3-2014

Effects of Nitrogen Deposition on Nitrogen Acquisition by Sarracenia purpurea in the Adirondack Mountains, New York, USA

Katy M. Austin

Effects of Nitrogen Deposition on Nitrogen Acquisition by *Sarracenia purpurea* in the Adirondack Mountains, New York, USA

by

Katy M Austin (SUNY-ESF Honors Student)
Candidate for Bachelor of Science
Department of Environmental and Forest Biology
With Honors

March 2014

APPROVED

Thesis Project Advisor: ________________________________
Dr. Mark Teece

Second Reader: ________________________________
Dr. Greg McGee

Honors Director: ________________________________
William M. Shields, Ph.D.

Date: ________________________________

Abstract

Elevated human generated emissions to the atmosphere have historically increased inorganic nitrogen (N) deposition throughout the Adirondack Mountains of New York. Nitrogen is generally a limiting nutrient for the purple pitcher plant (*Sarracenia purpurea*). Our objective was to determine the dependence of *S. purpurea* on atmospherically-deposited and insect-derived N sources across an increasing nitrogen deposition gradient. Sampling was conducted at 10 sites, with 104 pitcher plants sampled. The impact of variations in nitrogen deposition on morphological characteristics and organic N content of *S. purpurea* and a non-carnivorous reference plant, *Chamaedaphne calyculata* (Leatherleaf), were examined. Pitcher plant flower and leatherleaf tissues were analyzed for stable nitrogen isotope ($\delta^{15}N$), and foliar N content. Increased nitrogen deposition up to 4.1 $kgN*ha^{-1}*yr^{-1}$ was correlated with increased plant size and $\delta^{15}N$ values of *S. purpurea*; however, deposition exceeding these levels decreased overall plant size and $\delta^{15}N$ values. Nitrogen derived from assimilation of insects ranged from 55% to 90% of foliar N at higher N deposition levels. Plants that acquired the greatest amount of N from insect consumption were also the largest plants. These results reflect the importance of monitoring ecologically sensitive species, like the purple pitcher plant, in light of anthropogenic sources of pollution.

Table of Contents:

List of Figures

Appendix A:

List of Tables

Thesis Acknowledgements

I would like to extend a big thank you to Dr. Mark Teece, for his patience, direction, and continued guidance throughout the entire research process. Additionally, I would like to thank Jesse Crandall for his help with stable isotope and elemental analysis, Amy Sauer for her Adirondack insect data, Dr. Charley Driscoll and Habib Fakhraei for their assistance with N deposition maps, Dr. Greg McGee for reviewing multiple versions of the thesis, Theresa Baker for her guidance and Dr. William Shields for SUNY-ESF honors guidance.

Acknowledgements

This publication [article] was made possible by United States Environmental Protection Agency (EPA) fellowship number MA – 91751701-0 to KMA. Its contents are solely the responsibility of the fellow and do not necessarily represent the official views of the EPA. Further, the EPA does not endorse the purchase of any commercial products or services mentioned in the publication. Further funding provided by Northeastern States Research Cooperative (NSRC), New York State Energy Research and Development Authority (NYSERDA) EMEP Fellowship Program to AKS. We thank Theresa Baker, The SUNY-ESF Research Foundation, Mr. and Mrs. Austin, William Shields, staff and scientists at SUNY-ESF's Adirondack Ecological Center (Newcomb, NY) and Ranger School (Wanakena, NY), New York State Department of Environmental Conservation (NYSDEC), and the State University of New York College of Environmental Science and Forestry (SUNY-ESF) Honors Program for additional funding. We appreciate the help of Habib Fakhraei in preparing the map of atmospheric nitrogen deposition and comments of Greg McGee on an earlier version of the manuscript.

Overview of thesis

This thesis represents a two year study of pitcher plants in the Adirondack Mountains. During this time, I had the chance to collect plant data at some of the most beautiful ponds and bogs in northern New York State; in addition to spending a considerable amount of time preparing samples for stable isotope analysis. The thesis has since undergone 12 drafts and the resulting version was written concisely and specifically for submission to a journal. The thesis is a manuscript that was submitted to the journal *Botany* in February 2014. The title of the articles and authors are as follows:

Effects of Nitrogen Deposition on Nitrogen Acquisition by *Sarracenia purpurea* in the Adirondack Mountains, New York, USA

by

Austin KM[1], Teece MA[2], Crandall JB[2], Sauer, AK[3], Driscoll, CT[3]

1 – Department of Environmental and Forest Biology, SUNY-ESF, 1 Forestry Dr., Syracuse, NY, USA

2 – Department of Chemistry, SUNY-ESF, 1 Forestry Dr., Syracuse, NY, USA

3 – Department of Civil and Environmental Engineering, 151 Link Hall, Syracuse University, Syracuse, NY, USA

Submitted to *Botany* February 2014

Introduction

Anthropogenic emissions supply elevated quantities of reactive nitrogen to the atmosphere (Galloway et al. 2003). These pollutants are deposited to the Earth's surface in the form of wet and dry deposition, with the greatest deposition occurring in closest proximity to emission source areas, with increases with increasing elevation and in forests with elevated leaf area index values (Ito et al. 2002; Aber et al. 2003). The Adirondack Mountains of New York have been heavily impacted by atmospheric deposition because the regional surficial geology increases the sensitivity of overlying ecosystems to inputs of strong acids and nutrients (Jenkins et al. 2007). In the Adirondacks, wet deposition of inorganic nitrogen is distributed along a decreasing southwest to northeast gradient (McNeil et al. 2008). Nitrogen deposition is strongly correlated with emissions from the multi-state source area west of the Adirondacks (Aber et al. 2003; Driscoll et al. 2003). Nitrogen deposition has been shown to increase plant growth and foliar N in: *Betula papyrifera, Betula alleghaniensis, Acer rubrum, Acer saccharum, Pinus strobus, Tsuga canadensis, Abies balsamea, Picea rubens,* and more generally in both coniferous and hardwood stands across New England and the Adirondack Mountains (McNeil et al. 2007, Pardo et al. 2007). Evidence of increasing foliar N has sparked concern of the development of conditions of nitrogen saturation occurring across the forests of the northeastern United States (Aber et al. 2003).

Purple pitcher plants, *Sarracenia purpurea*, are wetland herbs known to face nutritional deficiencies, decreased growth, lessened cold tolerance, and increased regional extinction rates under chronic increases of nitrogen (Gotelli and Ellison 2002).

Compounding these risks, *S. purpurea* is a perennial that may survive for fifty or more years (Barthlott et al. 2004). *S. purpurea* obtains nitrogen from a variety of sources including atmospheric nitrogen deposition; nitrogen mineralized from captured prey; nitrogen derived from peat; and nitrogen remobilized from storage (Butler and Ellison 2007). However, the majority of nitrogen is obtained via direct precipitation into pitchers (Ellison and Gotelli 2002), or released into pitcher fluids from decomposition of captured invertebrates by plant-hosted trophic communities (Bledzki and Ellison 1998) and plant-synthesized enzymes. These communities include bacteria, rotifers, mites and mosquito, midge, and fly larvae (Butler and Ellison 2007) which aid in organic matter (OM) decomposition in the tubular pitchers. Nitrogen derived from decomposition is absorbed directly into plant leaves via diffusion (Slack 1979), and 18 - 43 mg N may accumulate in a plant with six new leaves per growing season (Bledzki and Ellison 1998). Hosted trophic communities are of greater importance in OM decomposition than any digestive enzymes, e.g. amylase, esterase, lipase, phosphatase, and protease (Slack 1979; Barthlott et al. 2004), as decomposing invertebrate OM contain substantial bioavailable nitrogen (Barthlott et al. 2004). Typically, less than five percent of a mature plant's (plant diameter > 10cm) total nitrogen budget is obtained from root interactions with the substrate (Gotelli and Ellison 2002. Ellison and Gotelli (2002) also found a relationship between nitrogen applied via aerial spraying and increasing pitcher morphologies. Consequently, increasing fluxes of atmospheric nitrogen deposition with steady phosphorus supply have been linked to decreased pitcher production relative to phyllodia (photosynthetic leaves) in *S. purpurea* (Ellison and Gotelli 2002), suggesting a decreased dependence upon carnivory under these conditions.

We developed a spatial model of atmospheric nitrogen deposition in the Adirondack Mountains of NY, and then tested the effect of nitrogen deposition on the growth and nitrogen acquisition strategies of *S. purpurea.* We specifically tested two hypotheses: 1) Overall growth of *S. purpurea* increases with increasing nitrogen deposition; 2) The contribution of nitrogen in mature pitcher plants derived from decomposing insects and biological materials captured in their pitchers decreases with increasing atmospheric nitrogen deposition. We sampled flower petals from 104 pitcher plants across a nitrogen deposition gradient. Petals are created new each growing season, providing an accurate depiction of current plant chemistries, unlike pitcher leaves which can resist senescence for several years (Gotelli and Ellison 2002). We measured a range of parameters to determine plant growth and stable nitrogen isotopic composition ($\delta^{15}N$) to assess nitrogen acquisition strategies.

METHODS

Sampling Methods

Research was conducted across a gradient of nitrogen deposition within the Adirondack Mountains of New York (Figure 1, see below). Samples were collected on NYS public lands, under New York State Department of Environmental Conservation (NYSDEC) TRP File #1851/#7010, and on State University of New York College of Environmental Science and Forestry (SUNY-ESF) properties.

Sampled plants were located in bogs and bog-like mats on the fringes of ponds and lakes. Ecological communities were commonly characterized by *Myrica gale,* *Chamaedaphne calyculata, S. purpurea, Vaccinium oxycoccos, Sphagnum spp.* and *Larix laricina*. A total of 11 sites were visited, and 104 purple pitcher plants were sampled. Sites include: Arbutus Pond [S1](Newcomb, NY), Deer Pond [S2](Newcomb, NY), Upper Chateaugay Lake [S3](Dannemora, NY), Raquette Lake – Inlet Flow [S4](Arietta, NY), Ferd's Bog [S5](Webb, NY), Eagle Lake – Paragon Brook [S6](Ticonderoga, NY), Long Pond [S7](St. Regis Canoe Area – Santa Clara, NY), "Unnamed" pond [S8](St. Regis Canoe Area – Santa Clara, NY), Esker Bog [S9](Wanakena, NY), Gull Pond [S10](Schroon, NY), and Mason Lake [S11](Lake Pleasant, NY). Flower petals from each *S. purpurea* plant were collected and leaves from a neighboring non-carnivorous plant, *Chamaedaphne calyculata* (leatherleaf), were collected at the same time and location. The isotopic composition of leatherleaf was used as a reference that recorded the stable nitrogen isotope composition of plants growing on non-carnivorous nitrogen in the local environment. At the end of each field day, tissue samples were frozen until later analysis. All samples were collected during two sampling windows in summer 2012 (6/30/2012 – 7/19/2012 and 8/16/2012 – 8/17/2012), thereby reducing temporal variation from sampling.

Morphological data including plant diameter (cm), number of pitchers, number of phyllodia, flower height (cm), pitcher opening width (mm), pitcher length (cm), keel width (mm), and total width of largest pitcher (mm) were measured with calipers or a ruler and recorded for *S. purpurea* plants (Ellison and Gotelli 2002), Figure 4. All recorded characteristics of pitcher morphology were based upon the pitcher with the largest opening width, and plants with multiple flowers were assigned an average flower height, calculated from all present flowers. GPS coordinates (Garmin Dakota 20) were also recorded for each pitcher plant sampled.

The pH of the plant's growing substrate and pitcher fluids were measured using a Hanna Instruments waterproof pH and temperature meter. Pitcher fluids were poured into a cup to facilitate sample collection because of the limited volume of fluids. In several instances however, not enough fluid was present in any of the pitchers for an accurate measurement. In these cases pH values were not recorded. Substrate pH measurements were also limited to locations where fluids were available. After pH measurements were completed, all fluids were returned to the substrate from which they were taken. Individual plants for morphological measurements were randomly selected to not bias selection based upon plant size or flower presence and only living plants were sampled. Highly unstable portions of *Sphagnum spp.* mats were not traversed for the safety of the researcher.

Representative invertebrates that we typically found in pitchers of *S. purpurea* were freshly collected from Bloomingdale bog and Madawaska flow in northwestern Adirondack Park. Samples from each order were pooled for analysis and included Hemiptera (n=5), Hymenoptera (n=7), Coleoptera (n=39), and Diptera (n=7).

Laboratory Methods

Samples for stable isotope analyses were randomly selected from collected tissues (*S. purpurea* petals and *C. calyculata* leaves) (Random Integer Generator [Random.org]). Five samples were selected from each site, unless fewer were available, in which case, all available samples were analyzed. Frozen tissue samples were dried (55˚C, 48 hrs.) and then crushed in clean polypropylene micro-centrifuge tubes (1.5 ml) to yield a fine powder. While processing *C. calyculata* (leatherleaf) leaves, the midrib was removed with scissors before pulverizing. A total of 43 leatherleaf samples and 22 pitcher plant flower tissues were used for isotopic analysis. Stable nitrogen isotope values ($\delta^{15}N$) and total nitrogen content of tissues were measured using a Costech Elemental Analyzer coupled to a ThermoFinnigan Delta XL Plus Stable Isotope Mass Spectrometer in the Environmental Stable Isotope Laboratory (EaSSIL) at SUNY-ESF. Samples were analyzed in triplicate and accuracy and precision of the stable isotope measurements (expressed in the standard per mil notation relative to atmospheric nitrogen for $\delta^{15}N$) were verified using National Institute of Standards and Technology RM8573 ($\delta^{15}N$ = -4.5 ± 0.3‰ [*n*=38]) and RM8574 ($\delta^{15}N$ = +47.6 ± 0.3‰ [*n*=38]). Daily precision of the instrument was verified by repeated analyses of internal laboratory standards including NIST1547 Peach Leaves ($\delta^{15}N$ =+2.0±0.1‰ [*n*=6]), acetanilide ($\delta^{15}N$ =-0.8±0.2‰ [*n*=16]), valine ($\delta^{15}N$ = -6.3±0.1‰ [*n*=3]), and daphnia ($\delta^{15}N$ = +17.9±0.1‰ [*n*=5]) during the sample runs.

The fraction of total nitrogen in *S. purpurea* tissues derived from consumption and assimilation of invertebrates (f_{invert}) was calculated utilizing a mass balance mixing model approach similar to that used in other carnivorous plant studies (Schulze et al. 2001):

$$\delta^{15}N_{S.\ purpurea} = f_{\mathrm{invert}} * \delta^{15}N_{\mathrm{invertebrates}} + (1-f_{\mathrm{invert}})\ \delta^{15}N_{C.\ calyculata}.$$

The $\delta^{15}N$ value of *S. purpurea* ($\delta^{15}N_{S. purpurea}$) is related to the fraction of total plant nitrogen assimilated from invertebrates (f_{invert}) and the fraction of total N acquired from soil nitrogen ($1-f_{invert}$) which is represented by the $\delta^{15}N$ value of a nearby plant reference plant ($\delta^{15}N_{C. calyculata}$). The fraction of plant N derived from invertebrates was calculated for each of the three N deposition ranges where *S. purpurea* were sampled, and the specific $\delta^{15}N$ values of *S. purpurea* and *C. calyculata* from each range were used in the calculation. The average $\delta^{15}N$ value for invertebrates (+2.8 ‰±0.4) was applied across all ranges of N deposition and a fractionation factor (+3‰) was applied prior to using the mass balance model, as suggested by Philips and Gregg (2001). We used the IsoError program in Microsoft Excel (Philips and Gregg 2001) to calculate standard errors and confidence intervals while accounting for variability in the $\delta^{15}N$ values of plants and invertebrates. We report the contribution of N from invertebrates as a percentage of total N (i.e. 100 x *f*) for ease of understanding.

The effect of nitrogen deposition on plant morphological characteristics including pitcher length, flower height, pitcher opening width, keel width, and total pitcher width (Figure 2) were analyzed using one-way ANOVAs and post hoc Tukey HSD tests with $\alpha = 0.05$. Residuals were examined for heteroscedasticity and data were $\log_{10}$ transformed and tested for normality with a Kolmogorov-Smirnov test. Correlations between plant size including diameter and nutrient concentrations were assessed with Pearson correlation tests. All statistical analyses were performed with R (R Core Team 2012).

Development of spatial model of atmospheric nitrogen deposition

We developed spatial regression models to extrapolate wet deposition measured at Huntington Forest to study sites in the Adirondacks for the sampling year (Fakhraei et al. in review). These spatial regression models were developed using meteorological data from the National Climatic Data Center (NCDC), and using wet deposition data from the NADP (http://nadp.sws.uiuc.edu/) and NYSDEC (http://www.dec.ny.gov/chemical/8422.html) monitoring sites inside and near the Adirondack Park. Our spatial models cover more meteorological and wet deposition sites and a longer record than the spatial models previously developed by Ito et al. (2002) and Ollinger et al. (1993). We estimated dry deposition of atmospheric nitrogen compounds based on dry to wet deposition ratios. A uniform dry to wet deposition ratios for NH_4^+ was estimated from throughfall studies at the Huntington Forest (Shepard et al. 1989). Dry to wet deposition ratio for NO_3^- was calculated from spatial models developed by Ollinger et al. (1993) and then modified via a model proposed by Chen and Driscoll (2004) to incorporate effect of forest composition (Cronan 1985). The forest composition for each study watershed was determined through a GIS data layer obtained from the National Land Cover Database (http://www.mrlc.gov/nlcd06_data.php).

RESULTS

Nitrogen deposition affects pitcher plant growth

Our spatial pattern of atmospheric nitrogen deposition is similar to the pattern reported previously (Ito et al. 2002; Ollinger et al. 1993), but with lower NO_3^- deposition values due to decreases in emissions of nitrogen oxides that have occurred since 2003 in the eastern U.S. to control tropospheric ozone concentrations (Driscoll et al. 2010). Total nitrogen deposition was highest in the southwestern Adirondacks 6.1 to 7.0 kg N ha^{-1} yr^{-1}) and decreases from southwest to northeast (Figure 1). As a result of this spatial pattern, the study sites experience a gradient of atmospheric N deposition.

Increasing nitrogen deposition corresponded with changes in plant growth of *S. purpurea* including the mean pitcher length, keel width, and total pitcher width (Table 1) and plant diameter (Figure 3). Up to a N deposition level of 4.1 kg $N*ha^{-1}*yr^{-1}$, *S. purpurea* plants grew larger throughout Adirondack study sites. Over this range, increasing N deposition was positively correlated with plant diameter, pitcher opening width, pitcher length, keel width, and total pitcher width (Table 2). Above the N deposition level of 4.1 kg $N*ha^{-1}*yr^{-1}$, plant size decreased (Figure 2; Table 1).

S. purpurea morphologies correlated to plant diameter

Plant diameter of *S. purpurea* was positively correlated with the number of pitchers, pitcher length, pitcher opening width, keel width, total pitcher width, and foliar

N content (Table 2). Average flower height was the only morphological characteristic without a significant correlation (Table 2).

Foliar N and stable isotope values

An overall increase in the foliar N (%N) content of both *S. purpurea* flower tissues and *C. calyculata* occurred across the spatial gradient of increasing inorganic wet nitrogen deposition (Table 2). Foliar N in *C. calyculata* decreased at the highest levels of deposition.

Although the δ^{15}N values of *C. calyculata* spanned a considerable range, the δ^{15}N values were not significantly different (Table 1; Figure 3) among levels of N deposition. In contrast, the δ^{15}N values of *S. purpurea* were significantly different and were most enriched (+4.8‰) between levels of 3.8 to 4.1 kg N*ha^{-1}*yr^{-1} N deposition (Figure 3). The δ^{15}N values of *S. purpurea* flower tissues ranged from + 0.1‰ to + 7.3‰ and were consistently enriched in ^{15}N relative to leaves of *C. calyculata* (-0.7‰ to - 14.1‰). The δ^{15}N values of *S. purpurea* were similar to or enriched in ^{15}N compared to the average δ^{15}N value of + 2.8 ‰ ± 0.5 SE of invertebrates. The δ^{15}N values of invertebrates in commonly collected orders were equal to: Hemiptera -0.28 ‰ ± 1.6 SE, Hymenoptera +0.1 ‰ ± 1.0 SE, Coleoptera + 4.3 ‰ ± 0.6 SE, and Diptera +4.9 ‰ ± 1.2 SE.

Using the mass balance approach, the nitrogen derived from assimilation of invertebrates ranged from 55% to more than 90% of total foliar N in *S. purpurea*. The highest level of N derived from carnivory was at N deposition levels of 3.8 to 4.1 kg

$N*ha^{-1}*yr^{-1}$ N, with considerably lower levels of carnivory in the lower N deposition areas (Figure 3).

DISCUSSION

Effects of nitrogen deposition on plant size

The plant size and $\delta^{15}N$ value of *Sarracenia purpurea* increased across the spatial gradient of increasing total N deposition up to 4.1 kg $N*ha^{-1}*yr^{-1}$ (Table 1). When total nitrogen deposition levels exceeded 4.5 kg $N*ha^{-1}*yr^{-1}$, *S. purpurea* size decreased (Table 1). Studies by Ellison and Gotelli (2002) suggest that keel width and pitcher size, are responsive to variations in nitrogen deposition. When we analyzed these characteristics, positive correlations were found between nitrogen deposition and pitcher length, pitcher opening width, total pitcher width, and keel width, up to nitrogen deposition of 4.1 kg $N*ha^{-1}*yr^{-1}$ (Table 1). We suggest that *S. purpurea* is impacted by variations in atmospheric nitrogen deposition, with growth increasing up to approximately 4.1 kg $N*ha^{-1}*yr^{-1}$. Similar patterns of increased growth were documented in trees of Adirondack Park, New York by Bedison and McNeil (2009) and McNeil et al. (2007). Increased growth by trees of young age classes (2.0 – 14.9 cm dbh) was most profound in *Acer rubrum*, while canopy trees of older age classes (> 15.0 cm dbh) exhibited species dependent neutral and increased growth impacts in response to increased nitrogen availability.

Nitrogen acquisition strategies

The foliar N content of *S. purpurea* and *C. calyculata* increased with increasing nitrogen deposition. These results are mirrored in elevated foliar N values in temperate forest trees surveyed by McNeil et al. (2007) in the Adirondack Mountains. Eight of nine tree species, including deciduous and evergreen species (*Betula papyrifera*, *Betula alleghaniensis*, *Acer rubrum, Acer saccharum*, *Pinus strobus*, *Tsuga canadensis*, *Abies balsamea, Picea rubens*), displayed an increase in foliar N with increasing inorganic wet nitrogen deposition.

Carnivorous plants such as *S. purpurea* are often co-limited by nitrogen, phosphorous (Chapin and Pastor 1995) and potassium in the absence of anthropogenic deposition (Ellison 2006). These plants are also known to uptake trace nutrients like potassium, manganese, and iron from prey captured in their pitchers (Adlassnig et al. 2009). Gotelli and Ellison (2002) demonstrated that high levels of nitrogen deposition or high N and P deposition have the highest likelihood of impacting the health of *S. purpurea*. Our observations of decreased plant sizes (pitcher length, total pitcher width, keel width, and flower height) at high nitrogen deposition levels are consistent with this observation.

Nitrogen is advantageous to plant growth before other nutrients become limiting; a benefit which likely has higher thresholds in *S. purpurea* because of the nutrients, like phosphorus (Adlassnig et al. 2009, Bledzki and Ellison 1998) obtained from digested insects, which would be co-limiting in similar plants. Excessive nutrient additions have the potential to cause ecosystem community shifts (Tomassen et al. 2003), as carnivorous plants are outcompeted by native or invasive species better adapted to elevated nitrogen concentrations in areas naturally more mesotrophic (Gotelli and Ellison 2002). Bobbink et al. (1998) came to similar conclusions when examining alterations in the distribution of *Sphagnum spp.* through multiple ombotrophic bogs across Western Europe, and accompanying shifts toward species best adapted for high nitrogen environments. In carnivorous plants, phenology and competitive advantage against other species is dependent upon the costs and benefits associated with the formation of carnivorous features; a beneficial strategy typically successful when in a wet, high light, oligotrophic environment (Ellison and Gotelli 2001).

S. purpurea acquires nitrogen from consumption of insects and other invertebrates. The $\delta^{15}N$ values of *S. purpurea* were significantly enriched in ^{15}N relative to *C. calyculata* (leatherleaf) tissues growing in the same ecosystems. The $\delta^{15}N$ values of *S. purpurea* were similar to and in some cases more enriched than the $\delta^{15}N$ value of insects (Figure 3), a pattern that has been observed in other pitcher plant species (Schulze 1997). We calculated that *S. purpurea* can obtain more than 90% of its total N content from insect consumption in areas of high N deposition. Our mass balance based acquisition calculation of 55 to 92% of nitrogen obtained from carnivory is much larger than if the value is calculated using the indirect method of insect capture rate and total nitrogen budget in these plants (10% assimilation) (Chapin and Pastor 1995). The plants that acquired the greatest amount of N from insect consumption were also the largest plants (e.g., plant diameter, number of pitchers, other morphological measurements), and contained higher foliar N content. We suggest that both increasing foliar N content and increasing $\delta^{15}N$ values observed with increasing levels of nitrogen deposition are primarily related to a shift to higher reliance on insect derived nitrogen at high levels of N deposition. Therefore, increasing N deposition may be impacting invertebrates in the ecosystem, specifically rotifers that digest invertebrates in pitcher communities (Bledzki and Ellison 1998). Increased N content of pitcher fluids may be increasing rotifer metabolism resulting in increased decomposition of trapped invertebrates. In ant-carnivorous plant interactions in other pitcher plant species, namely *Nepenthes bicalcarata,* and *Camponotus schmitzi*, the presence of ants resulted in the assimilation of more than 100% of N from insects as compared with 78% when no ants were present (Scharmann et al. 2013). The extent to which similar mutualistic ant-plant relationships exist in *S. purpurea* remains to be discovered. However, the strong reliance of Hymenoptera, mostly composed of ants, as a source of prey in *S. purpurea* (Heard 1998) may indicate that ant- *S. purpurea* interactions may not play a key role in N acquisition strategies in this pitcher plant species.

Plant diameter proxy for other plant morphologies

As an extension of this study, we propose that future studies on pitcher plants are best served by utilizing plant diameter as a proxy for other plant morphologies. A single measurement would be simpler for field researchers and also less stressful for the plant and the fragile plant community underfoot. There is a positive correlation between plant diameter and the number of pitchers, pitcher length, pitcher opening width, keel width, and total pitcher width. These characteristics are variable in both “size and shape” depending upon climate, nutrient and water availability/chemistry, and plant genetics across the plant's range (Ellison et al. 2004, Karberg and Gale 2010, Karberg and Gale 2013). However, pitcher plants grow outwards in a spiraling fashion, adding new leaves with increasing growth; thus diameter increases with growth. Healthier plants, potentially those benefiting from increased nitrogen resources, would be expected to increase their diameter faster than plants of poorer quality/health (Figure 2). This metric is a standard feature of plant growth based on the number of leaves produced. The regional shape differences attributable to climate by Ellison et al. (2004) should have no bearing on the number of leaves (phyllodia and pitchers combined) produced. The use of plant diameter should also eliminate changing morphologies with nutrient availability at local scales (Ellison et al. 2004). In previous studies, nitrogen and phosphorus enrichment had no effect on the number of leaves produced, mean leaf biomass and total above-ground plant biomass in pitcher plants from a single fen in Minnesota (Chapin and Pastor 1995), even though there was high variability within multiple plots of the same treatment type (nutrient and/or insect treatments). However, Karberg and Gale (2013) found a significant correlation between plant size (basal rosette diameter * number of leaves) and dissolved ammonium concentrations in the wetland water. Therefore, we recommend the use of plant diameter as a proxy for growth and other plant morphologies.

Note that atmospheric nitrate deposition has decreased markedly in the eastern U.S. since 2003 associated with decreases in emissions of nitrogen oxides from coal-fired powerplants (Lehmann et al. 2005; Driscoll et al 2010). Our investigation was conducted over a short period and therefore represents a snapshot of an ecosystem experiencing changes in nitrogen dynamics. Future research might be conducted to examine the response of pitcher plants (*S. purpurea*) and its nitrogen metabolism to this decrease in atmospheric nitrogen deposition.

CONCLUSIONS

Pitcher plants (*S. purpurea*) are able to tolerate and even grow to larger sizes with increasing N deposition. At higher N deposition, pitcher plants acquire greater amounts of nutrients from assimilation of insects in their pitchers. However, the advantage conferred from increased nutrient availability is only advantageous to a point. When deposition exceeds a threshold (> 4.5 kg $N*ha^{-1}*yr^{-1}$) in the Adirondack ecosystem, the excess nitrogen is no longer advantageous and plant size decreases. Plant diameter is a good indicator of the plant's morphological characteristics for studies of plant health, as it is a measurement of growth through leaf production (phyllodia and pitchers). Continued monitoring will further equip managers to adequately preserve unique species like *S. purpurea* in light of a rapidly changing environment and anthropogenic sources of pollution.

References

Aber JD, Goodale CL, Ollinger SV, Smith M-L, Magill AH, Martin ME, Hallett RA, Stoddard JL (2003) Is nitrogen deposition altering the nitrogen status of northeastern forests? Bioscience 53(4): 375-389

Adlassnig W, Steinhauser G, Peroutka M, Musilek A, Sterba JH, Lichtscheidl IK, Bichker M (2009) Expanding the menu for carnivorous plants: Uptake of potassium, iron and manganese by carnivorous pitcher plants. Applied Radiation and Isotopes 67: 2117 - 2122

Barthlott W, Porembski S, Seine R, Theisen I (2004) The curious world of carnivorous plants – A comprehensive guide to their biology & cultivation. Portland (OR): Timber Press Inc. p. 174

Bedison JE, McNeil BE (2009) Is the growth of temperate forest trees enhanced along an ambient nitrogen deposition gradient? Ecology 90(7): 1736-1742

Bledzki LA and Ellison AM (1998) Population growth and production of *Habrotrocha rosa* Donner (Rotifera: Bdelloidea) and its contribution to the nutrient supply of its host, the northern pitcher plant, *Sarracenia purpurea* L. (Sarraceniaceae). Hydrobiologia 385: 193-200

Bobbink R, Hornung M, Roelofts JGM (1998) The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. Journal of Ecology: 86(5): 717-738

Butler JL, Ellison AM (2007) Nitrogen cycling dynamics in the carnivorous northern pitcher plant, *Sarracenia purpurea*. Functional Ecology 21(5): 835-843

Cabana G, Rasmussen JB (1994) Modeling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature 372: 255-257

Chapin CT, Pastor J (1995) Nutrient limitations in the northern pitcher plant *Sarracenia purpurea*. Canadian Journal of Botany 73:728-734

Chen L, Driscoll CT, Gbondo-Tugbawa S, Mitchell MJ, Murdoch PS (2004) The application of an integrated biogeochemical model (PnET-BGC) to five forested watersheds in the Adirondack and Catskill regions of New York. Hydrological Processes 18: 2631-2650

Cronan CS (1985) Biogeochemical influence of vegetation and soils in the ILWAS watersheds. Water Air and Soil Pollution 26: 355-371

Driscoll CT, Cowling EB, Grennfelt P, Galloway J, Dennis R (2010) Integrated assessment of ecosystem effects of atmospheric deposition: Lessons available to be learned. EM Magazine November 2010: 6-13

Driscoll CT, Whitall D, Aber J, Boyer E, Castro M, Cronan C, Goodale CL, Groffman P, Hopkinson C, Lambert K, Lawrence G, Ollinger S (2003) Nitrogen pollution in the northeastern United States: Sources, effects, and management options. BioScience 53: 357-374

Ellison AM (2006) Nutrient limitation and stoichiometry of carnivorous plants. Plant Biology 8(6): 740-747

Ellison AM, Buckley HL, Miller TE, Gotelli NJ (2004) Morphological variation in *Sarracenia purpurea* (Sarraceniaceae): Geographic, environmental, and taxonomic correlates. American Journal of Botany 91(11): 1930-1935

Ellison AM, Gotelli NJ (2001) Evolutionary ecology of carnivorous plants. TRENDS in Ecology & Evolution 16(11): 623-629

Ellison AM, Gotelli NJ (2002) Nitrogen availability alters the expression of carnivory in the northern pitcher plant, *Sarracenia purpurea*. Proceedings of the National Academy of Sciences 99 (7): 4409-4412

Fakhraei H, Driscoll CT, Selvendiran P, DePinto JV, Bloomfield J, Quinn S, Rowell C (in review) Development of a total maximum daily load (TMDL) for acid-impaired lakes in the Adirondack region of New York. Atmospheric Environment

Galloway JN, Aber JD, Erisman JW, Seitzinger SP, Howarth RW, Cowling EB, Cosby BJ (2003) The nitrogen cascade. BioScience 53: 341-356

Gotelli NJ, Ellison AM (2002) Nitrogen deposition and extinction risk in the Northern pitcher plant, *Sarracenia purpurea*. Ecology 83(10): 2758-2765

Heard SB (1998) Capture rates of invertebrate prey by the pitcher plant, *Sarracenia purpurea* L. The American Midland Naturalists 139(1): 79-89

Ito M, Mitchell MJ, Driscoll CT (2002) Spatial patterns of precipitation quantity and chemistry and air temperature in the Adirondack region of New York. Atmospheric Environment 36: 1051-1062

Jenkins J, Roy KM, Driscoll CT, Buerkett C (2007) Acid Rain and the Adirondacks: An Environmental History. Cornell University Press

Karberg JM, Gale MR (2010) Exploration of meaningful subspecies definitions of the carnivorous Northern pitcher plant (*Sarracenia purpurea*) throughout its geographic distribution. Conservation Genetics 11: 2369-2378

Karberg JM, Gale MR (2013) Influence of surface water mineral nutrition on the plasticity of *Sarracenia purpurea* in *Sphagnum* fens, marl wetlands, and sand savannahs. Wetlands 33: 631-639

Lampman G, et al (2009) A long-term monitoring program for evaluating changes in water quality in selected Adirondack waters: core program 2007 – 2011: NYSERDA. Data Summary Report 2009

Lehmann CMB, Bowersox VC, Larson SM (2005) Spatial and temporal trends of precipitation chemistry in the United States, 1985-2002. Environmental Pollution 135: 347-361

Matson P, Lohse KA, Hall SJ (2002) The globalization of nitrogen deposition: Consequences for terrestrial ecosystems. AMBIO 31(2): 113-119

McCutchan JH, Lewis Jr. WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. OIKOS 102(2): 378-390.

McNeil BE, Read JM, Driscoll CT (2007) Foliar nitrogen responses to elevated atmospheric nitrogen deposition in nine temperate forest canopy species. Environmental Science & Technology 41(15): 5191-5197

McNeil BE, Read JM, Sullivan TJ, McDonnell TC, Fernandez IJ, Driscoll CT (2008) The spatial pattern of nitrogen cycling in the Adirondack Park, New York. Ecological Applications 18(2): 438-452

Ollinger SV, Aber JD, Lovett GM, Millham SE, Lathrop RG, Ellis JM (1993) A spatial model of atmospheric deposition for the northeastern U S. Ecological Applications 3: 459-472

Pardo LH, McNulty SG, Boggs JL, Duke S (2007) Regional patterns in foliar ^{15}N across a gradient of nitrogen deposition in the northeastern US. Environmental Pollution 149: 293-302

Phillips DJ, Gregg JW (2001) Uncertainty in source partitioning using stable isotopes. Oecologia 127:171-179

R Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Scharmann M, Thornham DG, Grafe U, Federle W (2013) A novel type of nutritional ant-plant interaction: Ant partners of carnivorous pitcher plants prevent nutrient export by dipteran pitcher infauna. PLoS ONE 8(5): e63556

Schulze W, Schulze ED, Pate JS, Gillison AN (1997) The nitrogen supply from soils and insects during growth of the pitcher plants *Nepenthes mirabilis*, *Cephalotus follicularis* and *Darlingtonia californica*. Oecologia 112: 464-471

Shepard JP, Mitchell MJ, Scott TJ, Zhang YM, Raynal DJ (1989) Measurements of wet and dry deposition in a northern hardwood forest. Water, Air, and Soil Pollution 48: 225-238

Slack A (1979) Carnivorous Plants. London: Ebony Press. p. 15-50

Tomassen HBM, Smolders AJP, Lamers LPM, Roelofs JGM (2003) Stimulated growth of *Betula pubescens* and *Molinia caerulea* on ombotrophic bogs: role of high levels of atmospheric nitrogen deposition. British Ecological Society Journal of Ecology 91(3): 357-370

USDA-NRCS PLANTS Database/Britton, N.L., and A. Brown (1913) *An illustrated flora of the northern United States, Canada and British Possessions. 3 vols.* Charles Scribner's Sons, New York. Vol. 2: 202. Available from: http://plants.usda.gov/java/largeImage?imageID=sapu4_001_avd.tif

Yao FY, Wang GA, Liu XJ, Song L (2011) Assessment of effects of the rising atmospheric nitrogen deposition on nitrogen uptake and long-term water-use efficiency of plants using nitrogen and carbon stable isotopes. Rapid Communications in Mass Spectrometry 25(13):1827-1836

Appendix A

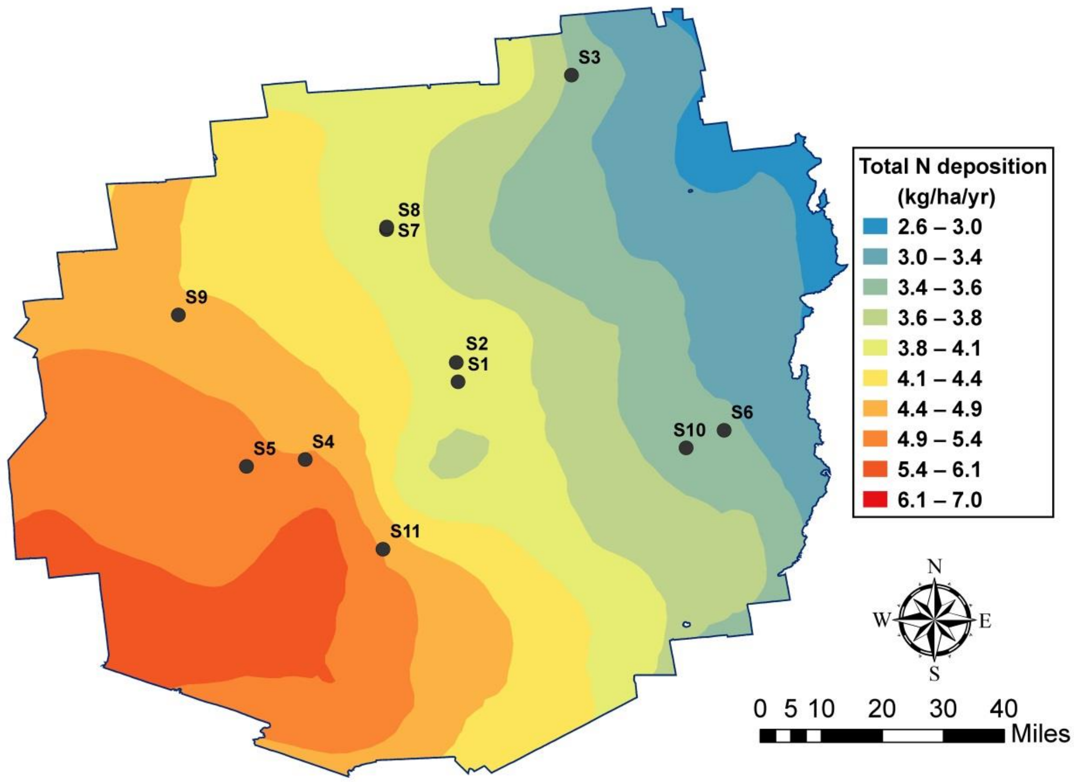

Fig. 1 Spatial model of atmospheric nitrogen deposition for the Adirondack Mountains and locations of our sampling sites (S1-S11).

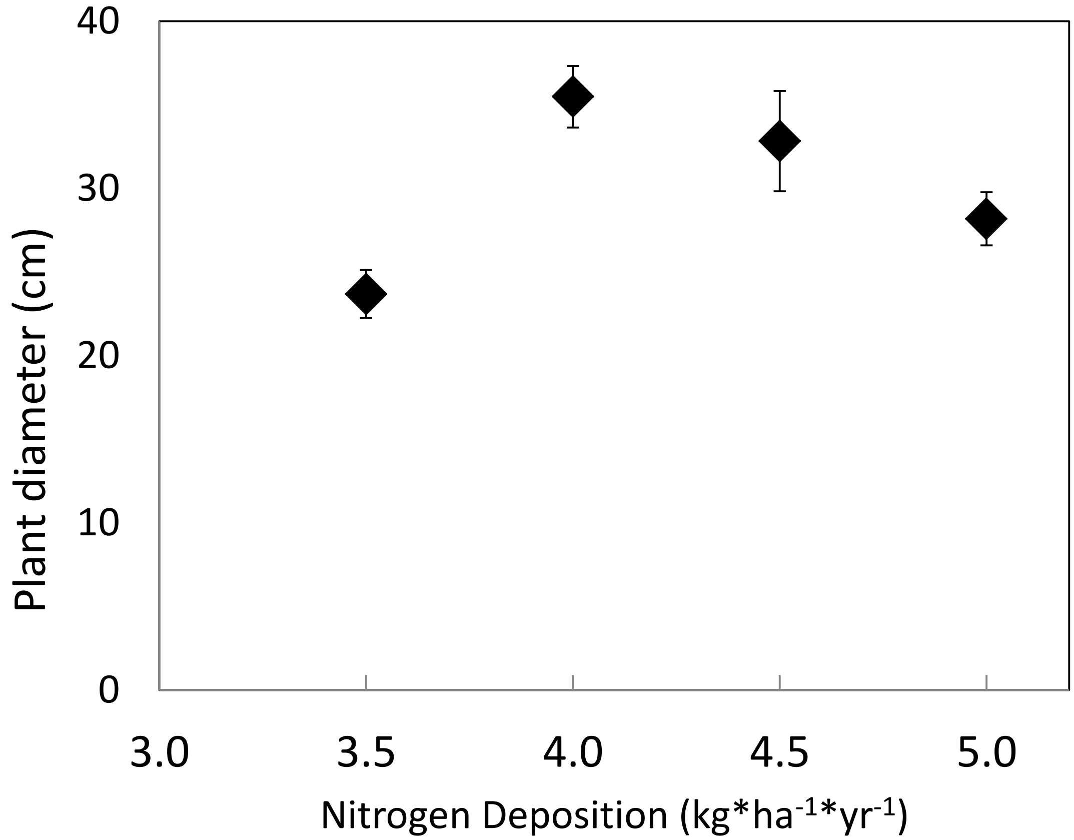

Fig. 2 Changes in plant size (mean plant diameter ±SE) of *S. purpurea* with increasing total nitrogen deposition gradient (kg*ha^{-1}*yr^{-1}).

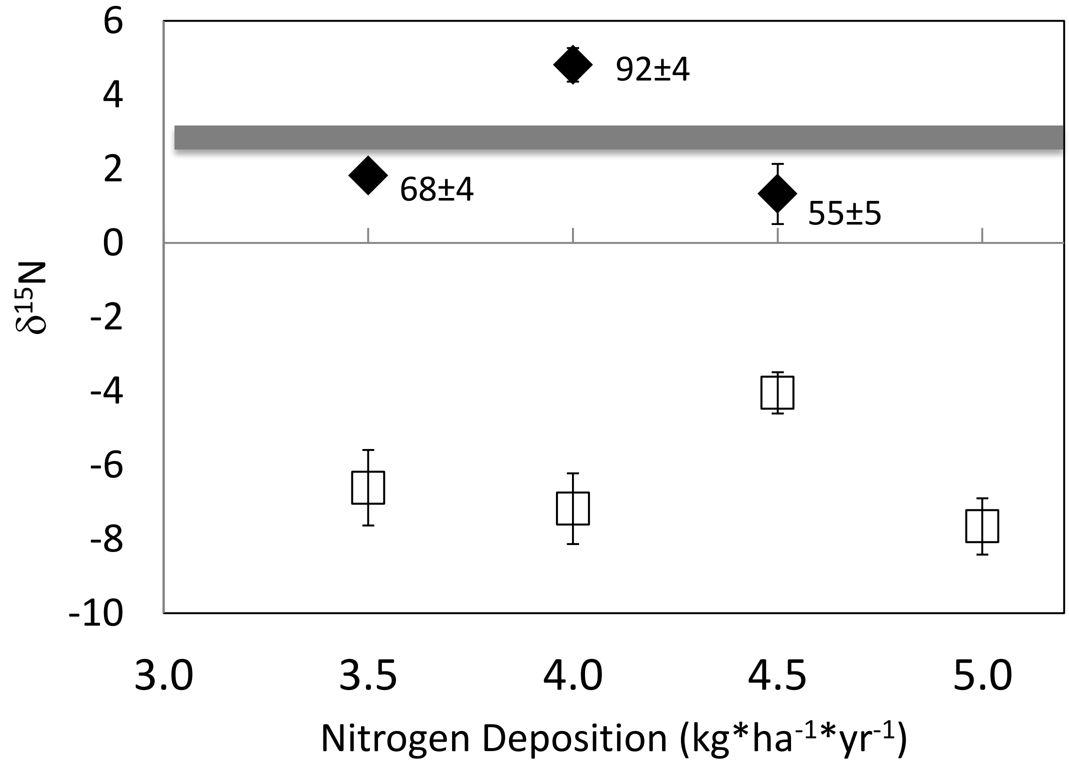

Fig. 3 Changes in the average $\delta^{15}N$ values (±SE) of *S. purpurea* (◆) and *C. calyculata* tissues (☐) in relation to total nitrogen deposition gradient. Bold values represent the amount of total N (%±SDEV) acquired by *S. purpurea* from insect consumption. Shaded box represents the $\delta^{15}N$ value (+2.8 ‰ ± 0.5 SE) for insects sampled at Madawaska Flow and Bloomingdale Bog in the Northwestern Adirondacks.

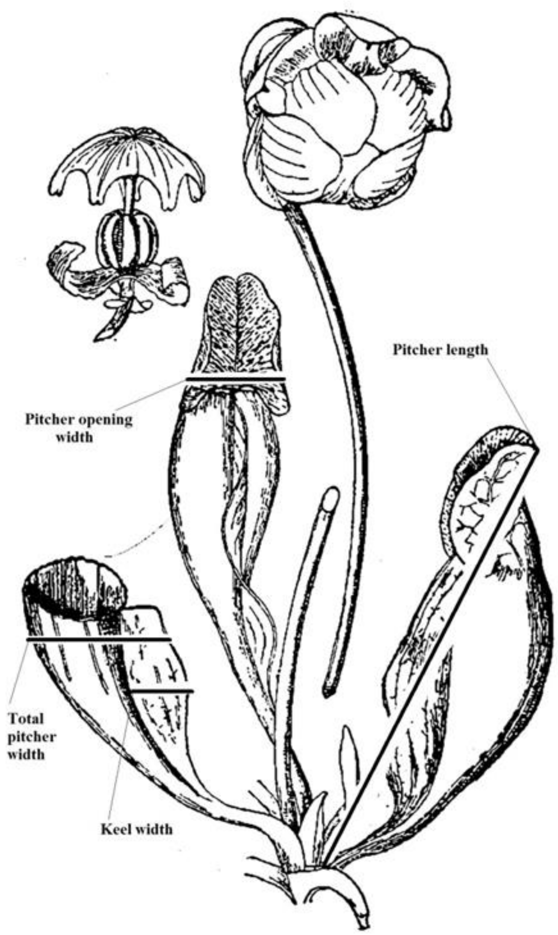

Fig.4 *Sarracenia purpurea* morphological measurements (USDA-NRCS PLANTS, 1913)

Table 1 Effect of increasing N deposition on average (±STDEV) foliar nitrogen content, stable nitrogen isotope composition, and plant morphology tested using a one-way ANOVA with post-hoc Tukey HSD test. Values that do not share a letter across a row are significantly different (n.m. = no samples of *S. purpurea* were collected or measured at the highest N deposition level; * Highlights results of statistical importance).

Nitrogen Deposition ($kg*ha^{-1}*yr^{-1}$)	3.4-3.6	3.8-4.1	4.4-4.9	4.9-5.0	F-value	P-value
S. purpurea $\delta^{15}N$	1.8 (0.9) A	4.8 (1.4) B	1.3 (1.6) A	n.m.	$F_{2,19}$	0.000*
S. purpurea foliar N	0.9 (0.2) A	1.6 (0.1) B	1.6 (0.1) B	n.m.	$F_{2,19}$	0.000*
C. calyculata $\delta^{15}N$	-6.6 (3.1)	-7.7 (3.7)	-4.1 (1.2)	-7.7 (3.0)	$F_{3,39}$	0.260
C. calyculata foliar N	1.2 (0.3) A	1.3 (0.0) AB	1.7 (0.1) AB	1.52 (0.1) B	$F_{3,39}$	0.008*
Plant diameter	23.7 (6.2) A	35.5 (10.6) B	32.8 (10.4) BC	28.2 (10.1) AC	$F_{3,100}$	0.000*
Pitcher length	16.8 (3.8) A	21.7 (5.4) B	19.9 (4.4) AB	18.0 (3.6) A	$F_{3,97}$	0.001*
Total pitcher width	40.9 (9.9) A	50.0 (11.7) B	50.9 (13.1) AB	43.7 (8.2) A	$F_{3,98}$	0.005*
Keel width	17.5 (6.6) AB	21.1 (9.3) AB	24.5 (8.1) A	16.8 (5.5) B	$F_{3,98}$	0.016*
# of pitchers	17.8 (17.0)	22.5 (13.2)	12.9 (7.6)	17.0 (11.2)	$F_{3,98}$	0.104
Flower height	37.0 (18.5) A	48.7 (7.7) AB	54.8 (9.3) B	48.3 (9.1) AB	$F_{3,63}$	0.019*
Pitcher opening width	27.4 (4.6) A	27.4 (5.0) B	26.7 (5.4) AB	26.5 (3.1) B	$F_{3,98}$	0.011*

Table 2 Pearson's Correlation Coefficient (PCC) and resulting p-values when plant diameter and N deposition levels (3.4 – 4.1 $kg*ha^{-1}*yr^{-1}$) were separately tested for correlation against the listed secondary variables (N/A = not applicable; * significant correlation at P<0.05).

Secondary variable	Plant diameter PCC	Plant diameter P-Value	N deposition PCC	N deposition P-value
# of pitchers	0.614	0.000*	0.028	0.846
Pitcher length (cm)	0.595	0.000*	0.359	0.011*
Pitcher opening width (mm)	0.475	0.000*	0.336	0.017*
Keel width (mm)	0.253	0.010*	0.174	0.226
Total pitcher width (mm)	0.442	0.000*	0.337	0.017*
Averaged flower height (cm)	0.153	0.218	0.381	0.024*
S. purpurea foliar N	0.560	0.007*	0.789	0.000*
S. purpurea $\delta^{15}N$ values	0.092	0.684	0.741	0.000*
Pitcher fluid pH	0.034	0.747	0.067	0.668
C. calyculata foliar N	N/A	N/A	0.293	0.164
Nitrogen deposition ($kg*ha^{-1}*yr^{-1}$)	0.463	0.001*	N/A	N/A