

The Seedling Germination Niche: Characterizing microhabitat structure of *Pinus flexilis* in a lower subalpine forest warming experiment at Niwot Ridge, Colorado

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ABSTRACT

Changing global climate conditions threaten to alter the habitat niche necessary for seedling germination, establishment, and recruitment. Although there is well documented research examining tree distribution patterns at large scales in subalpine forests, very few studies on small scale, microhabitat characteristics necessary for seedling germination currently exist. In this study, I characterized the microhabitat niches of the Lower Subalpine (LSA) site of the Alpine Treeline Warming Experiment at Niwot Ridge, CO. Utilizing already established experimental plots, I measured eight abiotic variables in 900 microsites from June to mid-August 2011. I surveyed substrate cover types, soil moisture and temperature, and litter depth in each 10 x 10-cm microsite within five heated (experimental) and five unheated (control) plots. To assess the seedling germination niche, I noted the number of low elevation (10,000 ft) adapted *Pinus flexilis* (limber pine) in all microsites. Soil moisture, soil temperature, and duff layer depth consistently emerged as the strongest contributors to characterizing the observed microhabitat niches. Germinants tended to cluster in microhabitats characterized by thicker litter layers and higher soil moistures. It is likely the presence of deeper litter layers may enhance the germination niche by improving water conditions. The measured abiotic variables provide a rough but general characterization of the physical microhabitat gradients for this subalpine forest. Such a baseline for understanding the abiotic variables most significant to creating microhabitat provides an important basis for comparing against larger scale tree distribution patterns and for understanding the implications of such patterns in the face of climate change.

KEYWORDS

plant-environment interactions, soil temperature, soil moisture, litter, climate change, Alpine Treeline Warming Experiment (ATWE), seedling demography

INTRODUCTION

Species distribution in a landscape are the result of processes operating at both local and regional scales. Emerging studies have found that factors controlling tree distribution in subalpine forests may be strongly dependent on scale, where distribution and establishment of seedlings have a more complex and nonlinear response to climate and habitat at the localized level than at the regional or global level (Kupfer and Cairns 1996, Holtmeier and Broll 2005). Because the physical environment to which a seedling is subjected affects its probability of establishment and recruitment into a population, understanding the microhabitat niche that facilitates germination is an important area of study (Harper et al. 1965).

Assessing physical variables and environmental conditions in the immediate vicinity of seedlings provide a more scale-appropriate approach to understanding the critical factors affecting seedling germination, known as the seedling germination niche (Stueve et al. 2011). In general, seedlings in the early stages of their life cycle do not cope well with abiotic stresses, affecting their germination and establishment capabilities (Humphries et al. 2008). Because seedlings are in their critical stages of development, they are also more prone to mortality as a result of unsuitable environments (Caspersen and Saprunoff 2005). Compared to adult trees, conifer seedlings are considerably more sensitive to even the smallest changes in abiotic factors such as soil moisture and sunlight availability (Germino et al. 2002). As a result, exposure to high temperatures and lack of moisture tend to limit seedling germination and establishment (Stöcklin and Bäumler).

Microhabitat niches, on the other hand, are the result of small-scale heterogeneity, though the exact environmental conditions that create these niches—and the ways in which they interact—are not fully understood. Studies suggest that a variety of important microhabitat features including temperature, sky and wind exposure, snowmelt timing, soil moisture conditions, drainage, and substrate cover create microhabitat niches observed (Smith et al. 2003, Holtmeier and Broll 2005). The presence of vegetation and microtopography might also be important contributors to creating these niches as they provide shade and trap water, expanding the limits of available environments and creating pockets of microhabitat (Harper et al. 1965, Jones and del Moral 2005). Physical features including the amount of litter, presence of rocks or

woody debris, and soil texture may provide additional important interacting factors that may be significant to characterizing microhabitat niches (Jones and del Moral 2005). In such a way, a variety of existing microhabitat conditions may potentially interact to create the microhabitat niche observed.

In order to fully grasp plant-environment relationships, aspects of both the microhabitat niche and the germination niche must be analyzed in concert. Despite the existence of general theories surrounding the microhabitat niche and the seedling germination niche, comparatively little exploration exists analyzing plant-environment relationships using microhabitat niches and seedling germination niches jointly. Specifically, the role of abiotic conditions in creating the microhabitat niches that in turn influence the germination niches of *Pinus flexilis* (limber pine) remains fairly unexplored in subalpine forests. As microhabitat and germination niches become more pronounced when climatic stresses increase, such as with increasing temperatures linked with global warming, the role of the microhabitat in facilitating seedling germination in relation to changing environmental limits also presents an important area in need of further exploration.

Using such a multivariate approach, I seek to determine the abiotic variables most suitable for characterizing the microhabitat niches of the lower subalpine coniferous forest in Niwot Ridge, Colorado. I also assess changes to abiotic conditions as a result of increasing temperatures linked with global warming. Lastly, I determine the microhabitat niches most central to promoting patterns of seedling germination at microsite spatial scales in the forest understory at Niwot. I expect that several interacting physical microsite factors will affect both the microhabitat niche and the seedling germination niche. In particular, though temperature-induced moisture deficits that hinder tree growth (Wilmking et al. 2004) may link soil moisture with seedling germination, I also predict that upper thresholds in litter depth may play an important role (Knapp and Smith 1986).

METHODS

Study Site

I conducted the study at the Lower Subalpine (LSA) site (40°2'N, 105°33'W) of the Alpine Treeline Warming Experiment (ATWE) at Niwot Ridge LTER located in the southern region of the Rocky Mountains, CO near Nederland, CO (Figure 1). The region on the whole

exemplifies the forest-alpine ecotone (FTE) with its striking spatial heterogeneity and unique climate gradient (Greenland and Losleben 2001). Spatially, the ecotone spans a dynamic transition zone between coniferous forest and alpine tundra ecosystems with elevations ranging from 9,600 m to 13,400 m. On a broad scale, the high elevation and mid-latitude location shape the larger climate patterns of the ecotone (Humphries et al. 2008), whereas easterly winds and low temperatures that prevail throughout the year tend to influence climate patterns at fine scales (Holtmeier and Broll 1992, Humphries et al. 2008). Most of the precipitation occurs in winter and spring, with secondary precipitation occurring in July and August (Holtmeier and Broll 1992). Annual snow cover also provides a source of soil moisture in the summer months between June and August, creating a variety of different plant communities uniquely adapted to each resulting microhabitat of the region (Greenland and Losleben 2001).

The plant communities of at LSA are composed of three dominant tree species—*Picea engelmannii* (spruce), *Abies lasiocarpa* (alpine fir), and *Pinus flexilis* (limber pine) (Batlori et al. 2009). For my study, I focused on *P. flexilis* because of its higher rates of germination and establishment compared to the other two species (Cristina Castanha, personal communication). This key germination and establishment characteristic of *P. flexilis* was important to ensure a large enough data set for later analysis. In addition, *P. flexilis* facilitates the emergence of late successional understory species (Baumeister and Callaway 2006). Because such a requisite understory exists exclusively at lower elevations of the FTE, I concentrated my study at the Lower Subalpine site (Humphries et al. 2008).



Figure 1. Lateral view of Niwot Ridge LTER. Elevational gradient from coniferous forest to open alpine meadow observed at Niwot Ridge near Nederland, CO. This study is focused at LSA site.

Experimental Design

Following snowmelt in late June 2011, I sampled already established experimental plots in the LSA site of the ATWE at Niwot Ridge. I sampled a total of ten plots: five control plots (UH) that reflected the natural forest ecosystem at LSA, and five experimentally manipulated plots (H) that mimicked projected global temperature increases within the next 100 years (Figure 2a, 2b). To simulate such climate warming, we heated the soil surface of the experimental plots to temperatures roughly 4°C higher than ambient temperatures (Karl and Trenberth 2003). Each plot contained 100 10 x 10-cm ‘microsites’ artificially sown in Fall 2010 with 10 *P. flexilis* seeds per microsite (Figure 2c). This size microsite was small enough to incorporate the physical habitat immediately surrounding seedlings while also large enough to include several measurable habitat parameters.

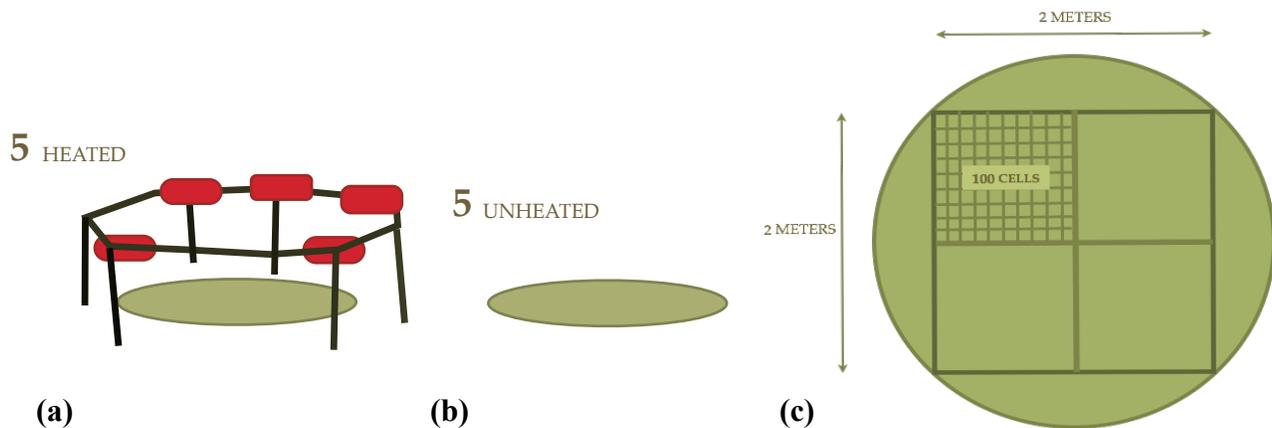


Figure 2. Plot Design. (a) Heated plots shown with infrared heating contraptions, (b) unheated plots demarcated by circle, and (c) a bird's eye view of plot layout with 100-cell grid of the microsites included.

Abiotic Variables

To characterize microhabitat conditions, I sampled abiotic variables in both the unheated plots and heated plots at LSA: substrate cover, soil moisture and temperature, and duff layer depth. To minimize time-dependent variability in abiotic variables within and among plots, for each plot, I collected all abiotic measurements on the same day within a two-hour time period corresponding to the strongest intensity sunlight hours of the day on cloudless days.

To measure soil temperature, I used an RTD handheld thermometer data logger (OMEGA, Stamford, CN) to take one temperature reading near the geometric center of each cell

at a depth of 2.5 cm below the duff and mineral soil layer interface (Cristina Castanha, personal communication). To measure soil moisture, I recorded one reading at the geometric center of each cell using the SM300 Soil Moisture sensor (DYNAMAX, Houston, TX) 5 cm below the duff and mineral soil layer interface (Cristina Castanha, personal communication). To quantify substrate cover, I visually categorized each cell into best estimates of percent cover based of five categories (rock, soil, plant, litter, and woody debris) (Simard et al. 1998). I measured duff layer depth by plunging a probe into the geometric center of each cell until the substrate penetrability changed. The length of probing was recorded as the duff layer depth for the cell. One measure for each abiotic variable was recorded, as it provided adequate replication while allowing rapid measurement under uniform conditions.

Table 1. Summary of methods for abiotic variables. Eight variables used to characterize the microhabitat niche of newly emergent *P. flexilis* seedlings in a lower subalpine forest of the ATWE.

Variable	Abbr	Measurement
Soil Temperature	SoT	One reading near the geometric center of each cell at a depth of 2.5 cm below the duff layer and soil layer interface.
Soil Moisture	SoM	One reading near the geometric center of each cell at a depth of 5 cm below the duff layer and soil layer interface.
Duff Litter Depth	DD	Use probe to drive into duff layer until substrate penetrability changes.
% Duff Cover	%D	Visual estimate of percentage of duff cover by area for each microsite, out of 100%..
%Woody Debris	%Woody	Visual estimate of percentage of woody debris cover, including particles of cones, bark, and twigs, by area for each microsite.
% Plant Cover	%Plant	Visual estimate of percentage of alpine plant cover, not including seedlings, by area for each microsite, out of 100%.
% Rock Cover	%Rock	Visual estimate of percentage of rock cover by area for each microsite, out of 100%.
% Soil Cover	%Soil	Visual estimate of percentage of soil cover by area for each microsite, out of 100%.

I repeated this sampling scheme for abiotic conditions in all plots, both unheated and heated. To assess short-term changes in abiotic conditions from the time of snowmelt to the end

of the summer season I also surveyed all ten plots twice over the course of the summer, once in late June and again in early August.

Biotic Variables

To collect biotic data, I conducted surveys of newly emergent seedlings, or germinants, observed in each microsite. At the time of survey of abiotic variables, I also recorded the number of newly emergent seedlings, or germinants, observed in each microsite for all unheated plots and heated plots in early and late summer.

Statistical Analyses

Abiotic Variables

To analyze the abiotic factors of each microclimate and determine correlations among abiotic variables, I first determined the mean and standard deviations for soil moisture, soil temperature, and duff layer depth for early and late summer surveys of the unheated, control plots (R Development Core Team 2011). Using a paired t-test, I determined statistically significant differences between early and late summer surveys for each abiotic variable from the unheated plots to determine immediate temporal changes to the abiotic factors (R Development Core Team 2011). I analyzed differences between unheated and heated plots for the late summer survey using the same numerical summaries. To improve normality, I square root transformed continuous variables from the substrate cover surveys for all tests (Collins and Good 1987).

I also determined the correlation coefficients of the eight abiotic variables surveyed (soil moisture, soil temperature, duff layer depth, % rock, % soil, % vegetation, % woody debris, % duff) via a correlation matrix among the heated plots and among unheated plots of the late summer survey (R Development Core Team 2011). I retained abiotic factors with statistically significant correlations from both the unheated plots and the heated plots to further assess within plot correlations of all individual plots using correlation tests. From these tests, I retained significant correlation coefficients to create scatterplots for further analysis.

To further summarize the abiotic variables, I ran a Principle Components Analysis (PCA)

on the reduced matrices of the strongest correlations for the unheated plots and the heated plots, separately (Collins and Good 1987). Principal components analysis reduces the complexity of a multiple variables by combing original variables into a few new, orthogonal principal components. Correlation coefficients, or loadings, indicate the relationship of each original variable to each principal component. After running the PCA, I retained the strongest contributors for each PCA axis for further analysis. By using the microsite points obtained through surveys, I determined differences in microhabitats between heated and unheated plots as well as among individual plots (Collins and Good 1987). Using an eigenvector diagram, I determined the strength of significant contributors of each PCA axis to establish the best proxies for characterizing the physical microhabitats of the microsities.

Biotic Variables

Based on trends observed from analyses of the abiotic variables, plots that exemplified the microhabitat conditions at LSA were retained for further analyses with *P. flexilis* germinants. To explore the ways in which biotic conditions interact with seedling germination, I plotted the mean values of significant variables retained from the correlation matrices and the PCA against the number of newly emergent seedlings observed for those variables. Using an eigenvector diagram, I determined the strength of significant contributors of each PCA axis to establish the best proxies for determining the probability of seedling germination.

RESULTS

Abiotic Variables

Early and Late Summer

Microhabitat characteristics between early and late surveys were significantly different. Based on mean values for soil moisture, soil temperature, and duff layer depth, I found that a considerable range of microhabitats exist between early and late summer. Among unheated plots, mean soil temperatures from late summer surveys were significantly higher than mean soil

temperatures from early summer surveys (13.6 °C vs. 13.2 °C, $p < 0.0001$). Similarly, mean soil moisture levels from late summer surveys of unheated plots were significantly higher than mean soil moisture levels from early summer surveys (173 mV vs. 149 mV, $p < 0.0001$). Alternatively, mean values for duff layer depth were significantly lower during late summer surveys compared to early summer surveys in all unheated plots (Table 2). Substrate cover types did not change between early summer surveys and late summer surveys, and thus, I omitted values for substrate cover from Table 2.

Table 2. Comparison between early and late summer soil conditions. Mean, standard deviation (SD) and the results of the paired t-tests evaluating the difference between early summer and late summer for unheated plots. Mean soil temperatures (SoT) are in degrees Celcius (°C), soil moisture (SoM) units are in millivolts (mV), and mean duff layer depths (DD) are in centimeters (cm). N=858 for both early and late summer.

	Early Summer (July)		Late Summer (mid-August)		<i>t</i>	<i>p</i>
	Mean	SD	Mean	SD		
SoT	13.2	1.19	13.6	0.93	36.5	<.0001
SoM	149	42.3	173	66.3	11.4	<.0001
DD	1.91	1.26	1.85	1.22	10.6	<.0001

Unheated and Heat Plots

Based on mean values of the abiotic variables, marked differences exist in the physical habitats of the unheated and heated plots. In particular, a considerable range of mean values between the unheated and heated plots exists for soil moisture, soil temperature, and duff layer depth. Although duff layer depth had large error values (0.65–3.17 for H2, 0.63–3.07 for UH2)), all three of the variables were significantly different between heated and unheated plots.

Mean values of soil temperature were significantly higher ($p < 0.0001$) in heated plots compared to unheated plots from the late summer survey (Table 3, Fig. 3a). On average, soil temperatures were approximately 5.6 °C higher in the heated plots compared to the unheated plots. On the other hand, mean values of soil moisture levels were significantly lower ($p < 0.0001$) in heated plots compared to unheated plots from the late summer survey (Table 3, Fig. 3b). On average, soil moisture levels were 35 mV lower in the heated plots compared to the unheated plots.

I also observed significant differences between the duff layer depths of heated and unheated plots. Mean duff layer depths were significantly higher in the heated plots compared to the unheated plots (Table 3). Duff litter depth was approximately 0.8 cm higher in the heated plots compared to the unheated plots. In contrast, I found that substrate cover showed a pattern of continuous variation among heated and unheated plots. I observed no statistically significant differences in substrate cover types between heated plots and unheated plots.

Table 3. Comparing unheated and heated plots. Mean, standard deviation (SD) and the results of the paired t-tests evaluating the difference between heated and unheated plots from late summer. Mean soil temperatures (SoT) are in degrees Celsius (°C), soil moisture (SoM) units are in millivolts (mV), and mean duff layer depths (DD) are in centimeters (cm). Substrate cover types are given as percentages (%). N=429 for both UH2 and H2.

	Unheated Plots (Control)		Heated Plots (Experimental)		<i>t</i>	<i>p</i>
	Mean	SD	Mean	SD		
SoT	13.6	.93	19.2	2.80	36.5	<.0001
SoM	173	66.3	138	58.6	11.4	<.0001
DD	1.85	1.22	2.60	1.21	10.6	<.0001
%D	81.5	25.9	81.7	23.8	0.80	.43
%WD	3.72	7.53	5.58	10.5	0.08	.93
%S	3.63	14.1	0.21	3.98	0.52	.63
%P	5.84	14.9	6.59	15.7	0.81	.42
%R	5.39	13.9	6.03	17.2	2.31	.02

Across Plot Comparisons

Correlation coefficients on the nine abiotic variables surveyed suggested that on the whole, soil moisture and duff layer depth were the driving abiotic variables, as most variables were significantly correlated to soil moisture and duff layer depth (Table 4). Although I determined that a majority of the abiotic variables surveyed were only weakly correlated with one another ($r < 0.10$, $n = 429$, $p > 0.01$), I found that soil temperature and soil moisture among both unheated ($r = -0.42$, $n = 429$, $p < 0.01$) and heated ($r = -0.43$, $n = 429$, $p < 0.01$) plots were significantly and negatively correlated within late summer surveys (Table 4). Similarly, soil

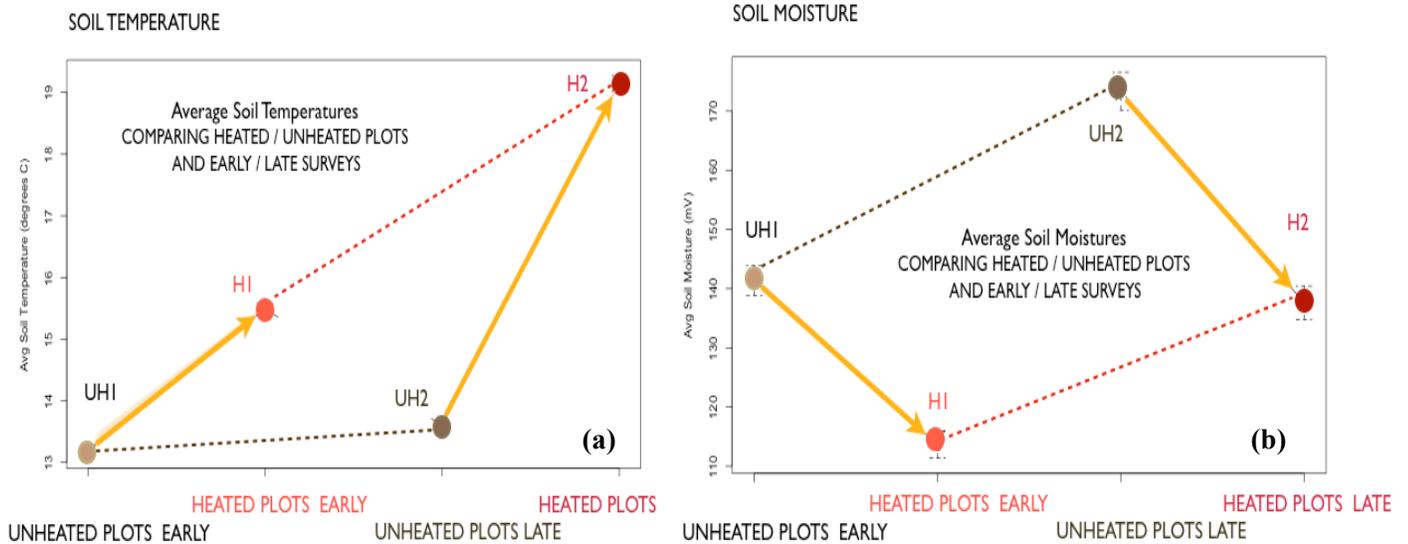


Figure 3. Summary of soil temperature and soil moisture across all plots. The four categories (H1, H2, UH1, UH2) are differentiated by dot color. Average soil temperatures consistently increase between unheated and heated plots as well as between early summer surveys and late summer surveys. Average soil moisture levels consistently decrease between unheated and heated plots but increase between early summer surveys and late summer surveys. P-values are reported in Table 3.

temperature and duff layer depth among both unheated ($r=-0.45$, $n=429$, $p<0.01$) and heated ($r=-0.31$, $n=429$, $p<0.01$) plots were significantly, negatively correlated within late summer surveys (Table 4). I also determined that soil moisture and duff layer depth were significantly and negatively correlated among the heated plots ($r=-0.15$, $n=429$, $p<0.01$) from late summer surveys (Table 4). I found significant positive and negative correlations between soil temperature, soil moisture, and duff layer depth and several substrate cover types summarized in detail in Table 4.

Because relationships between soil moisture and soil were the strongest, I retained both variables for further analysis. A scatterplot summarizes the relationships between soil moisture and soil temperature for UH1, H1, UH2, and H2 (Figure 4). Overall, there was a strong, negative relationship between soil temperature and soil temperature for UH2 and H2 plots, and only weak relationships for UH1 and H1 plots. As such, increases in soil temperature were related to decreases in soil moisture for both H2 and UH2 plots (Figure 5).

Table 4. Correlation matrix of abiotic variables. Correlation matrix of variables measured for heated (H2) and unheated (UH2) for the late summer survey plots at the lower subalpine (LSA) site of MRS. Only statistically significant relationships are bolded ($p < 0.01$).

H2	SoT	SoM	DD	%D	%WD	%S	%P	%R
SoT	1.000	-	-	-0.03	0.01	0.07	-0.03	0.05
SoM	-0.43	1.000	-	0.04	-0.01	0.00	0.06	-0.09
DD	-0.31	-0.15	1.000	0.05	0.06	-0.06	-0.14	0.06
UH2	SoT	SoM	DD	%D	%WD	%S	%P	%R
SoT	1.000			0.01	-0.08	0.02	-0.03	0.26
SoM	-0.42	1.000		-0.23	-0.07	0.16	0.23	0.06
DD	-0.45	0.03	1.000	0.22	0.11	-0.23	-0.16	-0.26

Within Plot Comparisons

Both within plot and across plot comparisons between soil temperature and duff layer depth were consistently negative, though trends observed across plots were stronger than trends observed within plots. Only three heated plots (4, 11, and 14) displayed statistically significant within plot correlations ($p < 0.01$). Relationships between soil moisture and duff layer depth were less conclusive among individual plots. Although I found that among all individual heated plots, comparisons between soil moisture and duff layer depth were consistently negative (with statistically significant correlations for plots 6 and 14), I found no strong relationships among the individual unheated plots. Within plot comparisons between soil moisture and soil temperature confirmed trends observed across all plot comparisons. With the exception of plot 1 and 14, soil moisture and soil temperature were negatively correlated. Plots 10, 15, and 18 were strongly, negatively correlated ($p < 0.01$).

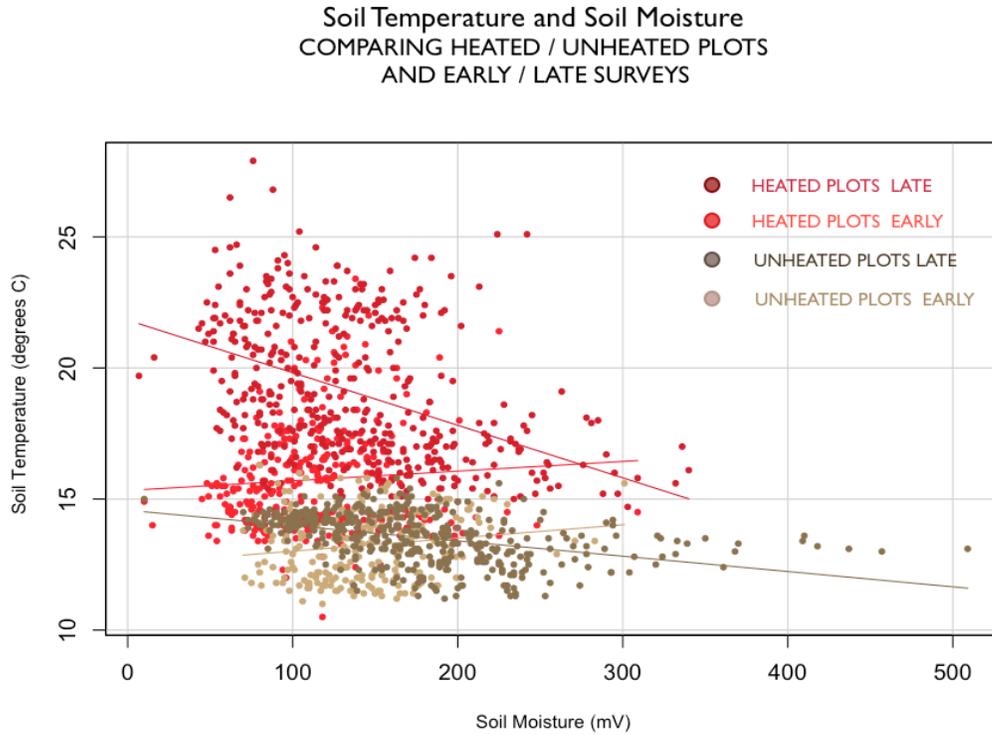


Figure 4. Relationship between soil moisture and soil temperature across all plots for all treatments and both survey groups. Four treatment groups (H1, H2, UH1, UH2) are differentiated by dot color. H2 and UH2 correlations are significant. R- and p-values are reported in Table 3.

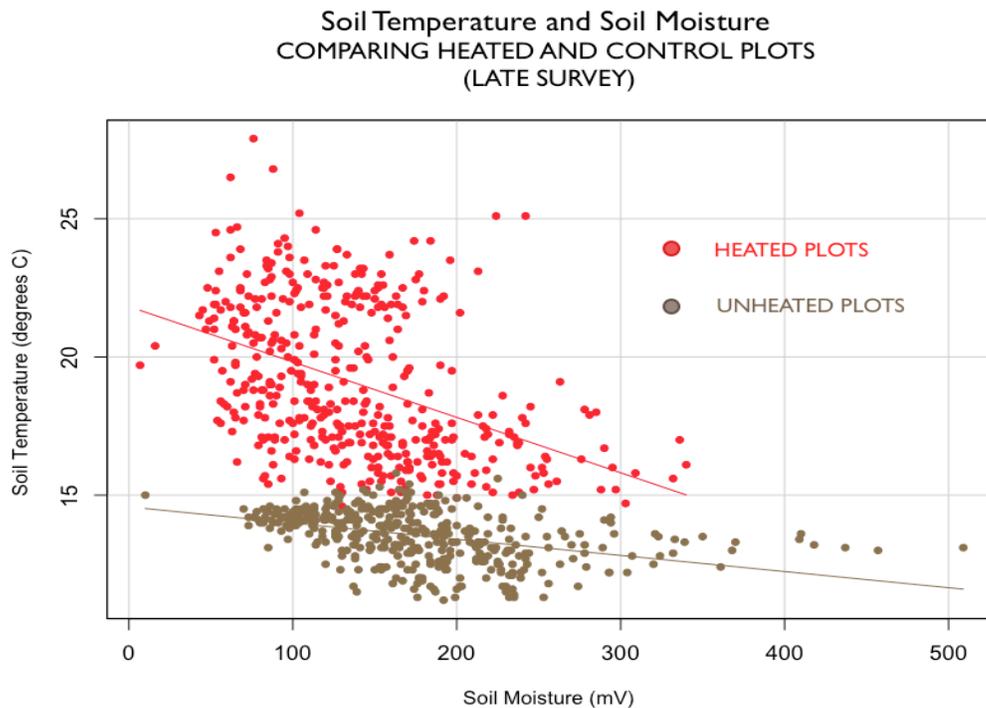


Figure 5. Relationship between soil moisture and soil temperature for late summer survey. Correlations are significant ($p < 0.01$). R-values are reported in Table 3.

Table 5. Within plot correlations. Summary of correlations for each individual unheated plot from the late summer surveys. The top five plots listed are the unheated plots and the bottom five listed are the heated plots. Only statistically significant relationships are bolded ($p < 0.01$).

PLOT	SoT, DD	SoM, DD	SoT, SoM
1	-0.22	-0.09	0.14
10	-0.20	0.12	-0.51
11	-0.36	0.04	-0.10
15	-0.10	-0.04	-0.33
18	-0.05	0.06	-0.26
4	-0.29	-0.02	-0.07
6	-0.05	-0.27	-0.01
14	-0.27	-0.45	0.41
16	-0.06	-0.08	-0.18
17	-0.14	-0.01	-0.04

Principal Components Analysis (PCA)

Five of the abiotic variables from the unheated plots and seven of the abiotic variables from the heated plots were used to run a Principal Components Analysis (PCA), which produced three factors with eigenvalues greater than 1.0, contributing to 73% the variances among microhabitats observed at LSA for the unheated plots (Table 6) and 64% of the variance among microhabitats observed for the heated plots (Table 7). PCU-1 (31%), PCU-2 (25%), and PCU-3 (17%) contributed to the majority of the variance among microhabitats observed for the unheated plots (Table 6), while PCH-1 (26%), PCH-2 (21%), and PCH-3 (17%) contributed to the majority of the variance among microhabitats observed for the heated plots (Table 7).

Unheated Plots. Soil temperature, duff layer depth, and rock cover were the strongest contributors to characterizing the microhabitat among unheated plots. Because soil temperature was strongly, positively correlated with PCU-1 while both duff layer depth and rock cover were strongly, negatively correlated with PCU-1, the PCU-1 axis defined the primary microhabitat gradient separating areas with high soil temperature from areas with high rock cover and deeper duff layer depths. Similarly, because soil moisture was strongly, positively correlated with PCU-2 while duff cover and soil temperature were strongly, negatively correlated with PCU-2, the PCU-2 axis defined the secondary microhabitat gradient separating areas with high soil moisture

from areas with high duff cover and high soil temperatures. The PCU-3 axis reflected a % plant cover gradient.

Table 6. PCA results for unheated plots. Principal Components Analysis (PCA) of the correlation matrix of six abiotic variables used to characterize microsite within the unheated plots of the second summer survey (UH2) at LSA. Eigenvalues and explained variance are also included. Significant correlations are bolded.

Variable	PCU-1	PCU-2	PCU-3
SoT	0.52	-0.42	0.13
SoM	-0.16	0.67	0.13
DD	-0.55	-	-0.19
%D	-0.37	-0.52	0.24
%P	-	0.18	0.90
%R	-0.51	0.29	-0.21
Eigenvalue	1.37	1.24	1.02
Explained variance (%)	31	25	17

Heated Plots. Percent substrate cover types were the strongest contributors to characterizing the microhabitat among heated plots. Because duff cover was strongly, positively correlated with PCH-1 while both plant cover and rock cover were strongly, negatively correlated with PCH-1, the PCH-1 axis defined the primary microhabitat gradient separating areas with high duff cover from areas with high rock and plant cover. Similarly, because soil moisture was strongly, positively correlated with PCH-2 while soil temperature was strongly, negatively correlated with PCH-2, the PCH-2 axis defined the secondary microhabitat gradient separating areas with high soil moisture from areas with high soil temperatures. The PCU-3 axis reflected a duff layer depth gradient.

Distribution of the microhabitats in space defined by the two principal components (Figure 6a) for the unheated plots shows that most microsite points occurred in habitats with deeper duff layer depths, high amounts of duff and rock cover, and higher soil temperatures. For the unheated plots, the distribution of the microhabitats in space defined by the two principal components (Figure 6b) reveals that most microsite points occurred in habitats with higher soil temperatures and higher amounts of duff cover.

Table 7. PCA results for Heated plots. Principal Components Analysis (PCA) of the correlation matrix of seven abiotic variables used to characterize microsite within the heated plots of the second summer survey (H2) at LSA. Eigenvalues and explained variance are also included. Significant correlations are bolded.

Variable	PCH-1	PCH-2	PCH-3
SoT	-0.18	0.68	-0.23
SoM	0.12	-0.63	-0.36
DD	0.23	-0.13	0.73
%D	0.68	0.17	-0.15
%WD	-0.13	-	0.37
%P	-0.47	-0.29	-0.22
%R	-0.44	-	0.27
Eigenvalue	1.35	1.21	1.09
Explained variance (%)	26	21	17

Biotic Features

Based on trends observed from analyses of the abiotic variables, plots 10, 15, and 18 were retained for further analyses with *P. flexilis* germinants, as these plots exemplified the microhabitat conditions of the lower subalpine (LSA). Results from the PCA analysis of the reduced matrices produced two factors with eigenvalues greater than 1.0, contributing to 59% the variances (Table 8). PCS-1 (33%) and PCS-2 (26%) contributed to the majority of the variance observed for the unheated plots (Table 6) (Table 8). For the primary microhabitat gradient of the eigenvector diagram, seedlings were located in areas of higher soil moisture and lower soil temperature (Figure 7). Seedlings also tended to be located in areas of deeper duff layer depths, as the shown by the secondary microhabitat gradient of PCS-2.

Further analysis of important abiotic variables from the PCA of germinants revealed trends between the average values of abiotic variables associated with observed number of germinants. In general a higher number of germinants were observed at lower levels of soil temperature (Figure 8a), although these trends were not statistically significant. A comparison of soil moisture and the relative number of germinants suggested an optimum soil moisture level facilitates seedling germination (Figure 8b). I found similar results for the comparison between duff layer depth and relative number of germinants (Figure 8c). The trends observed suggest a small range of plant cover (approximately 5% cover) facilitates seedling germination in general, with little influences of the relative number of germinants (Figure 8d).

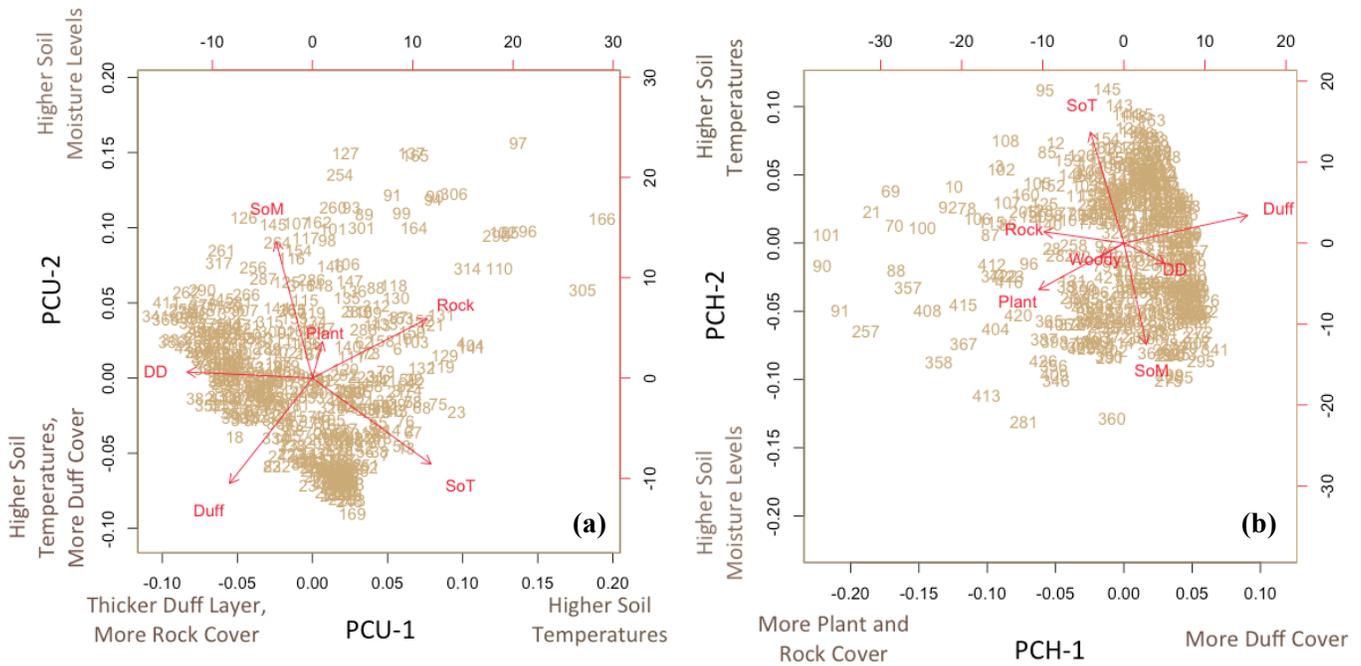


Figure 6. Eigenvector diagram of microhabitat characteristics. (a) Eigenvector diagram showing the coefficients of the linear function defining the two components in the microhabitat PCA for unheated plots. PC-1 (31% of the variance) is a gradient from soil temperature to duff layer depth and amount of rock cover. PC-2 (25% of the variance) is a gradient from soil moisture to soil temperature and amount of duff cover. (b) Eigenvector diagram showing the coefficients of the linear function defining the two components in the microhabitat PCA for heated plots. PC-1 (26% of the variance) is a gradient from amount of duff cover to amount of rock and plant cover. PC-2 (21% of the variance) is a gradient from soil temperature to soil moisture.

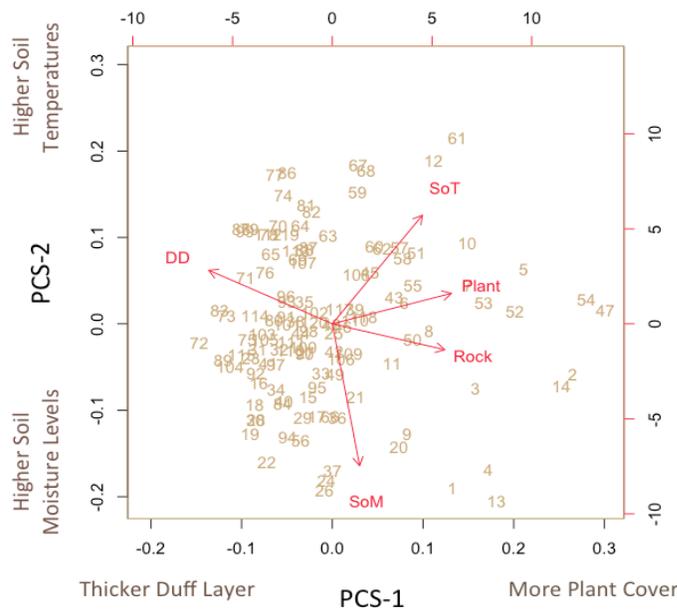


Figure 7. Eigenvector diagram of seedling germination distribution across microhabitats. Eigenvector diagram showing the coefficients of the linear function defining the two components in the seedling microhabitat PCA for the three unheated plots. PCS-1 (33% of the variance) is a gradient from duff layer depth to amount of plant cover. PCS-2 (26% of the variance) is a gradient from soil moisture to soil temperature. Seedlings tend to cluster in areas with higher soil moisture levels, lower soil temperatures, and deeper duff layer depths.

Table 8. PCA results for germinant analysis. Principal Components Analysis (PCA) of the correlation matrix of five abiotic variables used to characterize microsite within three unheated plots of the second summer survey (UH2). S stands for ‘seedling’ in PCS. Eigenvalues and explained variance also included. Significant correlations are bolded.

Variable	PCS-1	PCS-2
SoT	0.40	0.57
SoM	0.12	-0.74
DD	-0.55	0.28
%P	0.53	0.16
%R	0.50	-0.14
Eigenvalue	1.29	1.15
Explained variance (%)	33	26

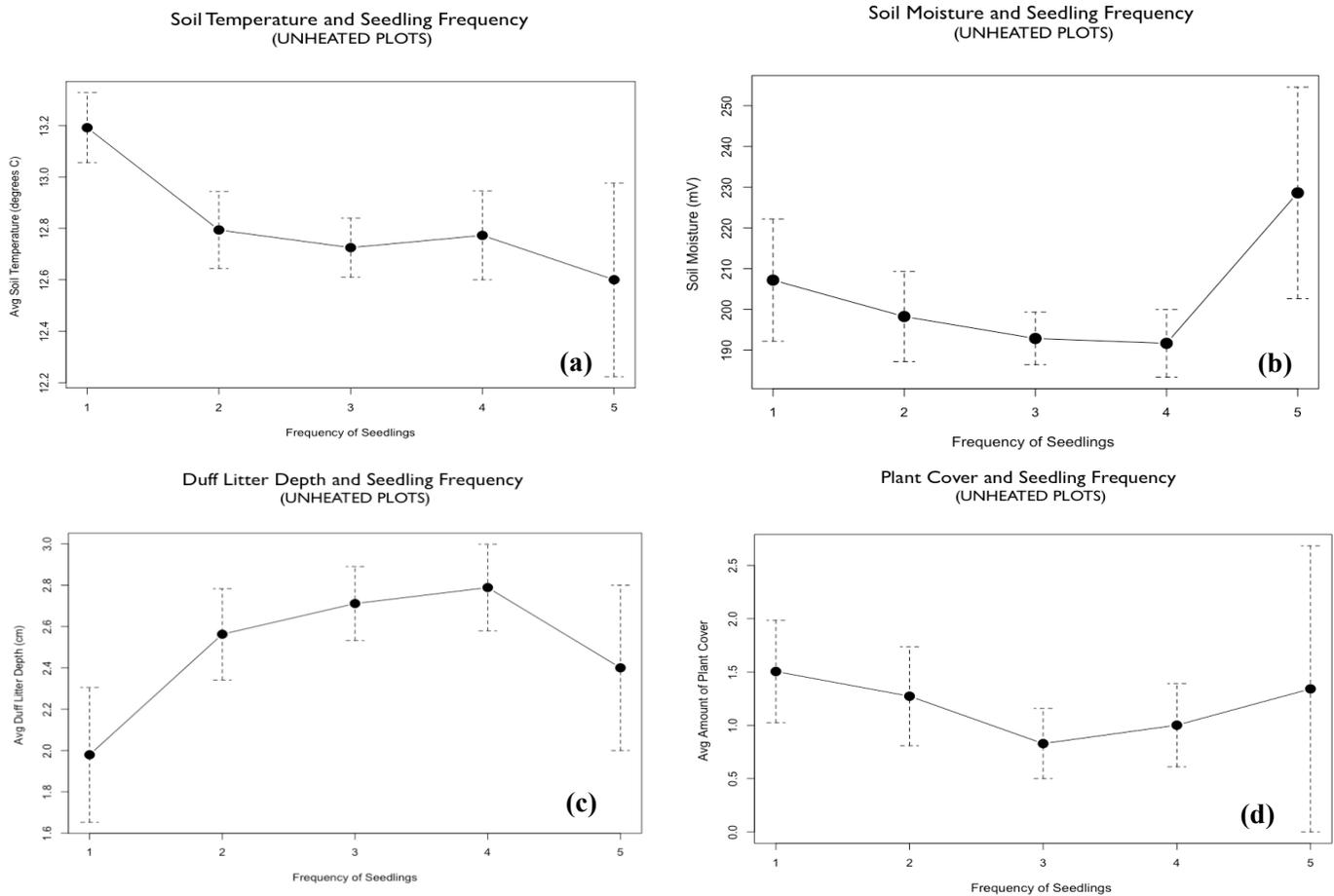


Figure 8. Influential variables and seedling germination counts. (a) Plot of average soil temperatures and number of germinants. Lower counts of germinants were found with increasing soil temperatures. (b) Plot of average soil moistures and number of germinants. Trends suggest optimum levels of soil moisture influence frequency of germinants. (c) Plot of average duff layer depths and number of germinants. Trends suggest optimum levels of soil moisture influence frequency of germinants. (d) Plot of average amount of plant cover and number of germinants. Though plant cover is an important variable for seedling germination, an optimum level of plant cover facilitates seedling germination in general, with no influence on the number of germinants.

DISCUSSION

Overall, the microhabitat variables I measured provide a rough but general description of the physical microhabitat gradients at the lower subalpine (LSA) forest of the study. Based on the eight abiotic variables used to characterize microhabitat, I found soil temperature, soil moisture, and duff layer depth consistently emerged as the strongest contributors to these observed microhabitat gradients. Seedling germination for *P. flexilis* generally tended to be highest in areas with higher relative soil moisture levels, lower relative soil temperatures, and deeper litter layers, although confounding interactions may complicate these simplistic trends.

Abiotic Variables

In surveying the abiotic variables most influential to characterizing the microhabitat at LSA, soil moisture, soil temperature, and duff layer depth accounted for statistically significant trends observed for unheated plots. However, the strength and direction of these interactions varied among unheated plots and within individual plots. Correlation coefficients from the PCA identified soil moisture and duff layer depth as the strongest contributors to explaining the variance among abiotic variables for unheated plots. A very similar study in an oak-pine forest found duff layer depth strongly contributed to habitat gradients observed, results that are consistent with the findings here in *P. flexilis* (Collins and Good 1987). In a study on microclimate and soil moisture responses in coastal Douglas fir forests in the Pacific Northwest, similar results indicated that soil moisture and soil temperature strongly influence the structure and composition of gap microhabitats (Gray et al. 2002).

Early and Late Summer

Comparing early and late summer surveys, it is evident that attempts to explore spatial variation of microhabitats must also consider temporal variations as well. The microhabitat differences between the two surveys suggest germination niches are dynamic and temporally specific. Because microhabitat differences between early summer and late summer survey data were statistically significant, I accounted for temporal changes by analyzing each survey group

separately, with special focus on the late summer survey, as the microhabitat data collected then most directly reflected microhabitat conditions influencing seedling germination (Castro 2006).

Soil temperature increased between early and late summer, as expected, due to the increase in ambient temperatures between the two surveys. As expected, soil moisture also increased between surveys since the early survey occurred before the snowmelt date, while the late summer survey occurred in the midst of snowmelt. As a result, the large volumes of water released from snowpack at the alpine (ALP) site likely contributed to the increased frequency of rain events between early and late summer surveys that in turn contributed to the increase in soil moisture during the late summer survey (Lapp et al. 2005). The factors influencing the decrease in duff layer depth observed between surveys was more complex, however. Because seasonal patterns of litter vary temporally, such that the greatest litter accumulation typically occurs in spring and early summer for coniferous ecosystems, litter was expected to change between surveys. Longer daylight days during early summer create high ambient temperatures, limited water availability, as well as nutrient changes within the litter, thereby increasing litter depths (Kueppers and Harte 2005). Moreover, mean annual temperature comparisons with decay rates of litter for pines suggest increasing temperatures up to a certain threshold increase the decay rate, consistent with the differences in mean temperatures between early and late summer observed here (Kueppers and Harte 2005).

Unheated and Heated Plots

In comparing heated and unheated plots, soil temperature, soil moisture, and duff layer depth again emerged as the variables with statistically significant differences. The results of the paired t-test comparing heated and unheated plots for soil temperature and soil moisture confer predictions by climate models and similar warming experiments. According to these predictions, soil temperature is expected to increase with increasing ambient temperatures, while soil moisture is expected to decrease (Shaver et al. 2000). Though the increases in soil temperature were expected, since the infrared heaters staggered around the experimental plots raised ambient temperatures roughly 1-4 °C, the recorded magnitude of increase (5.6 °C) in mean soil temperatures between unheated and heated plots was much greater than expected. Despite using the early and late summer surveys to normalize for temporally induced differences that may have

potentially interfered with trends recorded in the experimental and control plots, I still found the heaters, rather than temporal changes created by the seasons, accounted for the soil temperature increases between the unheated and heated plots.

Similarly, mean soil moisture in the heated plots was lower than the mean soil temperatures observed in early summer, suggesting the increased ambient temperatures induced by the heaters were partially responsible for the decreases in moisture observed between the unheated and heated plots. These decreases in soil moisture are consistent with similar alpine forest studies (Harte et al. 1995, Baumeister and Callaway 2006). However, as these studies indicate, the decreases between the heated and unheated plots cannot be solely attributed to the warming effect of the radiators. Complex feedbacks with soil albedo and soil respiration also complicate simplifications of the processes behind the observed decreases in soil moisture (Harte et al. 1995, Baumeister and Callaway 2006).

On the other hand, duff layer depth increased between the control and experimental plots. These trends are opposite of those observed between early and late summer surveys, suggesting that the duff processes observed in the early and late summer surveys are reversed by the microclimate conditions induced by the radiators. A study of carbon stocks in the region suggests that regional warming in the Rocky Mountain subalpine forests result in a net source of carbon to the atmosphere, a process potentially linked with the duff layer depth decrease observed between early and late summer here at LSA (Kueppers and Harte 2005). The study found a hump-shaped relationship between mean annual temperatures and duff decay (Kueppers and Harte 2005), suggesting that below a certain threshold, increases in temperature increase the decay rate of litter, but that above a certain threshold, the decay rate actually decreases. If these trends apply to the microhabitat at LSA, perhaps the duff layer depth trends between early and late summer occur as a result of the former trend, while the duff layer depth differences between the unheated and heated plots occurred as a result of the latter trend. Regardless, the underlying processes for litter decomposition, as measured by duff layer depth, are more likely to be driven by other confounding factors besides ambient soil temperatures, including carbon cycling dynamics, nutrient availability, resource quality, and microbial activity (Anderson 1991).

Correlation Matrices

Analysis of interactions among the various abiotic variables with correlation matrices confirms the complexities inherent to characterizing the microhabitat niche. However, the interacting relationships found among soil moisture, soil temperature, and duff illuminate underlying processes creating microhabitat at LSA.

Soil Moisture and Soil Temperature. The strong negative relationship between soil temperature and soil moisture across all plots, both heated and unheated, emerged as the strongest relationship retained from the correlation matrix. Soil moisture, in association with porosity, texture, and organic material content is a key factor influencing penetration of heat into the ground surface (Anderson 1991). Since all incoming heat to the plots is either distributed to raising surface cover temperatures directly or to causing evaporation of moisture from soil—the greater the soil moisture, the greater the amount of thermal energy necessary to increase the temperature of surface cover and the soil profile below it (Harte et al. 1995). Harte et al. refer to this phenomenon as the “moisture effect,” where higher levels of soil moisture infer that the incoming radiation goes less into raising soil temperatures and more into drying the soil (1995). In their meadow-warming experiment examining soil microclimate in the Rocky Mountains, higher soil temperatures were indeed observed in drier, less vegetated plots, as in Niwot, indicating plant cover might further interact with the aforementioned “moisture effect” (1995).

Soil Temperature and Duff Layer Depth. Litter depth can also influence soil temperature, as indicated by the strong negative relationship between soil temperature and duff layer depth. The characteristic low bulk density and thermal conductivity of the soil layer underlying deep litter cover result in considerable heat flux necessary to heat the active layer of the soil profile below (Anderson 1991). Thus, the deeper the litter layer, the more sunlight energy necessary to raise the temperature of the soil below. High litter mass is also known to buffer soil temperature fluctuations (Hogg and Leiffers 1991). Similar conclusions from a study on the role of litter in terrestrial ecosystems indicate the litter layer acts as a thermal insulator for the forest floor (Carson and Peterson 1990). Comparison of duff layer depth in the heated and unheated plots suggests deeper duff layers increase the amount of insulation in soils, thereby increasing the

absolute soil temperature of heated versus unheated microsites. Changes in soil temperature as a result of varied litter depths may directly influence vegetative cover, creating feedback loops with soil temperature and soil moisture (Facelli and Pickett 1991).

Soil Moisture and Duff Layer Depth. The effects of soil moisture and duff layer depth are interrelated in creating the microhabitat gradient of the coniferous forest at LSA as well. Although the presence of litter can increase soil moisture by decreasing surface evaporation (Heady 1956), soil moisture is also strongly influenced by water consumption, which is determined by traits such as plant phenology, water uptake per unit to biomass, and rooting morphology that in turn influence the rate of litter production (Ayres et al. 2009). Because litter depth is dependent on litter decomposition rates, and such rates in turn are regulated by carbon fluxes within the broader ecosystem, soil respiration provides the important link between soil moisture and litter. Both large- and small-scale studies have found that above ground litter production in forest ecosystems increase with mean rates of soil respiration (Raich and Schlesinger 1992, Kueppers and Harte 2005). Such processes are dependent on water availability and are intricately tied to carbon fluxes from the soil into the atmosphere (Raich and Schlesinger 1992). In alpine forests, decomposition is often moisture limited during the summer, although during times of increased moisture, as observed here, decomposition may actually be limited by waterlogging (Anderson 1991).

Principal Components Analysis (PCA)

Though relationships extracted from the correlation matrices characterize independent relationships within the microhabitat, the PCA run on the variables confirms that although certain variables contribute more to variances among microsites surveyed, characterizing the microhabitat niche involves consideration of the interaction among several abiotic variables. Overall, clear differences in microhabitat exist between the heated and unheated plots, though as expected soil temperature was the common overlap variable.

Unheated Plots. The results of the PCA indicate most of the natural microhabitats at LSA are characterized by thicker duff layers, more rock cover, lower soil temperatures and relatively

lower soil moisture levels. Though the timing of data collection may explain the deeper litter layers observed, litter plays an important role as an insulator from fluctuating temperature regimes in such subalpine forest environments (Stohlgren 1988). The presence of rock substrate has been found to be very important in creating niche habitats as well, as they provide substrate for roots and moisture pockets for promoting growth (Matthes-Sears and Larson 1995).

Heated Plots. On the other hand, greater litter cover and higher soil moisture levels dominated the microhabitat characteristics of the heated plots. Though one explanation for the greater duff cover might be a response to the warmer environments created by the radiators, another explanation might be related to the increased soil respiration and soil decomposition rates created by the warmer temperatures that contributed to increased litter production (Raich and Schlesinger 1992). High temperatures tend to create greater evaporative demand and greater levels of photorespiration (Waring and Schlesinger 1985),

The lack of plant cover found in most microhabitats of the heated plots coincides with the lower moisture levels observed, indicating that the warmer conditions created by the radiators inhibit vegetative growth and promote litter production. While the amount of vegetation in a given microsite influences soil moisture through its regulation of transpiration, soil moisture also influences vegetation establishment, survival, and productivity (Harte et al. 1995). Because soil temperature effects on both soil moisture and vegetation are interlinked, the lack of microhabitats with greater soil moisture in the heated plots further confirms these observations. In addition, because vegetation cover influences soil temperatures by shading incoming heat energy, thereby reducing soil warming, this trend may create positive feedbacks for microhabitat communities in this forest ecosystem. Consideration of these interacting processes suggests that microhabitats will shift to more homogeneous environments characterized by arid litter cover as temperatures increase.

Implications of Late Snowmelt

The very late snowmelt observed during the summer of data collection not only suggests the possibility of anomalistic data and therefore anomalistic trends, but also supports the emerging data on shifts in snowmelt times as a consequence of climate change (Adam et al.

2008). Such changes to snowmelt dates create changes in growing season temperatures and duration, with potentially profound effects on ecological and physical processes of the alpine tundra ecotone (Totland and Alatalo 2002). While predictions for changes in regional precipitation and its timing remain largely uncertain, warmer temperatures are predicted to advance snowmelt and increase evaporative demand during the growing season, both of which reduce growing season soil moisture (Germino et al. 2002).

Biotic Features

Differences in duff layer depth, plant cover, soil moisture and soil temperature correlate with microhabitat gradients along which the distribution of seedlings appears to be non-random. According to the results of the PCA, seedlings clustered in microhabitats characterized by thicker litter layers and higher soil moistures. The presence of litter may enhance the establishment of some species by improving water conditions (Fowler 1986). Collins and Good (1987) found that most pine seedlings in the New Jersey Pine Barrens occurred in patches with less litter depth. However, they concluded that litter depth was an important component of the regeneration niche of other species in the study. Though divergent microhabitat characteristics may account for differences in findings between their experiment and this one, it is also possible that seedling niches may be species-, in addition to site-, specific. Interestingly, the abiotic variables that most contribute to creating microhabitat at LSA also most influence patterns of seedling germination. Although it is unclear whether the seedlings germinate in these conditions as a result of microhabitat facilitation or because they are more adapted to these microhabitats, the results of the PCA still provide a meaningful description of the habitat niche of the germinants observed.

Limitations

Although my experimental design attempted to account for differences in sampling, the ‘snapshot’ approach of taking single readings of the abiotic and biotic variables did not fully account for error as a result of temporal differences among the surveys. As a result, unpredictable, large rain events or extended heat waves between the early and late summer surveys made across plot comparisons less consistent. Similarly, even within early or late summer survey groups, multiplicative effects on microclimate as a result of weather events could

have created inconsistencies among across plot comparisons of abiotic variables. Although all plots were sampled at the same time of day and with similar sunlight and weather conditions, the temporal variability of data collection likely introduced another dimension not accounted for in analysis. However, the high sampling size might have diluted error introduced by temporal variability. Within plot comparisons likely provide the most robust data set as minimal amounts of time differences occurred during surveys of microsites within individual plots.

Future Studies

Based on analyses of interactions among the microhabitat variables, it is clear that microhabitat gradients occur in the forest floor of coniferous forests at LSA. However, in general, relationships and gradients observed across plots were much more pronounced than those found within plots, suggesting the scale of data collection heavily influences the relationships found here. Thus, relationships between abiotic variables within plots were weaker than across plots, as the correlation coefficients of the matrices comparing abiotic variables within plots and across plots underscore. At the scale of the individual microsites (10-cm), relationships among variables were much more diluted compared to relationships at the plot scale (1-m). These findings point to the importance of determining the appropriate scale in studies attempting to characterize habitat and biotic niches. Future attempts to characterize seedling niches must address such concerns of scale within their study design.

Obviously, the suite of variables surveyed for in this study does not incorporate the complete environmental variables necessary for characterizing microhabitat in a coniferous forest. Numerous other microsite variables, including pH, microtopography, and nutrients all further interact to influence the microhabitat. Microclimate, as characterized here by interactions among soil moisture, soil temperature, and duff layer depth, are key variables attributed to understanding the distribution of plants and wildlife in forests, as well as for understanding forest ecosystem processes like soil respiration and nutrient cycling (Gray et al. 2002). Thus, in light of the strong interactions found among soil moisture, soil temperature, and duff layer depth, future studies should incorporate ways to measure nutrient levels and cycling in the soil in an attempt to capture processes connected with carbon cycling within the broader ecosystem. Specifically, the link between soil microclimate and soil respiration needs further exploration.

Broader Implications

The seedling germination niche plays a fundamental role in characterizing the structure, dynamics, and functions of the microhabitats the seedlings inhabit and offers a means to analyze community characteristics at small scales, including the distribution of species in response to environmental gradients across the landscape (Gosz 1992). In addition, large scale patterns of tree distribution may also be linked to interactions of emergent conifer seedlings and changing ecological thresholds that is only visible at smaller scales (Germino et al. 2002). Because patterns of seedling germination shed light on microhabitat niche characteristics and the boundaries of ecological limits, they serve as sensitive ecological monitors (Holtmeier and Broll 2005). Especially in light of the empirical evidence of the changes to microhabitat niches as a result warmer ambient temperatures that I provide from this study, monitoring changing patterns of seedlings germination will become increasingly important as changing global climate conditions threaten to alter the habitat niche necessary for seedling germination, establishment, and recruitment. This fine-scale study offers an important approach to simplifying the many climate feedback loops, complex biogeochemical cycles, and warming effects at smaller scales in an attempt to understand localized responses to climate change.

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REFERENCES

- Adam, J. C., A. F. Hamlet, and D. P. Lettenmaier. 2008. Implications of global climate change for snowmelt hydrology in the twenty-first century. *Hydrological Processes* 23:962-972.
- Anderson, J. M. 1991. The effects of climate change on decomposition processes in grassland and coniferous forests. *Ecological Applications* 1:326-347.
- Ayres, E., H. Steltzer, S. Berg, M. D. Wallenstein, and B. L. Simmons. 2009. Tree species traits influence soil physical, chemical, and biological properties in high elevation forests. *Plos One* 4:5964e.
- Batlari, E. J., M. Blanco-Moreno, J. M. Ninot, E. Gutierrez, and E. Carrillo. 2009. Vegetation patterns at the alpine treeline ecotone: the influence of tree cover on abrupt change in species composition of alpine communities. *Journal of Vegetation Science* 20:814-825.
- Baumeister, D., and R. M. Callaway. 2006. Facilitation by *Pinus flexilis* during succession: a hierarchy of mechanisms benefits other plant species. *Ecology* 87:1816-1830.
- Carson, W. P. and C. J. Peterson. 1990. The role of litter in an old-field community: impact of litter quantity in different seasons on plant species richness and abundance. *Oecologia* 85: 8-13.
- Caspersen, J. P., and M. Saprunoff. 2005. Seedling recruitment in a northern temperate forest:

- The relative importance of supply and establishment limitation. *Canadian Journal for Research* 35:978-989.
- Castro, J. 2006. Short Delay in Timing of Emergence Determines Establishment Success in *Pinus sylvestris* across Microhabitats. *Annals of Botany* 98:1233-1240.
- Collins, S. L., and R. E. Good. 1987. The seedling regeneration niche: Habitat structure of tree seedlings in an oak-pine forest *Oikos* 48:89-98.
- Facelli, J. M. and S T. A. Pickett. 1991. Plant Litter: Its Dynamics and Effects on Plant Community Structure. *The Botanical Review* 57:1-32.
- Fowler, N.L. 1986. Microsite requirements for germination and establishment of three grass species. *American Midland Naturalist* 115:131-145.
- Germino, M. J., W. K. Smith, and A. C. Resor. Conifer seedling distribution and survival in an alpine-treeline ecotone. Conifer seedling distribution and survival in an alpine-treeline ecotone. *Plant Ecology* 162:157-168.
- Gosz, J. R. 1992. Gradient analysis of ecological change in time and space: Implications for forest management. *Ecological Applications* 2:248-261.
- Gray, A. N., T. A. Spies, and M. J. Easter. 2002. Microclimate and soil moisture responses to gap formation in coastal Douglas-fir forests. *Canadian Journal for Forest Research* 32:332-343.
- Greenland, D., and M. Losleben. 2001. Climate. pp. 15-17 in W. D. Bowman and T. R. Seastedt, editors. *Structure and function of an alpine ecosystem: Niwot Ridge, Colorado*. Oxford University Press, Oxford, UK.
- Harper, J. T., Williams, and G. R. Sager. 1965. The behavior of seeds in soil. I. The heterogeneity of soil surface and its role in determining the establishment of plants from seed. *Journal of Ecology* 53:273-286.
- Harte, J., M. S. Torn, F. Chang, B. Feifrek, A. P. Kinzig, R. Shaw, and K. Shen. 1995. Global warming and soil microclimate results from a meadow-warming experiment. *Ecological Applications* 5:132-150.
- Heady, H. 1956. Changes in a California annual plant community induced by manipulation of natural mulch. *Ecology* 37:798-812.
- Hogg, E., and V. Lieffers. 1991. The impact of *Calamagrostis canadensis* on soil thermal regimes after logging in northern Alberta. *Canadian Journal of Forest Research* 21:387-394.
- Holtmeier, F., and G. Broll. 1992. The influence of tree islands and microtopography on

- pedoecological conditions in the forest-alpine tundra ecotone on Niwot Ridge, Colorado Front Range, U.S.A. *Arctic and Alpine Research* 24:216-228.
- Holtmeier, F. K., and G. Broll. 2005. Sensitivity and response of northern hemisphere altitudinal and polar treelines to environmental change at landscape and local scales. *Global Ecology and Biogeography* 14:395-410.
- Humphries, H. C., P. S. Bourgeron, L. R. Mujica-Crapanzano. 2008. Tree spatial patterns and environmental relationships in the forest alpine-tundra ecotone at Niwot Ridge, Colorado, USA. *Ecological Research* 23:589-605.
- Karl, T. R. and K. E. Trenberth. 2003. Modern Global Climate Change. *Science* 302:1719-1723.
- Kueppers, L. M. and J. Harte. 2005. Subalpine forest carbon cycling: Short- and long-term influence of climate and species. *Ecological Applications* 15:1984-1999.
- Kupfer, J. A., and D. M. Cairns. 1996. The suitability of montane ecotones as indicators of global climatic change. *Progress in Physical Geography*. 20:253-272.
- Jones, C. C. and R. del Moral. 2005. Effects of microsite conditions on seedling establishment on the foreland of Coleman Glacier, Washington. *Journal of Vegetation Science* 16:292-300.
- Lapp, S. J. Byrne, I. Townshend, and S. Kienzle. 2005. Climate warming impacts on snowpack accumulation in an alpine watershed. *International Journal of Climatology* 25:521-536.
- Matthes-Sears, U. and D. W. Larson. 1995. Characterization of the constrained growth of trees in a hostile environment: the role of water and nutrient availability for *Thuja occidentalis* on cliff faces. *International Journal of Plant Science* 156:679-686.
- R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Raich, J. W. and W. H. Schlesinger. 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus* 44:81-99.
- Shaver, G. R., J. Canadell, F. S. Chapin, J. Gurevitch, J. Harte, G. Henry, P. Ineson, S. Jonasson, J. Melillo, L. Pitelka, L. Rustad. Global Warming and Terrestrial Ecosystems: A Conceptual Framework for Analysis. *Bioscience* 50:871-882.
- Simard, M., Y. Bergeron and L. Sirois. 1998. Conifer seedling recruitment in a southeastern Canadian boreal forest: The importance of substrate. *Journal of Vegetation Science* 9:575-582.
- Smith, W. K., M. J. Germino, T. E. Hancock, and D. M. Johnson. 2003. Another perspective on altitudinal limits of alpine timberlines. *Tree Physiology* 23:1101-1112.

- Stöcklin, J., and E. Bäumler. 1996. Seed rain, seedling establishment and clonal growth strategies on a glacier foreland. *Journal of Vegetation Science* 7:45-56.
- Stohlgren, T. J. 1988. Litter dynamics in two Sierran mixed conifer forests: Litterfall and decomposition rates. *Canadian Journal of Forest Research* 18:1127-1135.
- Stueve, K. M., R. E. Isaacs, L. E. Tyrrell, and R. V. Densmore. 2011. Spatial variability of biotic and abiotic tree establishment constraint across a treeline ecotone in the Alaska Range. *Ecology* 92:496-506.
- Totland, Ø., and J. M. Alatalo. 2002. Effects of temperature and date of snowmelt on growth, reproduction, and flowering phenology in the arctic/alpine herb, *Ranunculus glacialis*. *Oecologia* 133:168–175.
- Waring, R. H., and W. H. Schlesinger. 1985. *Forest ecosystems: Concepts and management*. Academic Press, Orlando, FL.