

Assessing Two Plant Leaf Functional Traits across a Temperature Gradient

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Abstract:

In this study we attempted to discover some of the possible plant responses to global climate change, to assess plant responses we used two plant leaf functional traits: leaf maximum assimilation rate of CO₂ ($A_{\max}$) and Specific Leaf Area (SLA). To evaluate increasing temperature we used a natural elevation gradient and an experimental warming meadow. We found that six of seven species showed a higher SLA with increased elevation. $A_{\max}$ did not respond to elevation in a universal pattern; 5 of 6 species had a higher $A_{\max}$ at lower elevations, but a larger sample of species found no trend: only 6/12 species had higher $A_{\max}$ values at lower elevations. The Warming Meadow heating had no effect on the SLA of six species and no effect on the $A_{\max}$ of six species. Only two species responded, *Erigeron speciosus* and *Eriogonum subalpinum*, but this response agreed with previous studies on the system. We conclude that the relatively short termed *experimental* warming had a small effect on plant leaf functional traits, but natural warming along an elevation gradient shows more variation. As products of this study we suggest further investigation of these traits and acceptance of plant response as an integral part of global climate change science.

Introduction:

A central question in ecology and global change studies asks how do organisms and ecosystems respond to climatic change? Studies show that plants may provide a significant feedback to global climate (Saleska et al., 1999). Furthermore, inclusion of plant responses into models of climate change results in an accelerated global warming rate when compared to models that ignore biotic feedback (Cox et al., 2000). Many plant physiological processes respond directly to environmental conditions, such as temperature or precipitation. Therefore, as the climate changes, we would expect to see proportional changes in plant functional traits. This study will focus on leaf functional responses to climate change.

A functional trait can be any characteristic that has a profound impact on a plant's success, reproduction or evolutionary fitness (Reich et al., 2003). Changes in plant functional traits are often cited as the method by which plants inhabit a diverse range of environments; unique traits may arise for unique habitats (Enquist et al., In Prep). Leaf traits have even been used to characterize convergent evolution across the globe (Reich et al., 1997). If traits are so closely related to habitat and therefore climate, these traits may be the way in which plants respond to climate change.

Possible plant responses to climate change depend on the level of organization we are looking at, i.e. individuals, populations or communities. Individual plants may respond to climate change through two paths, acclimation or acclimatization. Acclimation occurs in a short period of time; plants undergo reversible physical changes. Acclimatization is a longer process, also involving plastic responses in physiology. Populations of plants can respond to climate changes through adaptation. This process is due to a specific phenotype (or genotype) being favored and selected. Through this method of response, modifications arise through generations rather than during individual lifespans. Entire communities of plants may undergo assembly changes in response to climate change, through species migration, extinction and changes in abundance. For example, Harte et al. (1995) found that the ratio of above ground biomass between forbs and shrubs changed between the heated and control plots on their experimental warming meadow.

Plant functional traits give vital insight into how plants respond to climate differences. For example, Kerkhoff et al.(2005), found that the ratio of nitrogen to phosphorous content of leaves, which is a relative measure of growth rate and photosynthetic potential, does not vary widely across a latitudinal (and therefore temperature) gradient. This result demonstrates that plant growth or photosynthetic potential is not affected by latitude and therefore temperature. In another study, Kerkhoff et al. (2005) used data from a global dataset (Distributed Active Archive Center) to evaluate the annual net primary productivity (ANPP) of eleven vegetation types across a temperature gradient. Their results showed no relationship between ANPP and temperature. These studies raise some very interesting questions:

If there seems to be a weak correlation between primary productivity and growing season temperature, then what is responsible for plants' seemingly plastic response to environmental change? Enquist et al. (In Prep) posit that adaptive changes in plant functional traits along broad scale gradients can potentially negate changes in climate. Many ecologists agree that discovering the method by which plants and ecosystems maintain a balance of productivity, and how functional traits relate to this homeostasis, is one of the fundamental steps toward understanding ecosystem dynamics (Enquist et al., In Prep; Westoby and Wright, 2006).

In this study I use both an observation framework and a manipulation experiment to test the effect of temperature on plant leaf functional traits. In the observational study, I measure traits along an elevation gradient, which functions as a temperature gradient. In the manipulation experiment I use an ongoing meadow warming manipulation established in 1990 by John Harte.

Hypotheses:

H₀: Plant leaf functional traits do not respond to temperature in a measurable way.

H₁: Plant leaf functional traits respond to temperature changes in measurable ways.

Methodology:

1. Plant Traits

The traits measured in this study are Specific Leaf Area (SLA) and maximum assimilation rate of CO₂ ($A_{\max}$). SLA is the ratio between leaf area when wet and leaf mass when dry. This ratio gives a measure of investment into photosynthetic processes as well as into a plants carbon and water cycles (Pierce et al. 1994). A high SLA value usually denotes short lived leaves, while a low SLA indicates long lived leaves. A low SLA also means plants are more water conserving, a high SLA usually denotes leaves that lose much water through evapotranspiration. $A_{\max}$ is proportional to a leaf's maximum photosynthetic capacity. This value can give great insight into the fitness of plant populations. This study will assess seven species that can be found in almost all of our study plots. *Artemisia tridentata*, *Delphinium nelsonii*, *Potentilla gracilis*, *Eriogonum subalpinum*, *Erigeron speciosus*, *Helianthella quinquenervis* and *Lathyrus leucanthus*. These species were chosen to cover a range of habits, both woody and herbaceous, and a range of habitat preferences (mesic to xeric). These species also represent six different plant families, and are thus phylogenetically diverse.

$A_{\max}$ will be determined using a Li-Cor 6400 equipped with a leaf chamber. This system uses a closed loop infrared gas analyzer (IRGA) to analyze a leaf's photosynthetic rate. A sample amount of CO₂ is pumped into the leaf chamber, and reference amount of

CO₂ bypasses the leaf cuvette, not being affected by the leaf assimilation. There are two IRGAs that measure the CO₂ concentrations in the sample line and the reference line. The difference between these two flow rate concentrations is calculated to be the assimilation rate of a leaf. Leaf A_{max} values will be determined by pumping a high reference concentration of CO₂ (400ppm) into the leaf chamber. At each plot, an individual from all species present is chosen and one leaf from that individual is clamped into the leaf chamber. Data is logged 3 times for each individual in the Li-Cor and the leaf is harvested. The leaf area that was in the chamber is determined using the CI-202 scanner. This area is then entered into the data tables produced by the 6400 and an assimilation rate is calculated. These measurements will be made during peak photosynthetic hours (0800-1100), to attempt to measure peak leaf assimilation rates.

Leaves used for SLA were harvested from healthy plants within the plots, from areas where the treatment affect is greatest (directly under the heaters in the upper zone of the plots). The leaves will be placed in 2.25"x3.5" manila envelopes labeled with species and date. Wet leaf area will be determined the same day as harvest, using a CI-202 Area Meter (CID Inc.). The leaves were dried at 65 degrees Celsius for 4-5 days in a Lindberg/Blue oven. Leaves were weighed using a Mettler BM400 (Mettler Instrument Corp.) scale.

2. Elevational Observations

In order to determine the effect of climate on functional traits I will examine species at 5 sites spanning a gradient of ~900 meters and ~3 degrees Celsius (NOAA June and July average temps 1971-2001). This study will use 2 years of previous data collected from 25 plots at 5 different sites (Table 1). SLA values for all of the elevational gradient sites were recorded by Dr. Enquist's lab in 2004 and 2005. In 2004, SLA values were calculated from the leaf samples used in the chamber of the Li-Cor 6400. A whole leaf was not harvested, only the area that was in the chamber was determined; the leaf section was then dried and SLA was calculated from subsequent values. A 2004 SLA value represented an average of three individuals per species per site. In 2005, whole leaves were sampled from three individuals per species per site along the elevational gradient; an average area and mass was calculated to give an SLA value for each species per site.

$A_{\max}$ measurements were also taken by Dr. Enquist's lab members in 2004 and 2005. An $A_{\max}$ value represents an average of three individuals per species per site.

3. Warming Meadow Manipulation

The warming meadow that will be used in this project was created in the early 1990s by John Harte and his field crew. The meadow consists of ten 3m x 10m plots spread longitudinally along a hillside (Figure 1). The plots are separated by a 3 meter swath to allow walking between plots. The plots have a great diversity of plant life, with over 80 species (Harte et al., 1995). Odd numbered plots are control plots, with no treatment, while even plots have three, 1.6m irradiative heaters (Kalglo, Lehigh, PA) suspended 1 meter above them. The heaters are meant to simulate the subsequent heating from a two-fold increase in CO₂ gas concentrations. This heating increase translates to about a 2 or 3 degree (C) increase of soil temperature in the heated plots (Harte et al., In Prep). SLA and $A_{\max}$ will be determined for one individual in all plots for each target species present.

4. Statistical Analyses

In order to determine whether leaf functional traits responded to increased temperature at the warming meadow I used one way ANOVAs to compare SLA and $A_{\max}$ values for species in treatment plots versus control plots. Data for each species were analyzed separately. SLA and $A_{\max}$ values can vary drastically from one species to another, so it was not helpful to do interspecific analyses. Many studies have shown that one of the major floral repercussions of the warming meadow treatment is the earlier snowmelt date in warmed plots (Dunne et al., (2001); Price and Waser (1998)). To test this possibility we performed an ANCOVA with snowmelt date as a covariate and treatment as the main effect.

The elevational gradient dataset is not large enough to do statistical analyses, so the data presented are preliminary findings.

Results:

The elevational gradient data show higher $A_{\max}$ values in lower elevations and higher SLA values in higher elevations (Figs. 3, 4). When we examined a larger species

list we found a 50 percent chance of a plant having a higher photosynthetic rate at a higher elevation (Table 6).

The warming meadow experiment did not significantly affect the SLA for 6 of 7 species sampled (Fig 5, Table 2). *Erigeron speciosus* had higher SLA values in the control plots than in warmed plots ($F_{1,8} 3.93 P=0.08$).

Experimental warming did not significantly affect the $A_{\max}$ of 6 out of 7 species (Fig 6, Table 3). In the species *Eriogonum subalpinum* the direction of the effect $A_{\max}$ was significantly greater in control plots ($F_{1,7} 4.58 P= 0.07$). As indicated in Fig. 6 *Eriogonum* seemed to be better suited (photosynthetically) to the control plots.

When snowmelt date was included in the analysis, neither snowmelt date ($F_{1,6} < 3 P>0.12$) nor warming treatment ($F<2.08 P>0.182$) affected SLA or $A_{\max}$ for any plant species (Tables 4,5).

Discussion:

Elevation Gradient:

The higher assimilation rate at lower elevations suggests that the species observed are more photosynthetically efficient than their higher elevation populations. When we observed a larger suite of species we found that there was not a predictable pattern in terms of $A_{\max}$ and elevation. The observed 50% chance of having a higher $A_{\max}$ suggests that the optimal photosynthetic range varies per species. This would lead us to believe that elevation does not have a consistent, across the board effect on photosynthetic potential.

Our SLA data show, on average, higher SLA values at higher elevations. This seems a bit counterintuitive because a high SLA value means that leaves have a large surface area in relation to their weight. Plants at high elevations tend to be water stressed (Enquist, personal communication). A high SLA value usually means that plants lose large amounts of water through evapotranspiration. Our samples may have come out of areas where water was not a limiting factor, or perhaps the short growing season at higher elevations and subsequent need to maximize potential photosynthetic surface area might explain the high SLA values we observed.

Warming Meadow Experiment:

The general trend that our Warming Meadow data show is that leaf traits are not highly affected by the warming treatment. The warming meadow experiment has been underway for 15 years, making it the longest running experimental warming treatment (John Harte, personal communication). However, in terms of plant adaptation, and acclimatization, fifteen years is a relatively small time period. Our data show that perhaps leaf level traits have not changed for most of the species studied. Within the species that did respond to the treatment there are some very interesting patterns.

Potential responses to warming seemed to occur on a per species basis, and the response was not directional, i.e. affected plants did not all have a negative reaction. For the two species that did show a response (*Eriogonum subalpinum* and *Erigeron speciosus*) our data seem to agree with previous studies done in the Warming Meadow. In particular, de Velpine and Harte (2001) found that the above ground biomass and flowering percentage for their species set was highly affected by the warming treatment. This observation, along with our findings, suggest that the process currently taking place in the warming meadow may be changes in assembly and minor leaf level physiological changes (acclimation). Our data suggests that leaf functional traits may be linked to the relative success of species in the warming meadow plots, and may determine some of the patterns within the assembly process.

de Valpine and Harte (2001) found that the above ground biomass of *Erigeron speciosus* declined in warmed plots, and was much greater in control plots. Our leaf level data for *Erigeron* suggest that there was no difference in Amax ($F_{1,7} 0.4101 P=0.5423$) for warmed plots, but shows an SLA difference ($F_{1,8} 3.93 P=0.08$), with higher values in control plots. A lower SLA in warmed plots suggests that the plants are water and possibly carbon stressed, which is in agreement with previous warming meadow studies of water stress and carbon levels (Harte et al. In Prep). The higher observed SLA values in control plots match our conclusions from the elevational gradient. *Erigeron* had higher SLA values at higher elevations, where it is presumably colder, and higher SLA values in colder control plots. This finding implies that the warming meadow does effectively create a temperature gradient that closely mirrors an observed elevational/temperature gradient.

de Valpine and Harte (2001) also found that *Eriogonum subalpinum* produced more flowers in control plots. Our data show that *Eriogonum* has a higher photosynthetic potential ($F_{1,7} 4.58$ $P=0.07$) in control plots. This relationship demonstrates that the photosynthetic capacity of *Eriogonum* in warmed plots is much lower, and most likely leads to fewer flowering stalks. This phenomenon may be due to *Eriogonum*'s elevation range; *Eriogonum* is a sub alpine plant living in high, cold environs (Barrell, 1969 see Fig. 2). Artificially warming *Eriogonum* may be stressing the plant and therefore decreasing the fitness and number of flowering individuals.

Our results suggest that not all plants may be physiologically affected by short term climate change. Plant leaf traits do seem to vary per species among both study systems, but no general trend of trait direction was found. This may be a function of species ranges. Some of our study species have comfortable ranges spanning the entire gradient, so we may not see any changes in leaf physiology if the species is not disturbed by elevation changes (Barrell, 1969). Our preliminary elevation data show an effect of elevation on leaf traits. When we analyze Warming Meadow data we find not as strong of a response in leaf traits. For the majority of species we studied, temperature does not seem to change SLA and $A_{\max}$ in a short term (15 year) experiment, i.e. we find no acclimation response. The elevation gradient suggests that temperature may affect leaf traits but in a longer time frame, perhaps through adaptation. (In terms of making statements about adaptation of populations there are several limitations that this study has. In the time period that this study provided there was no time to differentiate between acclimation/acclimatization and adaptation. Adaptation implies changes between generations, and with long lived perennial species it would be impossible to discover adaptation without years of study.) The observed elevation trait variation could also be due to factors other than temperature that differ along the gradient such as soils or slope.

The preliminary elevational findings advocate re-visitation and larger sample sizes to pin down what leaf level changes are taking place across the elevation gradient. The preliminary findings agree with predictions by Enquist et al (In Prep) that functional traits may change with environment, and may therefore help plants cope with global climate change.

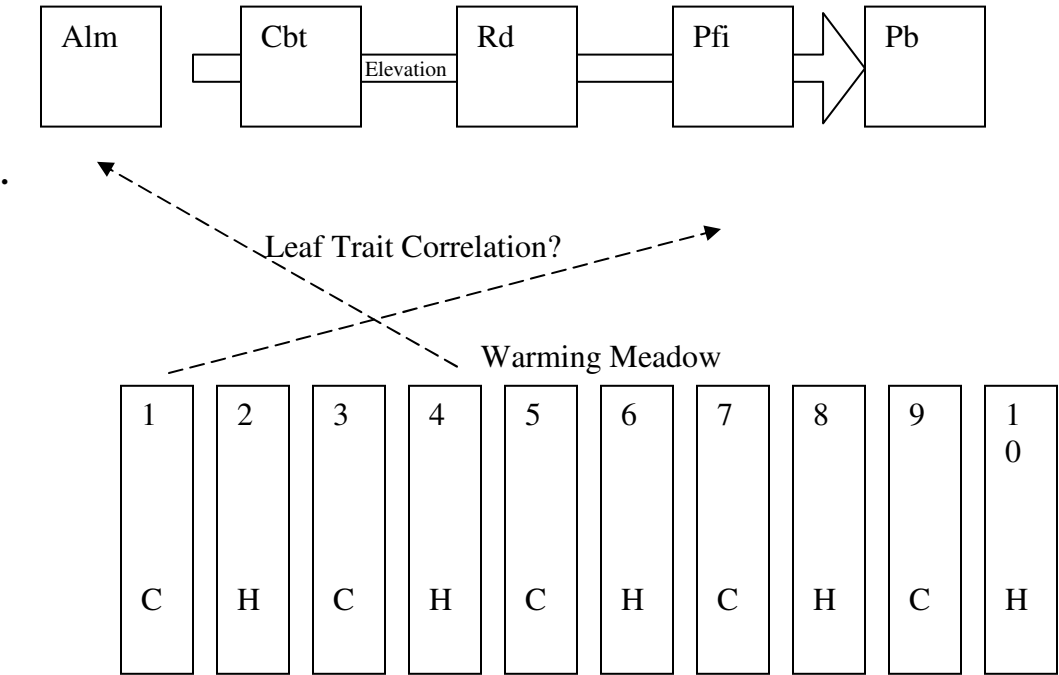
Our warming meadow results, along with previous studies done on the system, suggest that one of the first visible responses of plants to climate change may be an assembly response. Plant leaf functional traits seem to be directly linked to this assembly process. Plant leaf traits play a governing role in the assembly process. The traits a plant has will determine how the plant will respond to warming; if the main response to warming is a change in assembly, the traits will determine the assembly shifts. Plants with traits that favor warmer, drier climates (*Artemisia tridentata*) will most likely thrive in warmed plots or areas, while plants with traits favoring cooler, more moist environs will shift their dominance to control plots or cooler areas (*Eriogonum subalpinum*).

These findings have implications for climate change and its possible affects. We find that plants will play a significant role in climate change. As temperatures increase plant dominance may shift, possibly as a function of plant leaf functional traits. As plant dominance shifts, carbon cycles for a given area may change, as indicated by Harte et al. (In Prep). This shift in carbon cycling is exactly the type of biotic response that will impact global climate change. Our data along with that of Cox et. al. (2000) demonstrates that plant acclimation/acclimatization, adaptation, and assembly processes should be taken into account by climate change models and predictions.

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Tables, Graphs and Figures



Research Sites

Alm- Almont

Cbt- Crested Butte Land Trust

Rd- Allen property on Washington Gulch Rd

Pfi- Pfeiler property on Washington Gulch Rd

Pb- Painterboy Mine

Figure 1. Spatial Explanation of Research: Warming Meadow set up and gradient site progression. Arrows indicate where possible correlations may be drawn (warmed plots may correspond to lower elevations and vice versa).

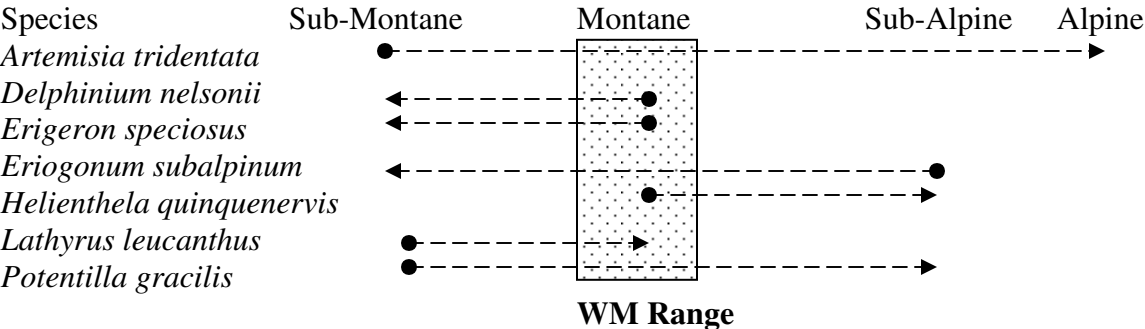


Fig. 2 Barrell's Elevation Ranges (1969) Dots are preferred habitat, lines to arrow a gradient of preference.

Site	Elevation (m)	mean annual precip (cm)	mean temp (Jun+Jul) (C)	Habitat	Type of Land
1	2485	25.273	14.6	Great Basin shrubland	BLM
2	2704	60.62	11.86	Montane Floodplain	Land Trust
3	2805	60.62	11.86	Montane Meadow	Allen Family
4	3172	no data	no data	Montane Meadow	Pfeiler Family
5	3366	no data	no data	Subalpine Meadow	State
6	2920	No data	No data	Warming Meadow	RMBL

Table 1. Physical Site Characteristics

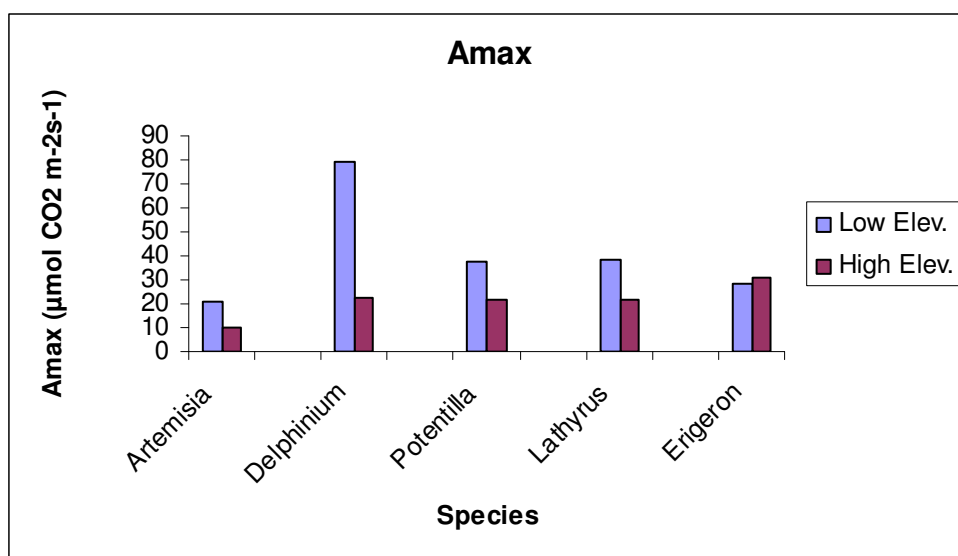


Fig. 3 A_{max} Elevation Observations

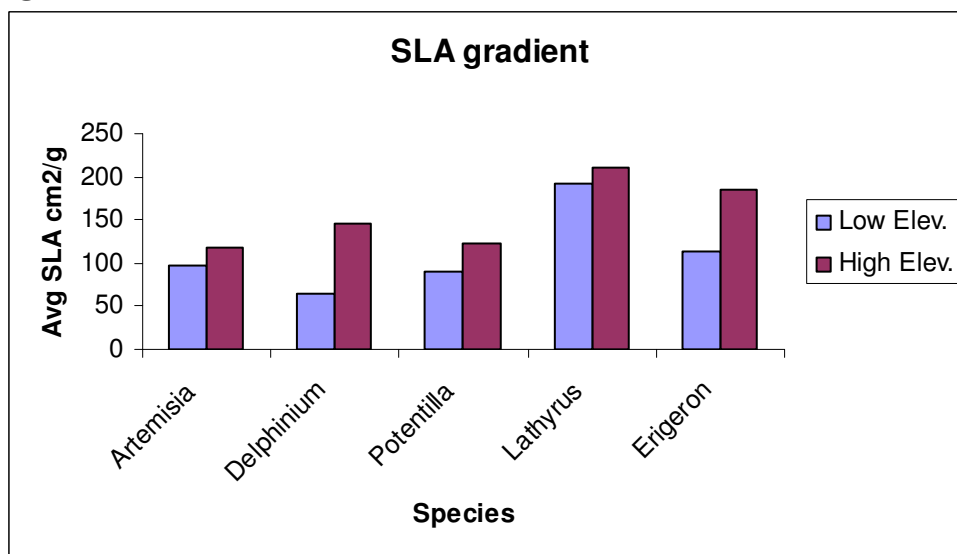


Fig. 4 SLA Elevational Gradient Results

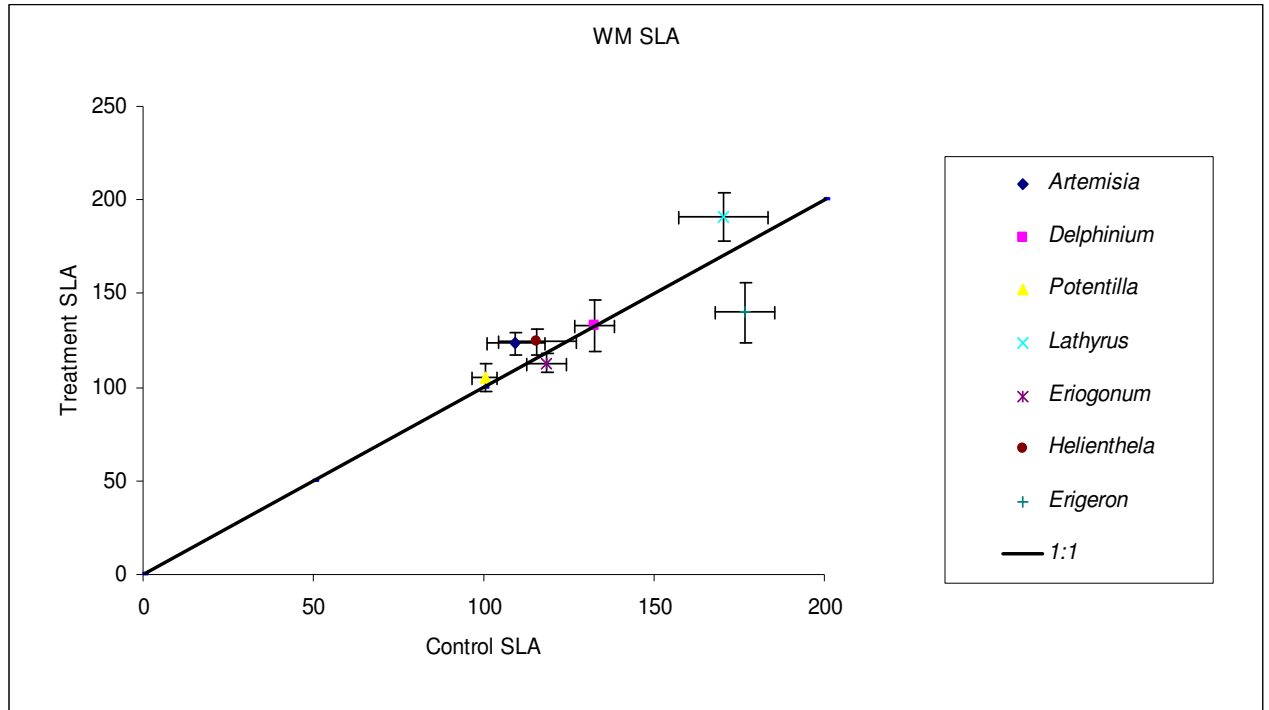


Fig. 5 SLA Warming Meadow Results

Species	F Ratio	P Value
<i>Artemisia tridentata</i>	F _{1,7} 1.84	0.22
<i>Delphinium nelsonii</i>	F _{1,8} 0.001	0.98
<i>Erigeron speciosus</i>	F _{1,8} 3.93	0.08
<i>Eriogonum subalpinum</i>	F _{1,8} 0.51	0.50
<i>Helianthella quinquenervis</i>	F _{1,8} 0.42	0.54
<i>Lathyrus leucanthus</i>	F _{1,8} 1.21	0.30
<i>Potentilla gracilis</i>	F _{1,8} 0.39	0.55

Table 2 SLA One-Way ANOVA Results

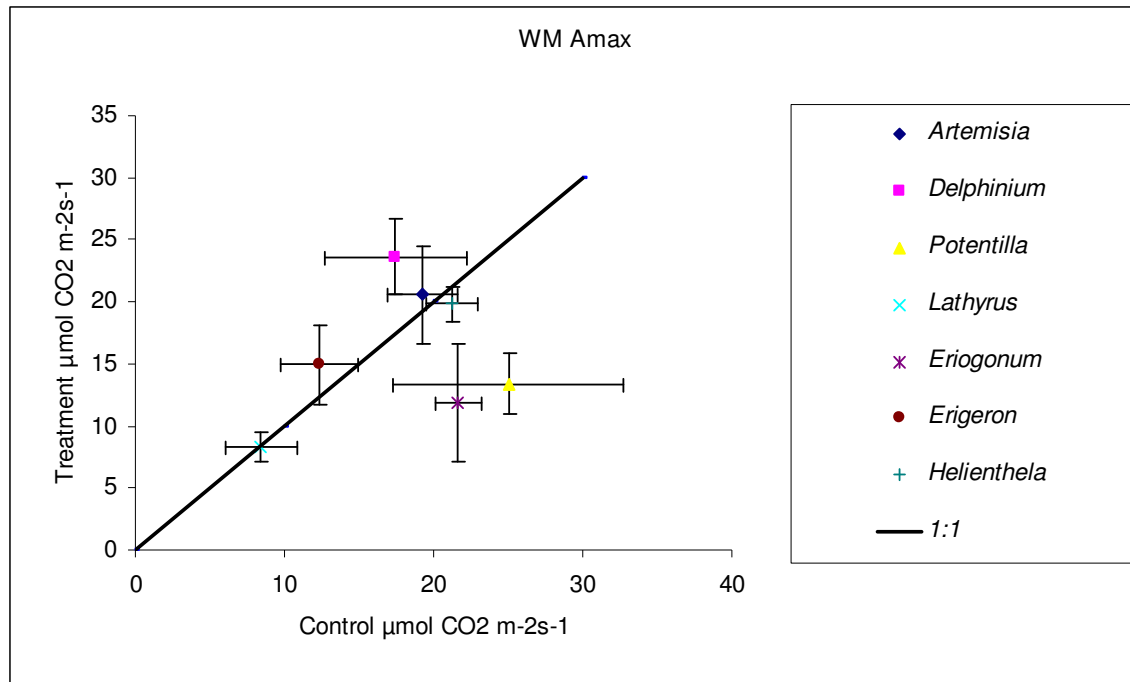


Fig. 6 Amax Warming Meadow Results

Species	F Value	P Value
<i>Artemisia tridentata</i>	$F_{1,7} 0.09$	0.78
<i>Delphinium nelsonii</i>	$F_{1,7} 1.05$	0.34
<i>Erigeron speciosus</i>	$F_{1,7} 0.41$	0.54
<i>Eriogonum subalpinum</i>	$F_{1,7} 4.58$	0.07
<i>Helienthela quinquenervis</i>	$F_{1,8} 0.42$	0.54
<i>Lathyrus leucanthus</i>	$F_{1,8} 0.003$	0.96
<i>Potentilla gracilis</i>	$F_{1,8} 2.08$	0.19

Table 3 Amax One-Way ANOVA Results

Species	F Value	P Value
<i>Artemisia tridentata</i>	$F_{1,5} 0.01$	0.91
<i>Delphinium nelsonii</i>	$F_{1,5} 0.93$	0.38
<i>Erigeron speciosus</i>	$F_{1,5} 0.39$	0.56
<i>Eriogonum subalpinum</i>	$F_{1,5} 1.97$	0.22
<i>Helienthela quinquenervis</i>	$F_{1,6} 0.93$	0.37
<i>Lathyrus leucanthus</i>	$F_{1,6} 0.66$	0.45
<i>Potentilla gracilis</i>	$F_{1,6} 0.44$	0.53

Table 4 Snowmelt ANCOVA Results (A_{max})

Species	F Value	P Value
<i>Artemisia tridentata</i>	F _{1,5} 0.03	0.87
<i>Delphinium nelsonii</i>	F _{1,6} 0.40	0.55
<i>Erigeron speciosus</i>	F _{1,6} 3.28	0.12
<i>Eriogonum subalpinum</i>	F _{1,6} 0.12	0.74
<i>Helianthella quinquenervis</i>	F _{1,6} 0.83	0.40
<i>Lathyrus leucanthus</i>	F _{1,6} 1.10	0.35
<i>Potentilla gracilis</i>	F _{1,6} 0.28	0.61

Table 5 Snowmelt ANCOVA Results (SLA)

Species	H/L photo rate at High Elevation
<i>Artemisia tridentata</i>	L
<i>Delphinium nelsonii</i>	L
<i>Potentilla gracilis</i>	L
<i>Lathyrus leucanthus</i>	L
<i>Eriogonum subalpinum</i>	
<i>Helianthella quinquenervis</i>	L
<i>Erigeron speciosus</i>	H
<i>Dugaldia</i>	H
<i>Frageria</i>	H
<i>Poa reflexa</i>	H
<i>Lupin</i>	H
<i>Linum</i>	H
<i>Valeriana capitata</i>	L

Table 6 Probability of Higher Photo Rate at Higher Elevation. Fifty Percent of plant species sampled had a higher photo rate at higher elevations.