

HIGH INTRASPECIFIC ELEMENTAL VARIATION IN PITCHER PLANTS
ACROSS A GEOGRAPHICAL GRADIENT

By

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THESIS ABSTRACT

High intraspecific elemental variation in pitcher plants across a geographical gradient

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Functional trait variation among individuals has become a fundamental component to understand how populations respond to spatiotemporal variation in the environment. Traits respond to varying environmental conditions and can influence ecosystem function. Among these functional traits, the elemental composition of living organisms (e.g., carbon, nitrogen, and phosphorus) relates and responds to its environment, playing fundamental roles in shaping population and community structure, and biogeochemical processes. Here we studied how multiple single elemental traits and functional trait hypervolumes respond to environmental drivers (e.g. pH, water capacity, habitat size, water temperature, and dissolved oxygen). To do this, we sampled populations of pitcher plants (*Sarracenia purpurea*) across a geographical gradient in New Jersey. We found that the bogs of New Jersey showed significant variation in environments but that environmental drivers were not strong predictors of trait variation. Variance for most elemental traits was higher within than among populations. Hypervolume niche size varied across populations but also displayed relatively large overlap and short centroid distances between populations. We found that environmental drivers influence

hypervolume size with larger hypervolumes in sites that display more heterogeneity in environmental conditions. We also found larger trait niche overlaps among environmentally similar sites. Overall, we found that variation in environmental conditions drive changes in functional trait values at the individual and population level and thus affected trait diversity and trait hypervolumes of pitcher plants. This study can help understand how trait variation may respond to environmental changes and affect the structure and functioning of ecological populations.

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Introduction

Functional trait approaches have emerged as a fundamental component to understand how ecological populations and communities are structured and function (McGill et al., 2006). Trait-based strategies focus on functional traits, which represent morphological, biochemical, phenological, physiological, and behavioral characteristics of individual organisms that influence an individual's performance (Nock et al., 2001). These traits also play critical roles in determining ecological strategies (i.e., a combination of trait values that may be favorable in a given environment), the responses of species to changes in their environment, and their effects on ecosystem processes (Díaz et al., 2013). Classical ecological approaches have focused on the differences in mean values of species functional traits; however, ecologists are increasingly focusing on trait variation within species (i.e., variation among individuals) (McGill et al., 2006; Violle et al., 2007, 2014). This approach can lead towards a more integrated and mechanistic understanding of the processes shaping the identity and abundance of species within ecological communities (McGill et al., 2006; Violle et al., 2007; Yeakel et al., 2016).

Variation in trait values within species or among groups of species is constrained by the role of abiotic and biotic filters that act upon individual organisms through selection on their functional traits (McGill et al., 2006; Violle et al., 2007). The identity and strength of how the environment acts on functional trait diversity may vary across spatial scales and the trait under scrutiny and may induce shifts, expand or reduce the possible functional trait diversity of a given population (Kraft et al., 2014; McGill et al., 2006; Messier et al., 2010, 2017; Vilà-Cabrera et al., 2015; Yeager et al., 2017). For example, abiotic drivers such as climate (e.g., temperature and precipitation) vary at

broader spatial scales. They can affect trait distribution at regional scales, whereas fine-scale variation in environmental conditions (e.g., pH, nutrient availability) might become more critical at local scales (Messier et al., 2010).

Recent approaches in trait-based ecology have revisited Hutchinson's multidimensional-dimensional concept of the niche (Hutchinson, 1957) to characterize the ecological niche using functional traits as major axes of ecological strategy variation (Carmona et al., 2016; Kirmer et al., 2008). This multidimensional trait niche quantifies the functional diversity and the hypervolume space that is occupied by groups of individuals in a local population or in a regional pool of species. This approach has often been utilized to study the leaf economics spectrum: the multivariate correlations of chemical, structural and physiological traits that constrain plant ecological strategies (Díaz et al., 2016; Bruelheide et al., 2018). Although this approach has helped understand the ecological and evolutionary processes that shape plant functional diversity across environmental gradients (Lamanna et al., 2014), studies addressing intraspecific variation on functional traits across spatial scales are still rare.

Among functional traits, the elemental composition of living organisms (i.e., carbon (C), nitrogen (N), phosphorus (P), potassium (K), and sodium (Na)) shapes the ecology of living organisms and plays a crucial role in determining ecological processes from organismal to population to biogeochemical cycles (Sturner and Elser, 2002; Jeyasingh et al., 2014; Carmona et al., 2016; Leal et al., 2017; Meunier et al., 2017; González et al., 2017). Despite the growing recognition that a stoichiometric trait (Helsen et al., 2020) focus has the potential to enhance our understanding of individual and population responses, the patterns and drivers of stoichiometric trait variation and the

distribution of these traits across spatial scales are still poorly understood (González et al., 2017).

Here, we aimed to characterize the spatial variability of stoichiometric traits (C, N, P, K, Na) of pitcher plants (*Sarracenia purpurea*) across a geographical gradient. We assessed variation in stoichiometric traits to address the following two questions: (1) how does elemental trait variation of pitcher plants change across a geographical gradient, and (2) how does the functional trait niche of pitcher plants change along environmental conditions?

We hypothesized that the trait variation of populations across the geographical (environmental) gradient would reflect the potential of pitcher plants to adjust to local-scale environmental conditions. We expected that if pitcher plants adjusted to local conditions, then they should display variance in their traits across the space and that those traits should then be correlated to environmental conditions. Further, if local environmental conditions influence the functional trait niche of local populations, we expect to find a relationship between the niche volume and critical environmental drivers such as pitcher water volume or pH gradients, which have been found essential for pitcher plant populations (Hamilton, 2010). We expect more environmentally homogeneous bogs to have smaller niches than more heterogeneous bogs. Finally, if the functional trait niche responds to the environment, we expected larger hypervolume overlaps and shorter hypervolume centroid distances between pitcher plant populations (i.e., bogs) under similar environmental conditions.

Methods

Study model: Pitcher plants

The pitcher plant *Sarracenia purpurea* is broadly distributed across North America and has a range from Canada east of the Rocky Mountains to the eastern part of the United States from Maine to Georgia (Schnell, 2002). This pitcher plant is also the most common in North America. This perennial plant can live up to fifty years and produces about 6–10 new leaves each year; the leaves (that are in the shape of a pitcher) lasting about 1–2 years (Karagatzides et al., 2009). The pitchers of these plants fill up with water and are often inhabited by an aquatic, detritus-based food web consisting of bacteria, protozoa, and macroinvertebrates (Addicott, 1974; Butler et al., 2008). Pitcher plants typically obtain nutrients from their prey, but also obtain them through nutrient deposition by the precipitation that is collected in their pitchers (Hemond, 1983).

Pitcher plant sampling

During 2017 we conducted a snapshot survey, sampling eight bog sites located across New Jersey (Figure 1). At each bog, we randomly sampled six to ten non-flowering medium-sized pitcher plants (4–12 pitchers) that were more than one meter apart in distance. We measured the height of the pitcher from the base to the top and diameter of the opening (following Gotelli and Ellison, 2006). In addition, we measured in situ physical-chemical parameters of the plants, such as water pH, water temperature, dissolved oxygen concentration, conductivity on each pitcher before collecting. Once we finished collecting morphological and physical-chemical parameters, we carefully removed the entire pitcher (and water inside) and put into plastic bags and placed in a

cooler for freezing. The samples were taken back to the laboratory and placed in a freezer at -20°C for later analysis.

In the lab, we measured the actual leaf pitcher liquid volume (i.e., a proxy for habitat size; hereafter “habitat size”) and maximum water holding capacity of pitcher plants (volume in mL). The pitcher water was filtered (following Louca et al., 2017) and stored at -20°C for future chemical analyses (NO₃ and PO₄). We washed the whole plant to remove all accumulated detritus and macroinvertebrates with fine forceps (following Buckley et al., 2003).

Elemental analyses

The chemical composition of pitcher plant tissue was determined for each pitcher within plants from all sampled bogs. To characterize the elemental content of pitcher plant leaves, we measured carbon and nitrogen content using a CN analyzer (Carlo Erba NC2500) at the Cornell University Stable Isotope Laboratory. Total phosphorus concentration of leaves was determined through a standard Kjeldahl digestion procedure and were analyzed colorimetrically using the ascorbic acid method (American Public Health Association, 1985). Sodium and potassium concentrations were determined by atomic absorption spectrometry following the methods of (Allen, 1989; Tuininga et al., 2002). Elemental ratios (e.g., C:N) are expressed in molar ratios.

Data Analyses

We tested six environmental variables (water capacity, habitat size, water temperature, dissolved oxygen, pH, and water conductivity) for their differences among bogs using

linear mixed-effects models (LMMs). We used LMMs with site (i.e., bog) as a fixed factor and plants and pitchers nested within sites as a random factor. We then ran an ANOVA to evaluate the significance of the LMM output.

We summarized the six environmental variables using a principal component analysis (PCA) using the PCA function in the package *FactoMineR* (Lê et al., 2008), and we retained only the most important components (PC1, 2, and 3) for further analysis. Environmental variables were log-transformed and scaled before the PCA. We also tested for spatial autocorrelation in our data using Mantel tests, but Mantel tests indicated a lack of spatial autocorrelation (except for potassium) among sampling sites ($p > 0.05$; Supplementary Material, Table S1).

To evaluate pitcher elemental trait-environment relationships we used linear mixed-effects models (LMMs) with the PCA scores of the first, second, and third components as environmental predictors. For each elemental trait (e.g., C or N content) we run independent models in which we included the first three PCA axes as fixed factors while geographical scale was included in the models as a set of hierarchical nested random factors (bogs/plants). We could not include pitchers nested within plants in these analyses because we did not have enough degrees of freedom with which to estimate residual error. To build the models, we used the dredge function of the *MuMIn* package (Barton, 2020) to create a set of models with all combinations of variables. We generated 12 candidate models for each element using the function `lme` in the *lme4* package (Bates et al., 2015) in R. We tested the significance of our candidate models against a null model, which did not include the fixed effects, but retained the random effects for the LMMs. All models were fitted using maximum likelihood for model comparison. We

ranked models based on Akaike's Information Criterion (AIC), using the `AICcTab` function in the *bbmle* package (Bolker and R Development Core Team, 2020), and selected the models with $\Delta AICc \leq 2$, which were considered equally plausible. Model assumptions were assessed using residual diagnostics and quantile-quantile plots. Each best model parameters were estimated using restricted maximum likelihood, which provides less-biased random-effect variance estimates (Pinheiro and Bates, 2002; Bolker et al., 2009).

To quantify the extent of intraspecific variation of the stoichiometric traits across scales (within plants, plants in a bog, and among bogs) we performed a variance partitioning analysis. We log-transformed the trait data and performed a nested ANOVA using the `anovaVCA` function in the *VCA* package (Schuetzenmeister and Florian, 2019). For each level, the function first calculates the group means and then compares the variance of the group mean to the mean of the next level.

To explore how the elemental traits of pitcher plants correlate among each other, and to collapse traits into fewer dimensions, we performed a principal component analysis (PCA) on the traits, using log-transformed and scaled, pitcher trait values. We extracted the first three PCA axes to use on the stoichiometric hypervolume analysis. To quantify the size, shape, and centroids of the stoichiometric trait space of the different pitcher plant populations, we used the *hypervolume* package (Blonder et al., 2014, 2018a). This approach uses mathematical algorithms that infer multidimensional volumes assuming a Gaussian kernel density estimation and provides hypervolumes expressed by-product of the axis units, i.e., the fourth power of standard deviations (sd^3). Based on these estimates, the niche volume reflects the stoichiometric diversity of the population

(the stoichiometric traits of plants within a bog) and is represented by the space itself (González et al., 2017). For instance, a large niche volume would show high variation among the traits found within the population. To test whether niche hypervolumes respond to environmental drivers, we used general linear models (GLMs) with the niche volume of local populations as the response variable and the degree of variation in environmental conditions as predictors. To determine the significance of our models we compared them against null models fitted to a constant. The variation in environmental conditions was estimated by the standard deviation of any given environmental variable within each bog. We also estimated the contribution of each variable (trait) to the total hypervolume with the function ‘hypervolume_variable_importance’. The observed shape (and hence which trait(s) are driving it) can suggest if an individual has an increased allocation of one of the elements or a combination of elements to structural or physiological processes (González et al., 2017). For example, spherical niche shapes exhibit an equivalent influence of each axis to the overall variance in the stoichiometric composition of a species.

To evaluate the degree of similarity in functional trait niches, we calculated the percentage of overlap among niches (i.e., hypervolumes) as the ratio of the niche volumes between sites (Villéger et al., 2011; Brandl and Bellwood, 2014) using the *hypervolume* package and the Sørensen index. In addition to the hypervolume overlap, we calculated the distance between pairs of centroids, which represents a multidimensional average of the cluster of data (i.e., within a bog). Finally, to test whether the niche overlap and centroid distance relate to the degree of environmental dissimilarity across bogs we used Mantel tests. Larger niche overlaps and shorter centroid distances should indicate a

smaller environmental difference between bogs. All statistical analyses and figures in this paper were implemented and generated in R ver. 3.6.2 (R Development Core Team, 2019).

Results

Environmental Variation Across Sites

Among the six environmental drivers, five (pH, water temperature, dissolved oxygen, water capacity, and habitat size) significantly differed among bogs while only one did not (water conductivity) (Supplementary Material Figure S1). The PCA of the environmental variables revealed three significant axes of variation, with roughly 72.5% of variation explained by PC1, PC2, and PC3 (Figure 2). Along PCA axes, we observed a strong correlation between environmental variables. PC1, which comprised of water capacity, habitat size, and pH explained 32% of the variation among sites, while PC2 explained 22% (dissolved oxygen concentration) and PC3 explained 18% (conductivity and water temperature) of this variation.

Single Trait–Environment Relationships

Trait variation was significantly explained by environmental drivers. Overall, our results show that the candidate models performed better in predicting the pitcher elemental contents than the null models, except for Na. We found that the best fitting C model (based on AIC selection) included the interaction between PC1 and PC2 (model 9; Table 3). The variance explained by the fixed effects (marginal r^2) of the chosen model was 6.4 %, while the variance explained by both fixed and random effects (conditional r^2) was

42.3%. Our analyses showed that there were three candidate models that were based on PC2, PC3, and PC1 (models 3, 7, and 5); that best predicted N content. The candidate model that included an interaction between PC2 and PC3 was the candidate model (model 11) that best predicted P and K content and C:P ratios of pitcher plants (Table 3). Na content was predicted by six models, however, none of these models ranked significantly better than the null model. The C:N and N:P ratios both had four candidate models based on PC2, PC3, and PC1 that best predicted these elemental ratios. The P:K ratio was best predicted by three models (2, 5, and 6) that were based on PC2, PC3, and PC1, while the K:Na ratio was best predicted by six models. Although most of the candidate models selected included the additive or interactive effect of environmental characteristics as PCA axes, these were neither strong nor consistent predictors of trait variation across populations of *Sarracenia purpurea*.

Trait Variance Partitioning

All ten stoichiometric traits showed variation at different ecological scales (Figure 3). Most elemental traits (C, C:P, N:P, P:K) showed greater variation at pitcher level, with 24-58% explained variation found at this scale overall. In contrast, K, Na, and K:Na varied more at the plant level, while N, P, and C:N showed larger variation at the bog level.

Elemental Trait Correlations

The PCA of the overall stoichiometric trait space revealed three significant axes of variation, with roughly 82% of variation explained by the first three PCA axes (Figure 4).

Along PCA axes, we observed a strong trait covariance between P content, C:P ratios varying along PC1, which explained about 33.5% of the variance of the trait space. K, P:K, and K:Na varied along PC2, explaining almost 33% of the variance of the trait space. PC3 explained 15.5% of the variance was comprised of C, N, Na, C:N, N:P (Table 2, Figure 3). The overall shape of the hypervolume, however, was driven by PC2, which correlates to K, P:K, and K:Na. Niche shape was itself was elliptical as K:Na and K correlated together and negatively correlated with P:K. The PCA for individual sites showed different trait correlations (Supplemental material Figure S2) across sites. The key driver in the majority of hypervolume niche across sites was either P or K and K:Na, while only one site (BHB) driven by C content. Trait correlations also changed across sites, with site HEL showing a positive correlation between C:N and C:P, site WMB displaying a negative correlation between P, N, and N:P and them negatively correlated with C:P and C:N. Finally, site TP showed a negative correlation between C and P:K. The total trait variation explained by the first three PCA axes also changed at the bog level and ranged from 78 to 94%.

Trait Functional Space-Environment Relationships

The results from the stoichiometric trait hypervolume approach showed that the stoichiometric space varied among bogs (Figure 5). Site HP had the largest hypervolume size, while site WL had the smallest. The analyses looking at the relationship between hypervolume size and the variation in environmental predictors showed that there were only two environmental variables (water capacity and habitat size) that had a significant effect on the trait hypervolume (Table 4, Figure 6). The amount of overlap among trait

hypervolumes of pitcher plant populations was variable, ranging from 39 to 76% with sites PP-TP having the largest amount of overlap and sites HP-WL having the least (Figure 7a). The sites that had the largest centroid distance were HP-PP, while the smallest distance between sites was between TP-WMB (Figure 7b). The hypervolume overlap was highly correlated to environmental variables with overlap showing a significant association with all six environmental drivers (Table 5). The stronger correlation was observed between overlap and water capacity ($r = 0.626$, $p < 0.0001$). Environmental factors had little influence on the centroid distance between bogs as only two environmental variables were significantly correlated (water capacity and water temperature) to the centroid distances (Table 5).

Discussion

In this study, we quantified the relationships between elemental traits and environmental variables, as well as the stoichiometric niche space of pitcher plant populations, *Sarracenia purpurea*, focusing on multiple elemental traits and the stoichiometric trait hypervolume approach. We found that within a constrained geographical region, the bogs of New Jersey showed significant variation in environmental conditions (e.g. pH, water capacity, habitat size, water temperature, and dissolved oxygen). These environmental differences translated into changes in functional trait values at the individual and population level and thus affected trait diversity and trait hypervolumes of pitcher plants.

Trait variation at different scales and elemental trait correlations

We found that intraspecific variability accounted for a large proportion of the population level variation in leaf elemental traits. Variation in most traits occurred within an individual plant (among pitchers) and among plants was greater than the variation found among sites. We observed that elemental traits vary in different degrees across the geographical gradient, with N:P, C, and C:P being the most variable traits and P and C:N being the least variable traits across the geographical gradient. These results are in agreement with Isaac et al., (2017) who found that C:N was one of their least variable plant root traits. Further, our results do not agree with Sterner and Elser, (2002) and Lemmen et al., (2019) who found more variation in P content than in C and N content across populations, in contrast, we found more variation in C and N than P content. Large variation in C and N contents could be because nitrogen and carbon fluxes within the pitcher plants are the basis of the interactions between the plant the aquatic community

that inhibits them (Mouquet et al., 2008), and this tends to vary across pitcher plant populations (Mouquet et al., 2008). However, N variation could potentially also be explained by the presence of nitrogen fixing bacteria inhabiting the pitcher water (Prankevicius and Cameron, 2011). Nitrogen fixing species have been seen to display an intermediate amount of intraspecific variability of N content (Tautenhahn et al., 2019). Our study also showed that elemental traits vary across spatial scales with C content being highly variable within plants, N content varying more among plants within bogs, while P content varied more among plant populations (i.e., bogs). These results are in agreement with studies that found larger trait variation at smaller scales within traits because they are more likely to be affected by local factors (Karbstein et al., 2020; Messier et al., 2017; Stark et al., 2017). Further, plant functional stoichiometry appears to reflect the outcome of individual elements' changing in concert as a function of environmental conditions (Ågren and Weih, 2012). These results support the idea that plant stoichiometry is limited by the availability of nutrients, and that particular nutrients may limit the uptake of other elements (Güsewell, 2004, Ågren and Weih, 2012). Several lines of evidence have shown that although plants have a specific elemental composition, there is a high degree of flexibility in their stoichiometry along environmental gradients (Güsewell, 2004; Ågren and Weih, 2012; Sardans et al., 2016). Further, previous studies have shown that *Sarracenia purpurea* may be exhibiting higher stoichiometric flexibility in responding to environmental changes (Urbina et al., 2015), which is consistent with the findings from our study.

The trait-environment relationship

Individual traits and trait hypervolumes varied significantly along the environmental gradient, but the magnitude of that variation was highly dependent on the trait. We hypothesized that the trait variation of pitcher plant populations across the environmental gradient would reflect the potential of pitcher plants to adjust to local-scale environmental conditions and display trait variance in response to environmental conditions. Although we did find significant effects of environmental variables on pitcher plant traits (except for Na), environmental drivers were not strong predictors of trait variation. These results contrast with previous findings of trait variation predominantly being driven by local environmental factors (Bernhardt-Römermann et al., 2011; Bruelheide et al., 2018; Frances et al., 2020). This could potentially be because of the environmental drivers that we focused on. Although the environmental drivers we studied have shown to play an important role in pitcher plant biology (Hamilton, 2010; Bazile et al., 2015), these might not be the best drivers of pitcher plant elemental content. It might have been more informative to look at drivers such as precipitation (Han et al., 2011) or water-dissolved nitrate and phosphate in the bog (Bott et al., 2008) that have shown to heavily influence elemental trait variation such as N, P, and K (Sardans and Peñuelas, 2014). These drivers could be important to include because *Sarracenia purpurea* can acquire diverse nutrients directly from rainfall that collects in its pitchers, from the decomposition of the prey (Ellison and Gotelli, 2002) as well as from their roots (Bott et al., 2008).

Trait Functional Space-Environment Relationship

We hypothesized that more homogeneous bogs would display smaller niche sizes than more heterogeneous bogs. Bogs that were more similar in their local environments did have more similar hypervolume sizes than bogs that were more heterogeneous when viewing the results of the general linear models (GLMs). Indeed, functional trait variation within populations is most likely to respond to local habitat heterogeneity (Karbstein et al., 2020). Multitrait approaches by delineating niche hypervolumes have shown to be better descriptors of the overall niche, ecological strategies of populations such as plasticity, and adaptations to local environments (Skálová et al., 2012; Blonder et al., 2018b; Scherrer et al., 2019). We found that the overall niche size was only significantly affected by heterogeneity in water capacity and habitat size, which are essential for *Sarracenia purpurea* populations because these plants directly acquire more nutrients from rainwater (specifically N) than that from prey, which only accounts for 10% of the plant's nutrient budget (Chapin and Pastor, 1995; Ellison and Gotelli, 2001; Hamilton, 2010). A larger niche hypervolume corresponds with larger functional trait diversity, which has often been related to water availability (de la Riva et al., 2018; Fyllas et al., 2020). Water availability is important in pitcher plants as the amount of water in these plants directly relates to their ability to obtain nutrients. As *Sarracenia purpurea* can acquire nutrients directly from rainfall that collects in its pitchers, but also water volume within pitchers determines the diversity of the food web inhabiting pitcher plants, which is directly responsible for nutrient cycling (Ellison and Gotelli, 2002). These findings support our results, which highlight the importance of the heterogeneity in environmental conditions on the size of the niche hypervolumes. Our results also showed the key role of

environmental drivers on the degree of trait niche overlap and centroid distances. These findings partially support our predictions on larger hypervolume overlaps between local niches under similar environmental conditions, although centroid distances only responded to a few environmental drivers. These results are in agreement with previous studies showing that plant populations increase niche overlap and decrease centroid distances in response to similarity in environmental drivers across space (Mao et al., 2018). High overlap provides evidence that the ability to capture resources is enhanced by convergence on an ‘optimum’ trait value (Mouillot et al., 2007; Mason et al., 2011). Further, high degrees of overlap would be expected among populations of *Sarracenia purpurea* sharing similarities in the resource quality they have access to (Jeyasingh et al., 2014).

Broadly, this study demonstrates the importance of small-scale environmental conditions in driving within and across population functional trait variation. Our results indicate that within-population trait variation can represent a large component of the observed trait variation and can be driven in part by local environmental drivers. Our findings also highlight that together with shifts in mean trait values, approaches that involve multiple traits and that characterize niche hypervolumes in relationship to environmental drivers, help representing a more integrated view of plant phenotypic variation and ecological strategies, and may be key to understand the response of plants to changes in environmental conditions.

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