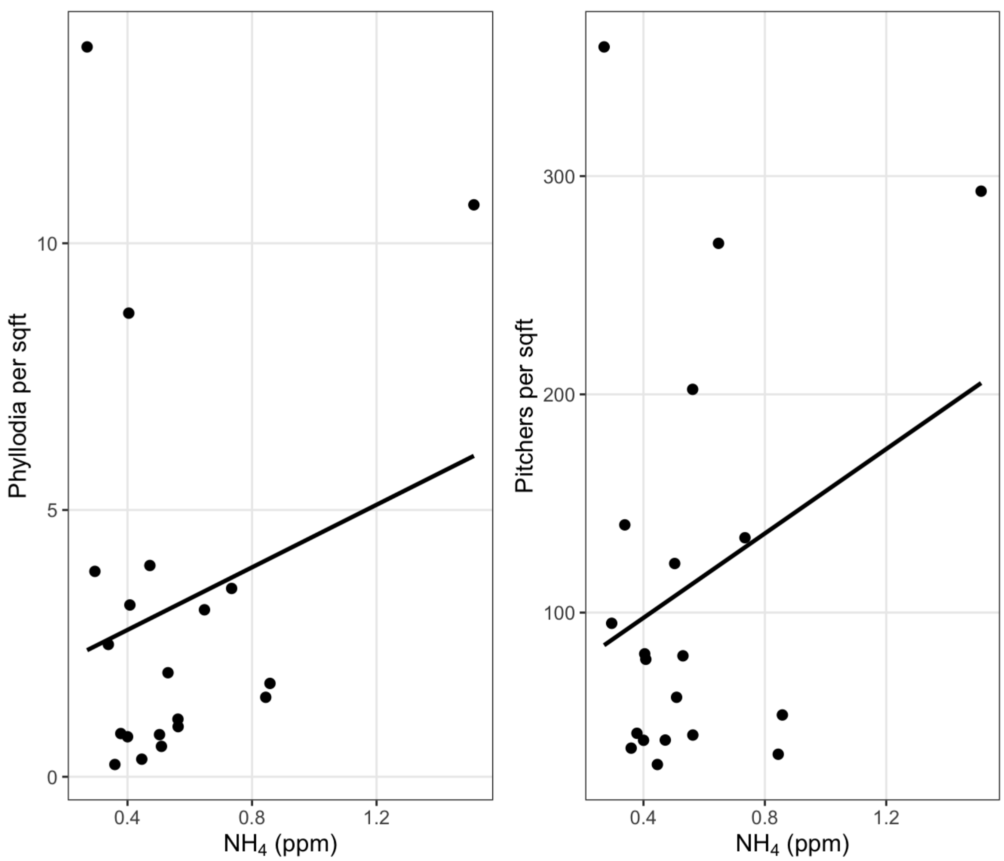

Figure 1. NH_4 (a plant-available form of nitrogen) and phyllodia production per square foot (sqft) did not have a statistically significant positive relationship (P= 0.59, slope= 0.35, r^2= 0.02) across all *S. jonesii* clumps. NH_4 and pitcher production per sqft did not have a statistically significant positive relationship (P= 0.57, slope= 0.2497, r^2= 0.02)

3.2. Soil nutrients

The soil at both subpopulations and the lone individual clump at the trailside site all had high total amounts of nitrogen (N) but low amounts of plant-available nitrogen in the form of NO_3 and NH_4 (Figure 2). The footpath subpopulation had an average % total carbon (C) of 6.48, an average % total nitrogen (N) of 0.37, and an average 0.65 ppm of NH_4. The lakeside subpopulation had an average % total carbon of 1.79, an average % total nitrogen of 0.09, and an average 0.45 ppm of NH_4. The individual clump at the trailside site had 1.8% total C, 0.09% total N, and 0.44 ppm of NH_4. Both subpopulations and the individual trailside clump had an average % total carbon of 4.15, % total of N of 0.22, and an average of 0.55 NH_4 ppm. All soil collected did not have enough NO_3 present to be detected; the soil of both subpopulations and the individual trailside clump had less than 0.10 NO_3 ppm (Table 1).

	Footpath	Lakeside	Trailside	All sites
%C	6.48	1.79	1.77	4.15
%N	0.37	0.09	0.09	0.22
NH_4 ppm	0.65	0.45	0.44	0.55
NO_3 ppm	<0.10	<0.10	<0.10	<0.10

Table 1. Summary of all nutrient levels for Robbert's Nature Preserve *Sarracenia jonesii* subpopulations.

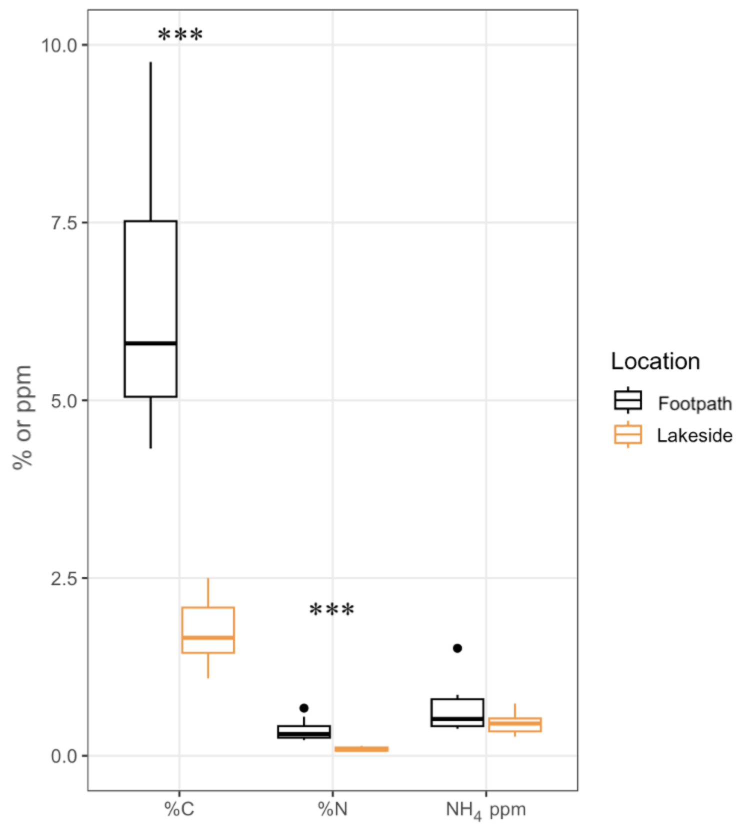

Figure 2. Comparison of % total C, % total N, and NH_4 (ppm) in the soil of both subpopulations of *Sarracenia jonesii* at the Robbert's Nature Preserve. Asterisks signify statistically significant relationships between locations for respective constituents.

3.3. Flowering

All clumps of *S. jonesii* in this present study had evidence of flowering. The number of flowers increased as the number of pitchers increased (Figure 3). Only one clump, which was at the lakeside subpopulation, had active flowers when the data were collected in October 2022. The footpath subpopulation had an average of 3 flowers per clump per sqft. The lakeside subpopulation had an average of 10 flowers per clump per sqft. The difference between these two has a P-value of 0.0094. The clumps at the lakeside population grow statistically significantly more flowers than the clumps at the footpath location. The clump at the trailside site had 74 flowers total.

The amount of NH_4 (ppm) in the soil did not have a statistically significant effect on the number of flowers grown (Figure 4). The two variables have a slight positive relationship, but there is not enough evidence to support the hypothesis.

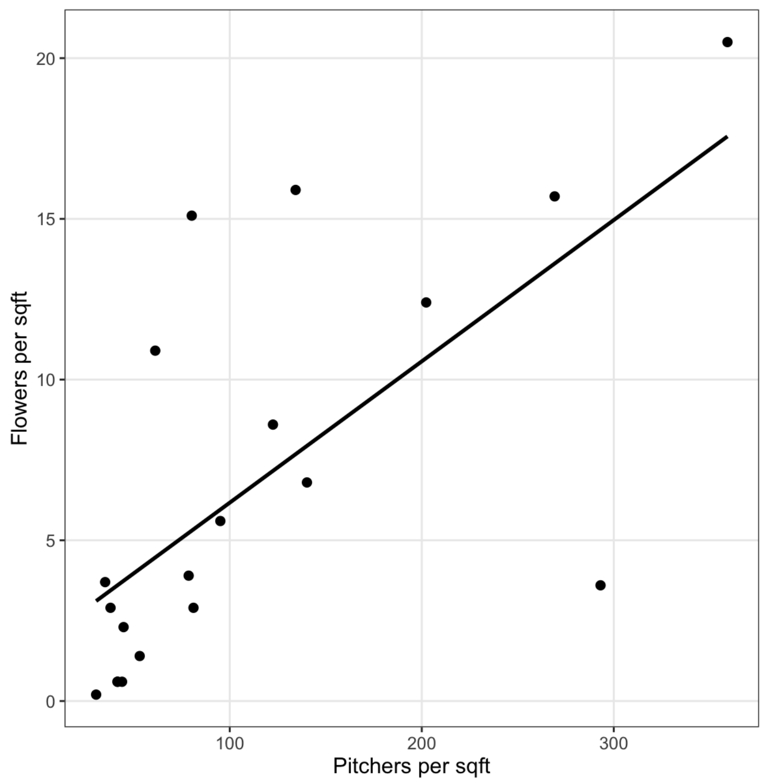

Fig 3. The number of flowers increases as the number of pitchers increase in *Sarracenia jonesii* clumps (P= <0.01, slope= 1.26, r^2= 0.53).

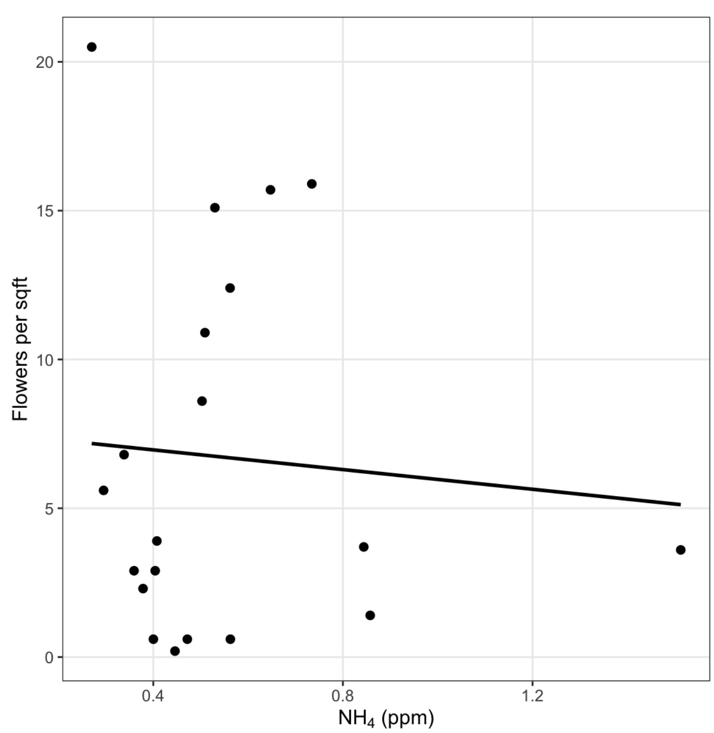

Figure 4. The number of flowers grown per sqft decreases as the amount of NH_4 (ppm) in the soil increases, but the relationship is not statistically significant (P-value= 0.99, slope= <0.01, r^2= <0.01).

3.4. Historical analysis

The RNP *S. jonesii* subpopulations had 17 recorded population surveys from 1979 to 2022 but have reportedly been mentioned in personal communication since the end of the 1930s (North Carolina Natural Heritage Program 2022, Casebeer and Caldwell n.d.). Conservation efforts were attempted by two botanical gardens; seeds were collected from the RNP in 1991 and 1997, grown in their respective botanical gardens, and planted as seedlings in the lakeside and footpath subpopulations in 1998. 22 of the 25 seedlings survived into the next year, but survival afterwards is unknown due to loss of label tags and poor recordkeeping (North Carolina Natural Heritage Program 2022, Casebeer and Caldwell n.d., United States Fish & Wildlife 2019).

All available consensus data, including the data from this present study, was combined for the first known time (Figure 5). Three trendlines compare the number of clumps, flowers, and pitchers over the past 43 years non-continuously. 1991 to 1998 are continuous, however, and are represented with the connected trendline. Following the trend of most *S. jonesii* populations, the RNP metapopulation is showing a decline in the number of clumps, specifically since 2013 (United States Fish & Wildlife 2019). There is a large gap of data between 1998 and 2013, and the decline could have started at any point in this period but was first shown by the 2013 census.

The pitcher plants showed an increase in the number of pitchers and flowers, however. Although the increase in flowers and pitchers between 2013 and 2022 might seem dramatic, the 2022 census was taken twice that year by different counters (including this study) and both supported each other’s findings. The 2022 numbers used in this figure, however, are calculated estimates based on the 21 clumps used in this study. From 1979-2022 non-continuously, there was an average of 27 clumps with the most recent count being 25 clumps. 1993 was an outlier with a count of 54 clumps; this is almost 20 clumps more than the year before and after it. If this outlier is removed, the average is 25 clumps. The average number of leaves was 1694 in this time frame, and the average number of flowers was 152.

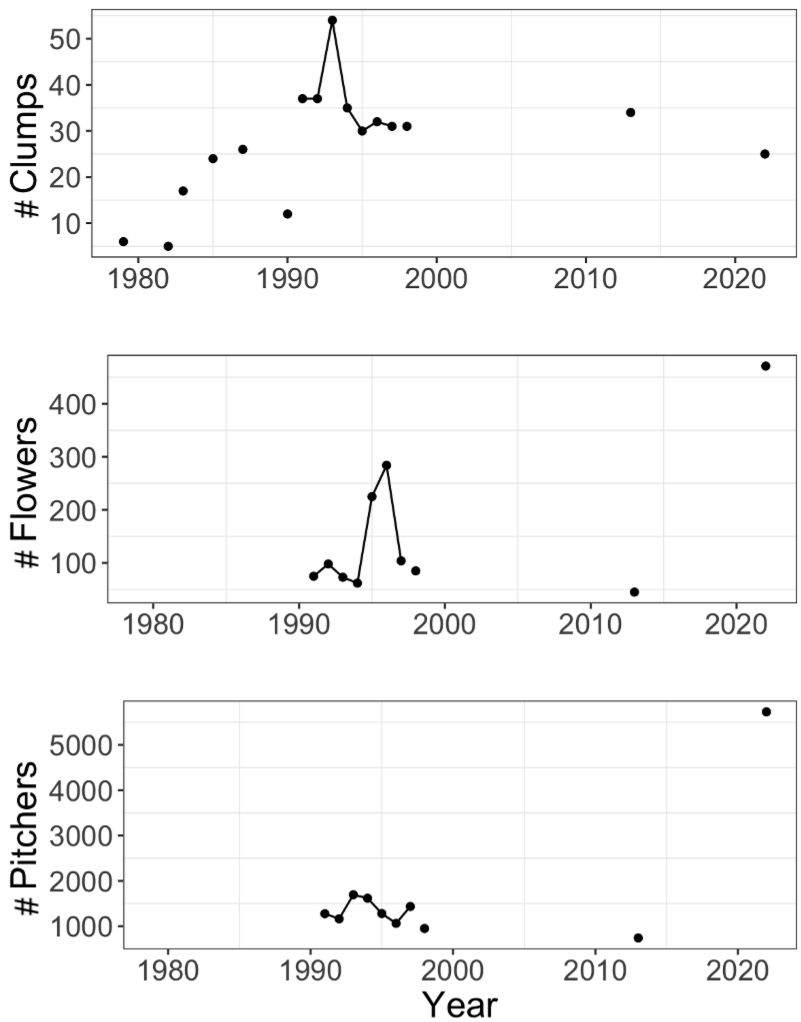

Figure 5. Historical trend lines of the Robbert's Nature Preserve pitchers, flowers, and clumps from 1979 to 2022, non-continuously.

4. Discussion

4.1. Leaf morphology

The data show that NH_4, a plant-available nitrogen, does not affect phyllodia growth, and this does not support our hypothesis. Although carnivorous plants, including those in *Sarracenia*, have been experimentally found to become less carnivorous as the amount of plant-available nutrients increased, other factors might have a stronger effect on why and when phyllodia grow, such as light (Ellison and Gotelli 2002, Thorén et al. 2003). Since phyllodia are more photosynthetically efficient than pitchers but more expensive to create, the extra investment in energy for growing a phyllodium might be paid back through carbon acquisition from photosynthesis (Karagatzides and Ellison 2009). The clumps at the RNP varied in size and the largest had a circumference of 480 centimeters; this large size meant that the outer larger leaves easily could have shaded out the center of heavily dense clumps. Most, if not all, clumps as defined in this study include multiple individual specimens and multiple rhizomes. Therefore, it is possible that rhizomes that are largely shaded by the clump itself are lacking in light which would encourage it to grow more phyllodia, regardless of soil nutrients. Another possible explanation for this lack of significant relationship between phyllodia and NH_4 could be from the experimental studies showing this relationship using more nutrients at a higher frequency than would be seen or expected in healthy bog and fen soils (Roberts and Oosting 1958).

Our results support the modification to the Givnish botanical carnivory model by Karlsson and Ellison (2009) by demonstrating that carnivorous plants can and do live in nutrient rich environments and plant-available nutrients in the soil may not influence carnivory (Givnish et al. 1984, Karlsson and Ellison 2009). Their findings show that this may be true only in environments with low competition such as the RNP, where invasive and encroaching plants are managed.

4.2. Soil nutrients

The high total nitrogen and carbon in the soil and low amounts of NO_3 and NH_4 (plant-available nutrients) in the soil of the RNP *S. jonesii* subpopulations support our hypothesis of the important nutrient difference in carnivorous plant soils. The high total amount of nitrogen in the soil supports our hypothesis that the wet fen soil conditions lead to higher organic matter accumulation with limited decomposition.The amount of NO_3 was below instrument detection limit which again supported the low amounts of plant-available nitrogen in the soil. Although the NH_4 was detected, the concentrations found were relatively low. Some NH_4 concentration was still expected to be found since carnivorous plants can and do take in some nutrients through their roots (Roberts and Oosting 1958, Butler 2007). The average NH_4 measured across all the soil samples was slightly below average for normal soils as hypothesized (leading to carnivorous adaptations). The average total nitrogen measured was slightly above the total nitrogen of an average agricultural soil as hypothesized because of the low rates of decomposition (Hornec 2011, McClaugherty 2001).

Future studies should compare the difference between total nitrogen and plant-available nitrogen for more carnivorous plant genera in other wetlands to help generalize this relationship for botanical carnivory. *S. jonesii* largely exists in highly managed areas; future studies should study the soil nutrient status of entirely wild and unmanaged sites as well (United States Fish & Wildlife 2019). We suggest a change of language when discussing soils carnivorous plants inhabit from “nutrient poor” to “nutrient unavailable” because instead of being “nutrient poor”, they can actually have more nutrients than the average soil.

4.3. Flowering

As shown in Figure 3, the more pitchers, or carnivorous leaves, a *S. jonesii* plant grows, the more flowers are present. This relationship was statistically significant. This figure includes the lakeside and footpath subpopulations, but not the single individual at the trailside site. The more pitchers the plant grows, the more potential the plant has for catching insects and gaining nutrients. Therefore, the more nutrients the plant has, the more likely it will be to have energy to create more flowers. This is the first known quantification of the flower and leaf relationship for *S. jonesii*.

The relationship between the number of flowers grown and the amount of NH_4, a plant-available nitrogen, did not have a statistically significant relationship as hypothesized. The trend had a negative relationship but more data is likely needed to better analyze it. It is possible that the other nutrients the plants need have a greater effect on flowering than nitrogen, and future studies could measure other nutrients in the soil. Other processes not mentioned in this study such as biological and microbial activity, rain, water movement, and other nearby plant competition might impact the amount of plant-available nitrogen in the soil.

4.4. Historical analysis

If the number of leaves and pitchers are used as a measure of plant vigor, the current remaining clumps are vigorously growing despite a general decline in population. This decline supports our hypothesis. *Sarracenia* rhizomes can grow for multiple decades, and this increase of growth over time might be attributed to plant maturity and also greater environmental protection. The RNP is a protected property, but there are still multiple factors working against the pitcher plants. Unknowing pedestrians visiting the preserve could easily harm these sensitive plants by walking on them, and illegal poaching for the private market is always a concern for a species as rare and desirable as this one (Casebeer and Caldwell n.d.). Herbicide and fertilizer use near the fen could easily harm the plants. The RNP continues to manage the canopy and overhanging vegetation near the clumps (shading can prove deadly to *Sarracenia* and hinder flower growth), but previous management methods have not been focused around protecting *S. jonesii* (Casebeer and Caldwell n.d.).

Future efforts should focus on this species specifically, especially as invasive species continue to outcompete the native plants. Fire has been used for management on this preserve in the past, but, unlike other species in the genus, fire may harm *S. jonesii* and should likely be avoided (Benjamin and Sutter 1993, Murdock 1990). Despite these factors, the attention given to this metapopulation by multiple independent and governmental agencies and the education given to the property owners is likely a reason why it has not yet been extirpated. For example, storm water management strategies have been employed to prevent runoff pollution that would add too many nutrients to the soil (Wilcox 2020).

5. Conclusions

The amount of NO_3 and NH_4 in the *Sarracenia jonesii* soils at the Robbert's Nature Preserve did not influence the growth of phyllodia (non-carnivorous photosynthetically efficient leaves) as hypothesized. The experimental studies showing a positive relationship between these variables commonly used a higher concentration of nutrients at a greater frequency than would be reflected in the natural fen system of this study.

The soil in all subpopulations at the RNP had a relatively high percentage of total nitrogen but low amounts of plant-available nitrogen. This supports our hypothesis that the fen's soil is not "nutrient poor" but rather "nutrient rich". We suggest that with more similar studies, carnivorous plants soils are instead referred to as low in plant-available nutrients.

We found a statistically significant relationship that shows that the more pitchers, or carnivorous leaves, a *S. jonesii* plant clump grows, the more flowers are grown. The number of flowers grown and the amount of NH_4 in the soil did not have a statistically significant relationship as hypothesized; more data might strengthen the negative relationship.

The *S. jonesii* population at the RNP is slightly declining in number of clumps, but the clumps are growing more leaves and flowers than in the past, possibly due to increased maturity and conservation efforts. The RNP property managers should continue to invest in conservation efforts and hydrological maintenance.

S. jonesii has the lowest genetic diversity in *Sarracenia* making it the species most at-risk for extinction in the genus (Furches et al. 2013). The results of this study should be used in future conservation strategies to better understand the soil conditions where transplanted specimens should thrive; this study can also inform propagation efforts when raising seeds and seedlings ex-situ. Planting more populations using clumps from multiple sites can help increase the species' dwindling genetic diversity.

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