Discerning Differences in the Plant Traits of the Floral Communities of Mt. Washington, NH, by Incorporating Intraspecific Variability Analysis

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**Abstract**

Plant functional traits allow ecologists to assess the ways floral communities respond to abiotic and biotic factors. By analyzing these traits, we can then ultimately assume the factors that control species distribution and community composition. Here, I analyzed plant traits (plant height, leaf area, specific leaf area, and leaf dry matter content) of four herbaceous plants found growing atop Mt. Washington, NH in sheltered snowbanks. They are also found in the sub-alpine understory. I examined baseline differences between the alpine and sub-alpine sites, analyzed differences in intraspecific variability, and also measured the sub-alpine sites' trait differences associated with canopy closure (light availability). Comparing plant traits along this elevational gradient, from alpine to sub-alpine using measures of intraspecific variability, allows us to investigate any underlying effects. These include differences in air temperature, light availability, and solar radiation. As a result, compared to the sub-alpine, we observed lower SLA, smaller leaf area, and higher LDMC in the alpine snowbed. Further analysis of the sub-alpine with a comparison of light availability also revealed differences in SLA, LDMC, and leaf area for some species. Overall, intraspecific variability detailed each populations' underlying response to environmental conditions. This approach will be critical to continue studying in terms of expected environmental changes to occur in the region.

**Introduction**

In plant ecology, a central goal is to understand and ultimately predict the factors that control species distribution and community composition (Jiang and Ma 2015). Plant functional traits have been used in studies to do just that, typically at a species-specific level. Traits are useful indicators for ecosystem function and environmental changes (Kichenin *et al.* 2013). When environmental conditions change, dominant functional traits in the plant community will

change as well (Jiang and Ma 2015).  Not only do they represent environmental strategies, they also directly influence species interactions.  This establishes plant traits as fundamental drivers of community assembly (Luo *et al.* 2016).

Variability among plant functional traits is often determined in order to analyze plant communities further.  Specifically, intraspecific variation represents the variation within a species' trait values compared to interspecific variation, which is the variation among species' mean trait values in a plot.  Intraspecific variation is increasingly useful in scientific studies as an indicator for underlying processes of plant responses to the environment and competition. Intraspecific shifts in trait values may be reinforcing interspecific patterns of trait convergence, which makes it an important factor to differentiate when assessing plant communities (Siefert 2012).  It also could be influencing community stability, assembly, and function (Albert *et al.* 2010).  A greater influence of intraspecific variability indicates greater resistance to both environmental changes and plant community structure (Kichenin *et al.* 2013), which with expected changes to the current populations being studied, reinforces an analysis of intraspecific variation.

There are a number of ways that trait-based studies can be carried out in order to predict patterns for an unmeasured area.  One method is evaluating traits along an environmental gradient.  These are measured by abiotic factors including, nutrients, rainfall, light, or elevation. An elevational gradient in particular can be described as an indirect gradient, one that induces variation on direct factors such as air temperature, length of growing season, and solar radiation (Fontana *et al.* 2017).  Indirect gradients therefore may mask ecological patterns because they do not directly affect functioning and plant physiology (Fontana *et al.* 2017).  However, an elevational gradient proves to be a strong gradient because it offers a stark contrast in

environmental conditions (Luo *et al.* 2016). Strong environmental gradients often provide a unique opportunity to analyze intraspecific variability of plant traits. Intraspecific variation, when accounted for, may detect the underlying short-term effects environmental gradients that are likely to induce community response through intraspecific variability caused by phenotypic plasticity of traits (Luo *et al.* 2016). Short-term effects include environmental conditions such as drought or flooding events. Because of this, it is important to understand these gradients further to understand their impact on community composition and function.

Many herbaceous plants being measured in the current study are found to be dominant in the snowbank communities atop Mt. Washington. These particular species have found refuge in these depressions sheltered from inclement weather conditions more than adjacent areas. Specifically, they experience similar microenvironments to the lower elevation populations given their protection provided by deeper snowpack and higher summer temperatures (Bliss 1963). These snowbank communities are the richest floristically containing species found in neighboring dwarf shrub heath but also the meadows and forests at lower elevations. 40% of species in the alpine zone are restricted to these areas and 84.7% of these species are vascular (Bliss 1963). Overall, comparing the alpine snowbed to the sub-alpine understory is advantageous to understand in the face of expected changes due to climate change. This has not been thoroughly examined in the Northeastern U.S. thus is it necessary to do so since the alpine zones on the mountains in the region are rather small in comparison to elsewhere and deserve scientific understanding.

Light is related to many of the traits being studied so it may be a considerable factor to explain any patterns of intraspecific variation in the sub-alpine. Some plants exposed to low levels of light availability may display a more conservative set of traits for acquiring and

maintaining resources (Laughlin *et al.* 2011). Specifically, the prevailing view is that plants in low light environments will show leaf traits that enhance light capture or carbon gain e.g. increased leaf area, specific leaf area, and/or will also show traits that minimize carbon loss e.g. increased leaf toughness, and increased leaf dry matter content (Gianoli and Saldaña 2013). Thus, it is important to examine variation of traits associated with shade tolerance and light availability at the intraspecific scale, in similarity with environmental gradients, in order to detect the selective processes involved in the interspecific patterns of trait variability (Gianoli and Saldaña 2013). Given that the alpine receives nearly full sun when un-impeded by clouds, it will be useful to use light as an explanation to the degree of variability that may be observed.

In the current study, plant trait variability was examined along the elevational gradient spanning the alpine and sub-alpine plant communities of Mt. Washington, NH. The four target species were measured for functional traits, with a focus on specific leaf area (SLA), leaf dry matter content (LDMC), plant height, and leaf area, as these all vary in relation to environmental changes (Kichenin *et al.* 2013). The first hypothesis tested was that alpine and sub-alpine plants will display different trait values, specifically alpine plants will have smaller and thicker leaves, shorter height, lower SLA, and higher LDMC. This will give insight to any baseline differences between communities. The second hypothesis examined was that intraspecific variability will be greater in the sub-alpine due to increased factors of variation. A third hypothesis then tests that in the sub-alpine, due to varying light levels, plants exposed to less light will be taller, display thinner and wider leaves, and higher SLA. This may explain some of the variation seen in the sub-alpine.

**Methods**

Study area:

Mt. Washington is a part of the White Mountain National Forest, with Mt. Washington State Park at the summit (1916 m). It boasts the largest alpine zone in the northeastern United States, with treeline ending around 1500 m. The sub-alpine communities were located around the Appalachian Club's Pinkham Notch Visitor Center on the southeast slope of the mountain. The sub-alpine sites did not exceed 823 m in elevation in order to retain lowland community composition. Elevation averaged at 664 m across all six sub-alpine sites. In the alpine, five snowbank sites were also sampled. Sampling occurred mainly in the Alpine Garden, which lies on the southeast face of the mountain. Both alpine and sub-alpine sites were marked using a handheld GPS unit (Garmin GPSMAP®64, Garmin Ltd., Olathe, KS). Sites were determined primarily by species composition, though no quantifications of abundance or richness were taken. All four target species (*Chamaepericlymenum canadense, Clintonia borealis, Coptis trifolia, Maianthemum canadense*) were present. Other plants in the understory included various rushes and sedges, hobblebush (*Viburnum lantanoides*), *Dryopteris spp.,* and starflower (*Lysimachia borealis*). In general, the canopy of the sub-alpine is dominated at some sites by American beech (*Fagus grandifolia*) and sugar maple (*Acer saccharum*). Yellow birch (*Betula alleghaniensis*), paper birch (*Betula papyrifera*), balsam fir (*Abies balsamea*), striped maple (*Acer pensylvanicum*) were also quite dominant at other sites typically at the expense of one another, though *A. saccharum* remained abundant throughout. Red spruce (*Picea rubens*), American mountain ash (*Sorbus americana*), and showy mountain ash (*Sorbus decora*) were present at some sites. Saplings of these canopy species also comprised the understory

vegetation.  Boundaries were set with transect tape and flags marking corners with any side no shorter than 5m to ensure sufficient sampling size.

Canopy Closure:

A canopy closure estimate was conducted at each sub-alpine site using a spherical densitometer.  Canopy closure was not estimated at the alpine sites because they receive virtually full sunlight.  Sites chosen displayed relatively open canopies in terms of canopy cover, which was important to retain uniform abiotic factors such as light in this case, but also slope, soil characteristics, water availability.  Canopy closure, in contrast to canopy cover, was estimated as it is better linked to ecological concepts such as canopy architecture, light regimes, and solar radiation (Paletto and Tosi 2009).  Measurements were taken at the relative center of the site with four estimates facing North, East, South, and West.  All squares on the surface of the densitometer are broken into 'quarters' or 'dots', with a dot representing a portion of discernable sky, totaling 96 dots. Holding the densitometer level and away from the body so the viewer was not in frame, dots that were composed of open canopy were added up, multiplied by 1.04 to obtain the percent not occupied by foliage, then subtracted from 100% to obtain the canopy closure estimate (Paletto and Tosi 2009).  The four estimates were averaged to retain the canopy closure percentage for the site.

Plant Sampling – Field

For five of the sites, three individuals of the target species were taken for sampling.  *M. canadense* is an exception, since vegetative *M. canadense* only grows one leaf.  Thus six individuals of the species had to be sampled.  The sixth site, referred to as the 'megasite', was sampled for 15 individuals of three of the target species, and 30 individuals of *M. canadense*.  To

measure height, I began from the relative center of the site, and utilized a random number generator to conduct random sampling in respect to amount of steps taken and direction. Whichever individual was closest was sampled, care always taken to not crush vegetation multiple times.  Height was measured in mm using a ruler.  The next individual to be sampled derived from the previous individual's location in order to prevent measurement confined to one area of the site.  *M. canadense* is the exception, where once one individual (A) was sampled, its counterpart (B) was the closest individual, again since vegetative *M. canadense* only grows one leaf.  Individuals that were sampled were dug out to retain some roots using a trowel.  The exposed root system retained some soil, was wrapped in a paper towel, and blotted with water (Cornelisson *et al.* 2013).  They were then stored in individual plastic bags, not fully closed, and placed in a cooler with some ice to retain freshness.

Lab – Trait measurement

To obtain measurements for all other traits besides height, plants were brought into the lab and stored at approximately $4^0$C until sampled, no longer than 24 hours to retain freshness (Cornelisson *et al.* 2013*).* Each individual was sampled for two of the youngest, fully-expanded, undamaged leaves, clipped at the base of the leaf, blotted dry, and massed on a digital balance to obtain its wet mass.  *M. canadense* was the exception where two individuals represented 'one plant', though leaves were not summed when measuring wet and dry mass because they were from two different individuals.  All traits for species besides *M. canadense* were averaged between both leaves of the plant, whereas in *M. canadense* they all represented two separate individuals.  Using a CanoScan LiDE 110, leaf scans were analyzed for leaf area, length, width, perimeter, and circularity using Image J (v. 1.51, National Institutes of Health, Bethesda, MD). Leaves were then placed in coin envelopes and stored in a drying oven for approximately 48

hours at $70^0$C. After drying, the individuals were measured for dry mass. While measuring, the remaining individuals were placed in a desiccator to ensure no water uptake was occurring. SLA values were obtained by dividing dry mass (g) from leaf area ($mm^2$). LDMC values were calculated by dividing wet mass from dry mass.

Data Analysis

To test the first hypothesis, that sub-alpine and alpine plant traits will differ from each other, two-sample t-tests were conducted. The tests compared individuals of species and an associated trait across populations. Data was log-transformed prior to analysis to fit normality. Phenotypic Plasticity Index (PPI) was calculated to quantify intraspecific variation in regards to the second hypothesis. PPI is represented by subtracting the minimum trait value of a species from the maximum trait value of a species, and dividing maximum trait value of a species ((max-min)/max). Two sample t-tests were then conducted in order to compare the alpine to the sub-alpine, disregarding site as only one PPI value per trait by species could be generated. The significance level for analysis of results was $p \leq 0.05$. To test the third hypothesis, that trait values of sub-alpine plants will display different values in response to light, a one-way ANOVA was conducted first, with $p \leq 0.05$ indicating significance. Tukey's post-hoc tests for pairwise comparisons was then utilized to detect specific differences across sites of significant ANOVAs. ANOVA analyses were conducted in Minitab (v. 18 Minitab, Inc., State College, PA). Data was log-transformed prior to analysis to fit normality. Linear regression was then utilized to test if trait values were the result of canopy closure, testing each species' trait to the canopy closure estimate at each site, again with $p \leq 0.05$ as the significance level.

**Results**

*Alpine and sub-alpine differences*

For all four species when testing for differences in plant height, there was no significant differences found as a result of the two-sample t-tests.  For SLA, *C. canadense* (t-stat=9.89, p<0.0001) and *M. canadense* (t-stat=3.98, p<0.0001) both displayed significantly larger values in the sub-alpine than the alpine (Fig. 1).  For LDMC, *C. canadense, C. borealis,* and *M. canadense* all displayed significantly lower LDMC averages in the sub-alpine compared to the alpine (t-stat=-3.59, p=0.0019; t-stat=-4.57, p<0.0001; and t-stat=-3.02, p=0.0059 respectively).  *C. trifolia* displayed the opposite, as the alpine LDMC values were significantly larger than the sub-alpine average (t-stat=7.58, p<0.0001).  For leaf area, *C. canadense*, *C. borealis*, and *C. trifolia* all displayed significantly wider leaves in the sub-alpine (t-stat=6.65, p<0.0001; t-stat=-4.52, p<0.0001; t-stat=3.84, and p<0.0001 respectively) (Fig. 1).  Although not significant, *M. canadense* showed the opposite in that leaves were wider in the alpine.

Species were generally more plastic (showed more variability) in the sub-alpine rather than the alpine as a result of two sample t-tests comparing PPI values, but differences were not significant (Table 2).  Noting specific differences in plasticity between species, *C. trifolia* and *M. canadense* showed more plastic responses in plant height in the alpine compared to *C. canadense* and *C. borealis*.  However, the latter two species demonstrated higher SLA PPI values in the alpine compared to *C. trifolia* and *M. canadense*.

*Light availability comparison among sub-alpine sites*

Table 1 shows canopy closure estimates listed in increasing order across sub-alpine sites. There were no significant differences in plant height for all species (Fig. 2).  For LDMC, *C.*

*canadense* values were greater at sites with greater canopy closure (F-stat=4.68, p=0.004) (Fig.

3). *C. borealis* also showed the same trend but no sites were significantly different. *C. trifolia*

showed no patterns and sites did not significantly differ from each other. *M. canadense* was the

same in that no patterns were detected, though sites were significantly different from each other

(F-stat=6.54, p<0.001). For SLA, *C. borealis* showed significantly lower values at sites that

were characterized by greater canopy closure (F-stat=4.53, p=0.005) (Fig. 4). *C. trifolia* also

showed significant differences across sites (F-stat=3.28, p=0.023), with a slight decrease in SLA

values at sites with greater canopy closure. *M. canadense* demonstrated significant differences

between sites (F-stat=11.25, p<0.001), but no discernable implications can be made from sites.

For leaf area, species generally displayed larger leaf area values at sites with greater canopy

closure (Fig. 5). Differences were significant across sites for *C. borealis* (F-stat=5.21, p=0.001).

Regression analyses were conducted testing canopy closure against species' trait values

in the sub-alpine (Table 3). Generally, plant height for all species besides *M. canadense*

displayed a direct relationship with canopy closure. *M. canadense* displayed the opposite. All

relationships were not significant for plant height. For all species, SLA values decreased in

response to increasing canopy closure. This trend for *C. borealis* was statistically significant

(F=12.75, p=0.023). For all species besides *M. canadense,* LDMC values increased with

increasing canopy closure, though again relationships were not significant. For leaf area, all

species besides *M. canadense* displayed a slight direct relationship with canopy closure,

however, relationships were not significant.

Regression analyses of canopy closure against PPI values were also conducted (Table 4).

Increasing canopy closure tended to elicit less plastic responses from species (low PPI values) in

terms of plant height, significantly in *C. borealis* (F=17.78, p=0.01). For SLA, besides *C.*

*canadense*, increasing canopy closure typically showed less plasticity from species, significantly in *C. trifolia* (F=11.83, p=0.03).  For LDMC, besides *C. borealis*, a slight decrease in plasticity was seen with increasing canopy closure.  For leaf area, another slight decrease in plasticity was seen with increasing canopy closure.  Apart from the mentioned significant trends, relationships between PPI and canopy closure were not statistically significant.

**Discussion**

*Alpine and sub-alpine differences*

The first objective within this study was to evaluate the baseline differences between the alpine and sub-alpine environments by comparing plant functional traits between the communities.  From the results, our initial hypotheses comparing the two populations was generally supported.  In the alpine, plants displayed lower SLA values, higher LDMC values, and smaller leaf area.  Smaller leaf area represents energy investment into structural support and also a reduction in light capture, as plants in the alpine receive nearly full light during the growing season.  SLA is often related to resource availability and energy investment.  Species with lower SLA values tend to have thicker/higher density leaves, correlating to leaf investments to structural defenses (Wright *et al.* 2001).  Thus, species with low SLA are likely to have a long leaf lifespan because such structural reinforcement is less susceptible to herbivory and physical hazards (Wright *et al.* 2001).  This trend is reinforced within our own study as a tradeoff – low SLA and high LDMC – is seen within the alpine population.  Higher LDMC values represent an individuals' ability to be more resistant to physical hazards and associated with less productive/resource poor environments (Cornelissen *et al.* 2003).

Species that display this type of tradeoff (low SLA/high LDMC) can be described as conservative species that imply individuals have dense leaf tissues and low growth rates, thus conserve resources (Albert *et al.* 2010). This describes the alpine populations. On the other end, there are exploitative species (high SLA/low LDMC) that corresponds with high photosynthetic rates and high resource acquisition, which defines the sub-alpine (Albert *et al.* 2010). However, the alpine and sub-alpine populations are not known to be members of different species that the resource strategies imply. This point requires further study to explore the differences found here, as they could be determined by phenotypic plasticity or genetic divergence of the two populations.

It was interesting to find no significant differences in plant height across populations. The alpine snowbed communities, comparatively across the alpine zone in general, are relatively resource-rich as they thrive off late snowmelt and retain more nutrients from decomposition processes (Björk and Molau 2007). They are also exposed to nearly full sun. However, they still experience extreme weather events, most notably wind, even though they are more sheltered compared to surrounding alpine communities. High wind typically selects for adaptations such as tough, thick leaves and short height, as wind can damage high structures, taking away heat and moisture at the same time (Bliss 1963). Although the results do not support the hypothesis, it does reflect the environmental conditions that each population is exposed to. We may be seeing a trade-off here between taking advantage of available resources in such a short growing season, especially full light, and protecting themselves from harsher conditions.

*Coptis trifolia* displayed opposite trends compared to all other species. It was found to have lower SLA values and higher LDMC values in the sub-alpine. The small, herbaceous plant retains two types of leaves, one evergreen leaf that is typically darker and leathery, as it is

sustained in winter, and two new growth leaves that are more delicate and brighter. There was

no discrimination between these leaves when sampling, thus this may have altered the results. It

may also be due that in the sub-alpine, *C. trifolia* is often found growing underneath other

vegetation. Since the species displayed higher plant height and larger leaf area in the sub-alpine,

this may be compensating for resource acquisition loss experienced through greater competition

in the sub-alpine. Larger sample size and selection of new growth leaves may reduce the

variability found.

*Intraspecific variability analysis*

Phenotypic plasticity indices (PPI) provide insight to plasticity, as higher values (close to

1) indicating that a species is able to grow in a number of conditions. Overall, it was seen that

species' traits were more plastic in the sub-alpine, which supports the hypothesis. It is

interesting to break down each trait specifically, as there are certain traits that displayed trade-

offs in plasticity due to environmental conditions. Particularly, looking at plant height, SLA, and

leaf area, plasticity varies from species to species. In both *C. canadense* and *C. borealis*, higher

PPI values are seen in the alpine for SLA and leaf area, and higher PPI for height in the sub-

alpine. On the other hand, for *C. trifolia* and *M. canadense*, higher PPI values are seen in the

sub-alpine for SLA and leaf area, and higher PPI for height in the alpine. This does not mean

that the actual trait measurements for these species represent taller plants or wider leaves, as

discussed in the previous comparisons. These values seem to indicate a trade-off in plastic

responses, as *C. canadense* and *C. borealis* are able to be more plastic to enhance light capture

by leaf size in the alpine, rather than height. *C. trifolia* and *M. canadense*, since they typically

are found growing underneath all vegetation even in the alpine snowbed, appear to be more

plastic to enhance light capture through height in the alpine, rather than through altering leaf size.

PPI accounts for intraspecific variability since it is analyzed within species. The intraspecific trait trade-offs determined here have been measured in other studies but prove to be less profound than trade-offs between species (Albert *et al.* 2010). However, intraspecific variability is an important influence on trait convergence suggesting that species are not just filtered by their community mean values (Siefert 2012). Intraspecific variability has been shown to account for upwards of 50% of variation along elevational gradients (Luo *et al.* 2016). Supporting results from Luo *et al.* (2016) also showed that plant height, along with leaf chemical traits, are more plastic than leaf physical traits (SLA, LDMC, leaf area) in sub-alpine forests. This limited range in plasticity among plant height in the alpine also represents the aboveground stress plants face including low temperatures, high wind, high UV radiation, and water limitations (Luo *et al.* 2016). Overall, intraspecific variability due to genetic variation and phenotypic plasticity is important to evaluate. These intraspecific responses and trade-offs have the potential to affect functional composition and assembly, which in turn influence ecosystem process such as productivity and nutrient cycling (Siefert 2012). Sampling strategy and choice of functional traits that are analyzed both prove to be essential when studying communities along gradients for influences of intraspecific variability (Albert *et al.* 2010; Jiang and Ma 2015). It is very important to narrow down environmental factors and plant functional traits when analyzing for the influence of either inter- or intraspecific variability as traits reflect the environmental conditions, thus making it possible to determine community processes such as environmental filtering or niche differentiation. Continued research on this topic is recommended for a variety of environmental gradients, as climate change threatens to alter the environmental conditions

currently, and to understand whether intraspecific shifts represent plastic responses or genetic variation.

*Canopy closure as an explanation to intraspecific variation*

For *C. borealis,* canopy closure is responsible for eliciting less plastic responses for plant height. Given the increase in plant height seen for this species with increasing canopy closure, it is indicative that greater height is required for growth in low light environments, and that trait convergence to taller plants within this species may be occurring. It can also be determined that for *C. trifolia*, increasing canopy closure is responsible for less plastic responses regarding SLA. SLA values generally decreased with increasing canopy closure, thus it may be that trait convergence to low SLA is also occurring in high light environments for this species. Calculating PPI values as a way of intraspecific variability analysis enabled the detection of these trends, whereas if just an interspecific approach was used with community means, no underlying trends would have been detected.

Yielding mostly not statistically significant differences of traits among sub-alpine sites, it can be concluded that our results do not fully support the third hypothesis. Supporting results show that with greater canopy closure, plants show greater height and wider leaves. However, it was found that plants had lower SLA values in areas of greater canopy closure. Light availability was measured because of its high influence and importance as a driver of changes of community composition and functional traits along gradients (Jiang and Ma 2015). Canopy openings also tend to influence other abiotic factors as well including increased nutrient availability, which tend to stimulate the growth of fast-growing herbaceous plants (Pages *et al*. 2003). All species besides *M. canadense* displayed slight increases in plant height and leaf area with increasing canopy closure. This shows that they are investing energy into enhancement of

light capture and carbon gain, as taller species are better at acquiring light (Laughlin *et al.* 2011), and species with wider leaves are also known to harvest light more efficiently (Bangguo *et al.* 2012). Lower SLA values and higher LDMC in response to increasing light represents a structural tradeoff in which a taller plant and wider leaves require more support from structural parts of the plant (Bangguo *et al.* 2012). However, these results cannot be completely attributed to just canopy closure since regression analyses were mostly not significant. Light availability is just one measure of the abiotic environment that affects a community, as soil nutrient content, elevation, slope, water availability, and temperature could also all be measured. The limited range of canopy closure estimates (~15% difference between min. and max. values) could also be contributing to a lack of significance, though for purpose of replication within the sub-alpine when comparing to the alpine, it was a goal to keep abiotic conditions rather similar.

*Climate change and conservation*

A common understanding of primary changes to occur in the alpine area is the upslope shift of climactic conditions (Gonzalo-Turpin and Hazard 2009), and with that, the encroachment of sub-alpine species into alpine areas. Because of this, alpine areas in the Northeastern United States are listed as 'critically imperiled', though also due to their rarity in general. With this shift, expected changes include higher average temperatures, earlier melt dates, decreased snowpack, and more precipitation falling as rain (Rawlins *et al.* 2012), further affecting abiotic factors in the sub-alpine.

Both the snowbank communities studied and the sub-alpine understory are important communities as they serve as indicators of environmental changes (Björk and Molau 2007; Bangguo *et al.* 2012). As mentioned, plant functional traits are the measurements that reflect species responses to biotic and abiotic factors, including temperature, moisture, soil nutrients,

and light availability (Amatangelo *et al.* 2014).  Plants that experience higher levels of intraspecific variation along elevational gradients are expected to show greater resistance to climactic changes (Jiang and Ma 2015).  Therefore, utilizing plant traits to anticipate plant responses to climate change has been noted as a crucial part of scientific study (Gonzalo-Turpin and Hazard 2009).  Phenotypic plasticity is also very important in terms of long-term adaptive potential because it allows species to rapidly respond to environmental changes (Gonzalo-Turpin and Hazard 2009).  The snowbank species studied here show a degree of plasticity as they can grow in each environment.  From this, it can be predicted that the species studied will continue to be successful amidst expected changes to the alpine zone.

While these herbaceous species may continue to inhabit the alpine zone in their current snowbed communities through expected changes, a different situation may be occurring for their current understory, sub-alpine populations.  There are expected changes for all abiotic factors including light availability, water availability, soil nutrients, and temperature.  In terms of water, the Northeastern U.S. will experience longer growing seasons but limited rainfall, and with reduced snowpack and warmer temperatures in winter, this provides less water to supply the water tables and streams (Rustad *et al.* 2012).  Because of this, water intake and evaporation will increase by plants, thus reducing soil moisture and prolonging droughts.  Drought will further reduce forest productivity and also increase its susceptibility to insects and disease (Rustad *et al.* 2012).  These changes have been already seen in the nearby Hubbard Brook Experimental Forest.  Specifically, annual air temperatures have increased within the range of 0.17°C–0.29°C per decade, attributed more to warming in winter rather than summer (Groffman *et al.* 2012).  Long-term measurements of snowpack have also experienced a decrease, roughly 4.8 cm per decade.  It is projected that the annual and winter precipitation levels are to increase 7%–14%

and 12%–30%, respectively, by 2100 (Groffman *et al.* 2012). Measured in the current study, one advantage with expected changes will be to have lower SLA values (thicker leaves with higher carbon investment), as this allows increased resistance to water loss during periods of drought (Royal Botanical Gardens 2017). The alpine communities and the sub-alpine individuals at low light level sites displayed lower SLA values, indicating that they may be more equipped for anticipated prolonged droughts. Because of this, it will be important to continue monitoring plant traits because of their ability to reflect environmental changes. It is recommended to monitor other suites of traits not included in this study, including nitrogen, carbon, phosphorus content, and respiration rates, in order to understand responses related to nutrient and water changes.

In regards to forest composition and light availability changes, the Northeastern U.S. is expected to experience the expansions of southern range tree including Red Oak (*Quercus rubra*) and Red Maple (*Acer rubrum*) at the expense of northern range trees (yellow birch and red spruce) as conditions become more suitable for the prior. Understory herbaceous plants will decrease as well, with invasive species becoming more present in canopy gaps such as honeysuckle (*Lonicera sp.*), trillium (*Trillium sp.*), and buckthorn (*Rhamnus cathortica*) (Rustad *et al.* 2012). With this change in forest composition, it may be useful in future study to consider a microsite approach, as the species studied currently were typically found growing underneath either one another or shrubs such as hobblebush. This would enable a more detailed understanding of the not only the light-trait relationships occurring, but any changes in water and nutrient exploitation with the involvement of other suites of traits as mentioned, as both are different with each type of forest (Maple-Beech/Oak-Hickory) (Pages *et al.* 2003).

Though the herbaceous species studied can be described as shade tolerant due to their ability to still show successful growth in shadier environments, decreased success is expected as plasticity among their traits decreases with increasing canopy closure. This decreased range variation of traits has been displayed in traits related to light harvesting in areas with limited sunlight (Bangguo *et al*. 2012). Given the threat that climate change has on the region in regards to diversity, productivity, and ecosystem functioning, trait-based studies will enable to reflect any changes, allowing conservation to be implemented appropriately. As discussed, alpine zones in the northeast are listed as critically imperiled, and both the alpine snowbank and sub-alpine understory are significant indicators of climate change, thus further study is encouraged.

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**Tables & Figures**

Table 1.) Canopy closure estimates (%) derived from a spherical densitometer at each sub-alpine site.

| Site | Canopy Closure Estimate (%) |
|------|------|
| SK1 | 73.74 |
| LPT2 | 78.42 |
| GG1 | 78.65 |
| SQL1 | 82.31 |
| DPL2 | 85.44 |
| SQL2 | 87.26 |

Table 2.) Results from two-sample t-tests comparing PPI values of the alpine and sub-alpine sites. Gray boxes indicate higher mean values among the alpine sites, whereas white boxes indicate higher mean values at the sub-alpine sites. * = significant difference (p<0.05). *CHCA Chamaepericlymenum canadense; CLBO Clintonia borealis; COTR Coptis trifolia, MACA Maianthemum candense.*

| Species | Plant height (mm) | SLA (mm2/mg) | LDMC (mg/g) | Leaf area (mm2) |
|------|------|------|------|------|
| CHCA |  | ▓ |  | ▓ |
| CLBO |  | ▓ |  |  |
| COTR | ▓ |  | * |  |
| MACA | ▓ |  |  |  |

Table 3.) Regression analyses of canopy closure and plant traits across the sub-alpine sites. Significant results are highlighted (p<0.05). *CHCA Chamaepericlymenum canadense; CLBO Clintonia borealis; COTR Coptis trifolia, MACA Maianthemum candense.*

| Trait | Species | F | Signficance F | r-squared |
|---|---|---|---|---|
| Plant Height (mm) | CHCA | 1.59 | 0.28 | 0.28 |
| | CLBO | 1.05 | 0.36 | 0.21 |
| | COTR | 0.72 | 0.44 | 0.15 |
| | MACA | 0.88 | 0.40 | 0.18 |
| SLA ($mm^2$/mg) | CHCA | 1.31 | 0.32 | 0.25 |
| | CLBO | 12.75 | 0.02 | 0.76 |
| | COTR | 3.00 | 0.16 | 0.43 |
| | MACA | 0.01 | 0.94 | 0.001 |
| LDMC (mg/g) | CHCA | 4.43 | 0.10 | 0.53 |
| | CLBO | 4.45 | 0.10 | 0.53 |
| | COTR | 0.54 | 0.50 | 0.12 |
| | MACA | 0.00 | 0.99 | 6.97E-05 |
| Leaf Area ($mm^2$) | CHCA | 0.72 | 0.45 | 0.15 |
| | CLBO | 0.21 | 0.67 | 0.05 |
| | COTR | 0.67 | 0.46 | 0.14 |
| | MACA | 0.02 | 0.89 | 0.01 |

Table 4.) Results from regression analysis of trait values and PPI values of the sub-alpine sites to tests if increasing canopy closure elicits plastic responses. Significant results are highlighted (p<0.05). *CHCA Chamaepericlymenum canadense; CLBO Clintonia borealis; COTR Coptis trifolia, MACA Maianthemum candense.*

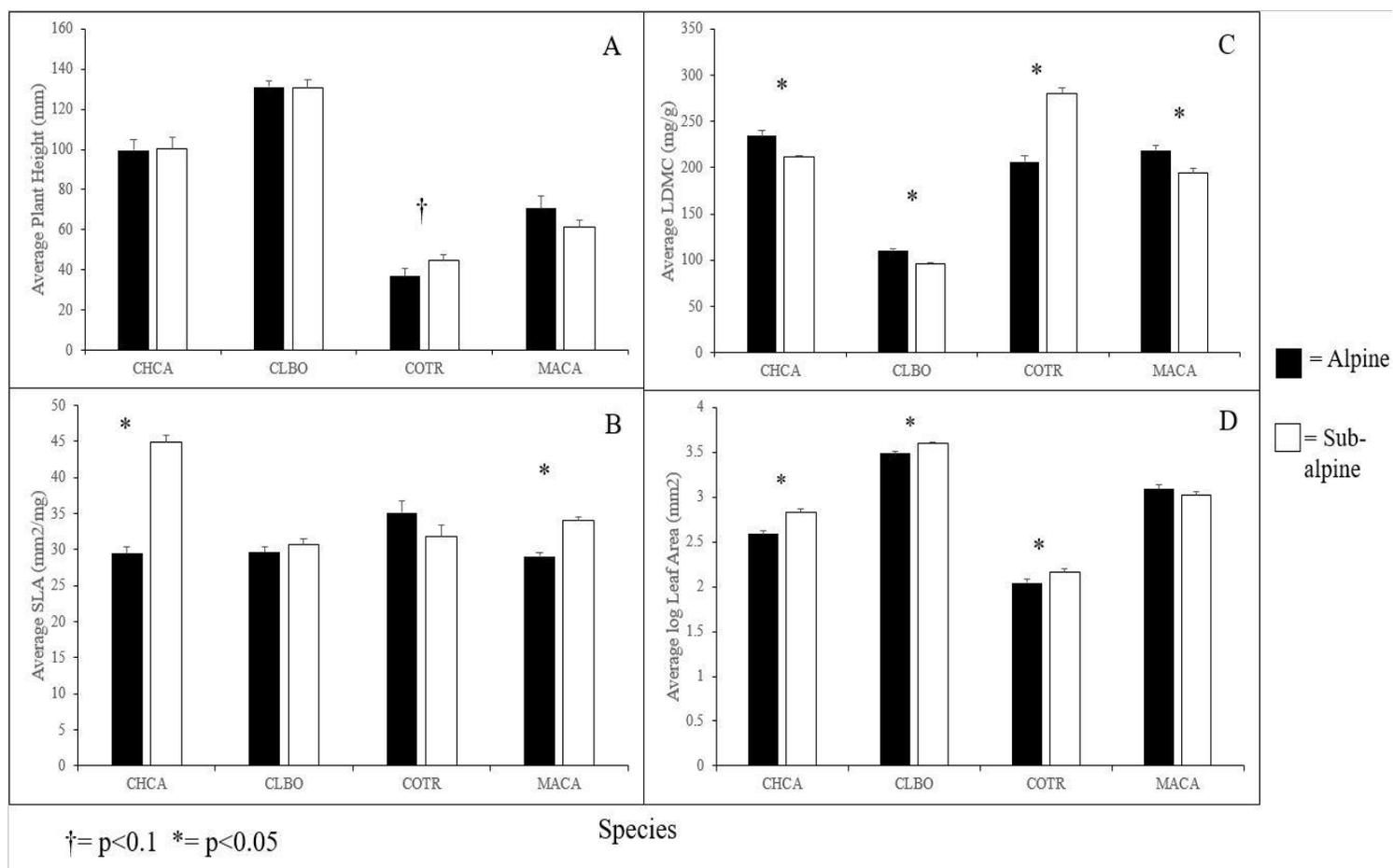| Trait | Species | F | Signficance F | r-squared |
|---|---|---|---|---|
| Plant Height (mm) | CHCA | 1.28 | 0.32 | 0.24 |
| | CLBO | 17.78 | 0.01 | 0.82 |
| | COTR | 0.04 | 0.84 | 0.01 |
| | MACA | 0.01 | 0.92 | 0.00 |
| SLA (mm$^2$/mg) | CHCA | 0.44 | 0.54 | 0.10 |
| | CLBO | 0.16 | 0.71 | 0.04 |
| | COTR | 11.83 | 0.03 | 0.75 |
| | MACA | 0.47 | 0.53 | 0.11 |
| LDMC (mg/g) | CHCA | 0.04 | 0.85 | 0.01 |
| | CLBO | 0.37 | 0.58 | 0.08 |
| | COTR | 0.98 | 0.38 | 0.20 |
| | MACA | 0.31 | 0.61 | 0.07 |
| Leaf Area (mm$^2$) | CHCA | 1.30 | 0.32 | 0.24 |
| | CLBO | 0.48 | 0.53 | 0.11 |
| | COTR | 0.28 | 0.63 | 0.07 |
| | MACA | 0.05 | 0.83 | 0.01 |

†= p<0.1  *= p<0.05

Figure 1.) Results from two-sample t-tests comparing the alpine 'megasite' (AG2) to the sub-alpine 'megasite' (SQL1).  'A' displays plant height (mm) comparison which no significant differences were found.  'B' represents SLA ($mm^2$/mg) comparisons in which significantly higher values were found in the sub-alpine for CHCA and MACA.  'C' shows comparisons of LDMC (mg/g) in which significant higher values were seen in the alpine for all species besides COTR, which displayed the opposite.  'D' refers to comparisons of log-transformed leaf area ($mm^2$), which all species besides MACA showed significantly higher values in the sub-alpine. *CHCA Chamaepericlymenum canadense; CLBO Clintonia borealis; COTR Coptis trifolia, MACA Maianthemum candense.*
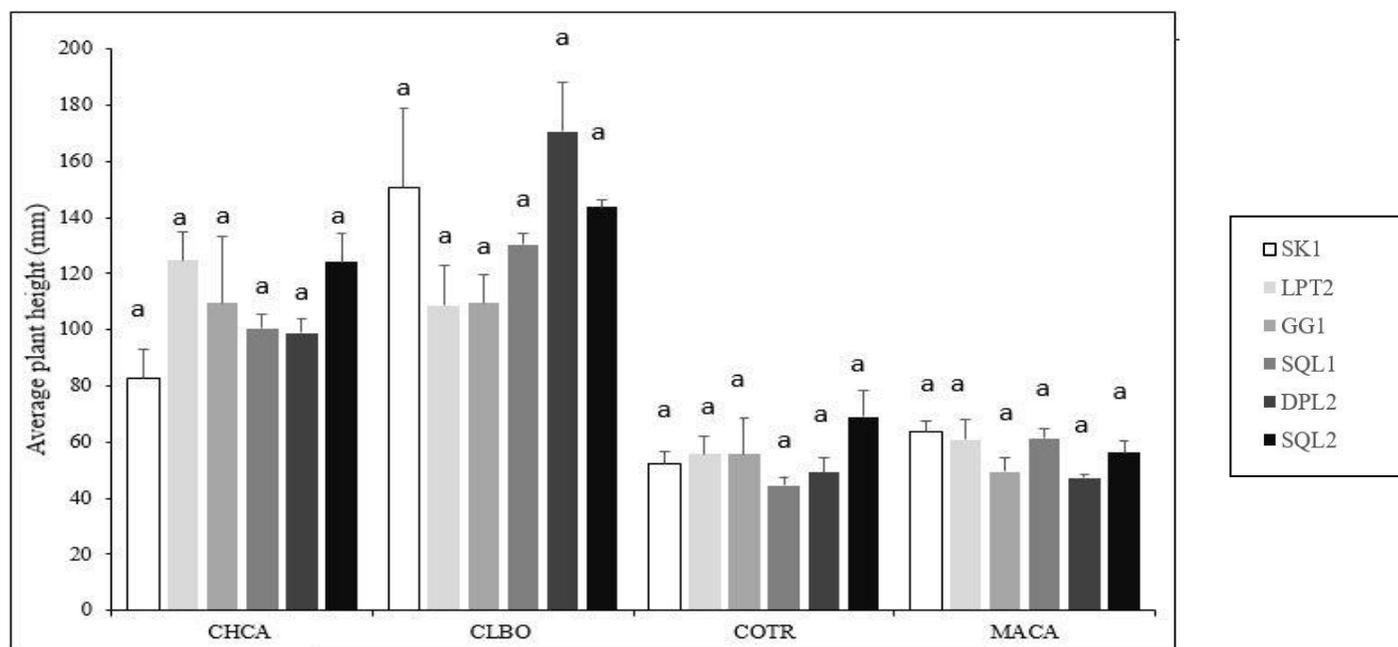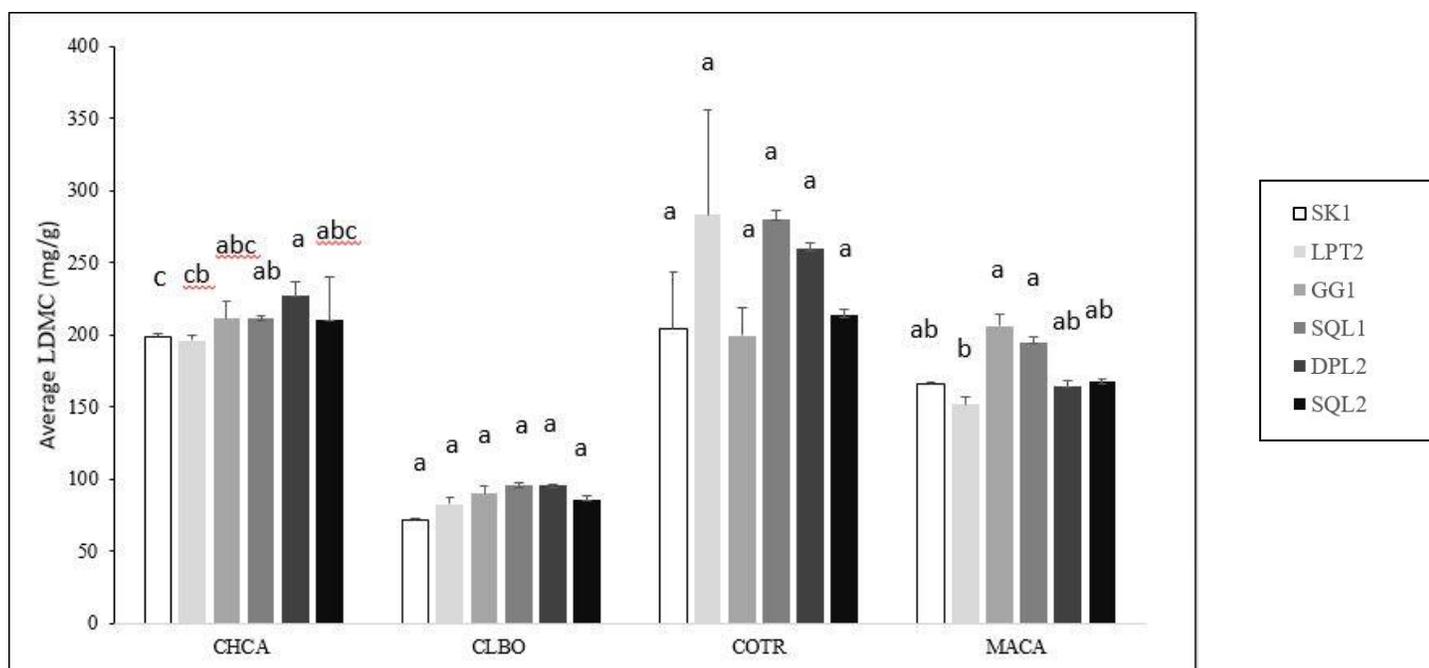
Figure 2.) Results from sub-alpine one-way ANOVA to test for differences between sites for plant height (mm). Bars are arranged by sites with least canopy closure to greatest canopy closure, from left to right (SK1 has least canopy closure, SQL2 has greatest canopy closure). No significant differences ($p < 0.05$) were found within species across sites, indicated by letter denomination from Tukey's post-hoc comparison results. *CHCA Chamaepericlymenum canadense; CLBO Clintonia borealis; COTR Coptis trifolia, MACA Maianthemum candense.*
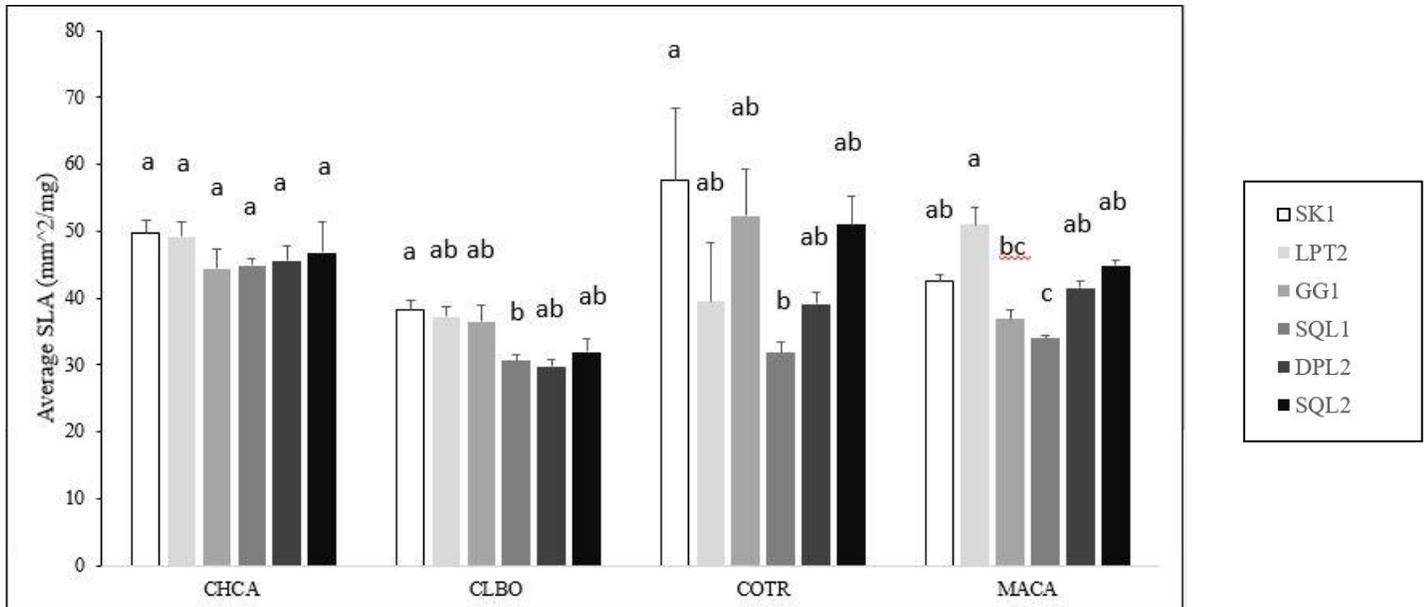
Figure 3.) Results from one-way ANOVA comparison within species across sub-alpine sites for LDMC (mg/g) values. Bars are arranged by sites with least canopy closure to greatest canopy closure, from left to right. Significant differences ($p<0.05$) within species across sites are indicated by letter denomination from Tukey's post-hoc comparison results. LDMC generally increases with increasing canopy closure. *CHCA Chamaepericlymenum canadense; CLBO Clintonia borealis; COTR Coptis trifolia, MACA Maianthemum candense.*

Figure 4.) Results from one-way ANOVA comparison within species across sub-alpine sites for SLA (mm$^2$/mg) values.  Bars are arranged by sites with least canopy closure to greatest canopy closure, from left to right.  Significant differences ($p<0.05$) within species across sites are indicated by letter denomination from Tukey's post-hoc comparison results.  SLA generally decreases with increasing canopy closure.  *CHCA Chamaepericlymenum canadense; CLBO Clintonia borealis; COTR Coptis trifolia, MACA Maianthemum candense.*
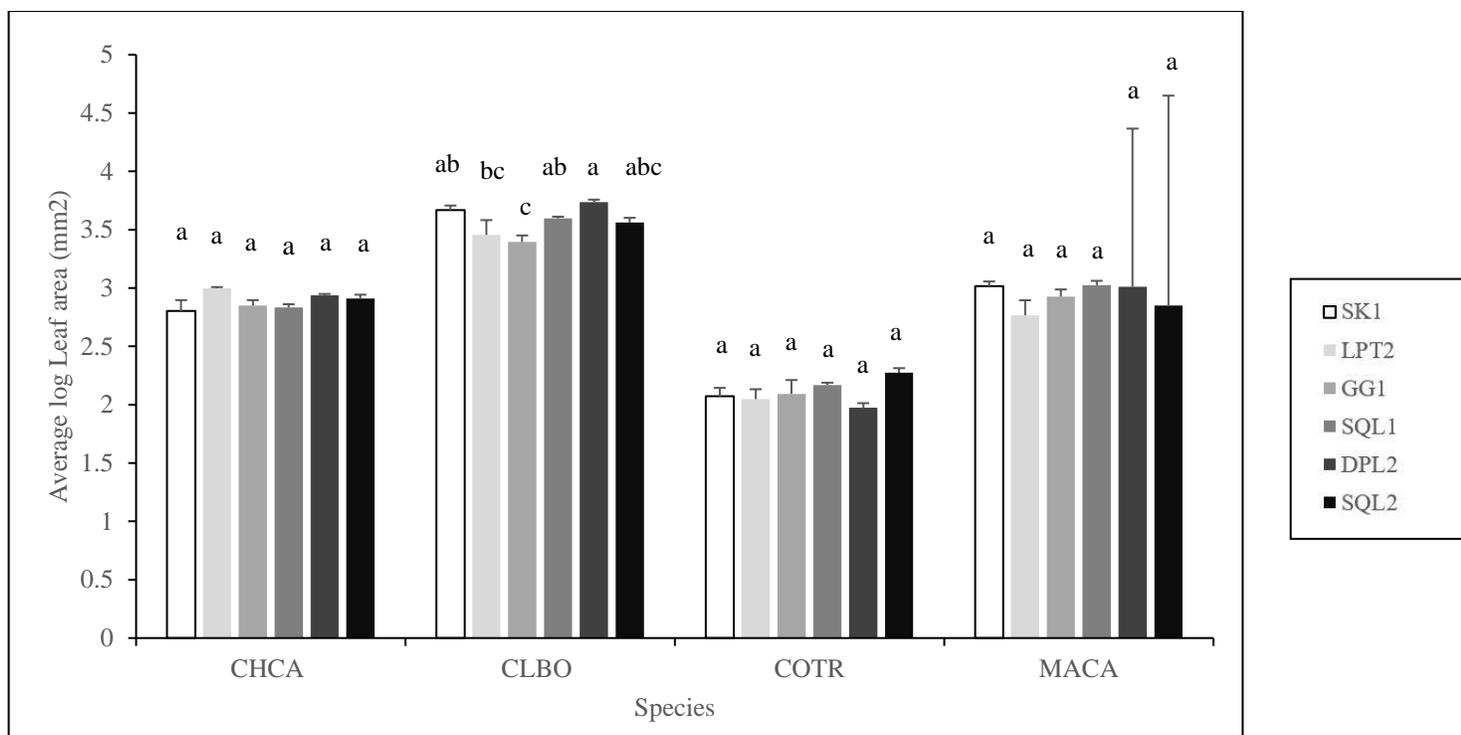
Figure 5.) Results from one-way ANOVA comparison within species across sub-alpine sites for log-transformed leaf area (mm$^2$) values. Bars are arranged by sites with least canopy closure to greatest canopy closure, from left to right. Significant differences ($p<0.05$) within species across sites are indicated by letter denomination from Tukey's post-hoc comparison results. *CHCA Chamaepericlymenum canadense; CLBO Clintonia borealis; COTR Coptis trifolia, MACA Maianthemum candense.*