

Indaziflam Experimental Use Analysis

Executive Summary

The City of Boulder created a multi-department experimental use process in 2022 to evaluate the efficacy and non-target impacts of the herbicide indaziflam (trade name “Rejuvra™”) for use in controlling tall oatgrass. The city contracted with multiple researchers at both Colorado State University (CSU) and the University of Colorado (CU) to assess both target and non-target impacts of indaziflam at two research sites. Studies took place over four years, with data gathered one to three years following a single indaziflam application. The overall findings were that indaziflam did not show significant efficacy in the intended mode of action (seed germination suppression) in controlling tall oatgrass. Conversely, significant non-target impacts were observed across multiple studies related to suppression of native seed germination. The chemical was also found to have longer persistence than indicated on the label and could have significant impacts on restoration and revegetation during this more extended timeframe. Based on these findings the research committee unanimously agreed that indaziflam should not be recommended for use in tall oatgrass control on City properties. Further consideration of the use of indaziflam would require additional research—ideally conducted collaboratively across multiple landscapes and jurisdictions — to further evaluate the issues identified in this study and formulate mitigation strategies to manage unintended and non-target impacts.

Section I: Background — Departmental Interest in Indaziflam

In 2021–2022, the City of Boulder Open Space and Mountain Parks (OSMP) Department initiated the experimental use of an herbicide with the active ingredient indaziflam (product label = Rejuvra™ developed by Bayer CropScience, now licensed by Envu) in response to growing challenges in managing tall oatgrass (*Arrhenatherum elatius* subsp. *elatius*), an invasive perennial grass species on OSMP lands. Indaziflam had failed an initial rapid assessment review to be an approved pesticide for use on City of Boulder properties. Since no process existed for considering experimental use of pesticides not on the city’s Approved Pesticide List (available in Supporting Documentation), OSMP and the Climate Initiatives (CI) Department developed a new experimental pesticide use process to allow the study to proceed with proper review and oversight. This project was the first use of this Experimental Use procedure. As part of the development of the Experimental Use process, a series of other issues of concern regarding indaziflam were identified and additional research was co-designed and contracted with other researchers to fill these information gaps. This broader set of research investigations and associated principal investigators are outlined in the next section and Table 1.

1.1 The invasive weed challenge: tall oatgrass and gaps in approved management methods

Tall oatgrass threatens biodiversity (D. L. Clark & Wilson, 1997), ecosystem functioning (D. L. Clark & Wilson, 1997; Maret & Wilson, 2005), and like many introduced grasses, can increase fine fuels and fire risk (Costanzo & Munson, 2026). Existing management on OSMP properties relies heavily on prescriptive livestock grazing, but staff were unable to sufficiently reduce or contain tall oatgrass using grazing at all sites where tall oatgrass was spreading. These limitations created a management gap and underscored the need to evaluate additional tools that could complement or improve upon current management strategies.

1.2 Rationale for indaziflam as a candidate: why this herbicide was identified; its approval status

Indaziflam emerged as a candidate herbicide based on findings from multiple studies indicating its effectiveness in reducing invasive annual grasses and in some situations increasing native species cover (Alba et al., 2024; Bradbury et al., 2024; S. L. Clark et al., 2019; J. Sebastian et al., 2025). OSMF was interested in determining whether those benefits might extend to the short-lived perennial, tall oatgrass, dependent on seed for reproduction (D. L. Clark & Wilson, 1997; Pfitzenmeyer, 1962).

1.3 Indaziflam background information

Indaziflam (currently marketed as Rejuvra™ by Envu) is a non-selective pre-emergent herbicide that was approved in 2020 for use on rangelands and natural areas to control invasive plant species (*Rejuvra Pesticide Registration*, 2020). Indaziflam is an herbicide that inhibits cellulose–biosynthesis; it works by limiting the production of cellulose, an essential structural component of plant cell walls (Brabham et al., 2014; *Rejuvra Pesticide Registration*, 2020; D. J. Sebastian et al., 2017). Indaziflam is designed to bind to soil organic matter particles and is intended to have little mobility in the soil profile, staying near the soil surface after application (Courkamp et al., 2022; Jeffries & Gannon, 2016). Under many conditions, due to its reduced mobility in the soil, indaziflam selectively impacts plants forming roots from seed at or near the soil surface, while established species with deeper roots are typically unaffected (S. L. Clark et al., 2019; Courkamp et al., 2022).

Section II: Development of the Experimental Use Process

2.1 Regulatory and procedural context

The City of Boulder uses an Integrated Pest Management (IPM) approach to address pest issues including invasive plant species. IPM uses an ecosystem management framework that includes a combination of approaches including cultural, mechanical, biological and when these alternative methods are not sufficient, chemical control including herbicides. Boulder’s pesticide decision-making framework is built around a precautionary, ecosystem-focused process where only pre-screened and approved pesticides on the City’s Approved Pesticide List (available in supporting documentation) can be applied to city-owned land. When a pesticide is requested by staff, they must justify its necessity, describe the failure or infeasibility of alternatives, and describe how exposure to people, wildlife, and sensitive habitats will be minimized.

Any pesticide under consideration goes through a two-stage hazard evaluation. An initial rapid screening process is designed to identify pesticides with issues such as high toxicity, persistence, bioaccumulation, eco-toxicity or evidence of potential adverse human health effects. Products that pass the initial screen then undergo a full hazard assessment to address any potential issues to determine if they can be mitigated. This assessment process reviews regulatory data, independent scientific literature, environmental fate, toxicity to non-target organisms, and potential ecosystem-level impacts. Based on this analysis, each pesticide approved for use is placed into a hazard tier—Allowed, Conditional, or Special Use—and any proposed use must comply with the restrictions of that tier (available in Supporting Documentation). The full process is described in the City’s IPM Operations Manual.

The City's pesticide assessment process was developed because pesticide regulatory systems, including federal pesticide registration through the EPA, frequently do not fully account for ecosystem-level effects, cumulative stressors, or long-term population impacts on wildlife or other species. The City of Boulder therefore applies a more conservative standard that prioritizes human health, ecological health, biodiversity, soil health, water quality, and long-term ecological resilience.

2.2 Design and approval of the experimental use framework

When city staff requested that the indaziflam herbicide product, Rejuvra™, be approved in 2021, it did not pass rapid screening. However, due to continued interest in indaziflam, an OSMP Funded Research Program proposal was approved by OSMP staff for a project that involved applying indaziflam on city land, a potential violation of the city's IPM policies because indaziflam was not on the City's Approved Pesticide List. This situation identified a gap in the pesticide approval process since the city had no provision for experimental pesticide use or research.

After consultation between Climate Initiatives (CI) and OSMP staff, it was determined that it was in the best interest of the city to move forward with a study if an experimental use process was developed and approved by the city manager. On this basis, the initial study was revised to meet the requirements of this new process.

2.3.1 Experimental Use Process

The experimental use process developed by staff and approved by the City Manager calls out four conditions that trigger allowing for the limited use of a pesticide through this process:

The pesticide fails the city's Approved Pesticide List process criteria;

- The city's pesticide risk assessment indicates too high a risk to be used on city properties;
- A pest issue is not being adequately managed with non-chemical practices or pesticides from lower hazard categories, **and** the pest poses a threat to ecosystem integrity, human health or another issue that cannot be addressed otherwise with existing resources. Further, staff experts, leadership and external experts agree that current management practices are not adequate, and the risk/benefit of the pest problem suggests exploring other measures that carry risk; and
- The scope of the known impacts of the pesticide is incomplete, and the city can conduct and/or fund researchers to study and provide more data to assist in determining if consideration for any use of the pesticide could be appropriate on city property in the future. This research may also help to answer questions about efficacy that are currently unknown and/or non-target impacts to organisms and ecosystems that can assist in estimating risk/benefit.

The process includes the following steps:

1. A more extensive risk assessment of the pesticide is conducted.
2. The IPM Coordinator and the staff team determine if a study is appropriate, which includes providing a scientific and ecological rationale that poses questions from the risk assessment that the study will address.
3. The IPM Coordinator drafts a recommendation of whether to proceed with the study to the directors from the requesting department. If the study is approved, once it's completed, the staff team reviews the results with three possible outcomes:

- The product can be approved with an appropriate management plan for the target species assessed;
- If more information is needed to make a decision, studies may be continued or new studies proposed; or
- The product will not be approved for use.

As part of the development of this experimental use review process, a multi-department team was created with representatives from both OSMP and the Climate Initiatives (CI) Department. The CI Department is responsible for the ongoing management of the City's Integrated Pest Management (IPM) policy and associated procedures.

2.3.2 Experimental Use Process for Indaziflam

Indaziflam's initial risk assessment flagged issues and questions that were used to guide the revision of the initial research proposal, which are summarized in Table 1. Initially, Dr. Caroline (Carrie) Havrilla from the Dryland Ecology and Management Lab at CSU was contracted by OSMP to conduct a study to monitor plant communities, the soil microbiome, and a seedbank study over the three subsequent years following indaziflam application. Through the process of developing the experimental use authorization, a series of other concerns were identified beyond the initial research scope including indaziflam persistence in soils and species-specific responses to indaziflam treatment. To address these concerns, additional researchers were engaged to develop and implement analysis designed to assess each of these considerations. The research focus and responsible research teams are described in Table 1.

Table 1. Summary of the identified concern, primary research focus and responsible research team and methodological approach.

Concern	Research Focus	Research Team	Research Approach
Efficacy and non-target effects on the plant community	Vegetation responses	Dr. Carrie Havrilla and Emily Swartz (Colorado State University)	Monitor aboveground vegetation community in treated and untreated plots repeatedly over time
Non-target soil microbiome effects	Soil microbiome responses	Dr. Carrie Havrilla and Emily Swartz (Colorado State University)	Sequence soil microbial samples using 6S (bacterial/archaeal) and ITS (fungal) rRNA methods over time.
Germination effects for target and non-target species	Natural seedbank dynamics	Dr. Carrie Havrilla and Emily Swartz (Colorado State University)	Evaluate soil seedbanks using field-collected soils in a greenhouse seed bank bioassay.
	Seed germination and growth dynamics: bioassay of tolerant species	Dr. Mike Thurman, Dr. Imma Ferrer and Dr. Tim Seastedt (University of Colorado)	Evaluate germination and growth responses of a tolerant species using a bioassay.
	Species specific germination responses to variable indaziflam rates	Dr. Cynthia Brown and Lauren Myers (Colorado State University)	Evaluate germination and growth responses of numerous species to varying rates of indaziflam using a greenhouse dose-response study.
Persistence, mobility and bioactivity	Indaziflam persistence at two soil depths	Dr. Cynthia Brown, Dr. Franck Dayan and Lauren Myers (Colorado State University)	Analyze indaziflam concentrations at two soil depths using liquid chromatography mass spectrometry (LCMS).
	Persistence and chemical breakdown products dynamics	Dr. Mike Thurman, Dr. Imma Ferrer (University of Colorado)	Analyze indaziflam concentrations and its degradates in soil over time using Liquid Chromatography - Quadrupole Time-of-Flight Mass Spectrometry.
Toxicity to invertebrates or other animals		The City was not able to contract a scientist to test the impacts of indaziflam on invertebrates or other animals.	NA

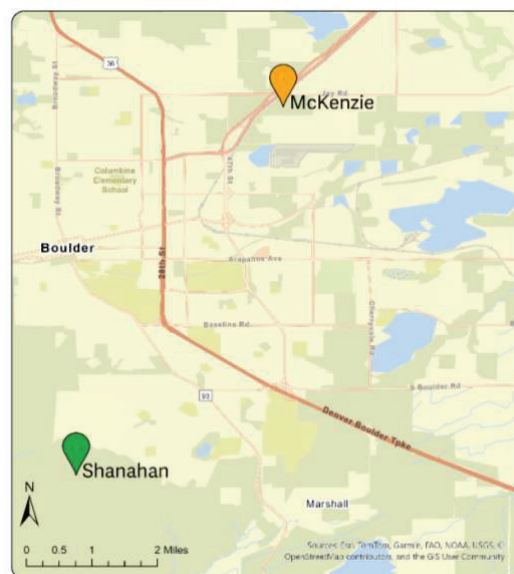
Section III: Research Program Description

3.1 Study sites

Two study sites (McKenzie, Shanahan Ridge; Map 1) on City of Boulder Open Space and Mountain Parks properties were chosen in consultation with former Vegetation Management Senior Coordinator, Kelly Uhing. Sites were chosen to have high density of the target species, tall oatgrass, and be away from areas of high trail and visitor activity. Only the Shanahan site is included in this report due to protocol deviations at the McKenzie site. This included the application of non-study herbicides prior to the start of the study, and an application rate of indaziflam during the study (7oz/acre, 102 g active ingredient/hectare) that was higher than the label recommended rate (5oz/acre, 72 g active ingredient/hectare) for post-agricultural areas with a history of soil disturbance (i.e. tillage)—a characteristic of the McKenzie site. The potential for injury to established plants on sites that have a history of soil disturbance prior to application of indaziflam is noted on the label (*Rejuvra Pesticide Registration*, 2020).

The vegetation response at the McKenzie site was near total aboveground mortality shortly following indaziflam application on two separate applications. This was not anticipated given the promotion of the product having a pre-emergent mechanism of control that asserts it primarily suppresses plants forming from seed. Results for the McKenzie site are included in Appendix 1 but should be interpreted with recognition of this unanticipated mode of action.

The Shanahan site is located on the urban periphery bordering both suburban neighborhoods and forested foothills. Tall oatgrass is common at the site but there is a diverse community of native and introduced grasses and forbs. While the area where study plots were established was historically grazed by cattle prior to 1970, no grazing of domestic livestock has occurred in the study plots since. However, the surrounding areas have been grazed annually since 2015 as part of an integrated approach to tall oatgrass control. The area that encompasses the study plots was burned during the NCAR fire in March 2022. Soil physical characteristics of control and treatment plots based on replicate samples collected in 2025 are provided in Table 2.



Map 1. Indaziflam Study Sites on OSMP managed lands.

Table 2. Soil physical characteristics of the Shanahan site. Physical soil samples were collected in July 2025 at a depth of 10cm and were analyzed by the Soil, Water and Plant Testing Lab at Colorado State University.

Indaziflam Application	Control	Treatment
Soil pH	5.5	5.5
Soil Organic Matter (%)	13.68%	11.25%
Sand (%)	54%	51%
Silt (%)	21%	22%
Clay (%)	26%	27%
Soil Class	Sandy Clay Loam	Sandy Clay Loam

3.2 Research components and methodology

At each site, ten 4m x 4m permanent plots were established in Summer 2022 and half the plots per site (n=5) were randomly assigned to be treated with the herbicide Rejuvra™ (active ingredient = indaziflam) or left untreated as controls (Appendix 2, Maps A2.1 and A2.2). Rejuvra™ was broadcast sprayed at 7 oz/acre (102 g active ingredient/hectare) on August 1, 2022, the recommended rate sites without soil disturbance history (*Rejuvra Pesticide Registration*, 2020). Study plots were revisited once annually in July to monitor the plant community in 2023, 2024, and 2025, and to collect soils for seedbank assays (March 2025), indaziflam persistence measurements (2023, 2024, 2025), soil physical characteristics (2025), and microbial analysis (2023, 2024, 2025). Specific research methodology (data collection and analyses) for each study is summarized in the methods sections of Section IV.

Section IV: Research Findings

Section IV summarizes the findings for six areas of consideration:

- **Efficacy and non-target effects on the plant community**—The effectiveness of indaziflam in controlling the target species (tall oatgrass) and impacts of indaziflam on non-target plant species.
- **Germination effects for target and non-target species**—Trials to assess germination inhibition effects from the natural seed bank.
- **Non-target seed effects**—Effects of indaziflam on native plant seed emergence in both treated field-sampled soils and in commercially sourced soils.
- **Persistence** —The period of biologically relevant levels of indaziflam post-treatment.
- **Non-target soil microbiome effects**—Effects of indaziflam application on soil microbiome dynamics.
- **Other environmental health considerations**—Review of the literature to assess other potential environmental health effects resulting from indaziflam use.

The determination at the end of each section reflects the City of Boulder’s precautionary approach to pesticide use. The concern level represents a risk-based judgment, where risk is defined as the potential for ecological or human health impacts, particularly longer-term effects that cannot be adequately assessed or managed with current information and capabilities.

4.1 Efficacy: Control of Tall Oatgrass and Non-Target Impacts on Native and Introduced Plant Cover

4.1.1 Overview

The efficacy portion of this assessment is based on work funded by the OSMP Funded Research Program and conducted by Dr. Carrie Havrilla and Emily Swartz from the Dryland Ecology and Management Lab at CSU (Swartz, 2026).

As a broad spectrum pre-emergent herbicide, indaziflam was hypothesized to have impact on the aboveground plant community by limiting the recruitment of new individuals from seed and impacting both tall oatgrass and non-target native and introduced species that propagate primarily through seed.

4.1.2 Methods

During annual monitoring each year in July, species level vegetation data were collected for all sampling plots using a quadrat point intercept method measuring four canopy layers at 100 points in a 1m² subplot. Final analysis included comparisons of tall oatgrass cover, native plant cover, and introduced plant cover.

4.1.3 Results

As a result of indaziflam treatment no significant difference in tall oatgrass cover was detected in any of the three monitoring years between control and treated plots (Figure 1). No evidence of damage to established plants, including tall oatgrass, was observed in late summer 2022 or at any point following indaziflam treatment. While recruitment of new individuals from seed was likely suppressed (see seedbank assay in section 4.2), previously established tall oatgrass likely persisted for the duration of the study period.

The impacts of indaziflam on the broader suite of non-native species were mixed. In general, non-native species cover trended down over time in plots treated with indaziflam. The first year following indaziflam treatment there was no significant difference in introduced species cover in treatment versus control plots. However, in years two and three there was significantly less introduced species canopy cover in indaziflam treated plots (Figure 1).

Further, no significant difference in native species cover between treated and control plots was detected during the study period (Figure 1). The expected increase in native species cover that was hypothesized to occur was not observed likely because of limited decreases in tall oatgrass and introduced species so competition from these non-native species remained relatively consistent throughout the study period.

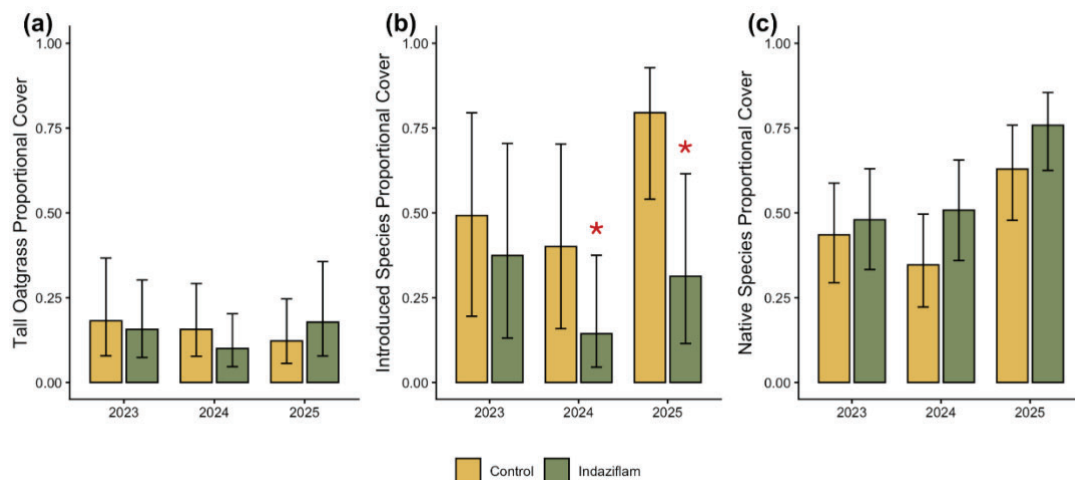


Figure 1. Bars show mean predicted canopy cover proportion of (a) tall oatgrass, (b) introduced species (including tall oatgrass) and (c) native species. Error bars show the 95% confidence interval. Asterisks (*) indicate significant differences ($p < 0.05$) between mean predicted values within site and year using a mixed effects beta regression model.

4.1.4 Issues to be Considered

Results should be interpreted with awareness of difficulty of evenly spraying small plots, potential edge effects, and limited time since treatment — particularly given tall oatgrass is a relatively short-lived perennial, and differences may develop as existing individuals begin to die off. Continued monitoring is recommended.

4.1.5 Key Results

There was no difference in tall oatgrass cover during the study indicating that indaziflam alone, without controlling established tall oatgrass through additional management actions, did not effectively control tall oatgrass, a perennial species where established individuals come back year after year. Further, indaziflam treatment did not lead to significant increases in native species cover where a robust native community was established prior to treatment likely because of limited changes in competitive dynamics.

4.1.6 Determination

Medium Concern

Indaziflam had limited effects on the aboveground plant community, no significant differences in tall oatgrass or native species cover were detected at any point during the three-year study demonstrating low efficacy in reducing the target species and low non-target impacts on established vegetation. Introduced species cover did decline significantly in years two and three.

4.2 Non-Target Effects: Analysis of impacts of indaziflam on germination from the natural seedbank

4.2.1 Overview

In addition to annual monitoring of the plant community, Swartz and Havrilla (2026) conducted a greenhouse study to assess levels of germination and emergence from the soil seedbank of indaziflam-treated field soils and comparable untreated control soils. While the species

composition in the soil seedbank often differs dramatically from the composition of above-ground plant communities, the persistent seeds in the soil seed bank serve as a source of community diversity and persistence for many species, particularly after disturbances and during active restoration of sites formerly invaded by non-natives.

4.2.2 Methods

On March 25, 2025, soil samples were collected during the dormant winter season from each of the treated and control plots, roughly two and a half years after indaziflam treatment. Four 500 cm³ soil samples were collected in a 10cm-by-10cm area at a depth of 5 cm in each plot. These four subsamples were then homogenized and stored in paper bags at ambient indoor conditions. The seedbank assay began on June 11, 2025, at the Colorado State University Plant Growth Facility in Fort Collins, CO, USA. Mesocosms were set up to grow out each homogenized plot sample, 13.25-liter plastic tubs were filled with 13 cm of field soil purchased locally. The field soil growing medium was autoclaved in small batches using a gravity cycle at 121 C (at 15 psig) for 60 minutes to eliminate any viable seeds (Steris, AMSCO 630LS, Model LS-136H). The seedbank soil samples collected from each plot were screened of any large vegetative material and spread in an even layer across the growing medium. Each mesocosm was watered every four days to field capacity. The top two inches of the mesocosms were typically dry prior to the next watering; however, this varied slightly with ambient greenhouse conditions. Monitoring occurred once a week after the first plants could be confidently identified. Any plants that could be identified were carefully removed by clipping the biomass off below the soil surface; these locations were marked and any resprouts were removed without recounting. The experiment ran for 15 weeks.

4.2.3 Results

Total seedling emergence, including both native and introduced species, from indaziflam-treated field soils was significantly reduced compared to untreated controls (Figure 2).

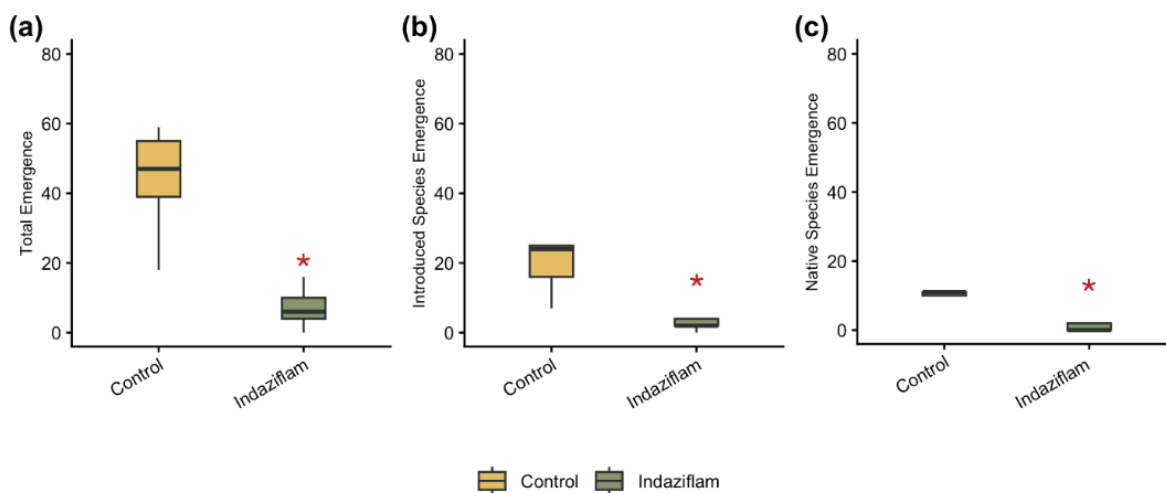


Figure 2. Box and whisker plots showing observed (a) total, (b) introduced, and (c) total emergence from seed bank assay mesocosms. Asterisks (*) indicate significant differences ($p < 0.05$) in predicted emergence between mesocosms sampled from control and treated plots in March 2025. Each response variable was modeled using a mixed effects negative binomial distribution.

4.2.4 Issues to be Considered

Results should be interpreted with awareness of difficulty of evenly spraying small plots, potential edge effects, and limited time since treatment. Greenhouse studies are not necessarily reflective of what happens in the field, because environmental conditions for germination and emergence are optimized. Further, this study looked only at species recruitment from seed and further work is needed to assess potential impacts to recruitment using other reproductive strategies such as by rhizomes or stolons.

4.2.5 Key Findings

Indaziflam had biologically relevant persistence that inhibited native seed germination from the soil seedbank roughly two and a half years after application, as demonstrated by significant reduction in both native and non-native species emergence in treated as compared to untreated soils.

4.2.6 Determination

High Concern

Native seedling emergence from the seedbank significantly declined from ~10 to ~1 when indaziflam-treated and untreated field soils were grown out in the greenhouse. Caution is advised when applying indaziflam in sites where recruitment from the natural seed bank or seed-based restoration is needed since all seed germination is suppressed while indaziflam remains at biologically relevant levels in the soil, at this study site at least two and a half years post treatment.

4.3 Non-Target Effects: Germination Bioassay using Field Collected Treated Soils

4.3.1 Overview

This bioassay assessed the germination and growth of a tolerant native grass species on field collected soil that had previously been treated with indaziflam. The study was funded by the Climate Initiatives department and conducted by Dr. Mike Thurman and Dr. Imma Ferrer from the CU Center for Environmental Mass Spectrometry and Dr. Tim Seastedt, Professor Emeritus from CU's Ecology and Evolutionary Biology Department. The impacts to a selected native species, western wheatgrass (*Pascopyrum smithii*), known to be tolerant to indaziflam (Mealor & Crose, 2024), were assessed from soils taken from the field plots that had been treated with indaziflam three years earlier. It was hypothesized that germination and emergence from western wheat grass seeds would not be suppressed by indaziflam that persisted in field soils three years after a one-time application.

4.3.2 Methods

The remaining soil from the 2025 soil indaziflam soil persistence analysis study (Section 4.5) was removed from the freezer, air dried and 20 grams from each field plot was placed in small plastic containers – 10 x 2 cm. The soil was re-wetted to field capacity and 5 western wheatgrass seeds added to each container. They were stored in darkness for 4 days and then placed next to a lab window with natural sunlight and regularly watered. Sprouting was observed and recorded for 51 days. At the end of the study, the grass was harvested, dried and weighed.

Once germination observations were completed, dried seedlings were ground up, extracted with a mix of methanol/water (80:20) and analyzed by LC/QTOF-MS for indaziflam and its degradates (breakdown products). Due to the small amount of plant tissue, data from multiple seedlings were combined by treatment group for analyses.

4.3.3 Results

Western wheatgrass is tolerant to indaziflam, and germination occurred in both treated and untreated soil as would be expected, with no major difference in germination rates between treatments. However, biomass of the resulting plants was reduced in treated soils compared to control soils by 17% (Table 3).

After the plants were dried and weighed to measure biomass, the plant tissue was analyzed for indaziflam, which was detected in the seedlings grown on the soil from indaziflam-treated Shanahan plots (Table 3).

Table 3. Effects on a tolerant native grass species grown on indaziflam-treated field soil. The germination rate, biomass and the amount of indaziflam in the tissue of western wheatgrass was measured in seedlings grown on soil collected from Shanahan field plots that were treated once with indaziflam in 2022. Due to the variable and small number of replicates and small quantity of plant tissue, biomass was combined from all replicates then divided by the number of pots rather weighing each replicate separately.

	Control	Indaziflam Treated
Germination (average)	84%	94%
Biomass (average)	22 mg/pot	18.2 mg/pot
Indaziflam concentration in soil (mean value)	0 ng/g	14 ng/g
Indaziflam concentration in plant tissue	NA*	2 ng/g

*There was not enough tissue to quantify indaziflam from the control plants.

4.3.4 Issues to be Considered

There was some emergence from the natural seedbank in addition to the seeded grasses, though these plants were not identified. The small quantity of field soil limited the scope of this germination bioassay and additional replication would be beneficial. In the future, more could be learned by testing both susceptible and tolerant species one, two, three or more years after indaziflam application.

4.3.5 Key Findings

Although tolerant plants can germinate, the vigor and biomass can be reduced as shown in western wheatgrass. This indicates that plants may be affected by indaziflam even when emergence is not suppressed. Indaziflam, a systemic herbicide, is also detected in plant tissue from tolerant plants that germinate in soil collected 3 years after a single application.

4.3.6 Determination

High Concern

Indaziflam was found to be present at biologically relevant levels in soil three years post-treatment. Additional findings include: 1) Indaziflam was found in the tissue of a small sample of grass germinated on field-treated soil; 2) The biomass of a tolerant species was lower than controls when grown on indaziflam field-treated soil.

4.4 Non-Target Effects: Species-specific emergence responses to variation in indaziflam concentrations

4.4.1 Overview

To better understand species specific responses to indaziflam applied at variable application rates and soil conditions, Dr. Cynthia Brown, Dr. Franck Dayan and Lauren Myers (CSU) conducted a dose-response greenhouse study to assess levels of emergence of 11 native species and three introduced species of concern from planted seed in response to increasing rates of indaziflam applications in two soil types (sand mix, topsoil) (Myers, 2025). Rates applied in the greenhouse reflected actual rates typically used in the field as well as lower rates, to explore if relationships between emergence decreased with increasing indaziflam concentrations or if relationships indicated thresholds at which inhibitory effects on seed emergence were not observed.

4.4.2 Methods

Agency staff supplied locally sourced seed, choosing native species typically selected for seed-based restoration, with an emphasis on native annual forbs, and key non-native species of concern. One or more seeds per species, depending on percentages of pure live seed content (PLS) per species, were planted in each planting cell in large trays. Species were randomly assigned to a row of cells in a tray, and trays were sprayed using a research grade track sprayer with a different concentration of Rejuvra including a zero-concentration as an untreated control on two soil types (screened and pasteurized grassland topsoil sourced from A1 organics, or a 50% volume to volume sand/topsoil mix). Each combination of species by soil type by herbicide concentration was replicated three times. Seedling emergence was recorded weekly for 45 days, after which greenhouse soils were analyzed for indaziflam concentration. Emergence for each species was compared across application rate of indaziflam, soil type and the interaction between these factors.

4.4.3 Results

Indaziflam lowered emergence of the native perennial grasses blue grama (*Bouteloua gracilis*) and bottlebrush squirreltail (*Elymus elymoides*). Native annual forbs meadow flax (*Linum pratense*), tansyleaf tansyaster (*Machaeranthera tanacetifolia*), viscid tansyaster (*Rayjacksonia annua*) and native perennial forb upright prairie coneflower (*Ratibidia columnifera*) exhibited lower emergence in response to indaziflam. Native perennial forbs fringed sage (*Artemisia frigida*) and subalpine gumweed (*Grindelia subalpina*) showed no significant reduction in emergence with increasing indaziflam treatments. Decline in emergence of native seeded species in response to indaziflam depended on soil type only for bottlebrush squirreltail and tansyleaf tansyaster (Figure 3). Emergence of non-native target species that indaziflam is used to control including tall oatgrass (*Arrhenatherum elatius*), feral rye (*Secale cereale*), and cheatgrass (*Bromus tectorum*) were all reduced by increasing indaziflam application rates and depended on soil type for cheatgrass and feral rye. Indaziflam concentrations in soils at the end of the study increased with application rate with higher rates detected in topsoil than the sand mix.

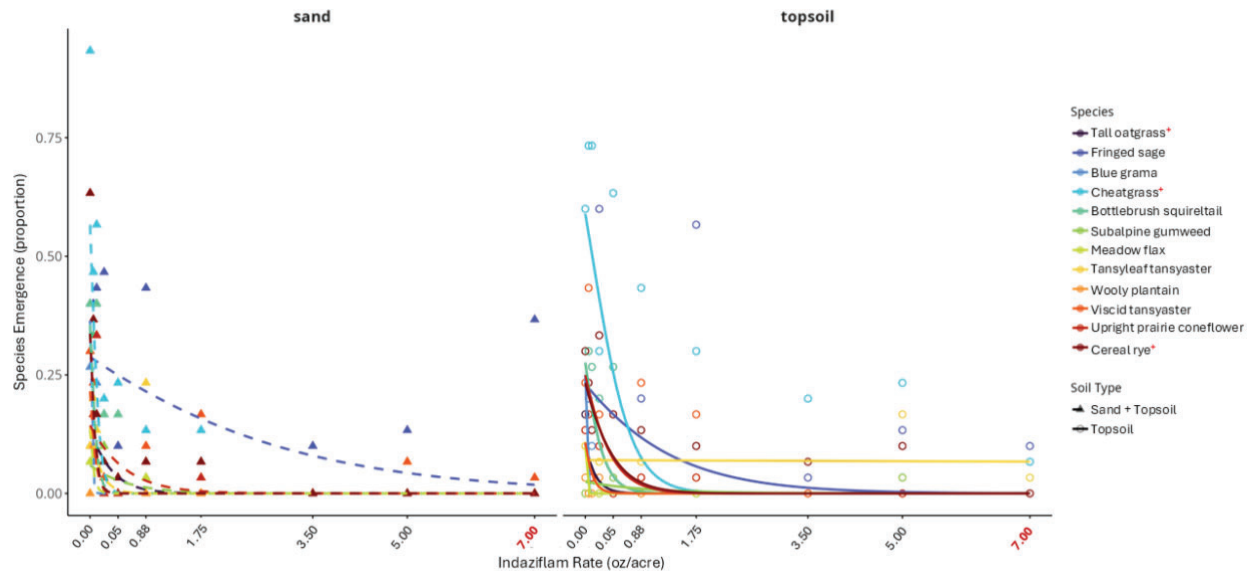


Figure 3. Relationship between emergence of the study species as a function of indaziflam rate (oz/acre) for the greenhouse study using a binomial generalized linear model separated by soil type. Emergence is the ratio emerged seedlings to planted seeds. Points represent untransformed proportions. Target invasive species are indicated by a red plus symbol (+) next to the species name in the legend). The rate used in this experiment and commonly in rangeland applications, 7oz/acre, is indicated in bold red text.

4.4.4 Issues to be Considered

Response was uncertain for some native species: six-weeks fescue (*Vulpia octoflora*), plains bee balm (*Monarda pectinata*), and woolly plantain (*Plantago patagonica*) with low or no emergence across all herbicide concentrations including no herbicide application in one or both soil types. Further study is warranted to determine if emergence from seed from these species is inhibited by indaziflam applications. While this study used pasteurized, screened commercial soils to reduce weed seed viability and variability among replicates in the greenhouse, these conditions do not represent the microbial and soil chemical properties of field soils *in situ*.

4.4.5 Key Findings

Emergence of native grasses, four of the six native forbs, and all three non-native target species decreased with increasing indaziflam application rates in the greenhouse dose-response study. This suggests that there are levels at which indaziflam residue creates an abiotic filter that could reduce populations of these species recruited from seed following indaziflam treatment, thus, altering natural plant community formation processes and seed-based restoration efforts. Dose response curves for the susceptible species show a range of shapes suggesting indaziflam application rates may have different thresholds for different species. For several species, the dose response curves suggest there may be a lower application rates of indaziflam (less than typically applied in the field) that do not inhibit seedling emergence for species studied. Testing additional species or rates may help fine tune potential for use in specific situations.

4.4.6 Determination

High Concern

Given that most native grasses and forbs tested showed decreased emergence of planted seeds with increasing indaziflam rates in this greenhouse study, we can expect limited recruitment of

some native species from added seeds or seeds accumulated in soil seedbanks when indaziflam persists and is bioavailable at high enough levels in soils.

4.5 Indaziflam Persistence in Soils: Concentration and Breakdown Products Over Time

4.5.1 Overview

This study was funded by the City of Boulder Climate Initiatives Department and conducted by Dr. Mike Thurman and Dr. Imma Ferrer from the CU Center for Environmental Mass Spectrometry. Indaziflam soil concentration and its breakdown products were assessed at one, two, three years after a one-time application.

Pesticide persistence is the length of time a pesticide or its breakdown products remain in the environment before they degrade through biological, chemical, or physical processes. A pesticide with high persistence can stay in soil for months or even years.

4.5.2 Methods

Thurman and Ferrer collected 10 cm soil cores and used the whole core for analysis. Sample preparation included drying soil for 24 hours, homogenizing and weighing out 2 grams and then spiking with labeled standards of similar molecules to quantify the amount of indaziflam in field samples. The samples were extracted with a methanol/water solution and further processed before analysis with liquid chromatography and mass spectrometry (LC/Q-TOF-MS). Samples were analyzed for both the parent compound, indaziflam, and its metabolites or breakdown products. Control samples (untreated field plots) were analyzed alone and spiked with known quantities of indaziflam into both soil samples and soil sample extracts to quantify the percentage of indaziflam that was recovered from treated field soil.

4.5.3 Results

Indaziflam concentration was tested at one, two and three years after application. Indaziflam and its residues were detected at all three time points. Six breakdown products/metabolites were consistently found, two indanones, three acids, and one fluoro-triazine. Because the concentration of indaziflam at time zero was not known, the half-life of indaziflam was calculated by measuring the concentration of the parent compound, indaziflam, over time and comparing it to the concentration of its breakdown products (using a ratio of metabolite/parent). When there is an equal amount of breakdown products to parent compound, this is considered the half-life. Indaziflam's half-life was calculated to be 4.6 years after application, which means that half of the indaziflam that was applied initially is still present in the soil 4.6 years later at this site.

4.5.4 Issues to be Considered

There was wide variation between replicate plots, which is to be expected due to variations of the spray during application and small-scale environmental variability.

4.5.5 Key Findings

Indaziflam is a pre-emergent herbicide designed to provide extended control of target species by suppressing seed germination and persisting in the soil. While the label (*Rejuvra Pesticide Registration*, 2020) recommends waiting at least 24 months to reseed following indaziflam application, this and other studies (Myers, 2025; J. Sebastian et al., 2025) indicate that indaziflam and its metabolites may persist for much longer, potentially inhibiting plant recruitment from seed. Indaziflam persistence, at biologically relevant levels, could have significant implications for native

plant communities and seed-based restoration efforts. This study also shows that indaziflam breakdown byproducts are highly persistent. These compounds have not been tested for herbicidal activity or non-target effects.

4.5.6 Determination

High Concern

Indaziflam and its degradates (breakdown products) remain at high levels for three years after one application with a half-life of at least 4 years for indaziflam at this site. This persistence could reduce the viability of subsequent restoration/reseeding efforts in treated areas. It could also inhibit or prevent native seedbank viability with unknown and unpredictable impacts to native plant communities. The persistence of breakdown products and their physiological or ecological impacts are unknown.

4.6 Indaziflam Persistence in Soils: Indaziflam Concentrations at Two Soil Depths

4.6.1 Overview

Dr. Cynthia Brown and Lauren Myers, a graduate student from the Brown Lab (CSU), were funded by OSMP to analyze indaziflam at two different soil depths 25 months after application. Indaziflam is designed to bind tightly to the soil particles and remain near the soil surface selectively impacting plants germinating from seed (Courkamp et al., 2022). This study tested the variability of indaziflam concentrations at two soil depths and hypothesized indaziflam concentrations would be greater near the soil surface.

4.6.2 Methods

Brown and Myers collected soils at two depths, 0-1 cm and 1-5 cm below the soil surface. Samples were collected using a 5 cm diameter by 10 cm deep metal core, keeping the 0-1 cm fraction separate from the 1-5 cm fraction. Two subsamples were taken from each treated or untreated plot and pooled, keeping the two depths separate. The investigators homogenized each soil sample and extracted indaziflam from a 5-gram subsample using acetonitrile as a solvent. Indaziflam concentrations in the extractant were determined with liquid chromatography and mass spectrometry without internal standards (LC-MS/MS); they used software to determine the concentration of indaziflam.

4.6.3 Results

Indaziflam concentrations tended to be higher at the shallower (0-1 cm below the soil surface) than the deeper (1-5 cm below the soil surface) depths but were not statistically different. This is likely due to large variation between replicate plots, which may reflect uneven application across the landscape and small plots and fine scale environmental variation (Figure 4).

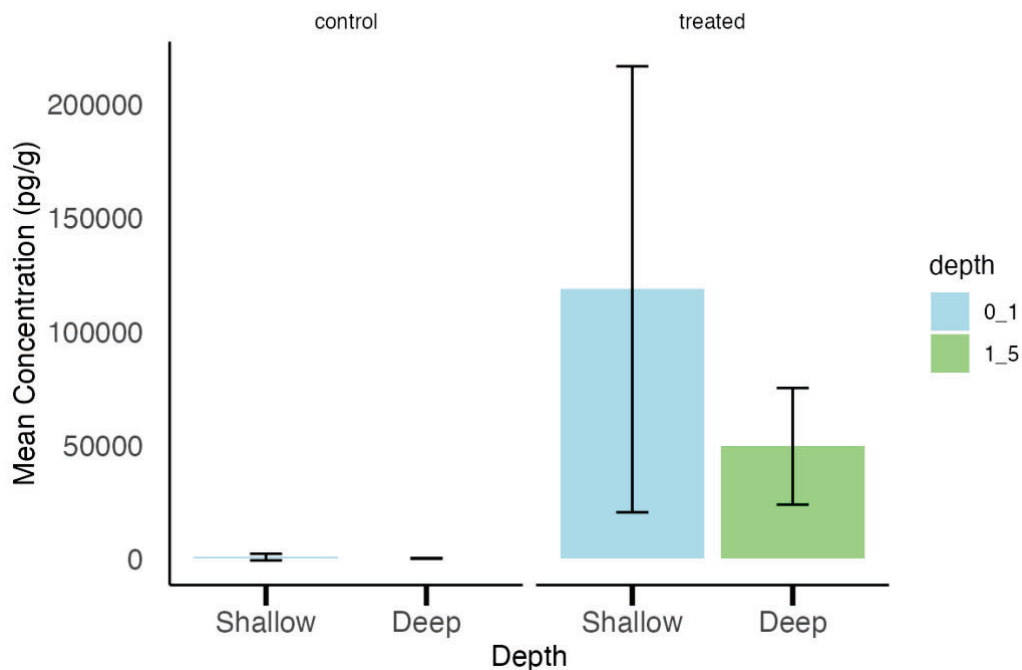


Figure 4. Indaziflam concentration (pg/g fresh soil) for Shanahan Ridge, a Boulder Open Space and Mountain Parks site at two sampling depths (shallow, 0-1 cm; deep, 1-5cm). The values for the untreated control plots are on the left, and for the treated plots are on the right. Bars represent the standard error of the mean.

4.6.4 Issues to be Considered

Detected indaziflam concentrations at both depths varied widely by replicate, potentially indicating the challenges of herbicide application in a small area and small scale environmental and soil differences. This study was designed as a small pilot study and a larger study with greater replication would be needed to detect potential differences in indaziflam concentrations at different depths. While efforts were taken to prevent the shallow soil from contaminating the deeper samples it is possible that some shallow soil fell into the deeper cores.

4.6.5 Key Findings

Mean concentrations of indaziflam were not significantly higher at the shallower soil depth (0-1 cm below the soil surface) compared to the deeper depth (1-5 cm). Additionally, the researchers sampled additional sites in Jefferson County (2 sites) and Boulder County (3 sites) at different time points post indaziflam application and found that generally concentrations of indaziflam were not significantly higher in the top centimeter of soil averaged across sites, which does not support the soil profile selectivity of indaziflam. Since indaziflam was detected up to 5 cm below the soil surface, additional research is needed to understand indaziflam movement and potential herbicidal activity throughout the soil column.

4.6.6 Determination

High Concern

It is of high concern that indaziflam was detected in soils more than two years after application, including at soil depths up to 5 cm below the soil surface. However, the detection of indaziflam does not necessarily indicate that indaziflam is present at biologically relevant levels.

4.7 Non-Target Effects: Soil Microbiome Study. Analysis of impacts on soil microbial communities in treated areas

4.7.1 Overview

As part of the same study examining the aboveground plant community and seedbank impacts funded by the OSMP Funded Research Program, Dr. Carrie Havrilla and Emily Swartz also took annual soil samples for microbial sequencing. Soil microbial sampling was conducted to evaluate potential non-target impacts of indaziflam, given the concern that herbicide use could reduce soil microbial diversity or shift communities toward undesirable compositions.

4.7.2 Methods

The study had 10 plots total (5 replicates × 2 treatments). From each plot, three small soil cores were collected and homogenized into a single bulk sample per plot. Sampling was repeated across 3 years (2023, 2024, 2025), yielding 30 samples in total (10 plots × 3 years) (Table 4).

Table 4. Microbial sampling intensity, showing the number of replicates by site/treatment combination over time.

Site, Treatment	2023	2024	2025	Sum
Shanahan, Control	5	5	5	15
Shanahan, Treated	5	5	5	15
Sum	10	10	10	30

Soil microbial communities were assessed using high-throughput sequencing of 16S rRNA (bacteria and archaea) and ITS rRNA (fungi) using standard laboratory protocols. Differences were statistically evaluated between herbicide treatments and across sampling years.

4.7.3 Results

Indaziflam treatment did not significantly alter microbial diversity for either bacterial/archaeal (16S) or fungal (ITS) communities at any point during the study period. Diversity levels were similar between treated and control plots, indicating that a single application of indaziflam did not produce measurable shifts in diversity.

No significant differences in community composition were observed between indaziflam-treated and control plots for either bacterial/archaeal (16S) or fungal (ITS) communities over the three sampling events. Community structure also did not differ significantly across sampling years within treatment groups.

4.7.4 Issues to be Considered

Researchers noted that in previous studies, indaziflam effects on soil microbiomes appeared to occur indirectly, mediated through changes in plant communities and litter dynamics. In the current study, absent strong plant community shifts, such indirect pathways may not have occurred, resulting in little to no detectable microbial response at the time of sampling. Continued monitoring may clarify whether indirect, plant-mediated microbial changes emerge over longer timescales.

4.7.5 Key Findings

Preliminary interpretation suggests that indaziflam did not produce direct, detectable effects on soil microbial diversity or composition during the study period. This aligns with observations that plant community composition also did not differ substantially between treatments.

Overall, indaziflam did not measurably alter microbial diversity or structure during the three-year study period.

4.7.6 Determination

Low Concern

There were no differences in microbial diversity or community composition based on indaziflam herbicide treatment.

4.8 Environmental Health Considerations

4.8.1 Overview

The previous sections summarize the results from City-sponsored field and lab research conducted on indaziflam efficacy and specific non-target impacts. This section summarizes regulatory data and other independent studies.

The Environmental Protection Agency (EPA) assesses pesticide risks using lab toxicity tests and risk exposure models for humans and a few surrogate species. Data are limited when a new pesticide first enters the market, and there are not independent, peer-reviewed studies available to assess risk at that stage; long-term impacts may not be understood for many years. As a result, the EPA pesticide registration framework provides only a partial picture, missing real-world conditions such as mixtures, chronic and sublethal effects, ecosystem-level dynamics, and climate-related stressors. Consequently, the city uses a precautionary approach, assessing the potential health and ecosystem risks for any pesticide being considered for use by conducting an extensive literature review that compiles the findings from a wide range of sources including both government tests and other human and environmental health research that may exist in the independent literature. The following section summarizes city's screening of indaziflam using this process.

4.8.2 Methods

The City's IPM process considers pesticides only after prevention and non-chemical management are determined insufficient to meet site management objectives. Because the goal is to reduce pesticide use and reliance, any proposed product undergoes a targeted review of toxicity, environmental risks, and whether safer alternatives exist before approval is considered.

The City of Boulder uses a hazard-based set of criteria (available in supporting documentation) to assess pesticides before they can be approved for use. Key criteria include toxicity and potential long-term health effects to humans and wildlife; risks to pollinators; environmental persistence and mobility; potential for water contamination; and the potential to increase the risk of cancer, birth defects, endocrine disruption, development and cognitive issues, and ecosystem-level impacts.

4.8.3 Results

There is very little research about the health impacts to people from indaziflam exposure. There are few independent studies about potential impacts to non-target invertebrates or other animals. EPA registration tests indicate several potential issues for human health and the environment (U.S. Environmental Protection Agency, 2010). Rejuvra™ (with active ingredient indaziflam) was initially approved for use with a “conditional registration,” which means that some tests were waived by the EPA. The additional safety factors that increase the thresholds of concern to account for children’s increased sensitivity were not applied.

These health concerns, which can apply to humans as well as non-target animals, were identified from regulatory testing (U.S. Environmental Protection Agency, 2010):

- Neurotoxic at both short- and long-term exposures
- Organ damage (kidney, liver, stomach, thyroid, seminal vesicles, ovaries)
- Potential endocrine disruptor (thyroid function)
- Possible reproductive or developmental effects

Non-Target Organisms:

EPA test results showed that indaziflam is extremely toxic to fish, aquatic plants and aquatic invertebrates.

Although EPA testing did not conclude that indaziflam is a carcinogen, a recent study showed that increasing concentrations of indaziflam lead to a genotoxic effect; genotoxicity tests detect changes in DNA that can initiate cancers or tumors (Könen Adıgüzel, 2023).

There are very few studies available about wildlife impacts. One Boulder County study—by Seshadri and Hardin (2021)—assessed pollinator populations from cheatgrass infested and indaziflam treated plots, but did not compare to untreated, native plant dominated areas. They concluded:

“Further studies are necessary to understand mechanisms that facilitate re-establishment of native flowering species, the long-term consequences of reducing invasive annual grasses and to document any residual effects of the herbicide on ground-nesting pollinators” (Seshadri & Hardin, 2021).

Two studies looked at the impacts from pesticides used for forest management, including indaziflam, on Oregon downstream watersheds. Indaziflam was detected in riverine and estuarine bivalve tissue in five of the eight watersheds (Scully-Engelmeyer et al., 2021). A toxicology study measured several parameters in soft shell clams (shell growth and condition, feeding rates, mortality, and chemical concentrations in tissue) showed that exposure to indaziflam caused a high mortality rate of 36% — alone and in mixtures. It also accumulates more than other pesticides in clam tissues (Tissot et al., 2022).

We were unable to conduct a study for this experimental use process to learn more about the impacts to soil, terrestrial or aquatic invertebrates from indaziflam or its metabolites. This remains an important and potentially consequential gap.

Indaziflam can move from one site to another. Regulatory documents include how indaziflam is likely to move offsite from three modes (*Rejuvra Pesticide Registration*, 2020):

- Surface water run-off: High potential for reaching surface water via runoff for several months after application
- Ground water contamination: May leach into ground water if used in areas where soils are permeable, particularly where the water table is shallow.
- High particle-bound transport: Adheres to soil particles and can blow offsite

Public Employees for Environmental Responsibility (PEER) recently conducted an independent study that detected the PFAS chemicals PFHxS, PFHxA, PFBS, and PFOS. PFHxS and PFHxA, in each of the 12 Rejuvra samples (Rosenthal & Jenkins, 2026). The source of these PFAS is unknown.

The Rejuvra™ label states that 80.95% of the formulation are ingredients other than indaziflam (Rejuvra Pesticide Registration, 2020). These ingredients are not disclosed. Indaziflam and its metabolites are fluorinated chemicals. As more fluorinated pesticides are being developed and released, the health and environmental impacts are largely unknown; there are significant gaps in the understanding of the short- and long-term risks, and the current regulatory framework does not adequately address these potential impacts (Jagani et al., 2025). The properties of fluorinated pesticides that provide effective and long-lasting pest control, are the same properties that create risk to non-target organisms and can in turn affect overall ecosystem function.

4.8.4 Key Findings

Indaziflam has potentially significant human health and non-target species health impacts, and the long-term impacts to ecosystem function are not known. The potential for indaziflam to move into waterways also indicates areas for both careful consideration and further research to determine the potential for both surface and groundwater contamination.

4.8.5 Determination

High Concern

Although there is limited data about the potential human and environmental health impacts of indaziflam, the data that does exist shows significant concerns about its use due to years-long persistence, and regulatory testing that shows potentially serious health impacts to people and wildlife, and high toxicity to invertebrates.

Section V: Determination, Conclusions & Opportunities for Future Research

Determination

Given the limited efficacy of indaziflam alone on tall oatgrass without using other methods to control established individuals (i.e. mowing, grazing or other herbicides), long persistence impacting seed germination, and risks to human health, indaziflam is not recommended for approval to manage tall oatgrass on City of Boulder properties at this time.

Conclusions

Indaziflam had limited impacts on the aboveground plant community at Shanahan Ridge (Section 4.1), there were no differences detected in tall oatgrass and native plant cover between treated and control plots at any point during the study period. However, cover of introduced species was significantly lower in treated plots in years two and three, likely reflecting the inhibition of

recruitment from seed of these species. Indaziflam did not significantly affect soil microbiome diversity or community composition at this site (Section 4.7). In contrast, a recent study reported belowground microbial shifts following indaziflam application that coincided with substantial changes in the aboveground plant community (Bradbury et al., 2024). Because comparable aboveground changes were not observed at our site, the absence of belowground microbial responses may indicate that soil microbial shifts are indirectly mediated by plant community changes that did not occur here.

Significant non-target impacts were found across the studies that investigated the impacts of indaziflam on seed germination and emergence. Indaziflam significantly reduced emergence of all species, both native and introduced, from the natural seedbank (Section 4.2) using field soils collected roughly two and a half years after indaziflam application. Similarly, a germination bioassay of a tolerant species showed impacts of indaziflam on plant performance and growth three years after treatment (Section 4.3). Section 4.4 investigated species specific responses to variable indaziflam concentrations using a greenhouse dose response study. Most species' emergence declined as indaziflam concentrations increased, especially at the rates commonly applied. However, some species were not significantly impacted and may be tolerant to indaziflam. These seed germination impacts will likely influence the way new plants establish and species composition changes over time through natural processes and may have significant implications for any seed-based restoration attempts following indaziflam treatment.

Related to the seed germination findings, indaziflam was shown to persist in soils for at least three years (Sections 4.2, 4.3, 4.5). As a pre-emergent herbicide, it is designed to persist in the soil to provide long term control. However, the half-life at the Shanahan Ridge site was calculated to be greater than 4 years — much longer than the typically cited 'greater than 150 days' half-life (U.S. Environmental Protection Agency, 2010) or the recommended 24-month reseeding interval (*Rejuvra Pesticide Registration*, 2020). In addition to an extended half-life, indaziflam degradants remained at high levels, while the herbicidal or non-target impacts of these byproducts are unknown. Indaziflam is designed to bind tightly to soil particles and remain near the soil surface impacting plants emerging from seed (Courkamp et al., 2022), however indaziflam was detected at depths up to 5cm with no significant differences in concentrations in the top centimeter of soil and depths up to 5cm detected (Section 4.6) and other studies have detected indaziflam at greater depths as well (González-Delgado et al., 2015; González-Delgado & Shukla, 2020). Studies finding indaziflam's migration into waterways and subsequent impacts on aquatic species are also an area of significant concern (Scully-Engelmeyer et al., 2021, Tissot et al., 2022). Further research is needed to understand the potential herbicidal activity, possible injury to established plants and possible movement in the soil column and potential migration into both surface and subsurface waterways.

Opportunities for Future Research

While this set of studies improves our understanding of indaziflam as a potential tool for managing introduced perennial species that depend on seed for reproduction, like tall oatgrass, and evaluates various non-target impacts, it also demonstrates clear knowledge gaps and areas that would benefit from further research including:

- Continued monitoring of these study plots to track if and how community composition changes as tall oatgrass, a short-lived perennial, naturally senesces.
- Evaluation of the potential herbicidal activity, persistence, impacts on ecosystem function and non-target impacts of indaziflam and its degradants/breakdown products.

- Quantification of indaziflam presence and potential associated herbicidal activity throughout the root zone of perennial species over time.
- Examination of the influence of seed germination inhibition on plant and microbial communities over longer timescales.
- Investigation of how indaziflam treatment influences recovery from other potential disturbances such as wildfire.
- Further analysis of the soil microbiome response to indaziflam, including both direct and indirect (e.g., plant community-mediated) effects.
- Effect of indaziflam on invertebrates, including soil macroinvertebrates and aquatic invertebrates.
- The presence and persistence of indaziflam in both ground and surface water.
- Evaluation of whether there are PFAS compounds present in either the active or inert ingredients of commercial indaziflam formulations.

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Appendix 1: McKenzie Site Results

Background

Indaziflam treatment first occurred on July 27, 2022 at McKenzie at a rate of 7oz/acre (Map 1 and Appendix 2, Map A2.2). Following the application, near total aboveground mortality of established plants was observed. This was unexpected since indaziflam is a pre-emergent herbicide designed to inhibit seed germination without impacting established individuals. Following this mortality, new plots were established and sprayed on September 20, 2022, again at a rate of 7oz/acre, due to concerns around potential post-emergent herbicide contamination of the sprayer used, however the same phenomena was observed following that application as well. As a result of this unexpected indaziflam behavior and other protocol deviations, we have included the McKenzie results in this appendix with a discussion of what may have driven the aboveground mortality, however, these results should be interpreted with caution.

The McKenzie site had a history of agriculture, tilling, prairie dog occupation and was dominated by a mix of nonnative invasive species, largely tall oatgrass (Table A1.1). Additionally, the McKenzie study plots were in areas previously treated with a broadcast application of the herbicide Envoy Plus (21 oz/ac) mixed with Panoramic 2SL (8 oz/ac) during the spring a year prior to the start of this indaziflam experimental study. Spot treatment of Milestone was directly applied to Russian knapweed on 7/19/2021 also overlapped with a couple of the indaziflam study plots. Little to no impact on tall oatgrass was observed in the study area during that growing season but it is possible that these previous applications interacted with or influenced the experimental observations at this site.

Table A1.1. Summary of site and physical soil characteristics at the McKenzie study site.

Variable	McKenzie	
Geographic Context	Urban edge	
Tall Oatgrass Abundance	Dominant	
Other Vegetation	Mixed grass prairie, but almost exclusively comprised of nonnative species including musk thistle, curly dock, Russian knapweed, and bindweed	
Land Use & Disturbance	Historically tilled pre-1995; prairie dogs last occupied study area in 2019; previous herbicide applications in the vicinity	
Indaziflam Application	Control	Treatment
Soil pH	7.7	7.8
Soil Organic Matter (%)	5.26%	5.16%
Sand (%)	55%	50%
Silt (%)	17%	19%
Clay (%)	28%	31%
Soil Class	Sandy Clay Loam	Sandy Clay Loam

Plant Community

At the McKenzie site, a post-agricultural area with minimal native species presence, tall oatgrass cover was significantly reduced in indaziflam treated plots for all three study years (Figure A1.1).

However, the effectiveness of indaziflam on reducing cover of tall oatgrass varied by site: no differences were detected at the Shanahan site during the study period and seemed to be influenced by differences such as soil organic matter and land use history. The decrease in tall oatgrass at McKenzie was primarily due to near total mortality of existing tall oatgrass plants and other aboveground vegetation shortly following indaziflam application in 2022. This was somewhat unexpected since indaziflam is designed to bind tightly to soil organic matter particles and stay near the soil surface after application selectively impacting plants germinating from seed and not impacting established vegetation as was observed at the McKenzie site (Courkamp et al., 2022; Jeffries & Gannon, 2016; D. J. Sebastian et al., 2017b). However, the label notes that damage to perennial vegetation may occur at rates above 5 oz/acre (7oz/acre was applied) in areas with a history of soil disturbance including agricultural history like the McKenzie site (*Rejuvra Pesticide Registration*, 2020). This study was not designed to assess these unexpected impacts of indaziflam, and more research is needed to understand how indaziflam interacts with variable site conditions and land use histories. Significant declines in introduced species, including tall oatgrass, were observed all three years in treated compared to control plots. There were minimal native species observed (3 out of 30 sampling units) so native species cover was not modeled.

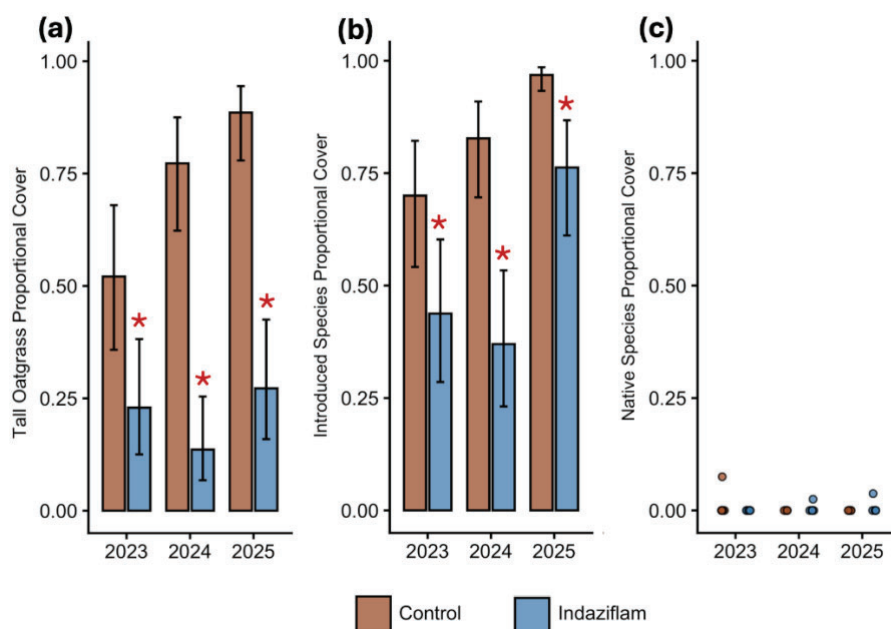


Figure A1.1. Bars show mean predicted canopy cover proportion of (a) tall oatgrass, (b) introduced species (including tall oatgrass) and (c) native species at the McKenzie site. Error bars show the 95% confidence interval. Asterisks (*) indicate significant differences ($p < 0.05$) between mean predicted values within site and year using a mixed effects beta regression model. Points show observed data for native species; too few native species were observed to be reliably modeled.

Seed Germination and Seedbank Dynamics

Seed Bank Assay (Swartz & Havrilla)

Like the Shanahan Ridge site, the seedbank assay shows that nearly all recruitment from seed was suppressed from the naturally occurring seedbank using soil samples collected roughly two and a half years post indaziflam treatment. The aboveground community at McKenzie is dominated by non-native species and no native species were observed in soil samples taken from either treated or control plots indicating that the seedbank lacks native species (Figure A1.2).

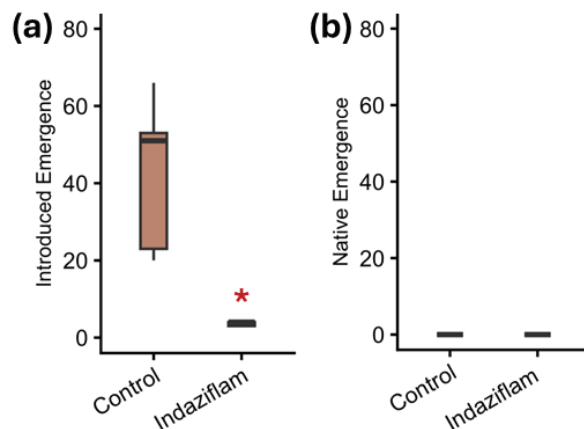


Figure A1.2. Box and whisker plots showing observed (a) introduced, and (b) native emergence from seed bank assay mesocosms from the McKenzie site. Asterisks (*) indicate significant differences ($p < 0.05$) in predicted emergence between mesocosms sampled from control and treated plots in March 2025. Each response variable was modeled using a mixed effects negative binomial distribution.

Seed germination bioassay of tolerant species on field collected soil (Thurman, Ferrer & Seastedt)

Western wheatgrass is tolerant to indaziflam. Seeds were placed on soil collected from study field plots. Germination occurred in both treated and untreated soil from McKenzie collected three years post indaziflam application. The McKenzie site had fewer emerging seeds on average, less healthy growth as seen in color and length of seedlings, and lower biomass at the end of the study compared to the Shanahan site across both control and treated soils reflecting the degraded state at McKenzie. Indaziflam treated soil reduced the seedling biomass by 38% compared to control soil from the McKenzie plots.

The researchers also tested indaziflam concentration in plant tissue grown on treated soils from both sites. The plant tissues grown on treated soils from the McKenzie plots had an average indaziflam concentration of 10 ng/g, and this was 5 times greater than plants grown on soil collected from Shanahan treated plots for this study. This likely indicates greater indaziflam bioavailability, reflected by plant tissue uptake of indaziflam at the McKenzie site than the Shanahan site. Additionally, the soil samples from the Shanahan site had two to two and a half times greater soil organic carbon than the McKenzie site soils. The researchers hypothesize that soil organic carbon binds to indaziflam, making it less available to plants, which is why Shanahan had a higher concentration of indaziflam in the soil, but it was less available to the plants.

Persistence

Brown & Myers

Mean indaziflam concentration trended higher at the shallow soil depth (0-1 cm below the soil) surface but the difference between the shallow and deeper (1-5 cm) was not statistically different (Figure A1.3). Importantly, the concentration of indaziflam detected at the two sites was vastly different, with far greater concentrations detected at the Shanahan Ridge site ($118,460 \pm 43,868$ pg/g fresh soil 0-1 cm below the soil surface) than the McKenzie site (40941 ± 7448 pg/g fresh soil 0-1 cm below the soil surface) at roughly the same post application time point (Myers, 2025). The lower concentration detected at McKenzie likely indicates lower sorption and greater bioavailability driven by soil characteristics and agricultural land use history, potentially explaining in part the aboveground mortality observed.

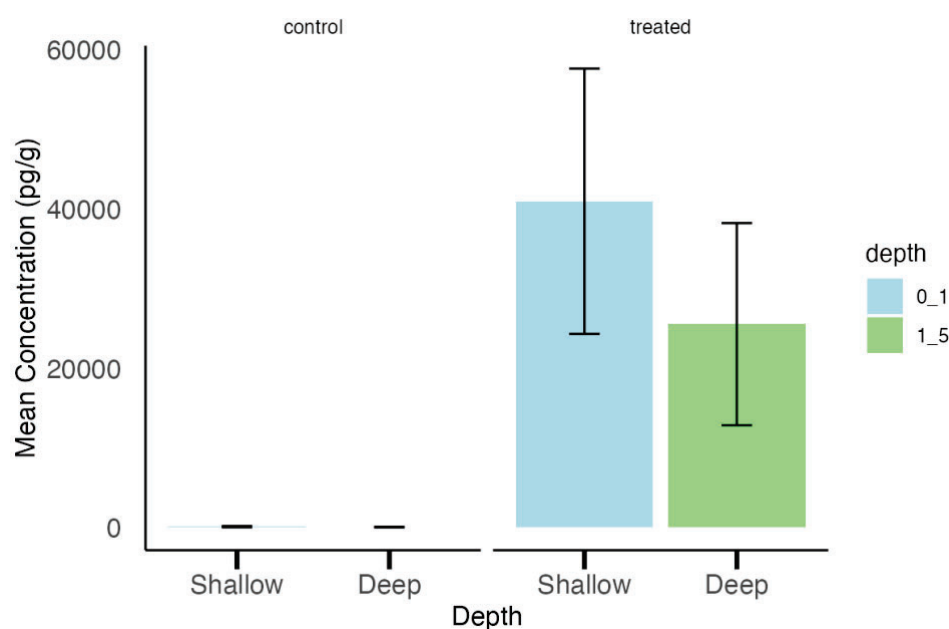


Figure A1.3. Indaziflam concentrations (pg/g fresh soil) for McKenzie, a Boulder Open Space and Mountain Parks site at two sampling depths (shallow, 0-1 cm; deep, 1-5 cm). For each site, the values for the untreated control plots are on the left, and for the treated plots are on the right. Bars represent the standard error of the mean.

Thurman & Ferrer

The metabolites/breakdown products detected at the McKenzie site were the same as the Shanahan site, two indanone, three acids, and one fluoro-triazine. The half-life for indaziflam calculated using the ratio of metabolites to parent compound was 4 years at the McKenzie site compared to 4.6 years at the Shanahan Ridge location. Both half-life measurements are significantly longer than the reported “greater than 150 days” half-life (U.S. Environmental Protection Agency, 2010).

Microbial Community Dynamics

Microbial diversity and community composition differed significantly between the Shanahan and McKenzie sites reflecting environmental and management contrasts. However, at McKenzie the differences in community composition and microbial diversity were not detected between indaziflam treated and control plots which match the findings at the Shanahan location.

Discussion

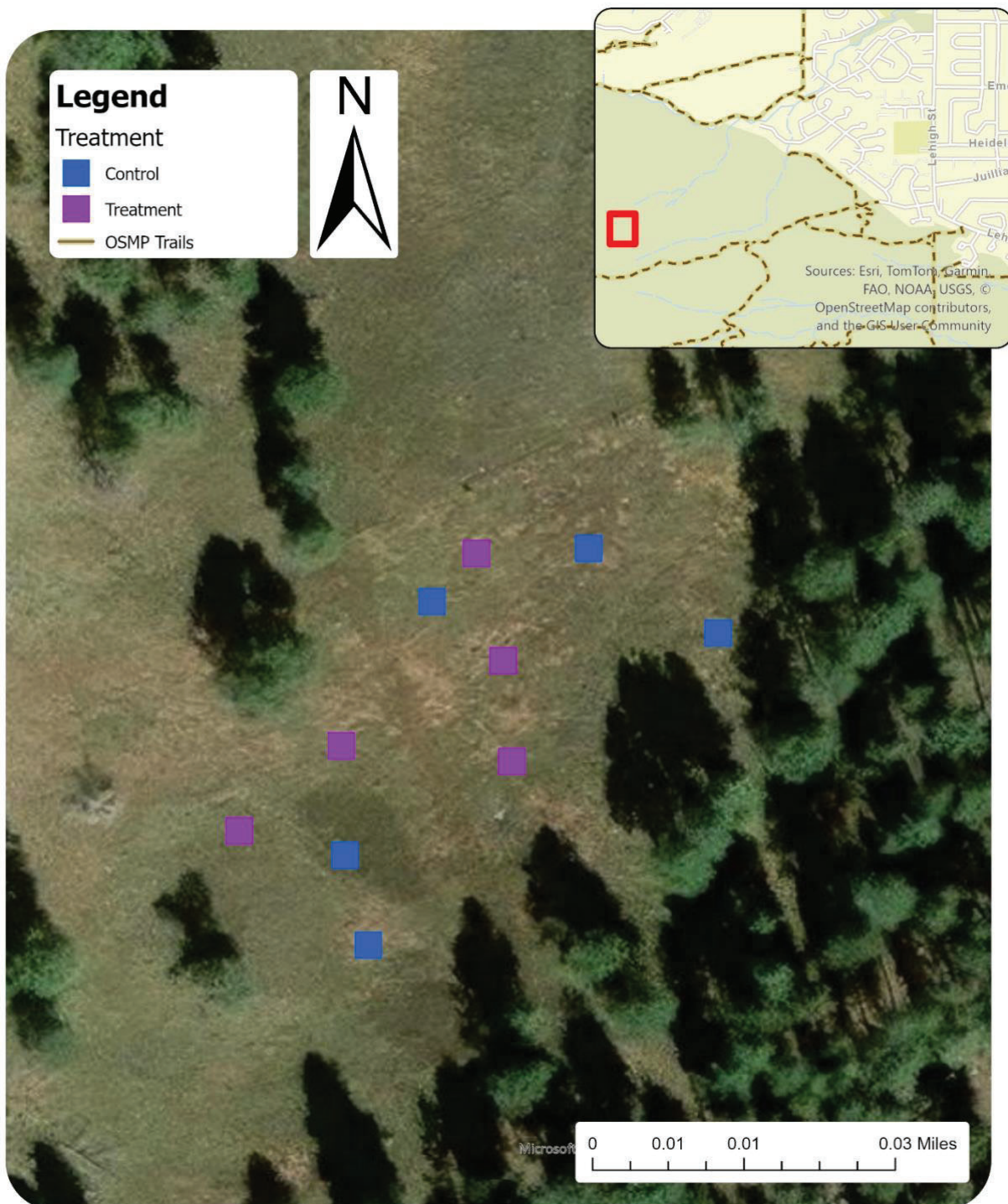
Indaziflam had vastly different impacts on established existing vegetation at the two sites, McKenzie and Shanahan Ridge. The near total aboveground mortality at the McKenzie site was likely driven, in part, by the use of a 7oz/acre rate of indaziflam which is higher than the label recommendation of a maximum rate of 5oz/acre on sites with a soil disturbance history including agriculture (*Rejuvra Pesticide Registration*, 2020). However, it is also clear that more research is needed on how indaziflam responds to different land use history and site conditions to prevent unexpected injury and assess recovery potential (e.g. remnant native plant communities).

In addition to the higher than recommended rate of indaziflam used, the aboveground mortality was also likely linked to site history and soil characteristics partially related to the agricultural history. At McKenzie, there is a history of tillage and much lower soil organic matter (SOM) than the Shanahan site. Average SOM at the McKenzie site is 5.21% while the Shanahan Ridge site averages 12.47%. While most studies report no injury to existing individuals (S. L. Clark et al., 2019; Dombro et al., 2025; D. J. Sebastian et al., 2017a), injury to established species has been documented following indaziflam treatment under both field and greenhouse conditions (González-Delgado et al., 2015; Jones et al., 2013; Schneider et al., 2015). Injury has been shown to be related to low SOM conditions that favor increased indaziflam-soil bioavailability (i.e. the indaziflam is less tightly bound and more available to plants for uptake) including at depths up to 7.5cm (Jeffries & Gannon, 2016; Jones et al., 2013; Schneider et al., 2015). While indaziflam has been shown to be present in greater concentrations near the soil surface, it also has been shown to move through the soil profile and is detectable at greater depths possibly increasing injury potential (González-Delgado & Shukla, 2020; Jeffries & Gannon, 2016). The lower concentration of indaziflam detected at the McKenzie site compared to Shanahan paired with the lower SOM and a soil disturbance history likely indicates lower sorption potential, greater bioavailability and potential leaching in the soil profile which may help explain the damage and mortality to established vegetation, observed following treatment at McKenzie but not at the Shanahan site.

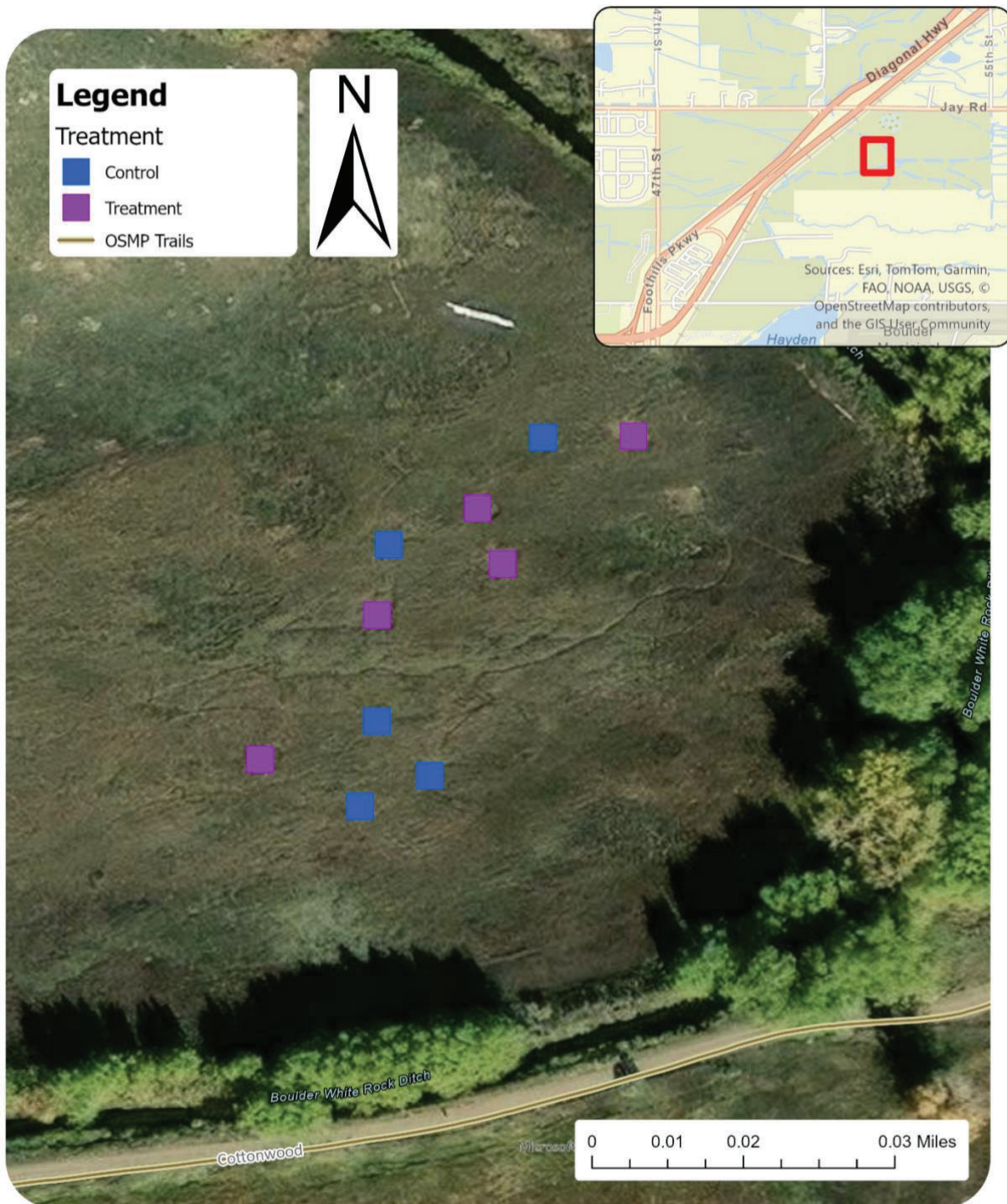
Opportunities for Future Research

The significant aboveground herbicide injury and mortality observed at McKenzie indicates a need for research focused on indaziflam behavior across variable land use histories, organic carbon concentrations in soil, and indaziflam treatment rates. Further, studies investigating the mechanisms behind the aboveground injury and mortality would be beneficial.

Appendix 2: Maps



Map A2.1. Plot layout at the Shanahan Ridge site, each plot is 16m² and randomly assigned control (blue) or indaziflam treatment (purple) as indicated by color.



Map A2.2. Plot layout at the McKenzie site, each plot is 16m² and randomly assigned control (blue) or indaziflam treatment (purple) as indicated by color.