Abating climate change impacts on Kincaid's lupine

2016

Report to the Bureau of Land Management, Eugene District

Report prepared by Erin C Gray and

Matt A Bahm

Institute for Applied Ecology

Institute for Applied Ecology

PREFACE

This report is the result of an agreement between the Institute for Applied Ecology (IAE) and the USDI Bureau of Land Management. IAE is a non-profit organization dedicated to natural resource conservation, research, and education. Our aim is to provide a service to public and private agencies and individuals by developing and communicating information on ecosystems, species, and effective management strategies and by conducting research, monitoring, and experiments. IAE offers educational opportunities through internships. Our current activities are concentrated on rare and endangered plants and invasive species.

Institute for Applied Ecology

Questions regarding this report or IAE should be directed to:
Matt Bahm
Institute for Applied Ecology
563 SW Jefferson Avenue
Corvallis, Oregon 97333

phone: 541-753-3099

fax: 541-753-3098

email: mattab@appliedeco.org

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Cover photograph: Monitoring at Fitton Green and Kincaid's lupine.

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EXECUTIVE SUMMARY

This document summarizes monitoring of reintroductions of Kincaid's lupine in multiple microclimates at three sites in the Willamette Valley, Oregon. Over the years of this study, we have found that seed source and microclimate both affect emergence, survival, and growth (height and number of leaves). Results will inform future management considerations regarding seed transfer zones and the perpetuation of this species in response to climate change.

Results from 2016 suggest:

- Success of Kincaid's lupine growth and survivorship is tied greatly to the location of out-planting, at both the site and microclimate scale. Considerations of site and microclimate quality could vastly impact the success of introduction efforts.
- Our data suggest that harsher microclimates can cause a decline in plant performance, though this was variable across sites and seed sources. This was most evident at Fitton Green, where survival declined with increasing microclimate harshness. As climate becomes increasingly harsher, Kincaid's lupine might be increasingly impacted throughout its range.
- Kincaid's lupine can be very plastic in its response to the environmental conditions it is planted into. This is evident in the differences in height and number of leaves across all of the sites and microclimates. Kincaid's lupine was much taller across all seed sources at Coyote Prairie, and tended to have fewer leaves, on average, at Fitton Green. These responses indicate that the plant shows potential to respond readily to the environment it is seeded into.
- Seed source was an important factor in growth and survival of Kincaid's lupine. Seed from Douglas County had higher rates of emergence than those from Eugene West and Corvallis West seed recovery zones, but survival in 2016 was variable and dependent upon site and microclimate. At Fitton Green, survival tended to decline with increasing harshness of microclimate.
- Survival declined across all sites from 2013 to 2016 to less than 5% at all sites, suggesting that maintenance of out-plantings and introductions would be essential to success.
- Kincaid's lupine from the southern extent of its range (Douglas County) can survive and thrive in the northern-most location, suggesting that if seed movement is needed to preserve this species, it would likely be successful.

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REPORT TO THE BUREAU OF LAND MANAGEMENT, EUGENE DISTRICT

INTRODUCTION

Current and ongoing climate change adds an additional threat to the preservation of native plant communities in the Pacific Northwest. Rare plant species, already at risk of extinction due to habitat loss and degradation, fragmentation, and loss of genetic diversity may have reduced capacity to respond and adapt to a rapidly changing climate. Recovery of threatened and endangered plants may require relocating or introducing populations in cooler and moister microclimates within potential habitat (Figure 1). In this project, we test the effects of climate on survival and growth of Kincaid's lupine (*Lupinus oreganus*, also known as *L. sulphureus* ssp. *kincaidii*), a federally listed threatened species and primary host plant for the endangered Fender's blue butterfly (*Icaricia icarioides fenderi*).

Figure 1. Collecting ibutton data in treeline habitat (above) at Doghead Meadow and open prairie habitat (below) at Coyote Prairie.

Kincaid's lupine, a rare legume found in prairies and oak savannas, is listed as threatened by the US Fish and Wildlife Service and the Oregon Department of Agriculture and as endangered by the Washington Department of Natural Resources (ORBIC 2016). Extensive land development and alteration in the prairies of western Oregon and southwest Washington have relegated remaining populations to small, isolated patches of habitat. Habitat loss is likely to continue as private lands are developed; the majority of Kincaid's lupine populations occur on private lands. Establishing new populations on protected lands is essential to long-term recovery of both Kincaid's lupine and Fender's blue butterfly.

Most Kincaid's lupine restoration efforts are focused on the historic habitats of this species: south-facing prairies and oak savannahs. However, we have observed that lupines growing in shade at forest margins are often more vigorous than lupines in full sun. Temperatures in the Pacific Northwest have increased by nearly 1°C since the early part of the twentieth century (Doppelt et al. 2009); thus, moderately shaded microhabitats may now provide more suitable climatic conditions for this species. The historic habitat of Kincaid's lupine may continue to become more inhospitable given that climate models predict a temperature increase of 1 to 2 °C by 2040 and up to 3 to 4° C by 2080 and decreased growing-season precipitation in the Pacific Northwest (Doppelt et al. 2009). These climate changes may be particularly detrimental to populations of rare species already stressed by a lack of connectivity and gene flow, as well as competition with exotic species. In addition, projected changes in climate may increase nitrogen availability in Pacific Northwest prairies, thus eliminating the main competitive advantage of nitrogen-fixing species like lupines. Despite these challenges, a number of factors suggest that the targets for recovery of Kincaid's lupine are achievable. Seeds have been collected from several populations in each recovery zone, protected sites with suitable habitat for emergence of new populations have been identified, and we have developed a large body of knowledge on the biology and ecology of this species (e.g. USFWS 2010). By determining how projected changes to climate affect emergence and survival of Kincaid's lupine, we can increase the potential for long-term persistence of introduced populations.

The objectives of this project are to:

1. Test for shifts in microclimate suitability given current climate change, by experimentally reintroducing plants in multiple microclimates at three sites.
2. Determine if there is a difference in emergence and survival within each microclimate between seeds from southern (hotter and drier) and more northern (cooler and moister) populations.
3. Develop recommendations for appropriate site selection.
4. Share findings with key land management partners and others involved in plant conservation.

METHODS

Three sites in the Willamette Valley, Oregon were selected for this project. Due to the need for frequent site visits, all were located within one hour of Corvallis (Figure 2). Sites were Coyote Prairie, west of Eugene (City of Eugene; Eugene West Recovery Zone), Doghead Meadow, northeast of Harrisburg (Eugene BLM; Eugene East Recovery Zone), and Fitton Green, west of Corvallis (Benton County; Corvallis West Recovery Zone). Fitton Green has a small population of recently introduced Kincaid's lupine at the site; Kincaid's lupine is not found at the other sites. There have been no observations of Fender's blue butterfly at any of the sites.

Our goal was to plant Kincaid's lupine in three potential microclimates (south-facing/full sun; south-facing/woodland edge; north-facing/full sun; and north-facing/woodland edge) at each site (Figure 1). Thus, at each site, we established three microclimate plots into which we seeded Kincaid's lupine (see below and Appendix A). Microclimate conditions are relative across each site and should not be considered replicates of each other.

1. **Coyote Prairie** (valley floor, thus no differences in aspect)
   1.1. Northwest exposure (SE treeline)
   1.2. Northeast exposure (SW treeline)
   1.3. Full sun- removed from study in 2015
2. **Doghead Meadow**
   2.1. SW aspect (N treeline)
   2.2. SW aspect (SW treeline)
   2.3. SW aspect, some shading
3. **Fitton Green**
   3.1. N facing aspect, full sun
   3.2. S facing aspect, full sun
   3.3. S facing aspect, north exposure (S treeline)

Figure 2. Kincaid's lupine microclimate study sites and seed source locations. Sites are in yellow and seed sources are in red. Wren=Corvallis West, Fir Butte = Eugene West, and China Ditch = Douglas County recovery zones

At Coyote Prairie and Doghead Meadow, 2.5 m by 10.5 m areas of each microclimate were delineated (also called microclimates), with 20, 0.5 m^2 plots nested within each (Appendix A). Microclimate areas were marked with orange-painted conduit in the southwest corner and rebar marked with yellow IAE rebar caps in the other corners. Each 0.5 m^2 plot was marked with 6 inch nails in the lower left and upper right corners. A unique plot tag was wired to each nail in the lower left corner of the plot. At Fitton Green, microclimate areas were 2.5 m x 15.5 m, with 30, 0.5 m^2 plots within. There was a 0.5 m buffer surrounding each plot. Within each microclimate, existing vegetation was clipped to 5-8 cm height and litter was gently raked out of the plots.

Seeds for this project were from China Ditch in Douglas County (Douglas County Recovery Zone), Fir Butte in the West Eugene Wetlands (Eugene West Recovery Zone), and a private site near Wren (used only at Fitton Green; Corvallis West Recovery Zone). China Ditch (Douglas County) is one of the southern-most occurrences of this species and its habitat is warmer and drier than that of the other two sources (Figure 10). The Wren (Corvallis West) population was added as a source at Fitton Green so that at least some of the introduced plants were from the same recovery zone as the site and could be left at the completion of the experiment. For China Ditch (Douglas County) and Fir Butte (Eugene West), we combined seed collected in 2011 with seed collected in previous years (predominately 2009-2010). We have found very little loss of viability in Kincaid's lupine seed that has been stored in cool dark conditions. Seed sources were randomly assigned to plots and we distributed 50 seeds per plot for China Ditch (Douglas County) and Wren (Corvallis West) and 40 seeds per plot for Fir Butte (seed was limited from this site) evenly over the surface of each plot. For the rest of the report, seed sources are named using the recovery zones: Eugene West, Corvallis West, and Douglas County.

We used iButtons (Maxim Integrated Products) to measure temperature and relative humidity in each microclimate. Hygrochrons, which measure both temperature and humidity, were attached to a stake that was inserted in the ground so that the hygrochron was approximately 30 cm above the ground surface (Figure 3). All hygrochrons were placed facing north. A plastic coffee cup lid was stapled to the top to protect the iButton from direct sun and rain exposure. Hygrochrons were programmed to measure temperature to 0.5 °C and humidity to 0.6 RH (%) every 30 minutes. A thermochron, which only measures temperature, was attached to a wire and buried approximately 15 cm near the base of the hygrochron. A unique tag was attached to the other end of the wire and was placed at the soil surface. Thermochrons were programmed to measure temperature to 0.5 °C every 15 minutes. iButtons were placed in the center of each microclimate plot in the buffer between the two rows at the 5 m mark on the x axis. Data were downloaded from the iButtons every 85 days.

Figure 3. A. Using the solar pathfinder to collect data on light availability. B. Hygrochron iButtons collect data on temperature and relative humidity in each plot every 30 minutes.

Plots were monitored in late May 2012- 2016. We counted number of leaves and measured the length of the longest leaf for each plant within a plot. Photopoints were taken of each plot during monitoring. At the time of monitoring, we used a solar pathfinder to measure light availability and canopy cover at each microclimate. The solar pathfinder (Swenson and Bielfuss 2001, Figure 3) provided an estimate of light availability during the entire year. Readings were taken at waist height in the center of each microclimate. We used the solar pathfinder thermal assist software to analyze data for the entire year.

To prevent hybridization of plants from different recovery zones, we count and then remove all inflorescences of flowering plants as they are formed. Prior to 2015, no plants were reproductive and in 2015 we observed one flowering plant, in 2016 no flowering plants were found. At the end of the experiment, all plants from sources outside of each site's recovery zone will be removed in consultation with land managers.

Data Analysis

Due to differences in population characteristics for all sites, and the lack of microclimate replicates across sites, we tested the effects of seed source and microclimate separately by site. Due to the complete lack of establishment at microclimate 3 at Coyote Prairie, we removed it from analysis. We used 2-factor ANOVA (R Development Core Team 2009) to test for the response of height of Kincaid's lupine, using seed source and microclimate as fixed factors. To test for the response of number of leaves, we used a general linear model with a quasipoisson distribution due to over-dispersion, using seed source and microclimate as predictors. Analyses on Kincaid's lupine growth (height and number of leaves) were conducted only on data for plants living in 2016. To test for effects of seed source and microclimate on the survival of Kincaid's lupine, we used logistic regression (family = quasibinomial), modeling each site separately. We considered $P < 0.10$ to be significant. For the regressions, when a significant main factor effect was found we modeled the response by that single factor. Data were analyzed separately to determine the effects of an unplanned herbicide application in microclimate 1 at Fitton Green (occurred winter 2013) on data collected from 2016.

Climate data [relative humidity (%), aboveground and belowground temperature (°C)] from the time of plot installation and seeding (November 2011 to August 2016) were extracted and monthly averages, maxima, and minima were calculated for each microclimate using R (R Development Core Team 2009). Standard error of the mean was also calculated for these summaries. Some microclimates experienced gaps in data due to failure of iButtons. At each site, microclimates were categorized as harsh, intermediate, or moderate dependent on environmental conditions measured at each microclimate (November 2011-August 2016, Appendix B). In future analyses, aided by climate data over the entire course of the study, these categories may change dependent on the environmental conditions observed.

RESULTS AND DISCUSSION

We categorized microclimates as either harsh, intermediate or moderate environmental conditions, based on a combination of slope, aspect, canopy cover, and climate data measured at each microclimate (November 2011-August 2016, Table 1, see Appendix B for a full description of microclimate differences). Relative environmental conditions remained the same from 2015. Differences between environmental conditions are relative within each site and are not meant to be compared between sites (Appendix B).

Table 1. Microclimates and their differences in relative environmental conditions at Coyote Prairie, Doghead Meadow, and Fitton Green. Note that microclimates have been re-ordered by the gradient of their environmental conditions over the course of the study (2011-2016). Note that Coyote Prairie only has two microclimates.

Site	Microclimate
Coyote Prairie (valley floor)	Microclimate 1: Moderate
	Microclimate 2: Intermediate
Doghead Meadow (SW aspect)	Microclimate 2: Moderate
	Microclimate 3: Intermediate
	Microclimate 1: Harsh
Fitton Green (N and S aspects)	Microclimate 1: Moderate
	Microclimate 3: Intermediate
	Microclimate 2: Harsh

For the remainder of the report, we will discuss the results in relation to the relative environmental conditions of the microclimates within each site: moderate, intermediate, and harsh.

Seedling emergence and survival of Kincaid's lupine

In 2012, Kincaid's lupine emerged in all sites and microclimates, with variable success. Fitton Green had the highest proportions of emergence, with 31% of seeds germinating and persisting (Figure 4). At Doghead Meadow, 14% of Kincaid's lupine emerged, while only 8% emerged at Coyote Prairie. Microclimate 3 at Coyote Prairie experienced flooding conditions each winter, which may have deterred emergence of the seeded Kincaid's lupine; only ten plants from each seed source emerged out of the 900 that were seeded there in 2012. From 2013-2015, no plants had survived in microclimate 3 at Coyote Prairie; due to this, microclimate 3 at Coyote Prairie was not monitored in 2016 and was removed from any analysis.

Figure 4. Kincaid's lupine at Fitton Green.

By 2016, seedling survival was less than 10% across the microclimates (Table 2, Figure 5) at all of the sites. At Coyote Prairie, survival in plots ranged from 0% to 30%, with a mean of 4%. There were 5 plants found in microclimate 1 (moderate) which was a decline from 2015 (28 plants), resulting in only 1% survival. Microclimate 2 (intermediate) had 89 plants in 2016, which was a slight increase from 2015 values (66 plants), resulting in 10% survival. The increase in plants seen between 2015 could be due to underground rhizomatous growth in the 5 year old plants. Survival did not differ by seed source (Figure 5), but was found to differ by microclimate (*P*=0.008), this was likely due to the very low survival observed in microclimate 1 (moderate). In microclimate 2 (intermediate), seed from Eugene West had higher survival (11%) than seed from Douglas County (9%).

Survival at Doghead Meadow was extremely low (Figure 5), with an average of 1%, and only 19 living plants recorded across the three microclimates. More plants were found at microclimate 1 (harsh) in 2016 (13 plants), which was an increase from the 4 seen in 2015. Microclimate 2 (moderate) declined to 2 plants as compared to 12 in 2015, and plants in microclimate 3 (intermediate) increased from 2 to 4 from 2015 to 2016. Similarly, the increase seen could be due to clonal growth. No plants from Douglas County survived in microclimate 3 (Figure 5). Survival was found to differ significantly by the interaction of seed source and microclimate ($P = 0.07$), which was likely influenced by the complete lack of survival of Douglas County plants in Microclimate 3 (intermediate), and the low numbers found in microclimate 2 and 3. Due to such low sample size, the statistical results should be interpreted cautiously.

While in previous years Fitton Green has had higher survival than the other sites, in 2016 survival at Fitton Green averaged 5%. From 2014 to 2015, survival within these plots declined by 36%, with a 50 % decline exhibited in previous years. Microclimate 1 (moderate) had the highest survival of the three microclimates (9%), with microclimate 3 (intermediate) having less (3%) and microclimate 2 (harsh) having the least survival (1%, Figure 5). Microclimate 1 (moderate) declined from 235 plants in 2015 to 121 in 2016. Microclimate 2 (harsh) had similar declines from 48 to 15 plants. We saw an increase in plants at Microclimate 3 (intermediate) from 11 plants to 59 plants in 2016. This increase is likely due to clonal growth. At Fitton Green, survival did not differ by seed source or microclimate, while in the past it has differed by microclimate. For microclimate 1(moderate) Eugene West plants had higher proportions of survival than Douglas County and Corvallis West plants. Microclimate 2 (harsh) had the greatest survival from both Corvallis West and Eugene West plants, with plants surviving from Douglas County; this microclimate had extremely few plants with only 15 surviving total. In Microclimate 3 (intermediate), Douglas County and Corvallis West had higher survival than Eugene West plants (Figure 5). Survival was the highest in microclimate 1 (moderate; 9%), which was open with full sun with a north facing aspect, and was vegetated predominately of native species. We saw increases in number of plants in a few of the microclimates from 2015 to 2016. While this is likely the result of clonal growth exhibited in 5 year old plants, our inability to truly determine if an individual was seeded or is a clone may be over-inflating true survival for each site.

Table 2. Characteristics of Kincaid's lupine, spring 2016

Site	Average # of leaves	Average Height (cm)	Mean proportion of survivorship 2016	Number of Plants 2012	Number of Plants 2013	Number of Plants 2014	Number of Plants 2015	Number of Plants 2016
Coyote Prairie (valley floor)	10.9	24.4	0.05	204	150	71	94	94
Microclimate 1:	5.0	17.8	0.01	77	33	24	28	5
Eugene West	5.0	17.8	0.01	38	18	11	21	5
Douglas County	0.0	0.0	0.00	39	15	13	7	0
Microclimate 2:	11.2	24.8	0.10	107	117	47	66	89
Eugene West	15.0	25.3	0.11	49	60	19	36	45
Douglas County	7.3	24.2	0.09	58	57	28	30	44
Doghead Meadow (SW aspect)	13.6	16.1	0.01	379	58	74	18	19
Microclimate 2:	11.5	24.4	0.00	94	20	22	12	2
Eugene West	8.0	17.4	0.00	48	9	11	4	1
Douglas County	15.0	31.3	0.00	46	11	11	8	1
Microclimate 3:	7.3	17.0	0.01	130	14	25	2	4
Eugene West	7.3	17.0	0.01	54	13	16	1	4
Douglas County	0.0	0.0	0.00	76	1	9	1	0
Microclimate 1:	15.8	14.6	0.01	155	24	27	4	13
Eugene West	18.0	15.7	0.01	58	11	14	4	4
Douglas County	14.9	14.1	0.02	97	13	13	0	9
Fitton Green	4.5	10.9	0.05	1299	934	482	294	195
Microclimate 1:	4.5	11.1	0.09	504	430	328	235	121
Corvallis West	4.5	13.0	0.09	133	114	100	74	45
Eugene West	4.9	10.0	0.11	148	128	107	90	45
Douglas County	3.7	9.9	0.06	223	188	121	71	31
Microclimate 3:	4.5	10.3	0.04	399	254	98	11	59
Corvallis West	4.8	11.4	0.05	115	93	34	5	27
Eugene West	3.8	8.6375	0.02	122	62	25	5	8
Douglas County	4.5	9.6	0.05	162	99	39	1	24
Microclimate 2:	5.1	12.1	0.01	396	250	56	48	15
Corvallis West	4.9	11.1	0.02	124	54	27	14	8
Eugene West	5.3	13.2	0.02	107	93	16	10	7
Douglas County	0	0.0	0.00	165	103	13	24	0

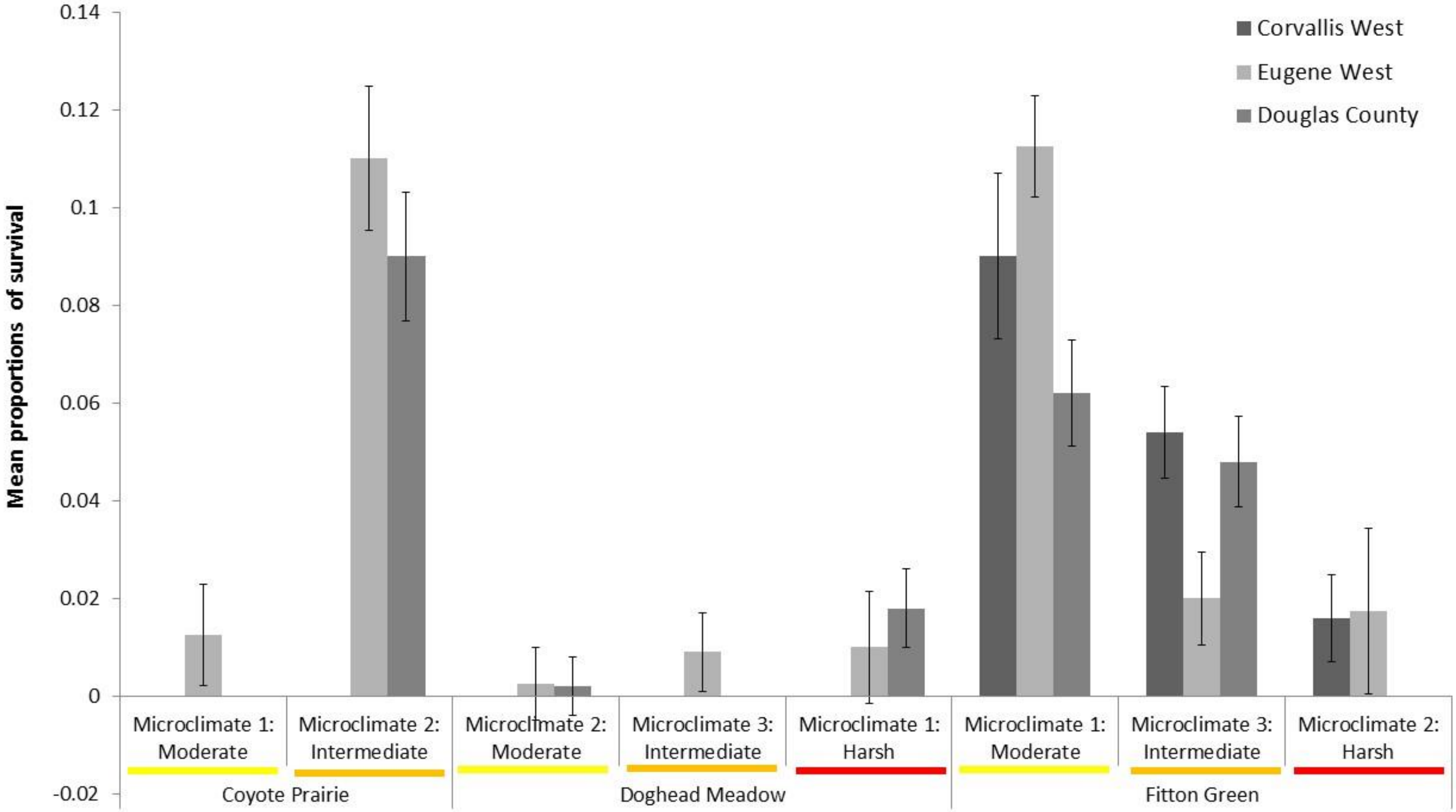

Figure 5. Mean seedling survival (proportions $\pm$ 1 SE) in June 2016, by microclimate and seed source. The Corvallis West seed source was only planted at Fitton Green. Microclimates are ordered according to their relative environmental conditions.

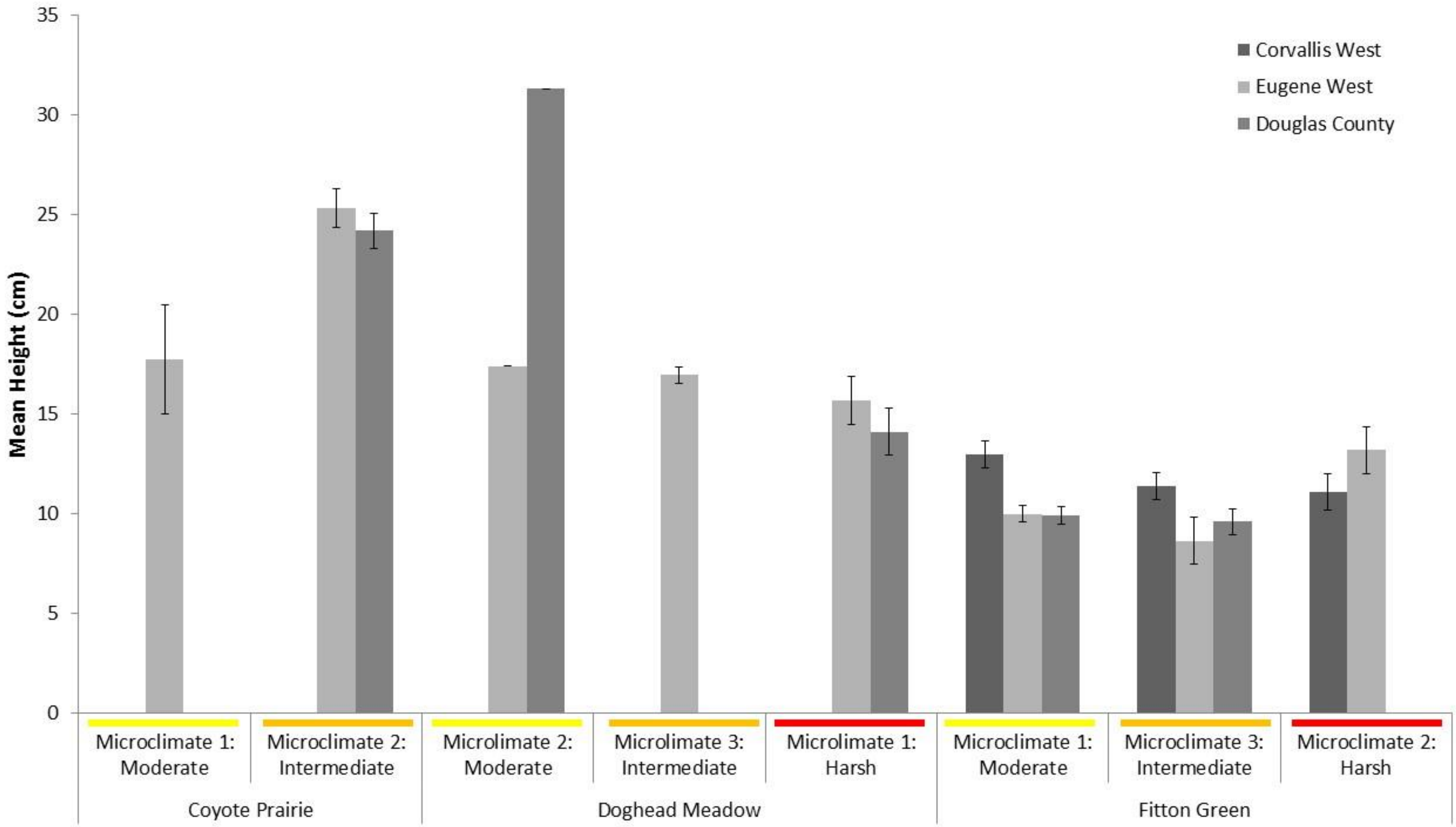

Figure 6. Mean (± 1 SE) height of Kincaid's lupine in 2016, by site and microclimate. Microclimates are ordered according to their relative environmental conditions. Corvallis West seed was only planted at Fitton Green.

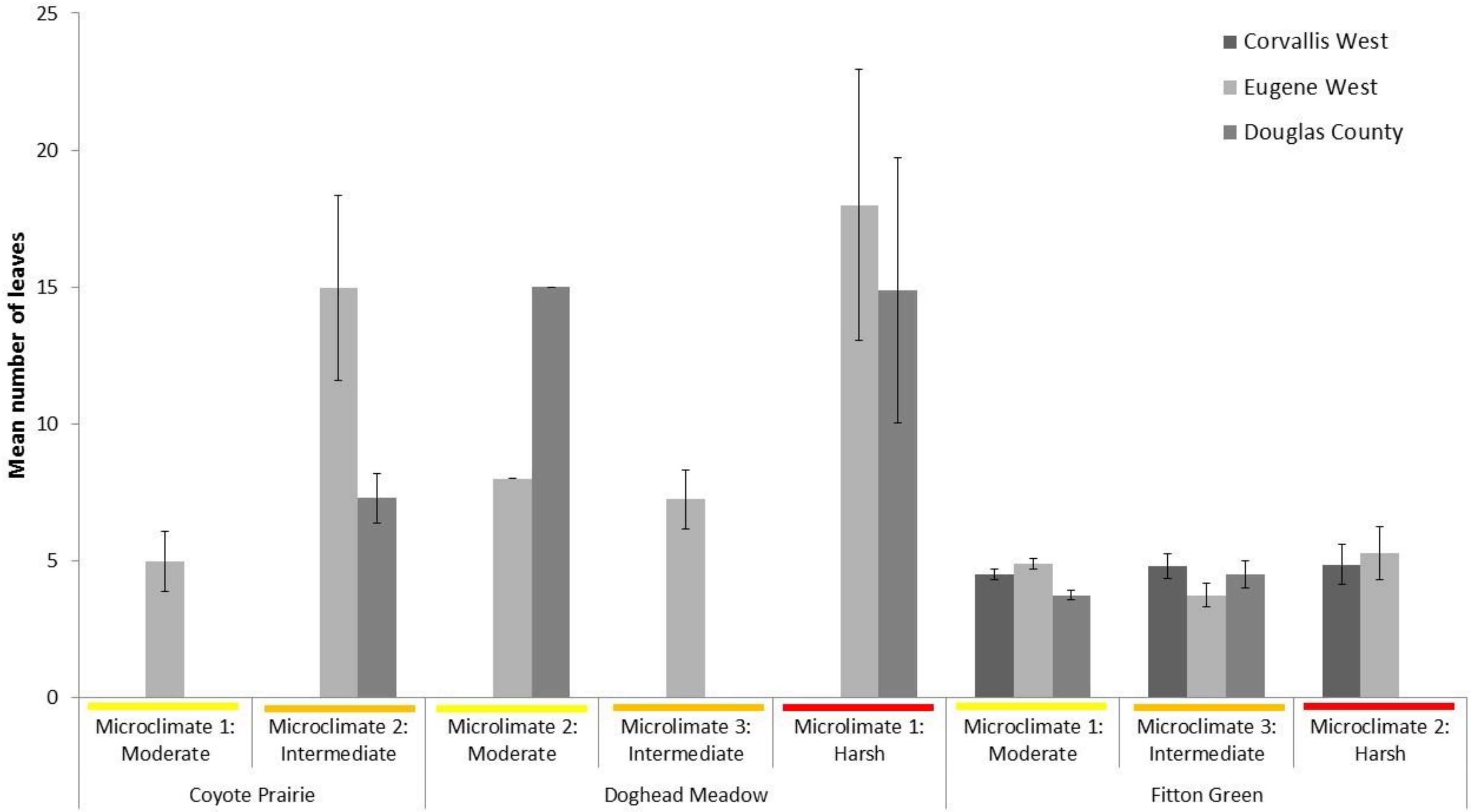

Figure 7. Mean (± 1 SE) number of leaves in 2016, by site and microclimate. Microclimates are ordered according to their relative environmental conditions. Corvallis West seed was only planted at Fitton Green.

Kincaid's lupine growth characteristics

Coyote Prairie

Height of Kincaid's lupine at Coyote Prairie was significantly affected by microclimate, but not by seed source ($P < 0.01$, Figure 6). There was a complete lack of survival at microclimate 3 (harsh) from 2013-2015, likely due to exposure to high standing water during winter months; this microclimate was not included in the analysis and is no longer monitored. Plants in microclimate 2 (intermediate) were slightly taller (average 24.8 cm) those in microclimate 1 (average, 17.8 cm; moderate). Plants were similar in height between seed sources in microclimate 2 (intermediate). Number of leaves was found to differ significantly by seed source, but not by microclimate at Coyote Prairie ($P = 0.02$, Figure 6), this was likely due to the lack of survival of Douglas County plants in microclimate 1. Douglas County plants tended to have fewer leaves than Eugene West plants in microclimate 2 (intermediate). Plants in microclimate 2 (intermediate) tended to have more leaves (average= 11) than plants in microclimate 1 (moderate; average =5). Though we found one reproductive plant in 2015, none were found in 2016. Monitoring in future years will enable us to see if reproductive effort will continue at this site.

Doghead Meadow

Only 19 plants survived at Doghead Meadow in 2016 so trends associated with size are based off of a very small sample size. At Doghead Meadow height of Kincaid's lupine was significantly affected by the interactions between seed source and microclimate ($P = 0.03$), and microclimate alone ($P < 0.001$). Mean height differed across microclimates, with the tallest plants occurring in microclimate 2 (average = 24.4 cm, moderate), followed by microclimate 3 (average = 17cm; intermediate), and the shortest occurring in microclimate 1 (harsh, average = 14.6 cm, Figure 6). The microclimate and seed source interaction was likely the result of one extremely tall Douglas County plant noted in microclimate 2 (height 31.3 cm). Number of leaves did not differ significantly by microclimate or seed source. Microclimate 1 (harsh) tended to have the greatest number of leaves (average = 16), followed by plants in microclimate 2 (moderate, average = 12), with the least amount of leaves in microclimate 3 (intermediate, average = 7). The small number of plants in each microclimate, particularly in microclimates 2 and 3, indicate that these trends in height and number of leaves should be interpreted cautiously.

Fitton Green

Fitton Green had three seed sources rather than two, including Corvallis West seed that was collected at a site in very close proximity to the out-planting site. Seed from Corvallis West and Eugene West came from populations which occurred in quite similar habitats (upland prairie in the Willamette Valley). Height of Kincaid's lupine at Fitton Green did not differ by microclimate or seed source in 2016 (Figure 6). In microclimate 1 (moderate), Eugene West plants tended to be the tallest, with Corvallis West and Douglas County plants being shorter and similar in height (Figure 6). Number of leaves did not differ by seed source or microclimate. In microclimate 2 (harsh), no plants from Douglas County survived.

In the fall/winter of 2013, some spot-spraying of *Crataegus* sp. occurred at Fitton Green and 12 plots (out of 30) in microclimate 1 were affected. The spraying was a combination of glyphosate and imazapyr and occurred in August 2013, while the lupine were dormant. During monitoring in 2014, we noted those plots that had been affected so we could compare sprayed and unsprayed plots and see if the lupine were responding. There was very clear differentiation between the plant communities within these plots, where sprayed plots had much less vegetative cover and more bare ground than unsprayed plots.

In the years following the spray (2014 and 2015), lupine growth did seem to be affected as mean number of leaves tended to be fewer in sprayed plots for all seed sources, and plants in sprayed plots were smaller than those in unsprayed plots, across all seed sources (Gray and Bahm 2015). In 2016, differences in number of leaves were not seen between sprayed and unsprayed plants. For height, unsprayed plants tended to be much taller for Corvallis West plants than sprayed, but the other seed sources did not differ between sprayed and unsprayed, and these differences were not statistically significant (Figure 8). These previous trends suggest the spray that occurred while the lupine were dormant could have affected growth of Kincaid's lupine at Fitton Green in the short-term, but with time these differences are not as apparent. Ten percent of plants survived in unsprayed plots, as compared to six percent that survived in sprayed plots, however this is not taking into account seed source. Survival in 2016 was similar in 2014 and 2015, suggesting that the effects of herbicide in these plots could be long-term. Differences in plant growth could be the result of a difference in plant community between sprayed and unsprayed plots, where sprayed plots had more bare ground and less cover of other species, at least initially.

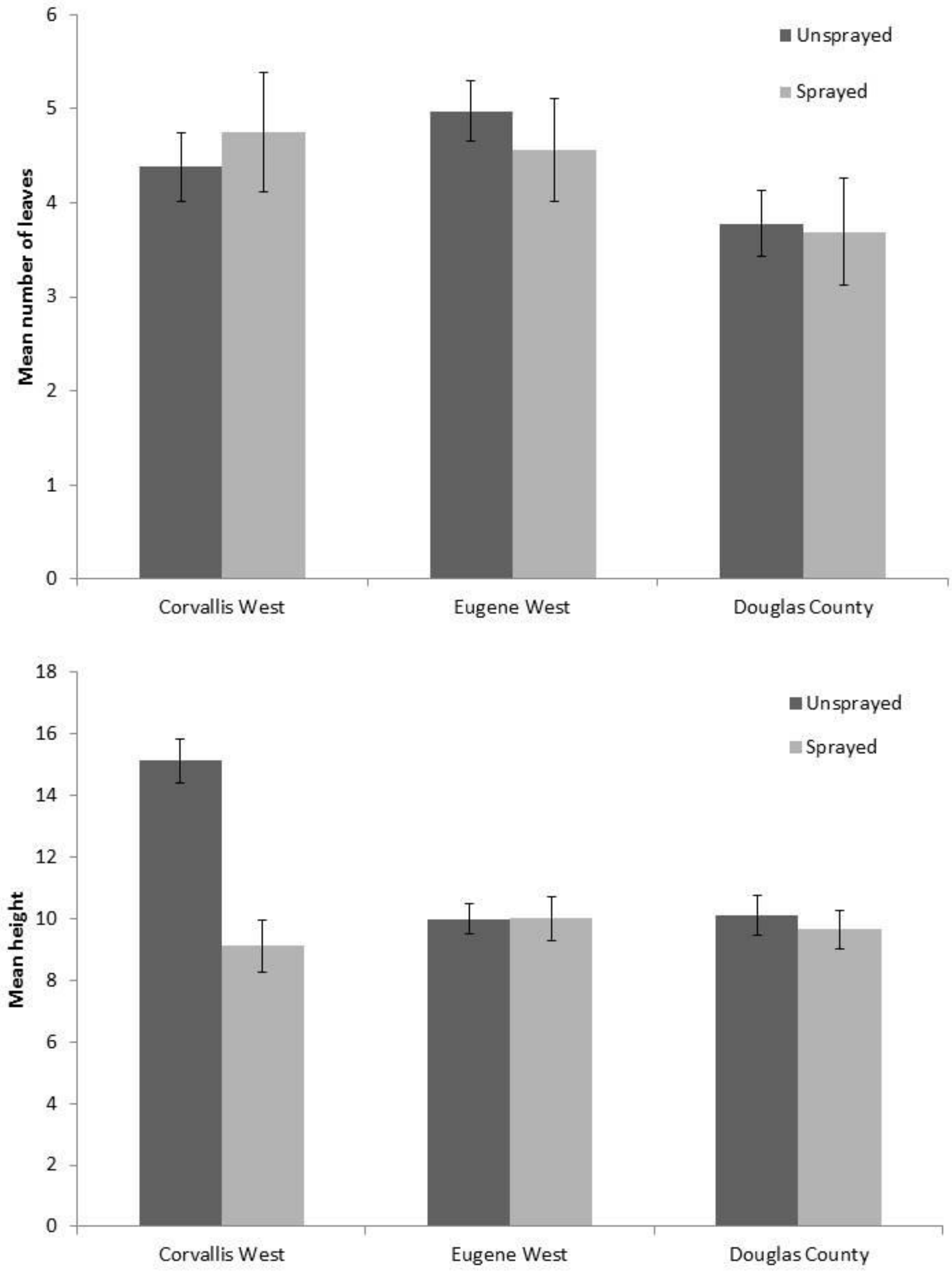

Figure 8. Mean number of leaves (above) and mean height (below) by seed source in microclimate 1 at Fitton Green in 2016. Means were calculated based on if the plots had been sprayed or unsprayed with herbicide in the fall of 2013.

Synthesis

Sites and microsites matter

In the fifth year of this study, our data suggest that site and microsite quality have strong impacts on growth and survival of Kincaid's lupine. Survival differed between site and microclimate over the course of this study. In 2016, however, survival has declined greatly across all sites, and this was with some increases in number of plants for a few microclimates, potentially from plants spreading clonally. Microclimate 1 at Fitton Green (moderate) had greater survival than the other microclimates, both within Fitton Green and across other sites. This microclimate had the most native-dominated plant community, supported by many native forbs and grasses, including nectar species (Figure 9). Despite these promising conditions, we observed a continued decline at this site suggesting that climate and other factors have impacted long-term survival from these seedings. In contrast to the higher survival at Fitton Green was the low survival that has occurred at Doghead Meadow throughout the course of this study. This was a higher elevation meadow with some quality native species components, and was located not far from the Oak Basin complex which supports not only Kincaid's lupine but also Fender's Blue Butterfly. Though from visual assessment Doghead Meadow seemed as if it would be a suitable site for Kincaid's lupine, survival there was very low for both seed sources from the very beginning. One environmental factor that differentiated Doghead Meadow from other sites was soils; some areas were composed of very rocky, shallow soils. We targeted areas for microclimates based on the plant community composition and while we chose areas that supported species such as *Festuca roemeri* and *Eriophyllum lanatum*, these areas might have been too dry or rocky for Kincaid's lupine. Coyote Prairie, in contrast, was much more of a wet, low elevation habitat than the other sites (Figure 9). Though the areas chosen were considered upland prairie, they tended to function more as a wet prairie, as compared to our other sites. Despite this, Kincaid's lupine had better survival in microclimate 2 (moderate) which was along a tree-line, and more protected than the other two microclimates. Microclimate 3 (harsh) turned out to be too harsh for this species and none of the 900 seeds survived in this area, most likely due to seasonal flooding. These results indicate that success of re-introduction efforts can differ greatly depending on placement of plant materials and that to ensure successful results, high habitat quality is necessary.

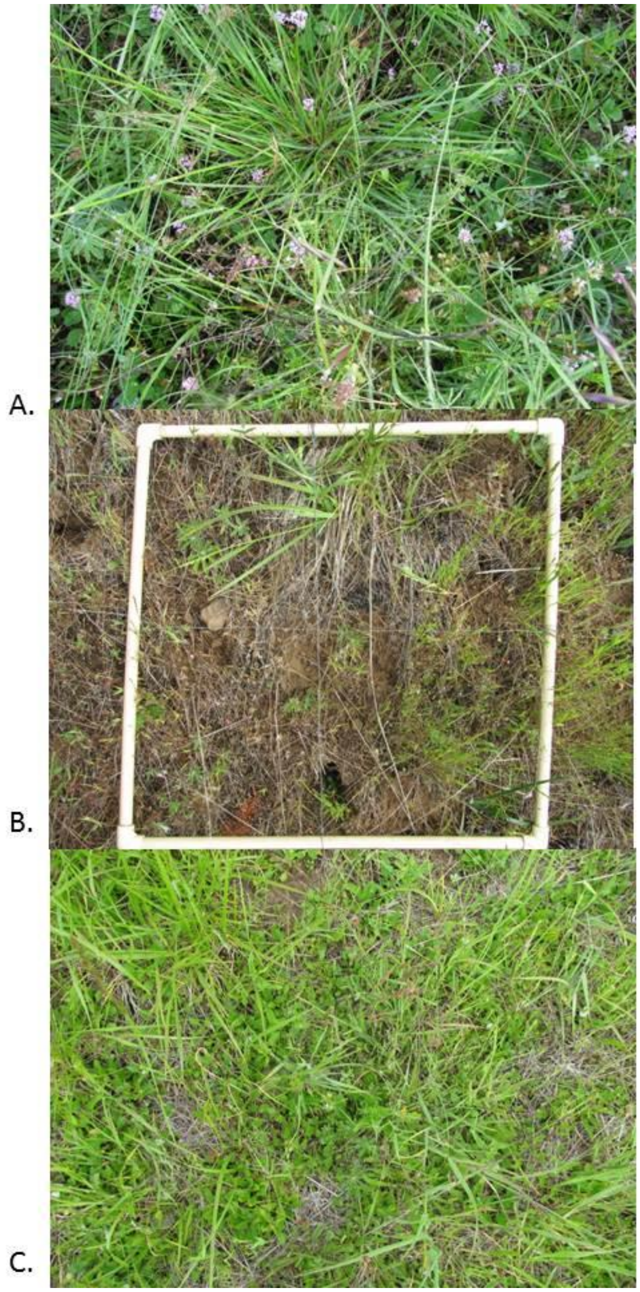

Figure 9. Comparison of plant communities within plots at A. Coyote Prairie, B. Doghead Meadow, and C. Fitton Green.

Implications for management: try multiple sites and microsites

These differences between site and microclimate suggest that land managers should incorporate spatial variability into their design if they are planning on introducing Kincaid's lupine. By evaluating various microclimates within sites, the potential for finding locations with optimal conditions for survival and growth of Kincaid's lupine will increase. Relative "harshness" between sites and microclimates can differ and the characteristics which may be beneficial in one site could yield less successful results in another. By testing potential microclimates within each site, land managers can determine the best possible areas for introduction success. Even at Fitton Green, the site with consistently higher survival and growth of Kincaid's lupine, location of out-planting mattered greatly.

Plants need continued management

While we had promising survival at Fitton Green early in our study, by 2016 survival at all sites had dwindled to a mean of 5%. This suggests that in order to have successful out-plantings and introductions, habitats should be managed to insure that the plants will continue to survive. While choosing an ideal site and microclimate within a site is important, continued maintenance in the form of weed removal and or watering would likely increase success.

Phenotypic plasticity

Our results suggest that Kincaid's lupine can be very plastic in its response to the environmental conditions into which it is planted. This is evident in the differences in height and number of leaves across all of the sites/microclimates. Kincaid's lupine was much taller, across all seed sources at Coyote Prairie, and tended to have fewer leaves, as a whole, at Fitton Green. These responses indicate that the plant shows potential to respond readily to its environment. The plant community at Coyote Prairie is dense and taller in stature than the other sites (Figure 9). It is likely that these community characteristics are an important factor in growth and physical characteristics for Kincaid's lupine and its accessibility to light. Within plots sprayed with herbicide in 2013, Kincaid's lupine were consistently shorter in stature and had fewer leaves in the short term, suggesting they readily adapt to the conditions after germination or re-emergence.

Seed source and climate

We found that characteristics of Kincaid's lupine differed by seed source. While in the past we have seen that Kincaid's lupine from Douglas County tended to be shorter in stature than those from the upper Willamette Valley, we did not see such trends in 2015 or 2016. Douglas County plants also had much higher rates of emergence than those from Eugene West and Corvallis West seed zones; though emergence rates did not necessarily translate into higher survival, which differed depending on site and microclimate. At Doghead Meadow, for example, plants from Douglas County initially had high emergence but had low survival in all microclimates. In 2016, very few plants from this seed source survived at Doghead Meadow, with none surviving in microclimate 2. We have observed consistent early germination of Kincaid's lupine from Douglas County seed in greenhouse trials as well (Denise Giles-Johnson, personal communication). Kincaid's lupine populations in Douglas County are at the southern extent of its range and may be adapted to much warmer conditions and a shorter growing period than those of the Willamette Valley (Figure 10). Adaptations to earlier spring climate might explain the more consistent emergence of Douglas County plants.

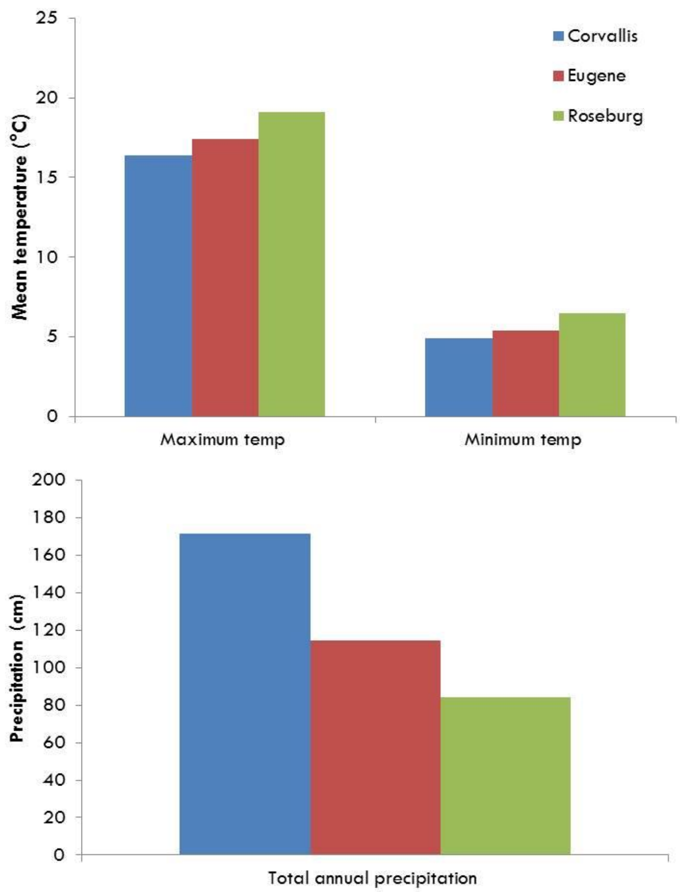

Figure 10. Annual means for maximum temperature (C), minimum temperature (C), and total annual precipitation (cm) for the three seed recovery zones: Corvallis West (Corvallis), Eugene West (Eugene) and Douglas County (Roseburg; Western regional climate center 2008).

We saw evidence that seed source can interact with microclimate to affect growth and survivorship of Kincaid's lupine. At Fitton Green, we saw decline in survival with increasing harshness, across all seed sources. The other sites had such low survival that it was difficult to decipher such trends. These data suggest that harsher climates could result in a decline in plant performance for all seed sources, with some affected more. One concern is the low survival we have documented across three sites where we were targeting habitat that seemed appropriate for Kincaid's lupine. Survival has been less than 5% for all sites. These numbers suggest that determining very specific criteria for appropriate habitat will be necessary for establishing new populations or expanding old ones.

Looking at these sites across a latitudinal gradient, Fitton Green, the northernmost site, had the highest survival across all three seed sources and microclimates. In previous years at Fitton Green, seed from Corvallis West and Eugene West (more local seed sources) tended to respond similarly in growth and survival. Fitton Green (close to Corvallis) experiences slightly cooler temperatures and greater precipitation than Eugene and Roseburg (Figure 10). While Douglas County plants are adapted to different conditions than Willamette Valley sourced Kincaid's lupine, we have determined that they can establish and can tolerate conditions in this northern out-planting site. While it was hypothesized at the beginning of this study that Douglas County seed would do better in more harsh conditions due to the environmental conditions it's adapted to, this was not the case. We found that plants from Douglas County consistently had fewer leaves than other seed sources, particularly in the harsher microclimates. These results suggest that Kincaid's lupine can tolerate a wide range of conditions and movement of seed sources in a northern trajectory may result in establishment of viable populations, however use of a more local seed source might be ideal.

Microclimate continues to play a role in growth and survival of this rare species as plants mature. These results suggest that the success of Kincaid's lupine survival and growth depends greatly on the site characteristics and the precise out-planting location. Results after monitoring in 2017 will enable us to explore further effects of microclimate on growth, persistence, and reproductive effort of this species.

FUTURE ACTIVITIES

Our tests of microclimate and seed-source effects on the growth and survival of Kincaid's lupine seedlings will provide crucial information to improve the success of future introduction efforts for Kincaid's lupine. By testing seed from throughout the range of Kincaid's lupine, we will be able to determine if seed transfer guidelines should be re-examined in order to provide necessary genetic diversity to adapt to climate change. While we are able to look at trends of growth and survival of this species, additional years will be beneficial for documenting effects of microclimate on reproductive effort. This study has identified many considerations that would inform future management of Kincaid's lupine, and could be addressed by a larger-scale, fully reciprocal common garden transplant experiment. We propose expanding this study by establishing multiple common gardens with a multi-agency collaboration throughout the range of Kincaid's lupine and utilizing more seed sources to more thoroughly tease apart the interactions between environmental conditions and genotype (including diploid vs polyploidy seed sources), and the implications for long term management and recovery under a changing climate. Potential locations of reciprocal common gardens include Douglas County, southern and northern Willamette Valley, the Puget Trough in Washington, and potentially Vancouver Island (no longer occupied but part of its historic range). By adding experimental plots throughout its range and increasing the number of seed sources used (tied with the common garden location), we can gain a greater understanding of the interactions between environment and genetics which will inform future introduction of this species in the face of climate change.

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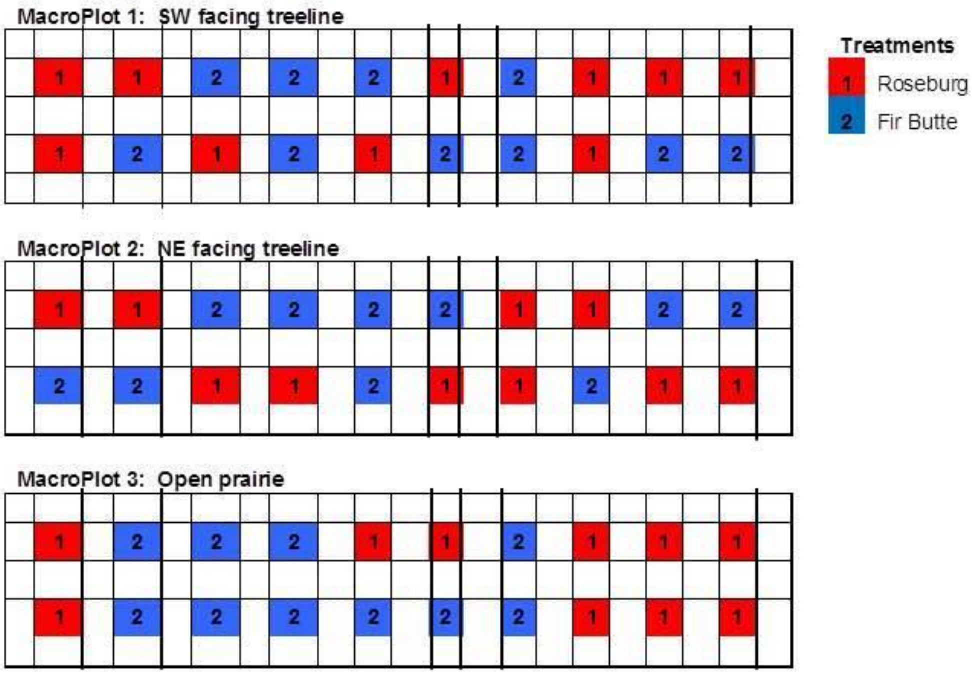
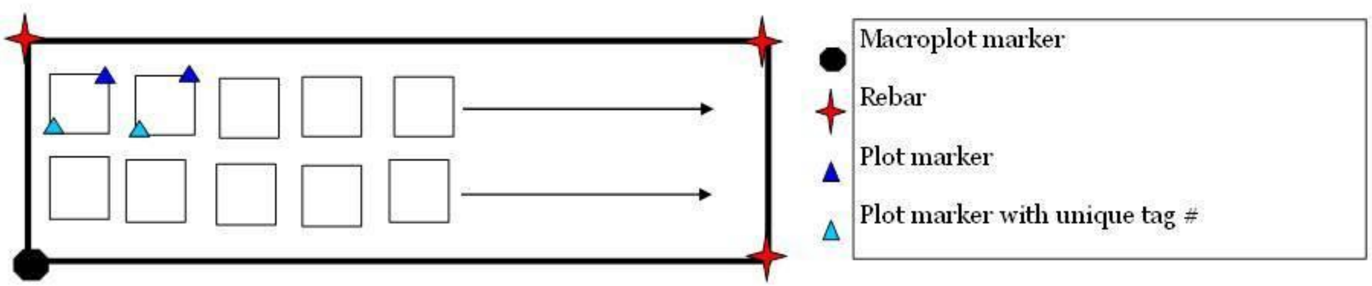
APPENDIX A. AERIAL PHOTOS AND SITE MAPS.

Coyote Prairie aerial photo with microclimate locations. Microclimate 1 = moderate exposure, Microclimate 2 = intermediate exposure, and Microclimate 3 = harsh exposure

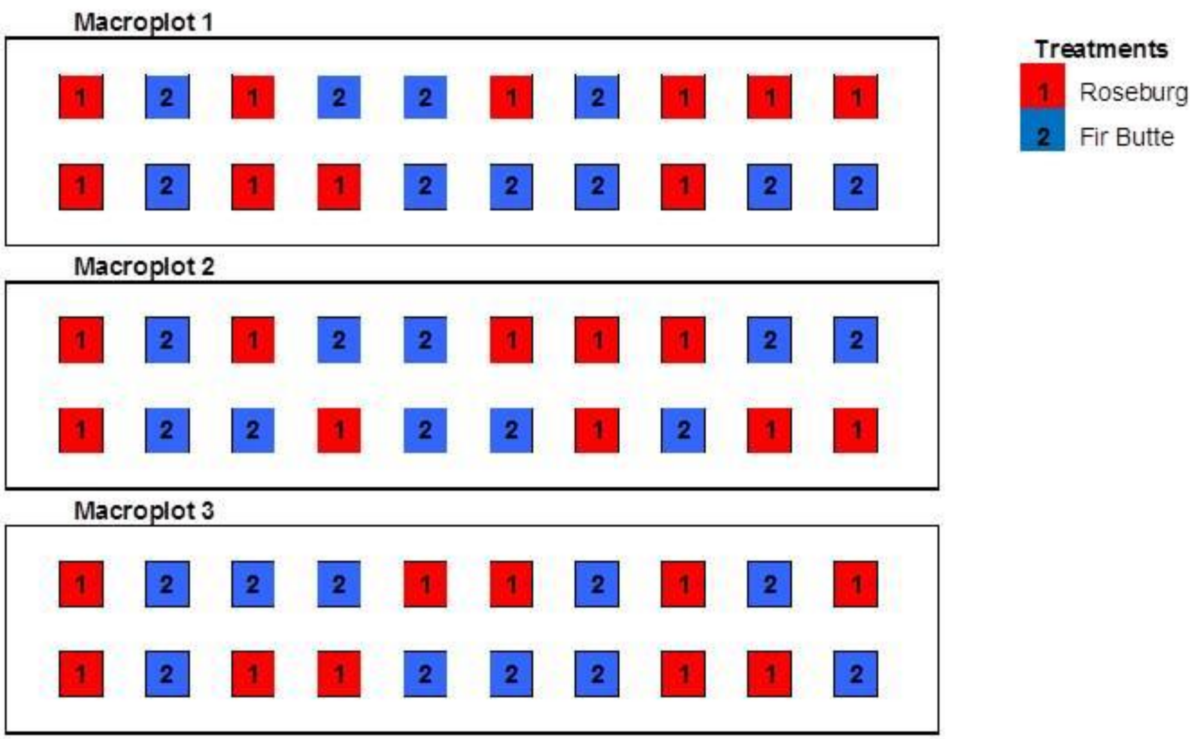
Coyote Prairie planting design

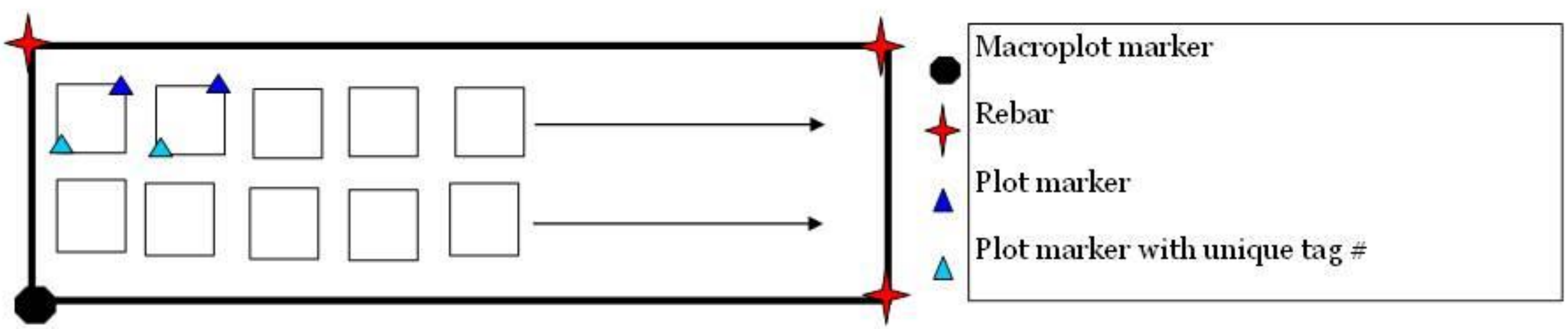




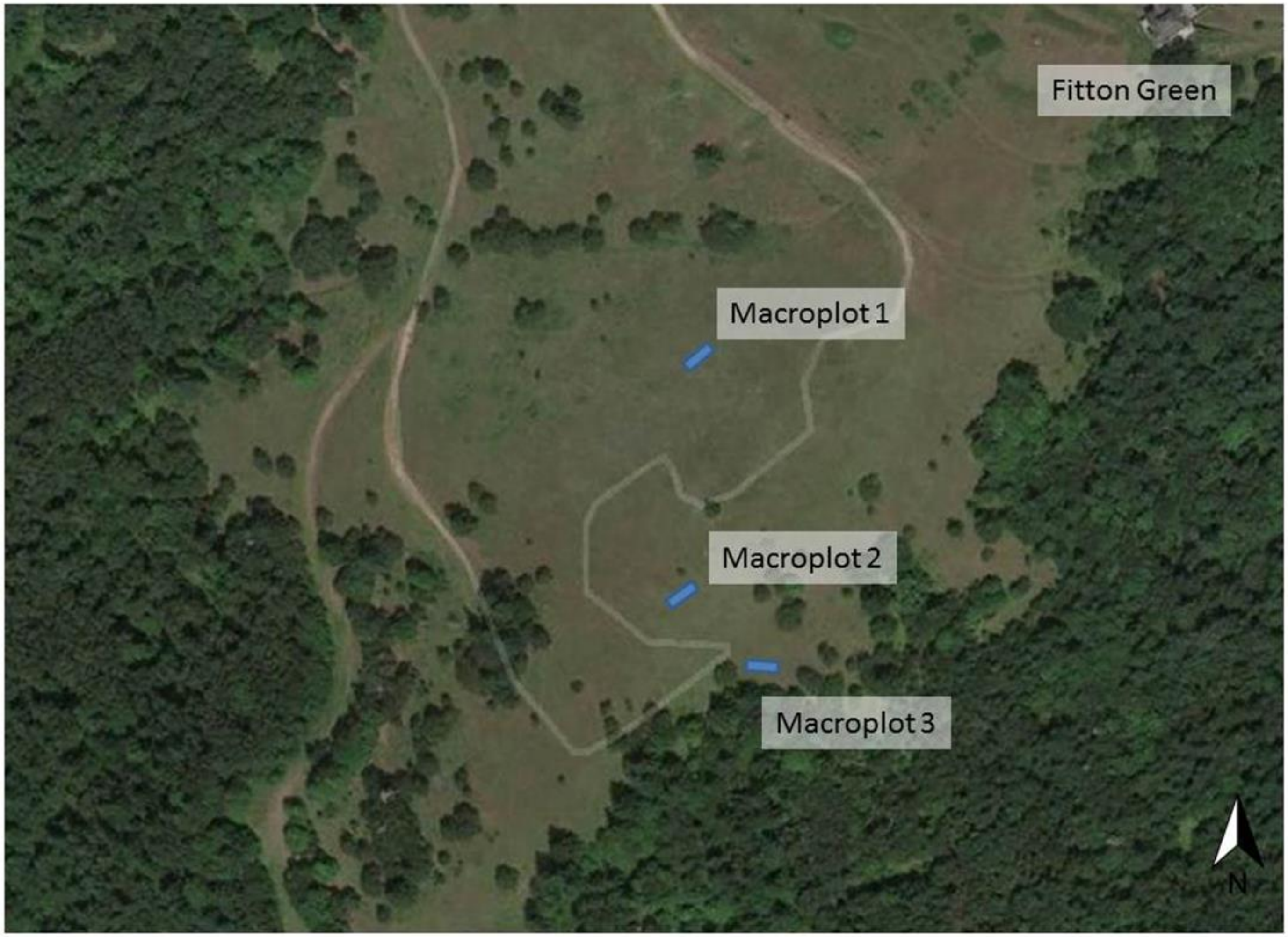
Doghead Meadow aerial photos with microclimate locations. Microclimate 1 = harsh exposure, Microclimate 2 = intermediate exposure, and Microclimate 3= moderate exposure.

Doghead Meadow planting design.





Fitton Green aerial photo with microclimate locations. Microclimate 1= moderate exposure, Microclimate 2 = harsh exposure, and Microclimate 3= intermediate exposure.



Fitton Green planting design

MacroPlot 1 (open, N facing)

1	3	3	2	3	1	3	1	1	3	2	3	1	1	2
3	2	1	3	1	2	2	1	2	2	3	2	2	3	1

MacroPlot 2 (open, S facing)

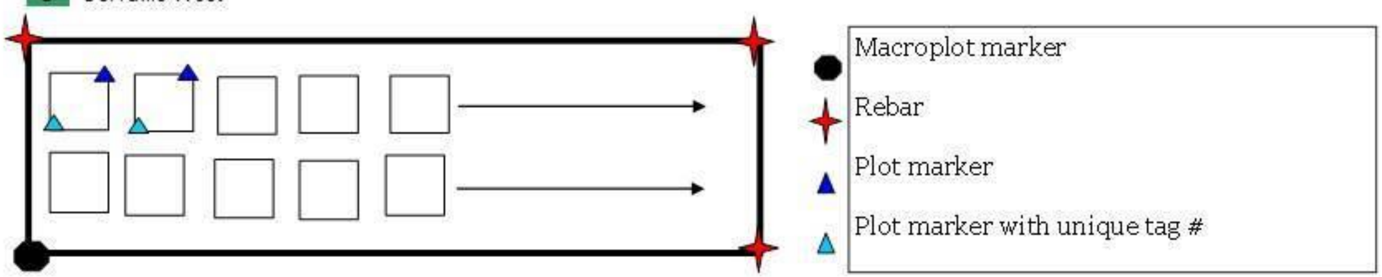
1	3	3	2	2	3	3	1	2	2	1	2	1	1	1
3	2	3	1	2	3	1	2	3	3	1	2	1	2	3

MacroPlot 3 (treeline)

1	2	2	2	1	3	2	3	3	3	3	3	3	1	1
3	2	2	2	2	2	2	1	3	1	1	1	1	1	3

Treatments

1 Roseburg
2 Fir Butte
3 Corvallis West



APPENDIX B. MICROCLIMATE DIFFERENCES

The various microclimates differed by canopy cover for all sites (Figure 11). At Coyote Prairie, microclimates 1 and 2 experienced similar levels of shade whereas and microclimate 3 experienced very little shade. Sun hours for microclimate 3 were slightly greater than for microclimates 1 and 2. The differences in exposure between microclimates 1 and 2 were relatively small, given they are both situated close to southern treelines (Appendix A). Microclimates at Coyote Prairie also differed by relative elevation. Though this area is considered upland prairie, it occurs on the valley floor and is lower in elevation than the other sites. Microclimate 3, while in full sun, was situated on a slight depression and experienced prolonged periods of standing water during the winter; the other two microclimates were situated on a slight rise near the treeline and were not affected by standing water (Appendix A). Microclimate 3 was removed from the study in 2015 due to the lack of survival of Kincaid's lupine. Relative humidity, and above- and belowground temperatures varied by microclimate at Coyote Prairie (Figure 12). Microclimate 2 tended to have higher temperatures during the growing season. Microclimate 1 had the highest relative humidity followed by microclimate 2.

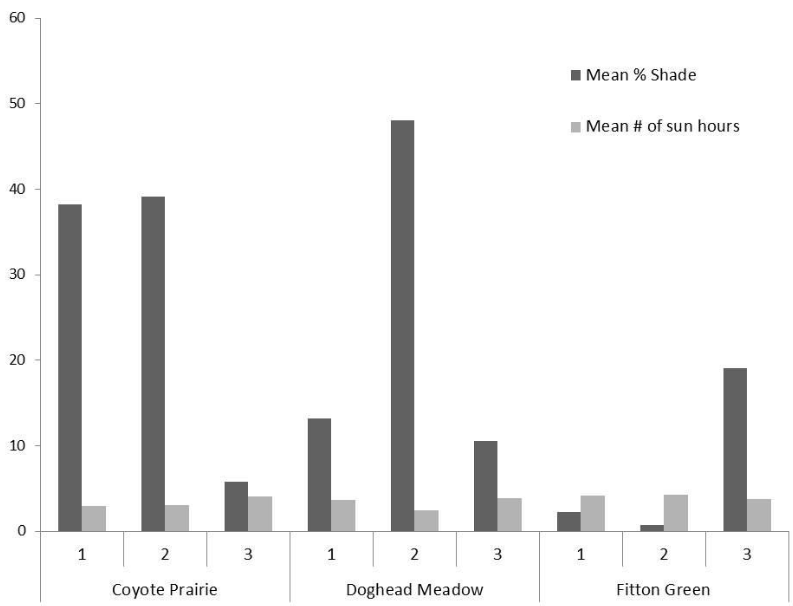


Figure 11. Mean annual % shade and number of sun hours at microclimate plots within sites. Microclimates are denoted by "1, 2, and 3".

Doghead Meadow had very strong differentiation between microclimates. Microclimate 1 was established on a north treeline with a southwest aspect, microclimate 2 was located along a SW treeline receiving a lot of shade (Figure 11, Appendix A), and microclimate 3 was located on a flat area with a slight SW aspect. Microclimate 1 had close to 12% shade. Microclimate 2 experienced the most shade (nearly 50%), and microclimate 3 experienced the least shade. Relative humidity was most differentiated during summer months between microclimates, where microclimate 1 had the lowest and microclimate 2 had the greatest relative humidity (Figure 13). Microclimate two is on a treeline and experiences much more shade (Figure 11), thus it would seem to hold onto humidity more easily. Microclimate 1experienced the highest temperatures across the years of this study, followed by microclimate 3 with the lowest temperatures occurring in microclimate 2 (Figure 13). Belowground temperature was widely differentiated between microclimates; microclimate 1 was the most exposed and the increased temperatures here seemed to persist in the soil (Figure 13). Similar to aboveground temperature, microclimate 3 had intermediate belowground temperature and microclimate 2 had the lowest (Figure 13)

Microclimates at Fitton Green were much more open than the other sites; microclimates 1and 2 differed by aspect but both were full sun and had no canopy cover, while microclimate 3 had greater shade because of its proximity along a treeline (Figure 11, Appendix A). While they were both in open prairie, microclimate 1 had a northwest facing slope whereas microclimate 2 had a south facing slope. At Fitton Green, microclimates followed interesting trends in relative humidity, where microclimates 1 and 2 had higher relative humidity than microclimate 3 (Figure 14). Interestingly, microclimate 3 had the most shade but was consistently the lowest in relative humidity. Temperature (both aboveground and belowground) was the greatest in microclimate 2, followed by microclimate 3; microclimate 1 consistently had the lowest temperature (Figure 14), most likely due to its northwest facing aspect.

All sites experienced a wide range of maxima and minima in relation to the mean values recorded by the iButtons (Figure 15). In general, monthly maximum temperatures (both aboveground and belowground) differed more from the mean than minimum temperatures. Relative humidity had more extreme minimum values than maximum values the other measurements across all sites. Temperature and relative humidity were similar between sites (Figure 15).

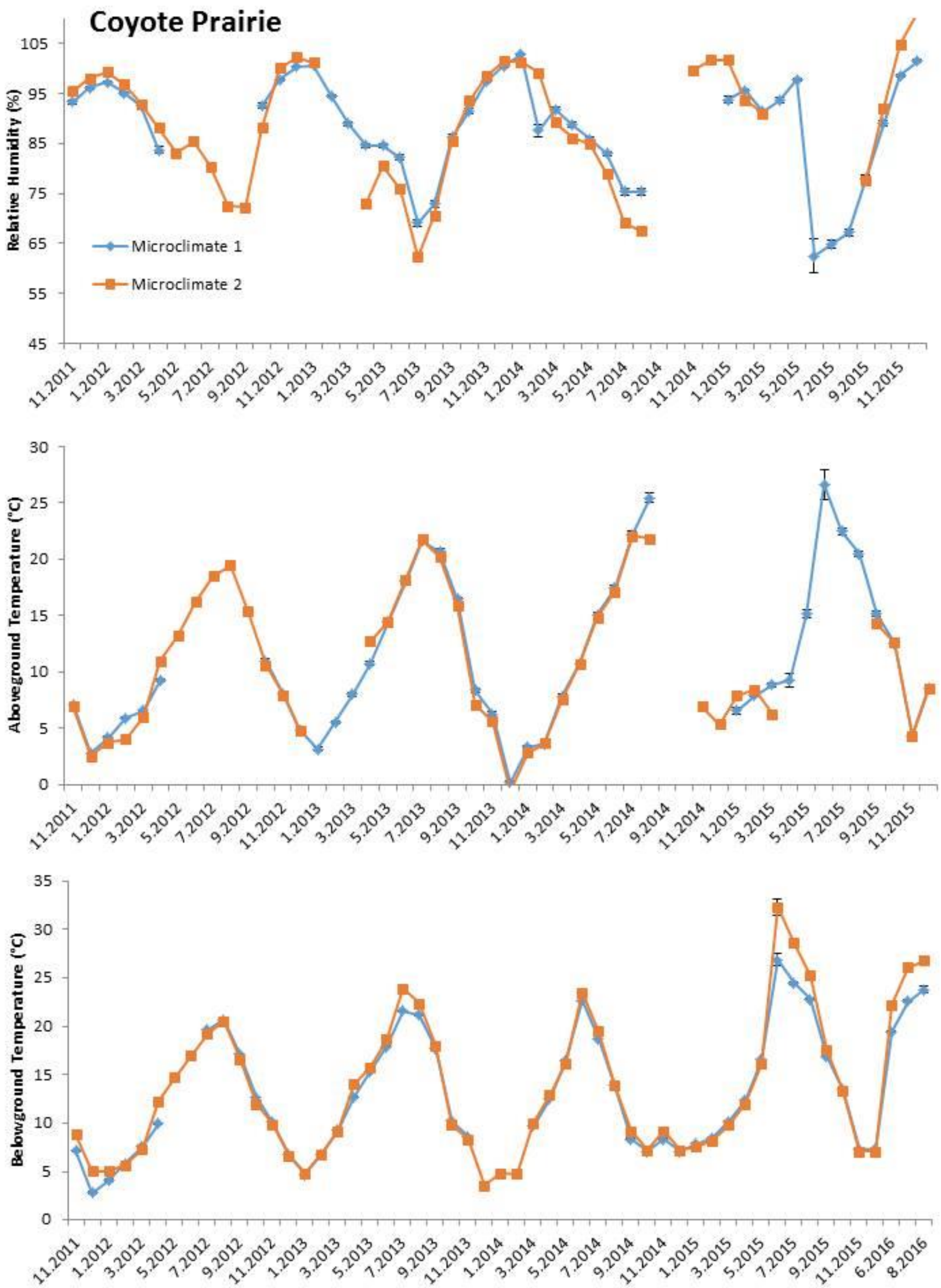


Figure 12. Relative humidity, aboveground and belowground temperature by microclimate at Coyote Prairie from November 2011-August 2016. Gaps in data are due to iButton malfunction.

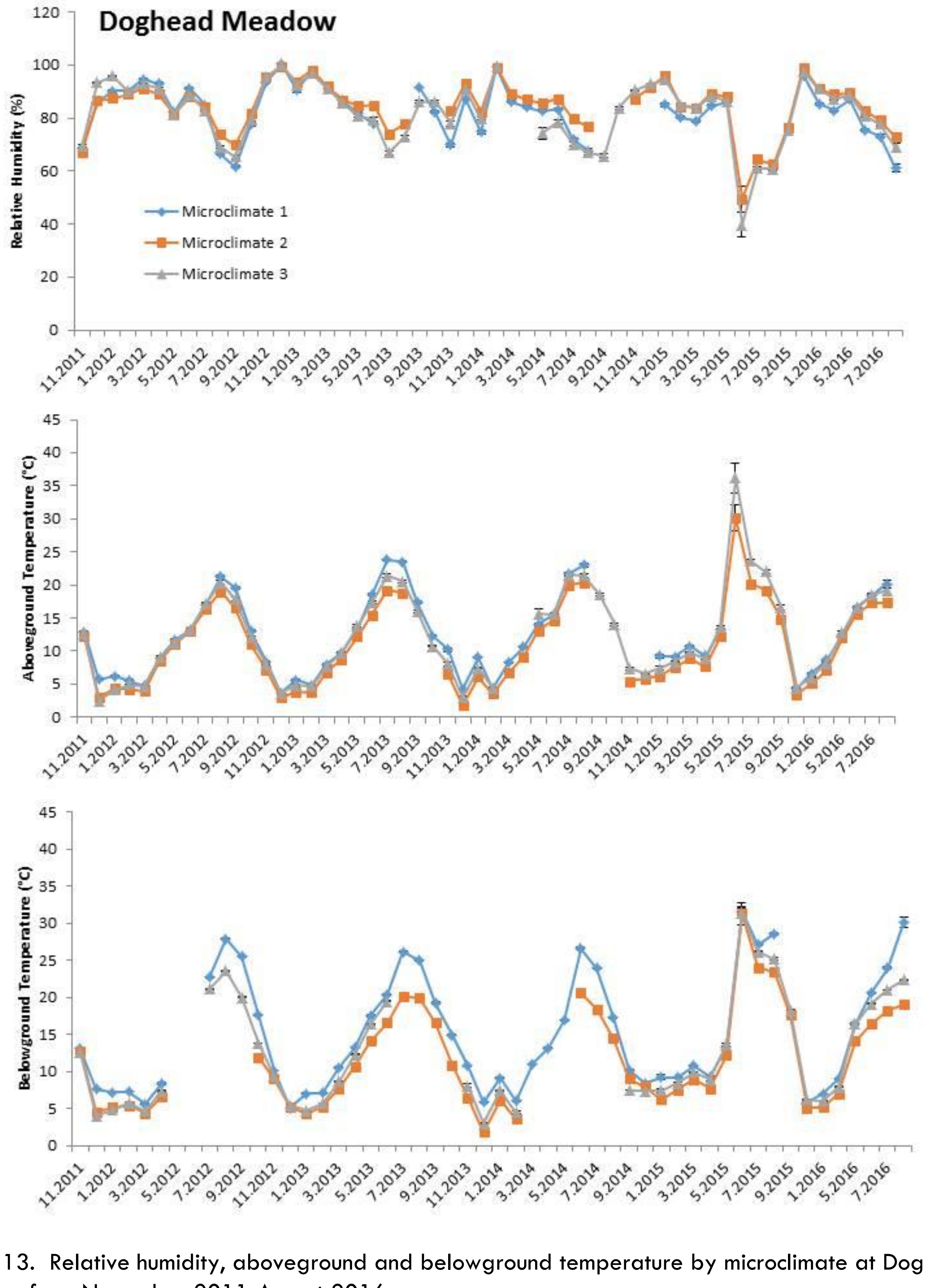


Figure 13. Relative humidity, aboveground and belowground temperature by microclimate at Doghead Meadow from November 2011-August 2016.

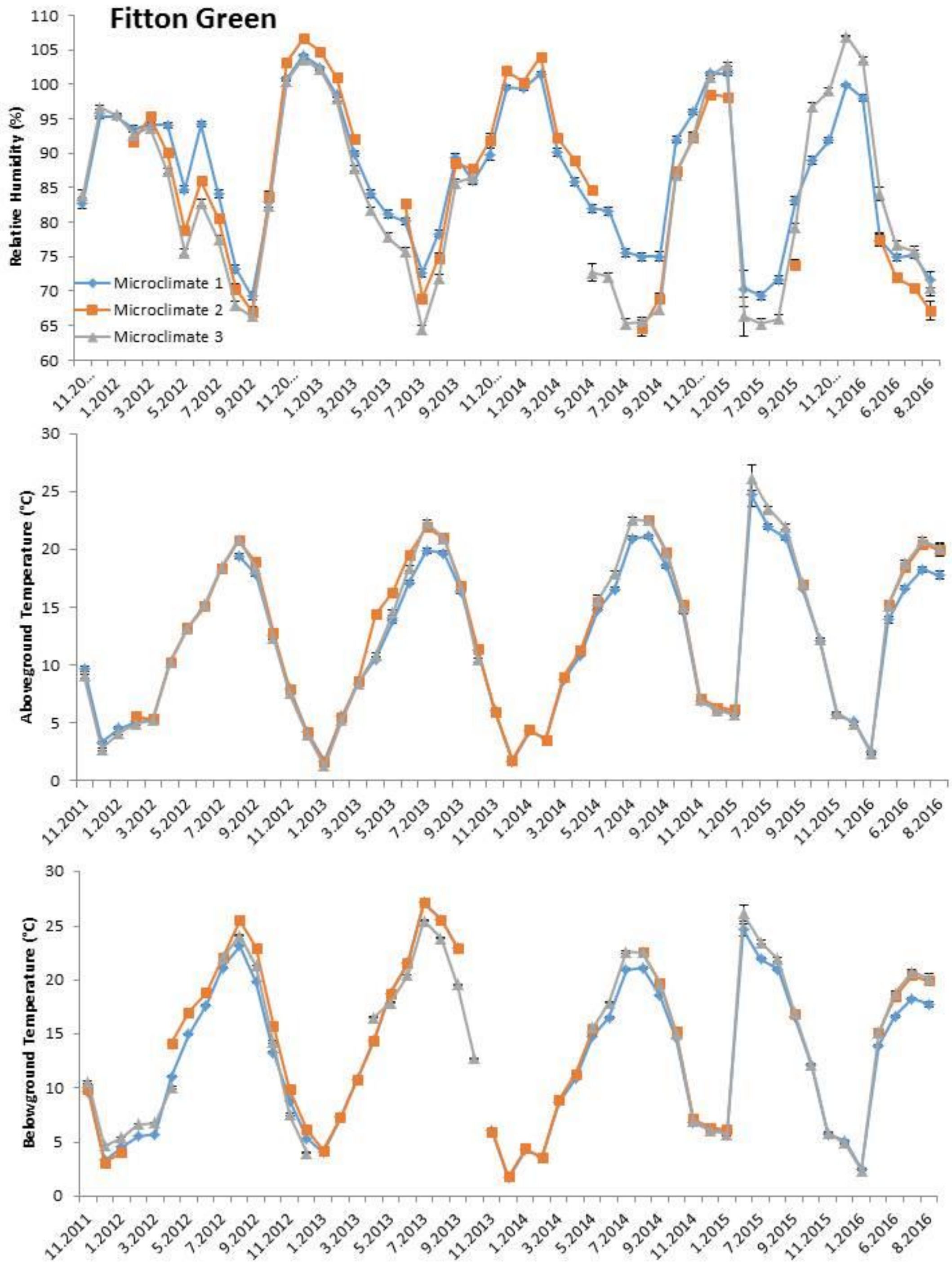


Figure 14. Relative humidity, aboveground and belowground temperature by microclimate at Fitton Green from November 2011-August 2016. Gaps in data are due to iButton malfunction.

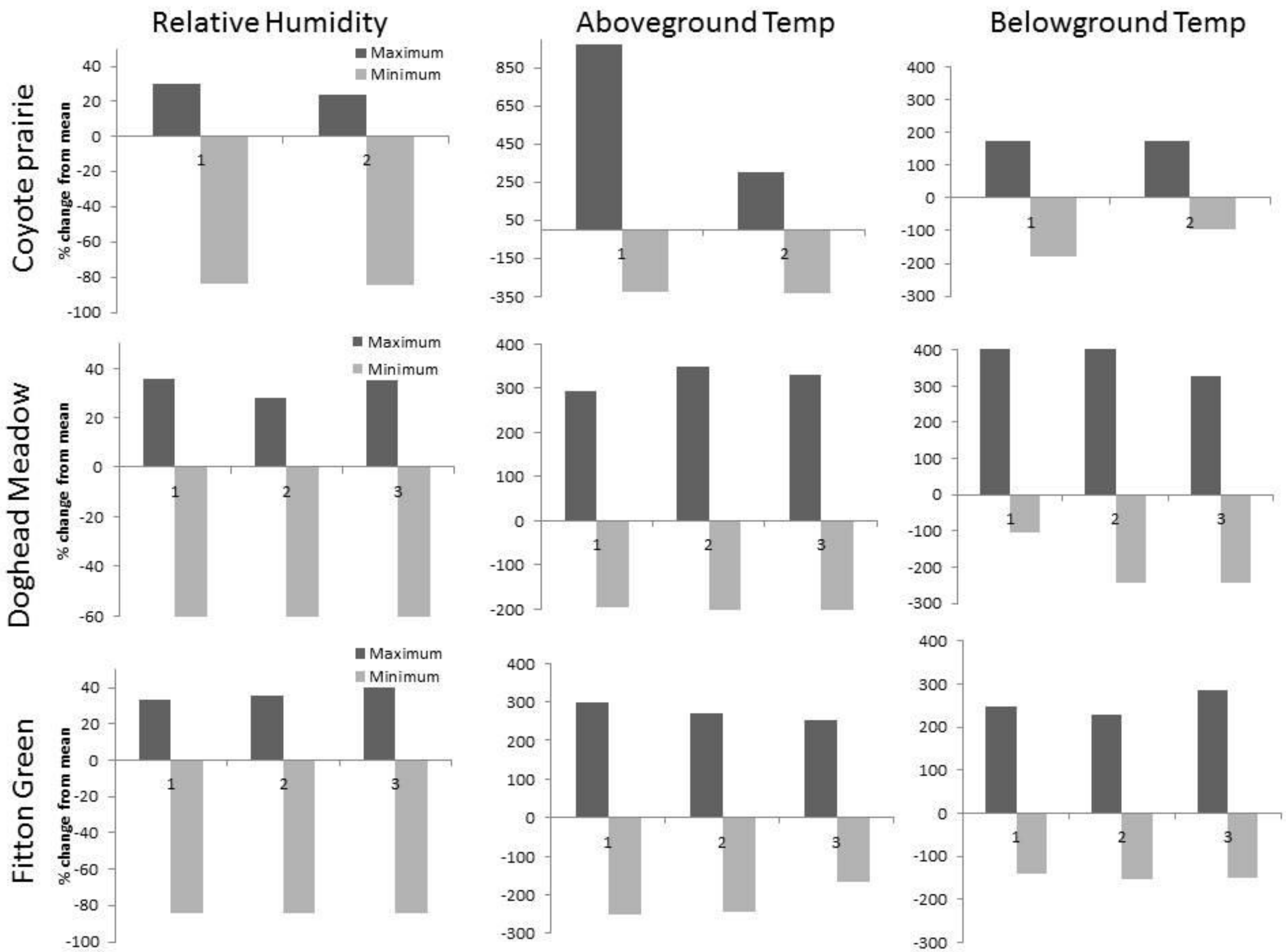


Figure 15. Percent change from mean for monthly maximum and minimum values for relative humidity, aboveground temperature, and belowground temperature at Coyote Prairie, Doghead Meadow, and Fitton Green 2011-2016 Microclimates are denoted by "1, 2, and 3". Note the difference in scales between graphs.