The effects of cheatgrass management with indaziflam on live fuel moisture content in rangeland plant communities along the Colorado Front Range

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Executive Summary

Cheatgrass invasion poses a major threat to rangeland plant communities along Colorado's Front Range (Front Range). However, the effects of invasive annual grasses, and other invasive annuals, on fire regimes and wildfire risks to people, property, and ecosystem integrity are less clear in these grassland ecosystems compared to sagebrush shrublands where the fire promoting effects of cheatgrass have been studied for decades. In addition, managers in Colorado have embraced the newer herbicide indaziflam (Rejuvra®, Envu) as a tool to reduce the abundance of invasive annuals in natural areas and open spaces, in many cases nearly eliminating invasive annuals for many years after treatment. Given (1) the relatively recent successes managers have achieved using indaziflam to manage cheatgrass and (2) the existing knowledge gap related to the fire promoting effects of cheatgrass in rangelands along Colorado's Front Range, the objective of our study was to measure the effects of indaziflam treatment, and subsequent reductions in cheatgrass abundance, on the live fuel moisture content (LFMC) of rangeland plant communities along the Front Range over the course of a summer fire season (approximately June-September). A measure of the amount of water contained in live vegetation, LFMC is a key driver of wildfire risk, with large and rapidly spreading wildfires becoming more likely as fuel moisture declines. We predicted that indaziflam treatment would increase LFMC in treatment plots compared to untreated control plots for at least a portion of the summer 2024 season.

To achieve our objectives we conducted bi-weekly biomass sampling at six parks and open spaces in Boulder County (four sites) and Jefferson County (two sites). Each location featured a treatment plot where indaziflam had been applied to manage cheatgrass sometime in the last several years (2018-2023) and a nearby untreated control plot. We used Analysis of Variance (ANOVA) to evaluate differences in LFMC between treatment and control plots, using each site as a replicate for the analysis (n=6). We found that indaziflam treatment significantly increased LFMC in treatment plots for six of the nine sample timings included in the study. Patterns in LFMC at each individual site were variable, but there were commonalities between sites that suggest precipitation timing during the 2024 field season played a key role in modifying LFMC trends. Data and photographs indicate that vegetation in treatment plots was better able to utilize precipitation later in the summer and increase LFMC, while more annual-dominated plant communities in control plots were less responsive to precipitation later in the summer. Our data also suggest that overall biomass production (standing crop) was increased in treatment plots, suggesting that interactions between increased LFMC and increased fuel amount may influence fire behavior and risk in indaziflam-treated communities.

Our results suggest that indaziflam treatment can reduce fire risks by increasing LFMC in treated areas compared to untreated plant communities with abundant cheatgrass. Research in other locations has identified thresholds of LFMC below which the likelihood of extreme fire is increased (e.g., 80 % LFMC in California chaparral). While we cannot be sure of the appropriate LFMC threshold for rangelands along the Front Range, LFMC was more than 30% higher in treatment plots for six of the nine total sample timings included in our study, and LFMC in the control plots never exceeded 70 % after 10-June. Our findings suggests that indaziflam treatment can mitigate the risk of ignition and rapid wildfire spread in treated plant communities, but it is important to note that the mitigating effects of increased LFMC are unlikely to blunt fire risk during extreme fire weather (hot, dry, windy conditions). When conditions are less than extreme, however, indaziflam treatment is likely to reduce wildfire risk. Our results also suggest that indaziflam treatment increases fuel amounts (standing crop) in treated areas and that the responsiveness of LFMC to precipitation is likely to differ based on treatment and the abundance of cheatgrass in a given area, indicating that more research is necessary to evaluate interactions between LFMC and fuel amounts and the effects of treatment on LFMC over multiple years.

Introduction

Exotic annual cheatgrass (*Bromus tectorum* L.) has invaded vast expanses of semi-arid rangeland in western North America (Bradley et al. 2018; Kleinhesselink et al. 2023), and increased fire frequency after cheatgrass invasion poses a threat to ecosystem integrity, public safety, and important infrastructure in invaded ecosystems. Cheatgrass litter provides a continuous layer of fine fuel that increases the likelihood of wildfire in invaded plant communities compared to those with little or no cheatgrass (Balch et al. 2013; Davies and Nafus 2013). While research clearly indicates that increased fire is linked to cheatgrass invasion in the sagebrush biome (Balch et al. 2013; Bradley et al. 2018; Davies et al. 2023), the impacts of cheatgrass invasion on wildfire in rangelands along the Colorado Front Range (henceforth Front Range) are less clear (Seastedt 2023). However, invasive grasses influence fire frequency and occurrence in a broad range of US ecosystems (Fusco et al. 2019) and it is possible that cheatgrass invasion has contributed to recent Front Range wildfires (e.g., Marshall Fire in Boulder County; Hogback Fire in Jefferson County).

Until recently, managers had few tools capable of achieving long term cheatgrass control. The herbicide imazapic (Plateau®, BASF) is often used to reduce cheatgrass abundance, but the long-term effects of imazapic treatment are variable, and reinvasion from the cheatgrass seed bank 2+ years after treatment (YAT) is typical (Mangold et al. 2013; Courkamp et al. 2022). Encouragingly, the newer herbicide indaziflam (Rejuvra®, Envu) has provided long-term (3+ years) cheatgrass control in several trials and operational treatments in the western US (Sebastian et al. 2016; Sebastian et al. 2017; Clark 2020), and more research is necessary to better understand the potential wildfire mitigation benefits of effective cheatgrass management. Live fuel moisture content (LFMC) is an important driver of rangeland wildfire behavior because water contained in vegetation acts as a heat sink that slows down or prevents ignition and reduces the likelihood of wildfire spread (Viegas et al. 1992; Plucinski et al. 2010; Parks et al. 2014). Thus, the potential for wildfire increases as the fuel moisture content of vegetation declines. Fuel moisture can decline in response to dry weather conditions and/or over the course of the growing season as vegetation senesces and dries out, but research has also shown that cheatgrass invasion reduces fuel moisture relative to non-invaded shrub-dominated plant communities in the Great Basin (Davies and Nafus 2013). This is likely due to the early senescence of cheatgrass compared to other plants; senesced cheatgrass provides a source of dry fuel that will readily burn when other native plants are greener and better able to resist ignition, due in part to their higher LFMC. Accordingly, Nafus and Davies (2013) estimated that cheatgrass invasion extended the fire season by more than a month in their study.

While the impacts of cheatgrass invasion on fire regimes in the sagebrush biome are well-documented (Balch et al. 2013; Bradley et al. 2018; Davies et al. 2023), plant communities along the Front Range differ from these ecosystems in important ways (e.g., precipitation timing; vegetative composition). For example, sagebrush-dominated shrublands typically feature more abundant bare ground for cheatgrass to exploit and fill in, whereas grass-dominated rangelands have less open space. Thus, fuel continuity may be altered to a lesser degree by cheatgrass invasion in grass-dominated rangelands along the Front Range compared to Great Basin shrublands, blunting the potential influence of effective cheatgrass management on wildfire mitigation in these ecosystems. However, the early phenology of cheatgrass is distinct from co-occurring native plants in both systems, suggesting that cheatgrass invasion may substantially alter fuel moisture in Front Range rangelands, with subsequent impacts on wildfire risk.

When one considers: (1) the destructiveness of recent wildfires; (2) recent advancements in cheatgrass management tools (e.g., indaziflam); (3) the potential effects of cheatgrass management on fuel moisture; and (4) the influence of fuel moisture on wildfire behavior, it is clear that more research is necessary to better understand the potential effects of cheatgrass management on fuel moisture content in Front Range rangelands. Accordingly, the objective of our study was to measure the effects of indaziflam treatment, and subsequent reductions in cheatgrass abundance, on the live fuel moisture content (LFMC) of rangeland plant communities along the Front Range over the course of a summer fire season (approximately June-

September). We predicted that indaziflam treatment would increase LFMC in treatment plots compared to untreated control plots for at least a portion of the summer 2024 growing season.

Methods

Study Sites

We selected six study sites for the project: four in Boulder County (Figure 1) and two in Jefferson County (Figure 2; Table 1). Treatment areas at all sites were treated with 102 g ai ha^{-1} of indaziflam (7 oz ac^{-1} Rejuvra®, Envu) in the last 6 years (2018-2023), but the details of treatment (timing, tank mix partner, application method) differ slightly between sites (Table 2). All sites were representative of Front Range grassland plant communities, and some of the more dominant native plant species across locations included western wheatgrass (*Pascopyrum smithii* [Rydb.] Á. Löve), needle-and thread (*Hesperostipa comata* [Trin. & Rupr.] Barkworth), big bluestem (*Andropogon gerardii* Vitman), fringed sagebrush (*Artemisia frigida* Willd.), wild tarragon (*Artemisia dranunculus* L.), white sage (*Artemisia ludoviciana* Nutt.), and slimflower scurfpea (*Psoralidium tenuiflorum* [Pursh] Rydb.). While cheatgrass was a major component of the non-native plant community at all sites, non-native annual *Alyssum* spp. were also present and abundant at most sites. Other non-native/invasive species that were present at varying levels of abundance in study plots included common mullein (*Verbascum thapsus* L.), dalmation toadflax (*Linaria dalmatica* [L.] Mill.), and musk thistle (*Carduus nutans* L.). All study locations featured areas treated with indaziflam for cheatgrass control (treatment plots) and nearby similar untreated locations (control plots), and cheatgrass and non-native *Alyssum* spp. were largely absent from treated plots and relatively abundant (>15% canopy cover) in untreated control plots. The one exception to this characterization was Mt. Galbraith Park (Table 1; Figure 2), where cheatgrass was more abundant in the treated plots compared to other locations, but still reduced overall relative to the control plots(Figure 4; see *Results*).

Table 1. Location and managing agency for the six study sites included in the project. All locations are UTM Zone 13N.

Study Site	UTM E	UTM N	Managing Agency
Rabbit Mountain	483053	4453221	Boulder County
Hall Ranch Mine	474742	4450387	Boulder County
Highway 36A	476799	4446150	Boulder County
Highway 36B	478409	4448007	Boulder County
Mathews-Winters Park	482414	4391633	Jefferson County
Mt. Galbraith Park	479571	4401347	Jefferson County

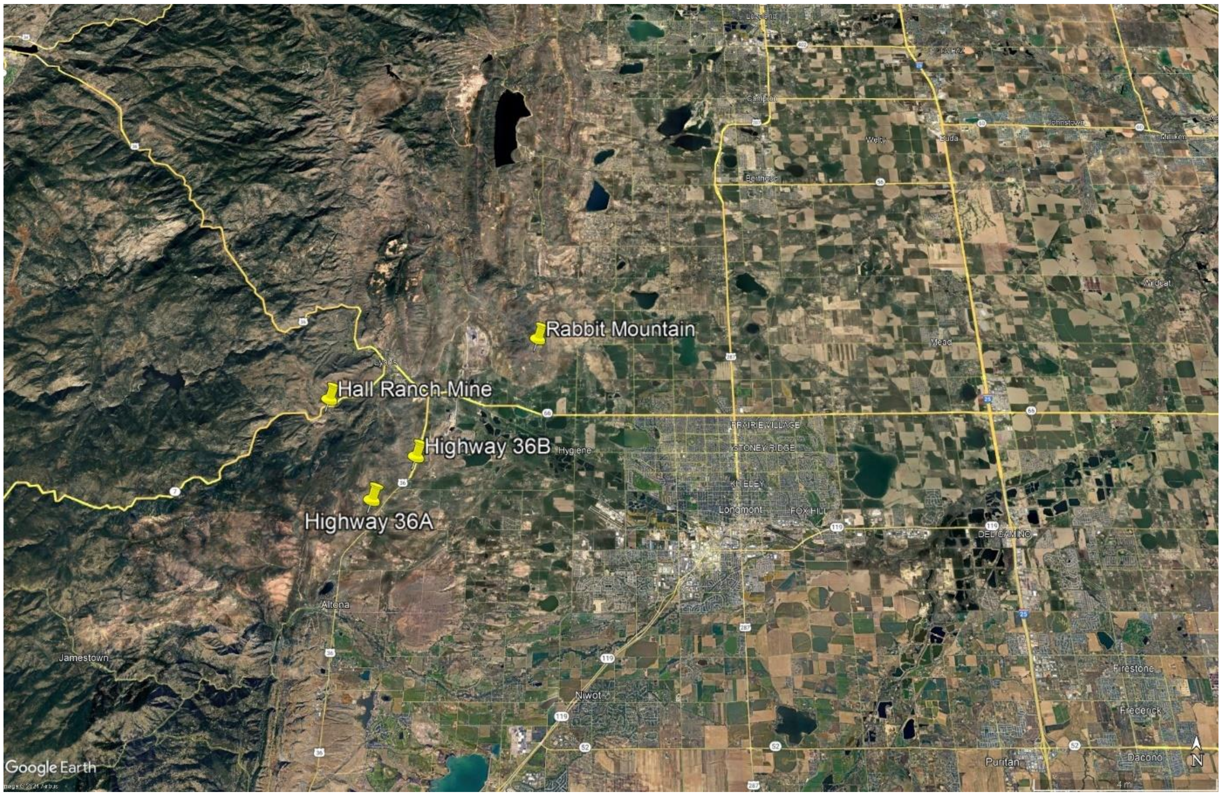

Figure 1. Map showing locations of the four Boulder County sites included in the study (Rabbit Mountain, Hall Ranch Mine, Hwy 36A, and Hwy 36B; Table 1).

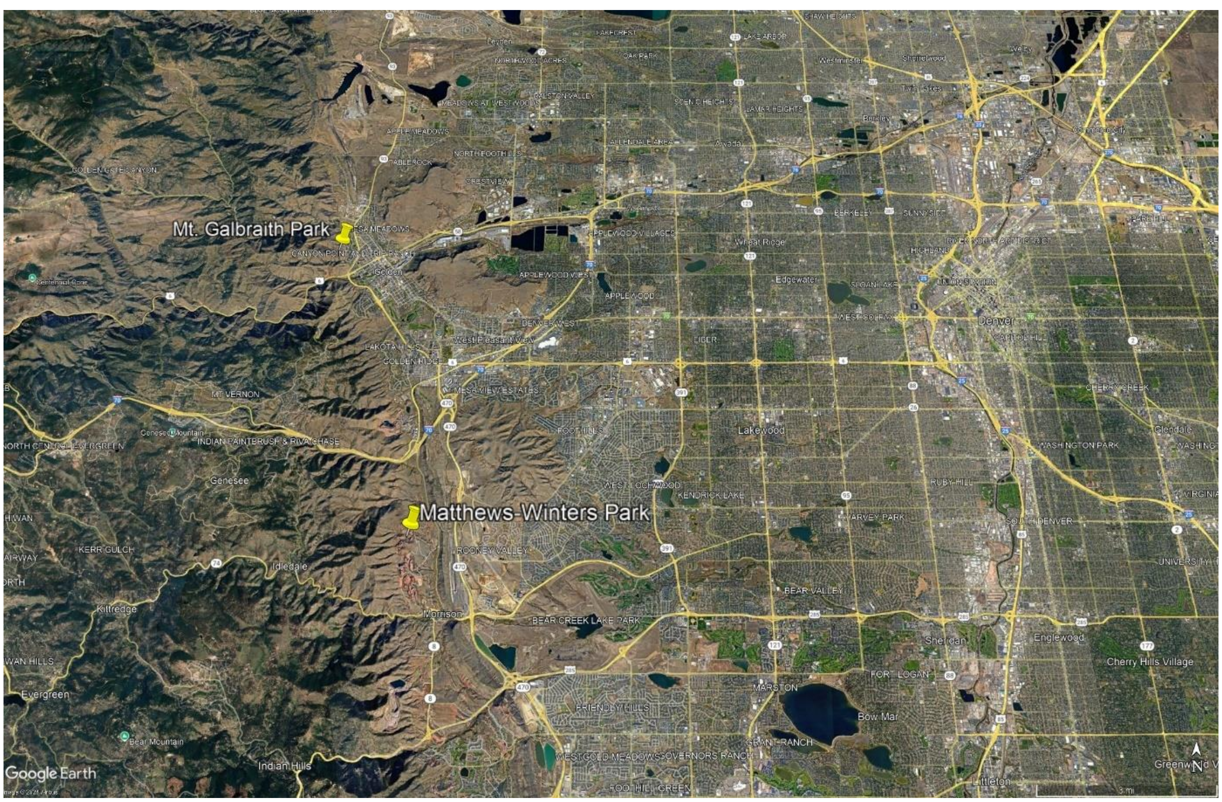

Figure 2. Map showing locations of the two Jefferson County sites included in the study (Matthews-Winters Park and Mt. Galbraith Park; Table 1).

Table 2. Treatment information for the six study sites included in the project.

Study Site	Treatment Date	Treatment Notes
Rabbit Mountain	1-December-2021	Aerial treatment (helicopter); 7 oz ac^{-1} Rejuvra (102 g ai ha^{-1} indaziflam) + 10 oz/ac glyphosate.
Hall Ranch Mine	3-April-2023	Ground treatment (fixed boom); 7 oz ac^{-1} Rejuvra (102 g ai ha^{-1} indaziflam).
Highway 36A	8-January-2018	Ground treatment (fixed boom); 7 oz ac^{-1} Rejuvra (102 g ai ha^{-1} indaziflam) + 12 oz/ac glyphosate.
Highway 36B	8-March-2021	Ground treatment (fixed boom); 7 oz ac^{-1} Rejuvra (102 g ai ha^{-1} indaziflam) + 10 oz/ac glyphosate.
Mathews-Winters Park	4-October-2022	Aerial treatment (helicopter); 7 oz ac^{-1} Rejuvra (102 g ai ha^{-1} indaziflam) + 5 oz/ac Plateau.
Mt. Galbraith Park	23-June-2021	Ground treatment (boomless); 7 oz ac^{-1} Rejuvra (102 g ai ha^{-1} indaziflam).

Field Sampling

At each study site, four randomly located 3 x 3-m plots (permanent plots) were installed in the both the control and treatment plot. The corners of each permanent plot were marked with pin flags and large steel nails driven into the soil surface until approximately 2.5 cm remained above ground level. We collected vegetation canopy cover and fuel moisture data from these plots nine times over the course of the summer 2024 field season, and it took 3-5 days to collect data at all sites each time depending on the weather and competing field work priorities (Table 3). Photographs were taken of all permanent plots and overall treatment and control plots each time sampling occurred.

Table 3. Dates associated with the nine bi-weekly LFMC sample timings included in the project. Note that sample timings for standing crop estimation differ from those shown in the table.

Sample Timing	Sample Initiation Date	Sample Completion Date
1*	13-May-2024	15-May-2024
2	28-May-2024	31-May-2024
3	12-June-2024	14-June 2024
4	24-June-2024	26-June-2024
5	8-July-2024	11-July-2024
6	23-July-2024	25-July-2024
7	5-August-2024	7-August-2024
8	19-August-2024	21-August-2024
9	4-September-2024	9-September-2024

*Data were not collected at Highway 36B for sample timing 1.

For cover data, we recorded ocular estimates of absolute percent canopy cover (cover) for grasses, forbs, shrubs, invasive annual grasses and non-native annual forbs (vegetation functional groups) in each permanent plot. We also recorded the cover of bare ground, litter, rock, and standing dead vegetation. For fuel moisture data, we clipped approximately 30 g of each vegetation functional group. We avoided collecting vegetation from inside permanent plots to prevent disturbing the vegetation and changing subsequent data collection, so vegetation samples for each functional group were collected in the immediate vicinity of each permanent plot. We attempted to roughly match the species composition of our biomass samples with the composition of the respective permanent plot, and native and non-native species were combined for each functional group, excluding invasive annual grasses and non-native annual forbs, which were collected separately. For shrubs, we collected both woody stem material and current-year's growth to approximate conditions of the shrub community in each plot. Invasive annual grasses included cheatgrass along with lesser amounts of Japanese brome (*Bromus japonicus* L.) and feral rye (*Secale cereale* L.), while annual *Alyssum* spp. comprised the totality of invasive annual forb samples. Biomass samples were immediately placed in pre-weighed sealed plastic bags after collection and stored in a cooler until transport to Colorado State University campus (CSU) later that same day.

In addition, we collected biomass samples to estimate standing crop by completely clipping four 0.25-m^2 sampling frames in each treatment and control plot at each study site twice during the 2024 field season; once in early-July (early-season) and once in late-September (late-season). Frames were subjectively located to match the approximate composition of each permanent plot, and all vegetation in each frame was clipped at ground level and placed in a single pre-weighed sealed plastic bag. After collection, samples were stored in a cooler until transport to CSU later that same day.

Lab Protocol

Biomass samples were weighed to the nearest 0.01 g in their sealed plastic bags immediately after transport to CSU, and wet weight was determined by subtracting the weight of the pre-weighed plastic bag containing each sample. Samples were then transferred to paper bags and placed in a drying oven for a minimum of seven days before being removed and weighed a second time (dry weight), again to the nearest 0.01 g. Live fuel moisture content (LFMC) for each functional group in each permanent plot at each sample timing was calculated as:

$$\text{LFMC} = ([\text{wet weight} - \text{dry weight}]/\text{dry weight}) \times 100$$

Biomass samples for standing crop estimates were also transferred to paper bags and placed in a drying oven for a minimum of seven days, after which they were weighed to the nearest 0.01 g to estimate production.

Data Summarization and Analysis

Data were summarized by multiplying the cover of each functional group by the corresponding LFMC and totaling up the resulting values to generate a weighted average LFMC for each plot and sample timing. Plots were considered subsamples for the purposes of analysis, thus values from each of the four treatment and control plots at each site were averaged to generate an overall LFMC for treatment and control plots at each site and sample timing ($n = 6$). These data were then used for repeated-measures Analysis of Variance (ANOVA) using the "LMER" package in R (R Core Team 2024). We used ANOVA to investigate the effects of treatment, week, and the treatment by week interaction (fixed effects) with plot and site included as random effects to account for repeated measures and the paired design of our study, respectively. Visual inspection of quantile-quantile and fitted vs. residual plots was used verify that our data met the assumptions of ANOVA, and effects were considered significant when $P \leq 0.05$. When ANOVA indicated that treatment differences existed, we conducted post-hoc pairwise comparisons of LFMC in treatment and control plots at each sample timing, with differences considered significant when $P \leq 0.05$.

We also conducted correlation analysis to evaluate relationships between vegetation functional groups and LFMC. Invasive annual grasses and invasive annual forbs were combined for the purposes of this analysis. Because vegetation data were not normally distributed, we investigated non-parametric correlations (Spearman's ϱ) between LFMC and perennial grass, perennial forb, shrub, and invasive annual cover. Relationships were considered significant when $P \leq 0.05$.

To investigate site-level patterns in fuel moisture, we visually inspected line plots showing mean LFMC and the 95% confidence interval for fuel moisture at each sample timing in the treatment and control plots at each site ($n=4$). We want to stress that, because there is only one treatment plot and one control plot at each study site, sites should be considered individual key areas (Elzinga et al. 1998), and any conclusions drawn from site-specific patterns are attributable only to their respective study sites and not suitable for extrapolation to other locations.

Student's t-tests were used to compare production in treatment and control plots. Similar to LFMC, data were summarized by site (n = 6), and data from the early-season and late-season sample timings were analyzed separately. Differences were considered significant when $P \leq 0.05$.

Results

Invasive Annual Cover and Control

Invasive annuals were anecdotally controlled in treatment plots at all study sites except Mt. Galbraith Park, where patches of dense cheatgrass existed in the treatment plots (Figure 4). Invasive annual cover never exceeded a trace (< 1% cover) in all treatment plots at all other study sites and sample timings, while invasive annual cover ranged from 6.5 to 20.75 % in the treatment plot at Mt. Galbraith Park. Mean invasive annual cover in control plots at all sites ranged from 17.75 to 61.5 % over the course of the season, with an average of 40.1 % across all sample timings. The almost complete lack of invasive annuals in treatment plots at five of the six study sites precluded traditional statistical comparisons, but photographic documentation of treatment effects is sufficient to establish that indaziflam treatment controlled invasive annuals at all sites except Mt. Galbraith Park (Figure 4), where invasive annuals were suppressed in the treatment plot, but not to the same degree as the other study sites. The treatment plot at Mt. Galbraith featured patches where cheatgrass was controlled and others where it was dense and abundant (Figure 4). We show an example of photographic documentation of cheatgrass control at the Rabbit Mountain study site in Figure 3, and photographs of treatment and control plots at all study sites and sample timings are included as supplemental material.

Figure 3. Photograph showing the boundary between treatment plot (right) and control plot (left) at Rabbit Mountain on 13-June-2024. Note the reduction in cheatgrass (reddish brown) in the treated area (see Table 2 for treatment details).

Figure 4. Photograph showing the treatment plot at Mt. Galbraith Park (Mt. Galbraith) on 12-June-2024. Note the patches of dense cheatgrass (reddish brown) in the treated area (see Table 2 for treatment details).

Fuel Moisture Differences and Relationships with Vegetation Functional Groups

All fixed effects and interactions were significant in our ANOVA (treatment $P = 0.016$; week $P < 0.001$; treatment x week $P < 0.01$; Figure 5), suggesting that both treatment and sample timing influenced LFMC. Post-hoc pairwise comparisons of LFMC indicated that LFMC was significantly increased in treatment plots compared to control plots at six of nine total sample timings, excluding both the earliest and latest sample timings in the season (13-May and 2-September) and 5-August (Figure 5). The greatest differences in LFMC between treatment and control plots occurred on 24-June (68% in control plots vs. 123% in treatment plots) and 19-August (44% in control plots vs. 88% in treatment plots).

Correlation analysis suggested that significant relationships existed between LFMC and the cover of several vegetation functional groups (Figure 6). Positive relationships existed between LFMC and perennial grasses ($P < 0.01$; $\varrho = 0.13$) and perennial forbs ($P < 0.01$; $\varrho = 0.24$), while a negative relationship existed between LFMC and invasive annuals ($P < 0.01$; $\varrho = -0.24$; Figure 6). This suggests that plots with higher perennial grass and perennial forb cover, and low invasive annual cover, typically had higher LFMC.

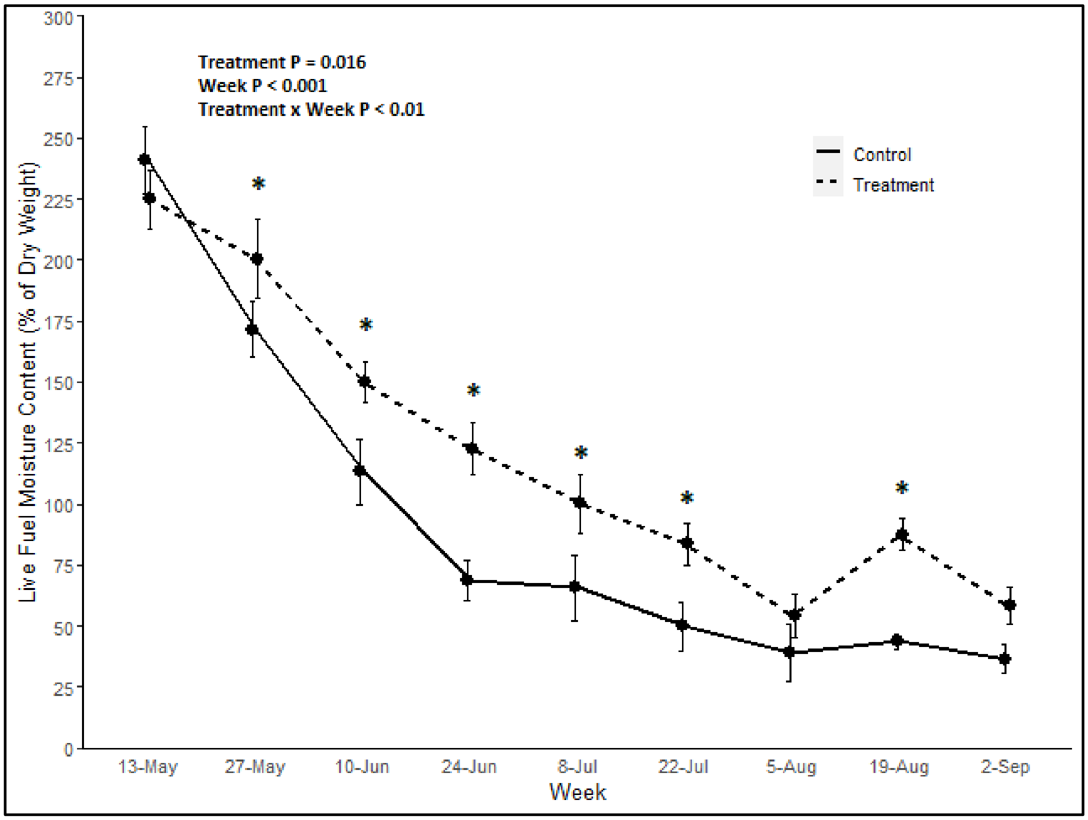

Figure 5. Bi-weekly live fuel moisture content (LFMC) in control plots (solid) and indaziflam treatment (dashed) plots at all six Front Range grassland study sites. Error bars correspond to ± 1 standard error. Annotations show results of analysis of variance (ANOVA) with significant fixed effects shown in bold, while asterisks indicate significant differences between treatment and control plots within sample timing (week) based on the results of post-hoc pairwise comparisons using a Tukey adjustment (n=6; P ≤ 0.05).

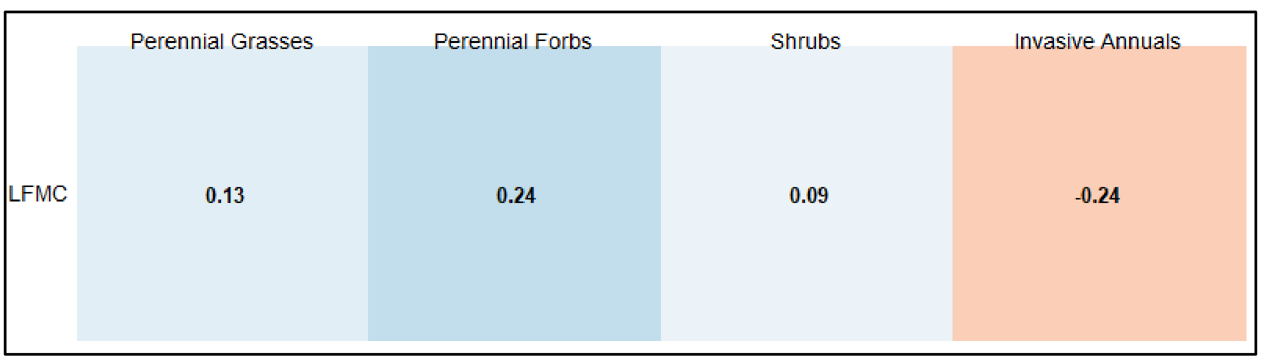

Figure 6. Non-parametric correlation coefficients (Spearman's ρ) between live fuel moisture content (LFMC) and vegetation functional group cover. Positive values (blue) indicate a positive relationship and negative values (red) indicate a negative relationship between LFMC and cover of each functional group. All correlations were statistically significant (P ≤ 0.05) except for relationship between LFMC and shrub cover.

Fuel Moisture Patterns by Site

The following sections describe patterns in LFMC at individual study sites. We want to stress that each study site should be considered an individual key area (Elzinga et al. 1998), and patterns attributed to a given study site should not be extrapolated to other locations.

Rabbit Mountain

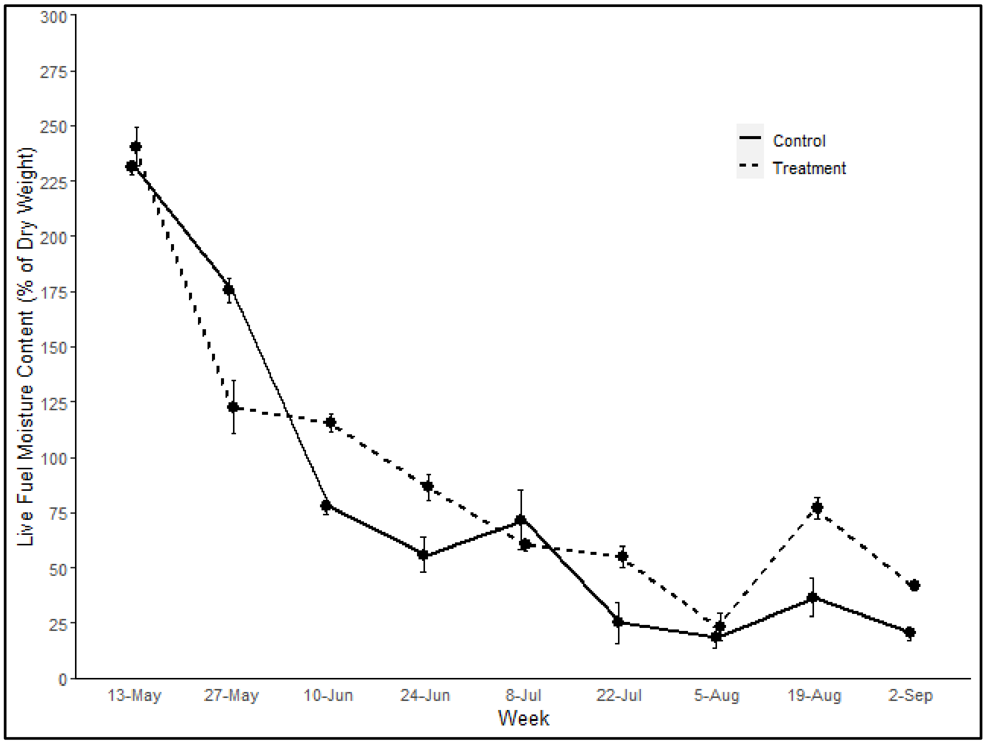

Figure 7. Bi-weekly mean live fuel moisture content (LFMC) in control (solid) and indaziflam treatment (dashed) plots at the Rabbit Mountain study site (BCPOS). Error bars correspond to the 95% confidence interval (n = 4).

At Rabbit Mountain, LFMC was highly variable, with LFMC in the treatment plot exceeding that of the control plot (non-overlapping 95% confidence intervals), for five of the nine total sample timings (Figure 7). The largest discrepancies between LFMC in the treatment and control plots occurred on 27-May, when LFMC was greater in the control plot (176 % in control plot vs. 123 % in treatment plot), and 19-August (36 % in control plot vs. 77 % in treatment plot).

Hall Ranch Mine

At Hall Ranch Mine, LFMC in the treatment plot exceeded that of the control plot (non-overlapping 95% confidence intervals), for seven of the nine total sample timings, with the only exceptions being the earliest two sample timings of the season (Figure 8). The largest discrepancies between LFMC in the treatment and control plots occurred on 24-June (107 % in control plot vs. 154 % in treatment plot), 5-August (29 % in the control plot vs. 70 % in the treatment plot), and 19-August (45 % in control plot vs. 84 % in treatment plot).

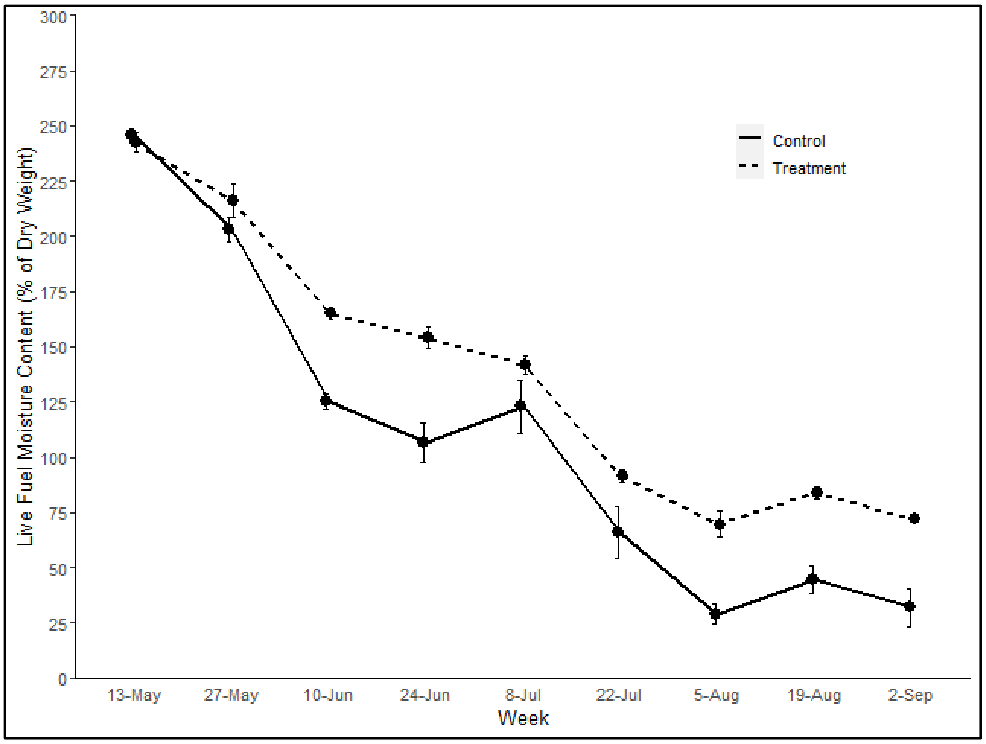

Figure 8. Bi-weekly mean live fuel moisture content in control (solid) and indaziflam treatment (dashed) plots at the Hall Ranch Mine study site (BCPOS). Error bars correspond to the 95% confidence interval (n = 4).

Highway 36A

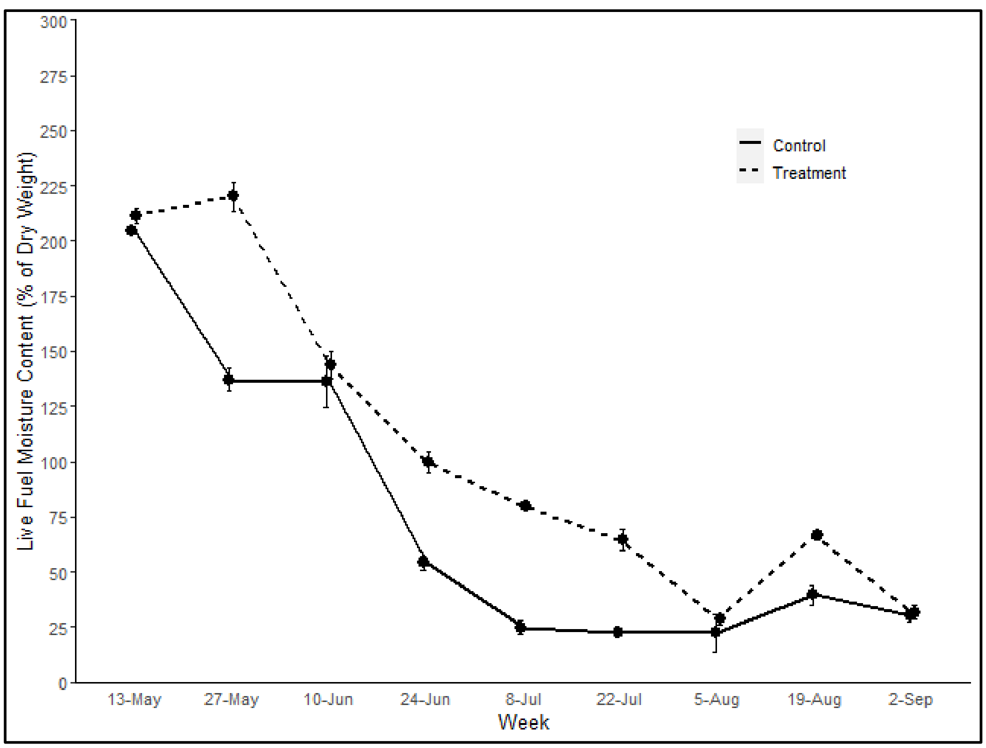

Figure 9. Bi-weekly mean live fuel moisture content in control (solid) and indaziflam treatment (dashed) plots at the Highway 36A study site (BCPOS). Error bars correspond to the 95% confidence interval (n = 4).

At Highway 36A, LFMC was highly variable, with LFMC in the treatment plot exceeding that of the control plot (non-overlapping 95% confidence intervals), for five of the nine total sample timings (Figure 9). The largest discrepancies between LFMC in the treatment and control plots occurred on 27-May (137 % in control plot vs. 220 % in treatment plot), and 8-July (25 % in control plot vs. 80 % in treatment plot).

Highway 36B

At Highway 36B, LFMC in the treatment plot exceeded that of the control plot (non-overlapping 95% confidence intervals), for seven of the eight total sample timings, with the only exception being 10-June (Figure 10). The largest discrepancies between LFMC in the treatment and control plots occurred on 24-June (62 % in control plot vs. 141 % in treatment plot), and 8-July (46 % in the control plot vs. 124 % in the treatment plot).

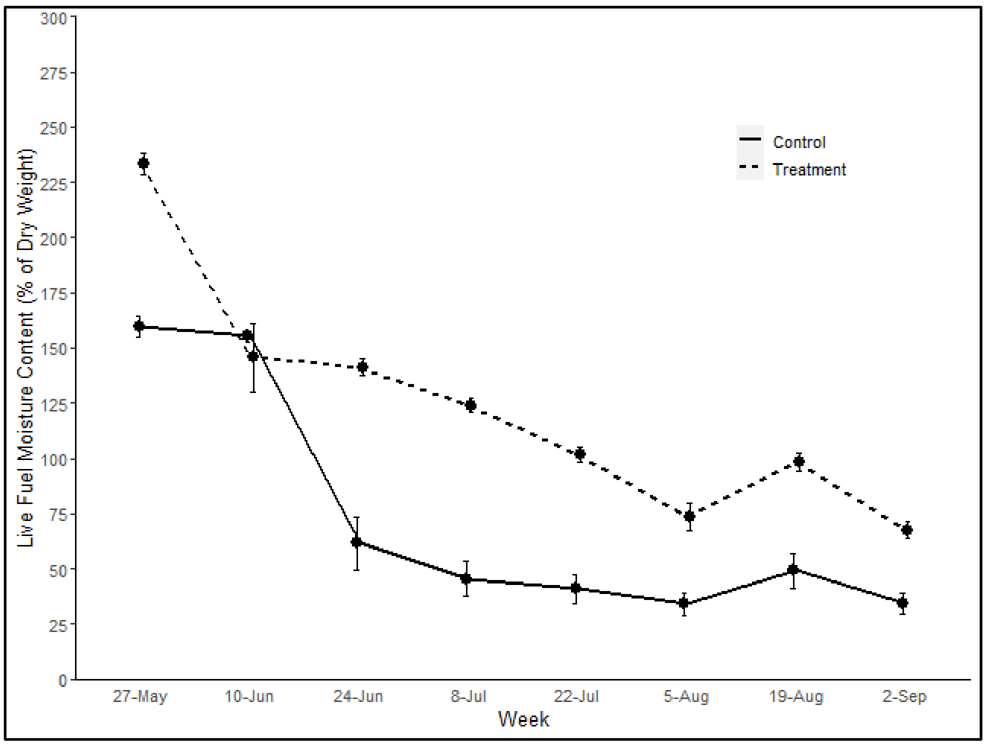

Figure 10. Bi-weekly mean live fuel moisture content in control (solid) and indaziflam treatment (dashed) plots at the Highway 36B study site (BCPOS). Error bars correspond to the 95% confidence interval (n = 4). Note that data were not collected at this site on 13-May.

Matthews-Winters Park

At Matthews-Winters Park, LFMC was highly variable, with LFMC in the treatment plot exceeding that of the control plot (non-overlapping 95% confidence intervals), for six of the nine total sample timings (Figure 11). The largest discrepancies between LFMC in the treatment and control plots occurred on 13-May, when LFMC was greater in the control plot (288 % in control plot vs. 183 % in treatment plot), and 24-June (77 % in control plot vs. 138 % in treatment plot).

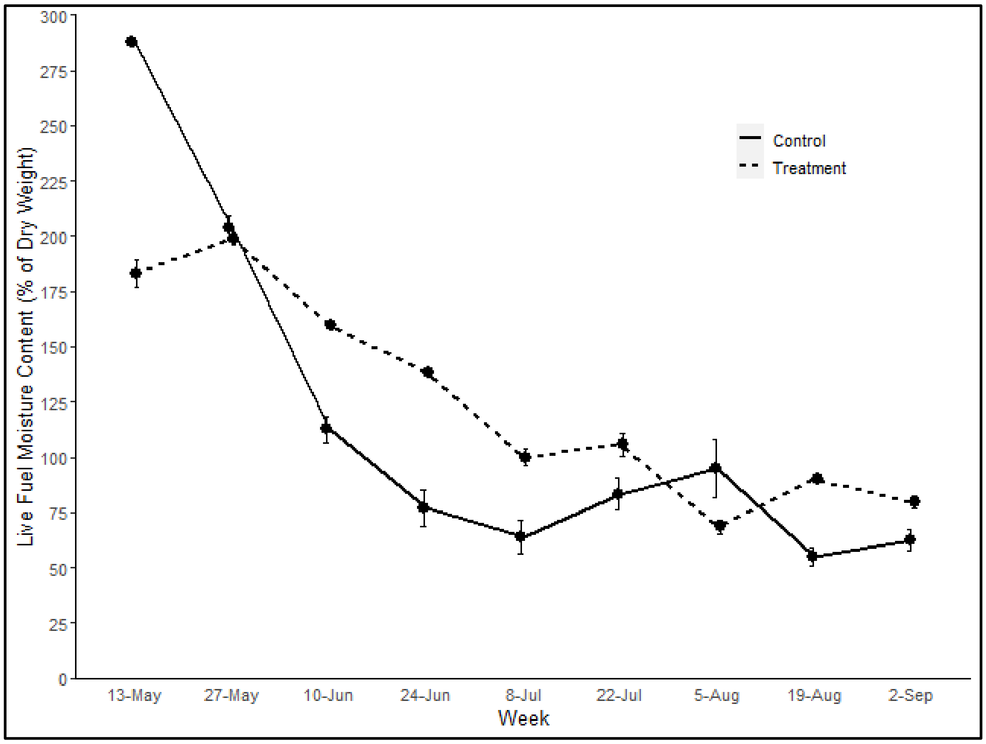

Figure 11. Bi-weekly mean live fuel moisture content in control (solid) and indaziflam treatment (dashed) plots at the Matthews-Winters Park study site (JCOS). Error bars correspond to the 95% confidence interval (n = 4).

Mt. Galbraith Park

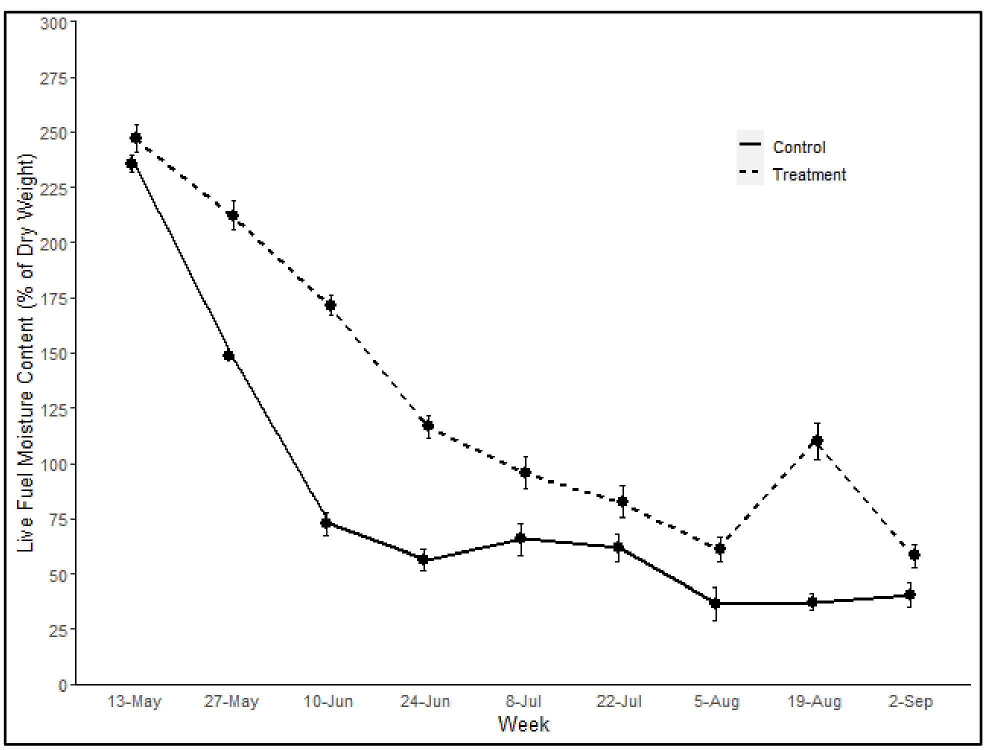

Figure 12. Bi-weekly mean live fuel moisture content in control (solid) and indaziflam treatment (dashed) plots at the Mt. Galbraith Park study site (JCOS). Error bars correspond to the 95% confidence interval (n = 4).

At Mt. Galbraith Park, LFMC in the treatment plot exceeded that of the control plot (non-overlapping 95% confidence intervals), for eight of the nine total sample timings, with the only exception being the earliest sample timing of the season (Figure 12). The largest discrepancies between LFMC in the treatment and control plots occurred on 10-June (73 % in control plot vs. 172 % in treatment plot), and 19-August (37 % in the control plot vs. 110 % in the treatment plot).

Standing Crop Comparisons

Standing crop was significantly increased in treatment plots at both the early-season ($P = 0.029$) and late-season ($P < 0.01$) sample timings (Figure 13). Overall, standing crop was slightly lower for the late-season timing compared to the early-season timing in both treatment and control plots (Figure 13), suggesting that vegetation may have been utilized by herbivores between sample timings (early-July to late-September).

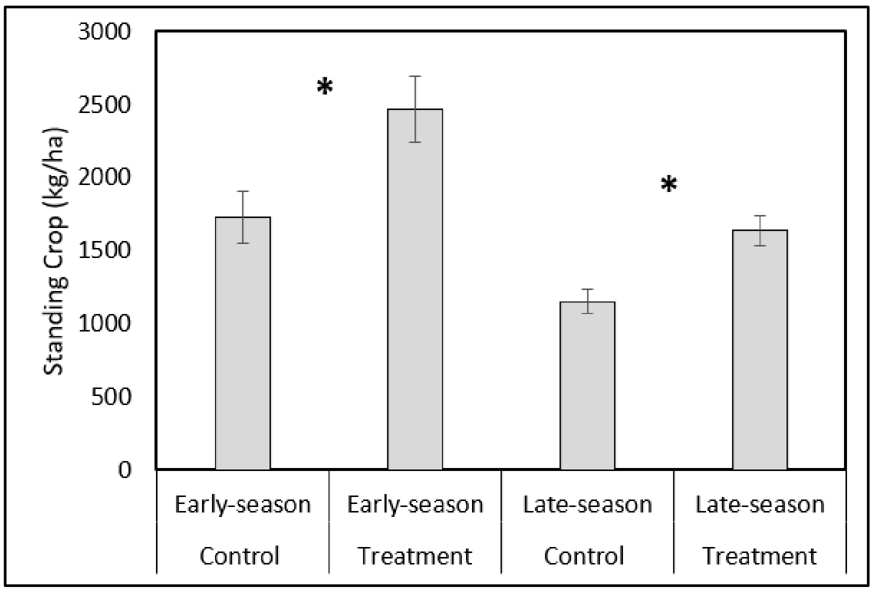

Figure 13. Mean total standing crop for treatment and control plots at all six Front Range grassland study sites for the early-season (early-July) and late-season (late-September/early-October) sample timings. Error bars correspond to ± 1 standard error. Asterisks indicate significant within-timing (early-season; late-season) treatment differences based on the results of a t-test ($P \leq 0.05$; n= 6).

Discussion

Our results indicate that indaziflam treatment increased LFMC at our study sites during the summer 2024 season, with significant increases in LFMC observed for six of the nine total bi-weekly sample timings (Figure 5). This supports our prediction that indaziflam treatment would increase LFMC for at least a portion of the season and suggests that effective management of invasive annual plants can reduce the risk of catastrophic wildfires. To put our results in context, several wildfire studies in chaparral ecosystems in California identified LFMC thresholds of 70-80 %, below which the likelihood of large wildfires increased substantially (Dennison et al. 2008; Dennison and Moritz 2009). In our study, mean LFMC in treatment plots fell below 80 % only twice during the season (5-August and 2-September), while LFMC in the control plots persisted below 80 % for all sample timings after 10-June (Figure 5). While Front Range grassland plant communities are different from chaparral ecosystems and the exact threshold may differ, indaziflam treatment substantially increased LFMC for most of the summer 2024 season, and increased LFMC is associated with reduced likelihood of ignition and wildfire size (Rothermel 1972; Davis and Michaelson 1995; Schoenberg et al. 2003; Chuvieco et al. 2009). It is important to note that extreme fire weather (hot, dry, windy conditions) is likely to blunt the effects of increased fuel moisture on fire behavior and real-world wildfire spread depends on conditions in a mosaic of plant communities with different fuel characteristics (Flannigan et al. 2024), but when conditions are less than extreme the increases in LFMC we observed in the treated communities we sampled are likely to reduce wildfire risk.

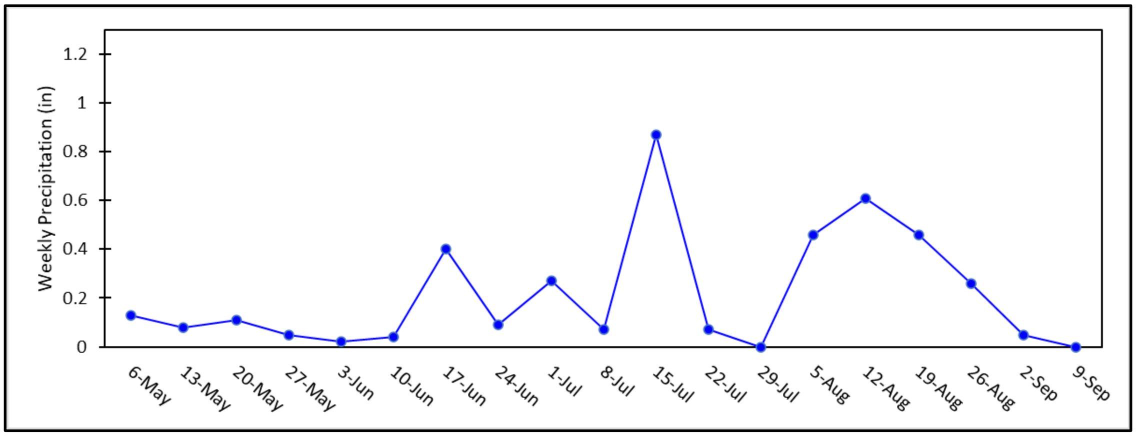

Figure 14. Weekly precipitation in Lyons (Station CO-BO-146; CoCoRaHS 2024). Total precipitation for the 2024 growing season (May-September) was 4.77 in compared to a 30-year average of 9.44 in (PRISM 2024).

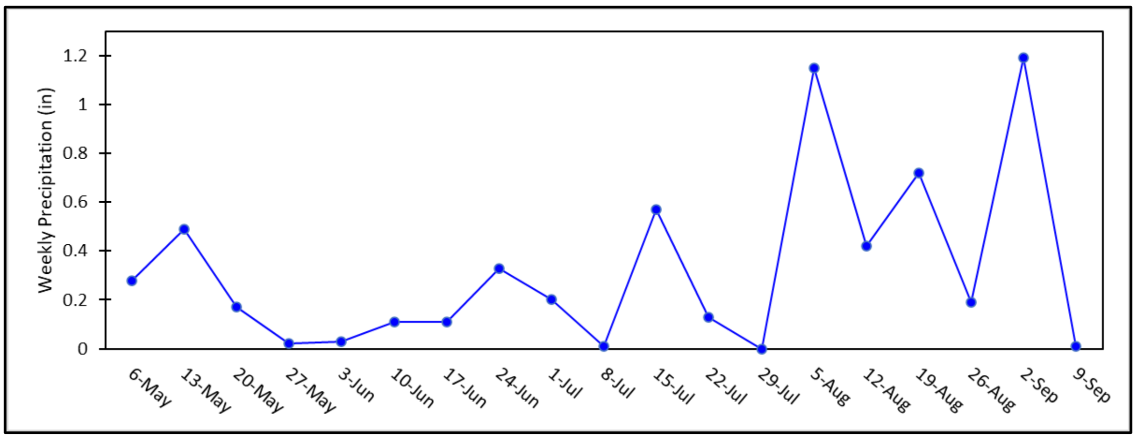

Figure 15. Weekly precipitation in Golden (Station CO-JF-147; CoCoRaHS 2024). Total precipitation for the 2024 growing season (May-September) was 7.03 in compared to a 30-year average of 9.92 in (PRISM 2024).

The patterns in LFMC we observed also may be related to precipitation timing during the summer 2025 season. Figure 14 and Figure 15 show weekly precipitation for Lyons, CO (Boulder County) and Golden, CO (Jefferson County), respectively (CoCoRaHS 2024). Note the sequence of two dry weeks in late-July (22-July and 29-July) at both locations (Figure 14; Figure 15). This dry period precedes a narrowing in the difference in LFMC between treatment and control plots observed at our study sites (except Hall Ranch Mine; Figure 8), and this is further reflected by the lack of a significant difference in mean LFMC between treatment and control plots for the 5-August sample timing (Figure 5). This likely reflects the drying out of perennial plants in response to dry conditions, as reduced antecedent precipitation and soil moisture is associated with reduced LFMC (Dennison and Moritz 2009; Sharma et al. 2021). However, after relatively wet weeks in early-August (Figure 14; Figure 15), LFMC increased in treatment plots while remaining relatively stable in control plots at several individual sites (Figure 7; Figure 9; Figure 10; Figure 12). The more pronounced response of LFMC to precipitation in treatment plots as opposed to control plots is also reflected in photos taken at each study site, and an example photo sequence showing disparate patterns of vegetation green-up is included in Appendix A. Our results suggests that invasive annuals have limited capacity to green-up in response to precipitation later in the summer, while herbaceous perennials can more effectively utilize water inputs later in the season and increase LFMC after precipitation occurs, reducing the period of time each fire season when vegetation is particularly susceptible to ignition and rapid wildfire spread. In addition, summer 2025 was relatively dry overall (May-September precipitation 51 % of 30-year mean in 2025 in Lyons, CO and 71 % of 30-year mean in Golden, CO; Figure 14; Figure 15; CoCoRaHS 2024; PRISM 2024), and the observed LFMC responses to precipitation suggests that increases in LFMC as a result of indaziflam treatment may be more pronounced in wetter years.

It is also important to note that we observed excellent invasive annual control in treatment plots at all sites except one, Mt. Galbraith Park, where patches of dense cheatgrass existed in the treated area (Figure 4). It is remarkable that we observed effective control across most of the different treatment timings and application methods implemented at our study sites (Table 2). This speaks to the versatility of indaziflam as a tool for managing invasive annuals, and this versatility is likely related to the long period of residual activity that is unique to indaziflam among other herbicides typically used for annual grass control (e.g., Plateau®). Our results further support the conclusion that indaziflam is an effective tool for annual weed control in rangelands and natural areas, a finding that has been replicated in many studies (Sebastian et al. 2016; Sebastian et al. 2017; Clark 2020; Courkamp et al. 2022). The one unique detail that sets apart the Mt. Galbraith Park study site is that indaziflam was applied with a boomless sprayer (Table 2), which may be related to the lack of complete control observed at this study site because even herbicide coverage is more difficult to achieve with boomless sprayers per the label for Rejuvra® (Envu 2024). Managers should use fixed boom sprayers whenever possible to promote even coverage, which is critical to maximizing indaziflam's effectiveness, and increase water volumes as much as possible when using boomless sprayers to avoid reduced herbicide performance (Envu 2024).

Our estimates of standing crop suggest that overall production was increased in treatment plots compared to controls for both the early- and late-season sample timings (Figure 13), indicating that indaziflam treatment increases wildfire fuel amount (standing crop) as well as LFMC. More research is necessary to better understand the combined effects of increased fuel amounts and LFMC, along with changes in the spatial distribution of fuel after indaziflam treatment, on wildfire behavior, and our results represent an important first step documenting some of these treatment outcomes. It is also interesting that standing crop was lower in both treatment and control plots for the late-season timing compared to the early-season timing (Figure 13). This suggests substantial utilization of these vegetation resources by wild herbivores between the early- and late-season sample timings, and more research understanding the effects of herbivory on LFMC and other key drivers of fire behavior in areas treated with indaziflam may help managers better understand the effects of treatment. In addition, future studies may wish to evaluate differences in the quality and quantity of forage for wildlife given that our study suggests utilization is occurring in treated areas.

Implications and Next Steps

Key findings from our study include:

(1) Indaziflam treatment exerted significant effects on LFMC in treatment plots, with treatment increasing LFMC for six of the nine total sample timings included in the study (Figure 5). The observed differences in LFMC suggest that relatively high levels of vegetation moisture content persist longer into the summer season after indaziflam treatment, reducing the likelihood of wildfire ignition and the potential rate of wildfire spread in treated areas.

(2) High LFMC was positively related to perennial grass and perennial forb cover, and negatively associated with invasive annual cover (Figure 6), suggesting that increased LFMC after treatment may result from increases in native perennial herbaceous plants in treated areas.

(3) Patterns in LFMC more closely aligned with precipitation in treatment plots compared to control plots at our study sites (Figure 5; Figures 7-12; Figure 14; Figure 15; Appendix A), suggesting that invasive annuals have limited capacity to green-up in response to precipitation later in the summer, contributing to relatively

low LFMC in areas infested with invasive annuals later in the season. Multi-year studies may help managers better understand year-to-year differences in the effects of indaziflam treatment on LFMC as summer 2024 was relatively dry (Figure 14; Figure 15), and differences between treatment and control plots may be greater in wetter years.

(4) Indaziflam treatment resulted in successful invasive annual control at all study sites except Mt. Galbraith Park, where invasive annuals were suppressed in the treatment plot, but not to the same degree as other study sites. The consistency of indaziflam treatment effects across various timings and application methods (Table 2) speaks to its versatility as a tool for managing invasive annuals. Mt. Galbraith was unique among our study sites because herbicide was applied with a boomless sprayer (Table 2), and this may have contributed to the lack of effective control observed at this site because it is more difficult to achieve even coverage with boomless sprayers and even coverage is critical to maximizing indaziflam's effectiveness.

(5) Standing crop was increased in treatment plots compared to control plots for both the early-season (early-July) and late-season (late-September/early-October) sample timings (Figure 13), suggesting that indaziflam treatment may increase fuel amount as well as LFMC. More research is necessary to better understand the combined effects of increased fuel amounts and LFMC, along with changes in the spatial distribution of fuel after indaziflam treatment, on wildfire behavior.

(6) Standing crop was lower overall in both treatment and control plots for the late-season sample timing compared to the early-season sample timing (Figure 13), suggesting that substantial utilization of vegetation by wild herbivores occurred between the early- and late-season sample timings (early-July to late-September). More research investigating the effects of herbivory on LFMC and other key drivers of fire behavior in areas treated with indaziflam, along with evaluating differences in the quality and quantity of available forage for wildlife, may help managers better understand the effects of management.

In addition, our results and experiences with this project suggest several topics that warrant further investigation. These include, but are not limited to:

(1) Our study focused exclusively on live fuels, and better accounting for dead fuels and litter while sampling could provide more detailed information on the potential effects of treatment on wildfire behavior.

(2) Anecdotal observations suggest that indaziflam treatment results in large increases in perennial forb abundance. In particular, we observed much more abundant slim-flower scurfpea (*P. tenuiflorum*) and white sage (*A. ludoviciana*) in treated areas. Future research may seek to better understand the mechanisms behind these apparent increases in perennial forbs (i.e., differences in new plant recruitment vs. release of existing plants over time), and their implications for wildfire risk and wildlife habitat quality.

(3) Invasive biennial and perennial forbs like common mullein (*V. thapsus*) and dalmatian toadflax (*L. dalmatica*) were common in many treatment plots and may have contributed to increased LFMC. We combined all perennial forbs (native and invasive) in our study, and future research should keep them separate to better understand the contribution of invasive perennial forbs to LFMC, and the implications of overall invasive plant control (i.e., not exclusively annuals) on wildfire risk.

Finally, this report represents a first step towards analyzing and presenting the results of this projects, but more analyses will be conducted in the future using additional data that is not presented in this report. For example, study plots were instrumented with temperature and humidity sensors that recorded data every 30 minutes for the duration of the summer 2024 season. Additional analyses will, at a minimum, investigate vegetation functional group-specific patterns in LFMC and the influence of relative humidity and temperature patterns on LFMC. All additional results will be shared with project partners in the future.

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Appendix A: Example photo sequence from the Rabbit Mountain study site. Photos from all study sites included as supplemental material.

Figure A1. Photo of boundary between control (left) and treatment (right) plots at the Rabbit Mountain study site on 28-May-2024.

Figure A2. Photo of boundary between control (left) and treatment (right) plots at the Rabbit Mountain study site on 13-June-2024.

Figure A3. Photo of boundary between control (left) and treatment (right) plots at the Rabbit Mountain study site on 29-June-2024.

Figure A4. Photo of boundary between control (left) and treatment (right) plots at the Rabbit Mountain study site on 11-July-2024.

Figure A5. Photo of boundary between control (left) and treatment (right) plots at the Rabbit Mountain study site on 29-July-2024.

Figure A6. Photo of boundary between control (left) and treatment (right) plots at the Rabbit Mountain study site on 5-August-2024.

Figure A7. Photo of boundary between control (left) and treatment (right) plots at the Rabbit Mountain study site on 19-August-2024. Note the fall green-up in treatment plot compared to Figure A6.

Figure A8. Photo of boundary between control (left) and treatment (right) plots at the Rabbit Mountain study site on 9-September-2024.