Final Report

2020 Small Grants Program

Boulder County Open Space

Impact of Esplanade® Herbicide on Pollinators and Pollinator Resources

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EXECUTIVE SUMMARY

Invasive winter annual grasses, such as downy brome, have impacted thousands of acres on Boulder County Open Space Properties, creating monocultures that outcompete flowering forbs critical for pollinators and non-pollinating insects that make use of floral resources. Although these impacts to floral resources from winter annual grass invasions imply negative impacts to pollinator habitat, research has not directly addressed this issue. Our study aimed to identify floral resources and floral visitation of arthropods in areas treated in Boulder County for downy brome with Rejuvra® herbicide compared to control (non-treated) areas. We found that all arthropod diversity measurements including floral visitor richness, abundance and Shannon's H Index were increased in sites treated for downy brome. Furthermore, pollinator abundance of native bees, butterflies/moths, and beetles was also increased in treated areas. Our data suggest that treatments for invasive winter annual grasses on Boulder County Open Space properties are increasing habitat and overall community diversity of pollinating and non-pollinating insects at the sites.

Management Implications for Boulder County Open Space Managers

- Managing invasive winter annual grasses on Boulder County Open Spaces increases floral resources for pollinators and non-pollinating insects.
- The floral visitor community diversity can be increased on Boulder County Open Space properties by treating winter annual grasses such as downy brome.
- Pollinating insect abundance, including native bees and butterflies/moths, can be increased on sites by treating winter annual grasses.
- Rejuvra® herbicide is a viable tool for Boulder County land managers to aid in the restoration process of critical pollinator habitat areas invaded by winter annual grasses.

ABSTRACT

Invasive winter annual grasses, including downy brome, impact thousands of acres on Boulder County Open Space properties. As these grasses form monocultures, they begin to impact several ecosystem processes, including pollination services. Rejuvra® herbicide has been shown to provide multi-year annual grass control with one application, allowing time for restoration of the native plant community. We hypothesized that applications of Rejuvra® to control winter annual grasses on Boulder County properties could increase floral resources and diversity of pollinators and other non-pollinating insects that use floral resources. Rejuvra® treatments to manage downy brome were made to six Boulder County Properties from winter 2017 to winter 2018. Transects were established in treated and control (non-treated) areas at each site, and floral visitors and flower abundance was evaluated over two sampling periods in summer 2020. Floral visitor abundance increased 2-fold while visitor richness increased 2.5-9x in treated sites. Shannon's H Index for community diversity of floral visitors was also higher in treated sites, increasing from < 1.0 in control plots to > 1.4 across treated plots. More pollinating insects, including native bees, butterflies/moths, and beetles were observed where downy brome had been managed. Lastly, there were significant over a 3.5x increase in native flower abundance and 1.5x increase in native flower richness across treated sites, indicating better floral resources for floral visitors. The results from this study indicate downy brome has a significant impact to floral visitors, including pollinating insects. Rejuvra® is a viable tool for Boulder County Open Space managers to restore critical pollinator habitat areas invaded by winter annual grasses.

Keywords: floral visitors, downy brome, indaziflam, bees, pollinator habitat, floral resources

INTRODUCTION

Invasive winter annual grasses, particularly downy brome or cheatgrass (*Bromus tectorum*), have had devastating impacts across the rangeland habitat of the western United States. They are highly competitive exotics that effectively displace native vegetation by depleting soil moisture and nutrients. Downy brome infests a combined area estimated at over 54 million acres (Sebastian et al. 2017), disrupting the ecosystem by restructuring historic fire regimes, displacing native flora and rendering the land less fit for grazing and other purposes (Mack and Pyke 1983. Recently, indaziflam (marketed as Rejuvra® by Bayer CropScience) has been approved for use on rangeland and open spaces infested by invasive annual grasses. Acting immediately following germination of downy brome seeds, Esplanade® provides a mode of action different from previously used herbicides (Sebastian et al. 2017). Beginning in 2015 Esplanade® (now named Rejuvra®) has been applied in areas of Boulder County Open Space lands and initial applications have shown effective control of invasive winter annual grasses, with a resulting increase in broadleaved forbs and native perennial grass species (Sebastian et al. 2017). Many of these forbs promote and sustain important ecosystem services including pollination as they provide food and shelter for pollinating bees, butterflies and flies. Most of these pollinators are native to this region of Colorado, and the bee fauna of Boulder County has been particularly well-documented (Kearns and Oliveros 2009a, Kearns and Oliveros 2009b, Goldstein and Scott 2015). Pollinating insects in general are facing increasing challenges due to widespread loss of habitat. The use of Rejuvra® has the potential to serve as a valuable tool in restoring the biodiversity of degraded lands.

Increased habitat loss and degradation have serious negative impacts on biodiversity and ecosystem functions, notably that of pollination. Pollinating insects are not only important for production of food crops, they are critical for the reproductive success of many native plant

92 species. Over 70% of flowering plants depend on pollinators for successful seed production
93 (Blitzer et al. 2012). While the impact of invasive species on the native vegetation is well known,
94 few studies explore the relation between herbicide use for invasive plant control and the
95 subsequent impact on pollinators.

96 In collaboration with Bayer CropScience and county agents, we conducted a preliminary
97 study at 6 sites (3 treated, 3 untreated) on Boulder County Open Space lands from June-
98 September 2017. In 2018 we received funding from the Boulder County Parks and Open Space
99 Small Grants Program, as well as from Bayer, which allowed us to expand the preliminary study
100 (Seshadri and Hardin 2018). Our studies documented an increase in flowering plants in treated
101 plots as compared to untreated sites. However, our timed, stationary survey methods resulted in
102 low recordings of pollinator visitation rates. Studies have shown that conducting walking
103 transects may be a more accurate method for estimating numbers of pollinators and pollination
104 visitation rates (Fijen and Kleijn 2017, Westphal et al. 2008). Therefore, in 2020 we modified
105 our methodology and conducted walking surveys for pollinators in treated and untreated sites at
106 6 locations where Boulder County conducted assessments of floral resources in 2019.
107
108 Our objectives were to:
109 1) Conduct pollinator surveys along transects at treated and untreated sites in Boulder County,
110 identifying pollinating insects to the lowest possible taxonomic level.
111 2) Document the floral resources (plant species diversity and richness) available at the time of
112 survey.
113 3) Assess the visitation rates of pollinating insects to quantify differences in floral habitat
114 quality in treated and untreated sites.

We hypothesized that we would find a greater diversity of flowering plants in treated plots, with a concomitant wider range of insect species visiting those flowers. We also anticipated that the treated areas would provide a wider range of pollinator resources, resulting in higher visitation rates than those observed in untreated plots.

METHODS

Experimental Design

Six sites on Boulder County Open Space Properties were selected to evaluate floral visitors in areas treated for downy brome with Rejuvra® herbicide (indaziflam, Bayer) compared to a non-treated (control) area on the same site with downy brome present (Figure 1). Sites were treated 2.5 to 3.5 years before sampling was conducted, allowing time for the native plant community to respond to treatments. At each of the six sites, three 50 m x 2 m transects were established in both the treated and control plots. GPS coordinates of transects are listed in Appendix Tables A1 and A2. Photos of transects on control versus treated sites can be found in Appendix Figures A1, A2, and A3. Sampling was done starting in mid-June 2020 through the end of August 2020, with two sampling periods at each site. Sites and sampling dates are listed in Table 1.

Timed Floral Visitor Observations

To determine floral visitor richness and abundance, observers walked each 50 m transect for 20 min and recorded all arthropods that contacted the reproductive structure of a flowering plant that occurred within 1 m of either side of the transect. To avoid trapping and killing insects, arthropods were identified to the lowest possible taxonomic level visually in the field (Appendix Table A3). The order in which plots were surveyed were changed on alternate visits in an effort to eliminate bias in timing of floral visitor activity and flowering phenology.

138 *Floral Resource Sampling*

139 After the timed floral visitor observations, all 50 m transects were walked and flowering plant
140 species and number of flowers were recorded for any plants occurring within 1 m from either
141 side of the transect line. All plant species recorded throughout the study period are listed in Table
142 2.

143 *Data Analyses*

144 For analyses, floral visitors were categorized into the following groups: native bees, hemipterans
145 (true bugs), hover flies (flower flies), dipterans (other fly species), lepidopterans (butterflies and
146 moths), wasps, coleopterans (beetles), ants, orthopterans (grasshoppers), spiders and other. All
147 floral visitor and plant species richness data were analyzed using a generalized linear mixed
148 model with a Poisson distribution with treatment and observation period included as fixed
149 factors. Site and transect replication were included as the random factors. Nonnative species
150 were excluded from all analyses except for the analyses directly evaluating nonnative species.
151 Any significant treatment effects were determined post hoc using pairwise comparison of least-
152 squares means test with Fisher's protected LSD ($P < 0.05$) (LME4 and EMMEANS packages; R
153 Core Team 2019). To analyze treatment effects on floral visitor and flower abundance as well as
154 Shannon's diversity index, a linear mixed-effects model was created with treatment and
155 observation period as fixed factors. Site and transect replication were included as the random
156 factors. Again, any significant treatment effects were determined post-hoc using the same
157 method as described above.

158 RESULTS

159 The community response of floral visitors in downy brome treated areas was evaluated
160 using several diversity measurements. During observations, all arthropods contacting the

reproductive structure of a flowering plant were recorded even if they were not considered a pollinating insect, as many non-pollinating insects still make use of the floral resources for hunting and other uses. Therefore, the term floral visitors is used throughout the results and discussion to describe all the arthropods observed in the study. Treatment significantly increased all diversity measurements: floral visitor richness, floral visitor abundance, and Shannon's H Index (all: $P<0.001$) (Table 3). Floral visitor richness was increased two-fold while floral visitor abundance was increased 2.5-9x in treated plots compared to control plots. The richness of plants with observed floral visitors was also increased in the treated plots ($P<0.001$). Floral visitor abundance (number of observations) was also evaluated by the individual arthropod categories. Shannon's H Index of floral visitors went from < 1.0 in control plots to > 1.4 in treated plots. Native bees ($P=0.005$), hemipterans ($P=0.0106$), lepidopterans ($P=0.0024$), and coleopterans ($P=0.0012$) were observed more often on the transects in the sites treated for downy brome compared to the control sites (Figure 2).

The flowering native plant community response to downy brome treatments was also evaluated to determine differences in floral resources. Treatments again significantly increased both native plant richness ($P=0.0014$) and native flower abundance ($P<0.001$) along the transects compared to the control plots (Figure 3). There was a 3.5x increase in native flower abundance and a 1.5x increase in native plant richness observed across treated sites. A representative photo of the Trevarton Tree site showing the contrast between the control and treated areas can be found in Appendix Figure 4.

Lastly, invasive flowering plant species were looked at to determine treatment impacts to these species as well as their potential impact on floral visitors. Overall, invasive plant richness and flower abundance was significantly higher in the control plots compared to the treated plots,

indicating the herbicide (indaziflam) had some impact to invasive forb species as well (Table 4). Floral visitor richness and abundance on invasive plants was also higher in the control plots although these data are somewhat confounded by several observations of seed-feeders introduced as biological controls contacting the reproductive structures of the plants, especially on *Centaurea diffusa* (Table 4).

DISCUSSION

The results of our study provide critical information about the impact potential of invasive winter annual grasses such as downy brome to native pollinators and other floral visitors, as well as floral resources. When evaluating the floral resource response to downy brome treatments with Rejuvra® herbicide, all diversity measurements were increased in the treated plots. By managing for downy brome on the six evaluated Boulder County Open Space sites, not only were more native flowers observed in treated areas, but more species of native flowering forbs were observed. Additionally, this trend continued across both sampling periods, showing increased floral resources even towards the end of the summer season as Colorado entered a severe drought, receiving roughly 50% of average moisture (CoCoRaHS 2020) This indicates that treatments are also allowing the savings of critical moisture that is normally used up by downy brome before native plants even break dormancy in the spring (Mack and Pyke 1983). This has increased the window for flowering, hence extending floral resources for pollinators and other floral visitors to later in the summer. An example of the contrast between the control and treated areas can be found in Appendix Figure A4.

An interesting finding from this study was that the herbicide treatments also reduced the richness and flower abundance of invasive plant species. Although invasive plants can provide some resources to pollinators, they do not provide adequate resources for native specialists,

207 pollinators that have evolved a specific relationship with a few or even just one plant species
208 (Rathcke 1983). Invasive plants also tend to form monocultures, providing pollinators with less
209 variety for a shorter time-period (Rathcke 1983). The decrease in invasive plant species and
210 increase in native plant species in treated areas suggests further benefits from using Rejuvra®
211 herbicide for downy brome control.

212 In addition to improved floral resources, this study indicated a significant increase in
213 floral visitors on sites treated for downy brome compared to control sites. This is the first study
214 to evaluate in-depth the effect of downy brome on floral visitor and pollinator richness and
215 abundance. We not only observed more floral visitors along transects in treated sites, but also
216 found more species of arthropods on flowering plants within these areas where downy brome had
217 been managed. The overall floral visitor community diversity, measured by the Shannon's H
218 Index, was also increased in treated areas. The increase in use of floral resources by pollinating
219 insects within downy brome treated areas was also documented with native bees,
220 butterflies/moths, and beetles all observed more in the treated sites. Example of floral visitors
221 observed during sampling can be found in Appendix Figures A5, A6, and A7.

222 Overall, these data suggest that downy brome is having a large impact on these critical
223 floral visitor communities in Boulder County, and hence the ecosystem services provided by
224 these pollinators and other non-pollinating insects. As native pollinators, especially native bees,
225 have faced population declines due to a myriad of factors (Kearns and Oliveros 2009b),
226 managing for critical pollinator habitat on Boulder County Open Space properties has become
227 even more important. Invasive winter annual grasses impact thousands of acres in Boulder
228 County, which our research has shown is significantly impacting pollinator resources. Rejuvra®

herbicide provides a tool for Boulder County managers to start the restoration process in these critical habitat areas.

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Table 1: Site and sampling information for study period.

Site	Observation	Date
Rabbit Mountain	1	16 June 2020
Rabbit Mountain	2	22 July 2020
Colp	1	24 June 2020
Colp	2	29 July 2020
Trevarton Rocky Hill	1	1 July 2020
Trevarton Rocky Hill	2	5 August 2020
Trevarton Gate	1	7 July 2020
Trevarton Gate	2	11 August 2020
Hall Ranch	1	9 July 2020
Hall Ranch	2	20 August 2020
Trevarton Tree	1	16 July 2020
Trevarton Tree	2	23 August 2020

289 Table 2: List of all flowering species observed in transects across six sampling sites. Not all

290 species occurred at every site.

Scientific Name[1]	**Nativity Status[2]**	**Observed w/ floral visitors**
Asclepias stenophylla	N	Yes
Allium cernuum	N	No
Asclepias stenophylla	N	No
Asclepias viridiflora	N	No
Astragalus flexuosus	N	No
Astragalus laxmannii var. robustior	N	Yes
Calochortus gunnisonii	N	Yes
Calylophus serrulatus	N	Yes
Carduus nutans	I	Yes
Centaurea diffusa	I	Yes
Cirsium undulatum	N	Yes
Convolvulus arvensis	I	Yes
Cryptantha virgata	N	No
Dalea purpurea	N	Yes
Eriogonum alatum	N	No
Erigeron divergens	N	No
Erigeron flagellaris	N	Yes
Eriogonum jamesii	N	Yes
Eriogonum umbellatum	N	Yes
Erodium cicutarium	N	No
Euphorbia marginata	N	No
Evolvulus nuttallianus	N	Yes
Gaillardia aristata	N	Yes
Gaura coccinea	N	No
Gaura mollis	N	No
Gentiana affinis	N	Yes
Geranium caespitosum	N	Yes
Geranium richardsonii	N	No
Glandularia bipinnatifida	N	No
Grindelia squarrosa	N	Yes
Gutierrezia sarothrae	N	Yes
Helianthus pumilus	N	Yes
Heterotheca villosa	N	Yes
Hymenopappus filifolius	N	Yes
Hypericum perforatum	N	Yes
Lactuca serriola	I	No

Liatris punctata	N	Yes
Linaria dalmatica	I	Yes
Linum lewisii	N	No
Linum pratense	N	No
Lupinus argenteus	N	Yes
Medicago sativa	I	No
Mertensia lanceolata	N	No
Mirabilis hirsuta	N	No
Mirabilis linearis	N	No
Onosmodium molle	N	Yes
Opuntia phaeacantha	N	Yes
Opuntia polyacantha	N	Yes
Physalis hederifolia	N	No
Potentilla hippiana	N	Yes
Psoralea (Psoralidium) tenuiflora	N	Yes
Ratibida columnifera	N	Yes
Rosa woodsii	N	Yes
Sisymbrium altissimum	I	No
Solidago missouriensis	N	No
Sphaeralcea coccinea	N	No
Symphyotrichum falcatum	N	No
Symphyotrichum porteri	N	No
Taraxacum officinale	N, I	No
Thelesperma megapotamicum	N	No
Tragopogon dubius	I	No
Verbascum blattaria	I	No

[1]Nomenclature based on U.S. Department of Agriculture PLANTS database: https://plants.usda.gov.

[2]N, native; I, introduced

300 Table 3: Floral visitor community response to treatments compared to control plots averaged

301 over the six sites.[1]

	Observation 1		Observation 2	
Diversity measurements	**Control**	**Treated**	**Control**	**Treated**
Floral visitor richness	2.89 a	6.5 b	1.61 a	3.61 b
Floral visitor abundance	6.83 a	15.17 b	0.78 a	9.11 b
Shannon's H Index	0.98 a	1.5 b	0.85 a	1.41 b

302 [1]Means followed by the same letter do not differ significantly between treatments ($P<0.05$).

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Table 4: Invasive plant response and subsequent floral visitor response to downy brome treatments compared to control plots averaged over the six sites and two sampling periods.[1]

Diversity measurements	Control	Treated
Invasive plant richness	1.5 a	0.71 b
Invasive flower abundance	51.63 a	4.25 b
Floral visitor richness-invasive plants	0.94 a	0.11 b
Floral visitor abundance-invasive plants	5.83 a	0.11 b

[1]Means followed by the same letter do not differ significantly between treatments ($P<0.05$).

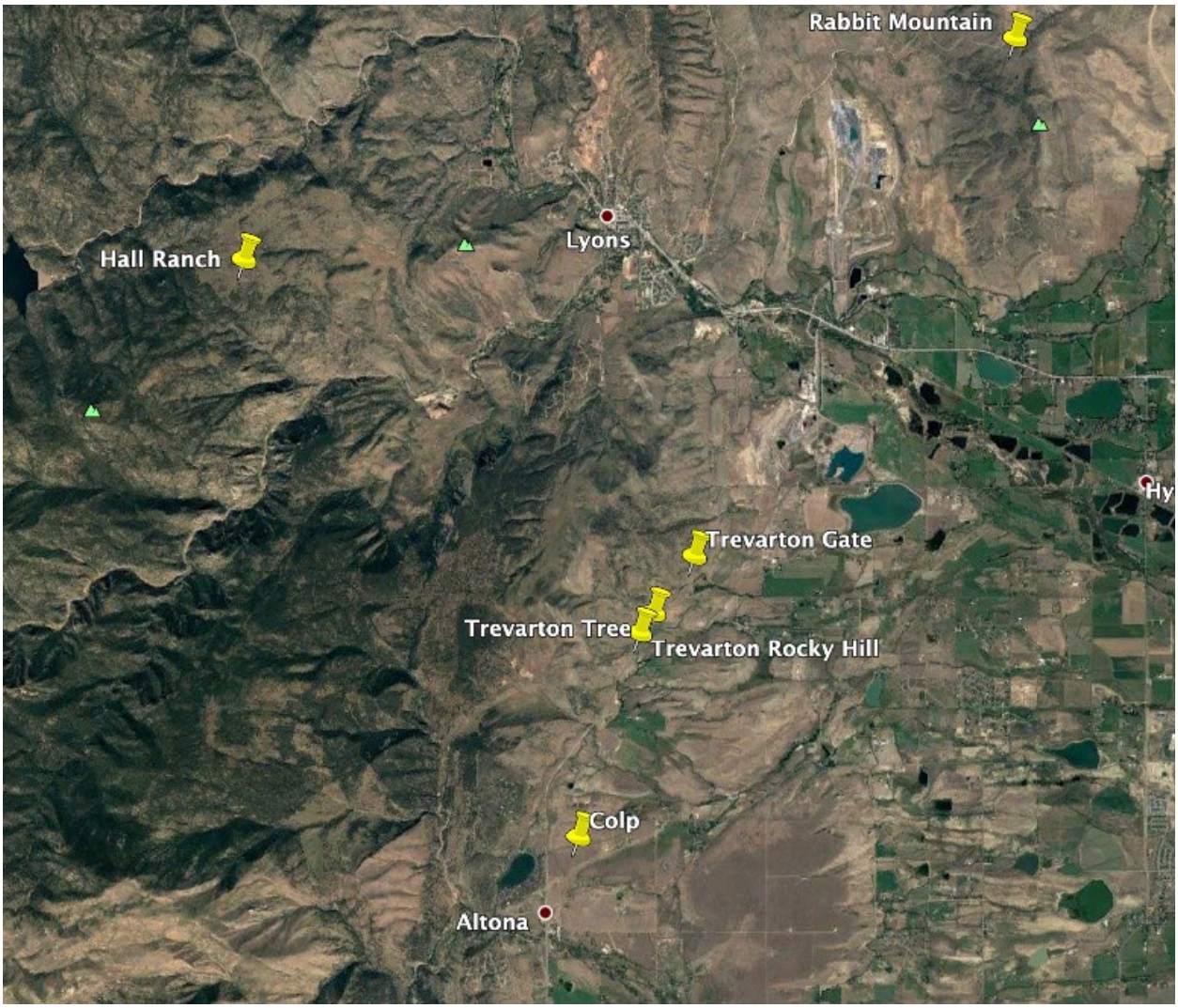


Figure 1. Locations of the six sampling sites in Boulder County used for the study.

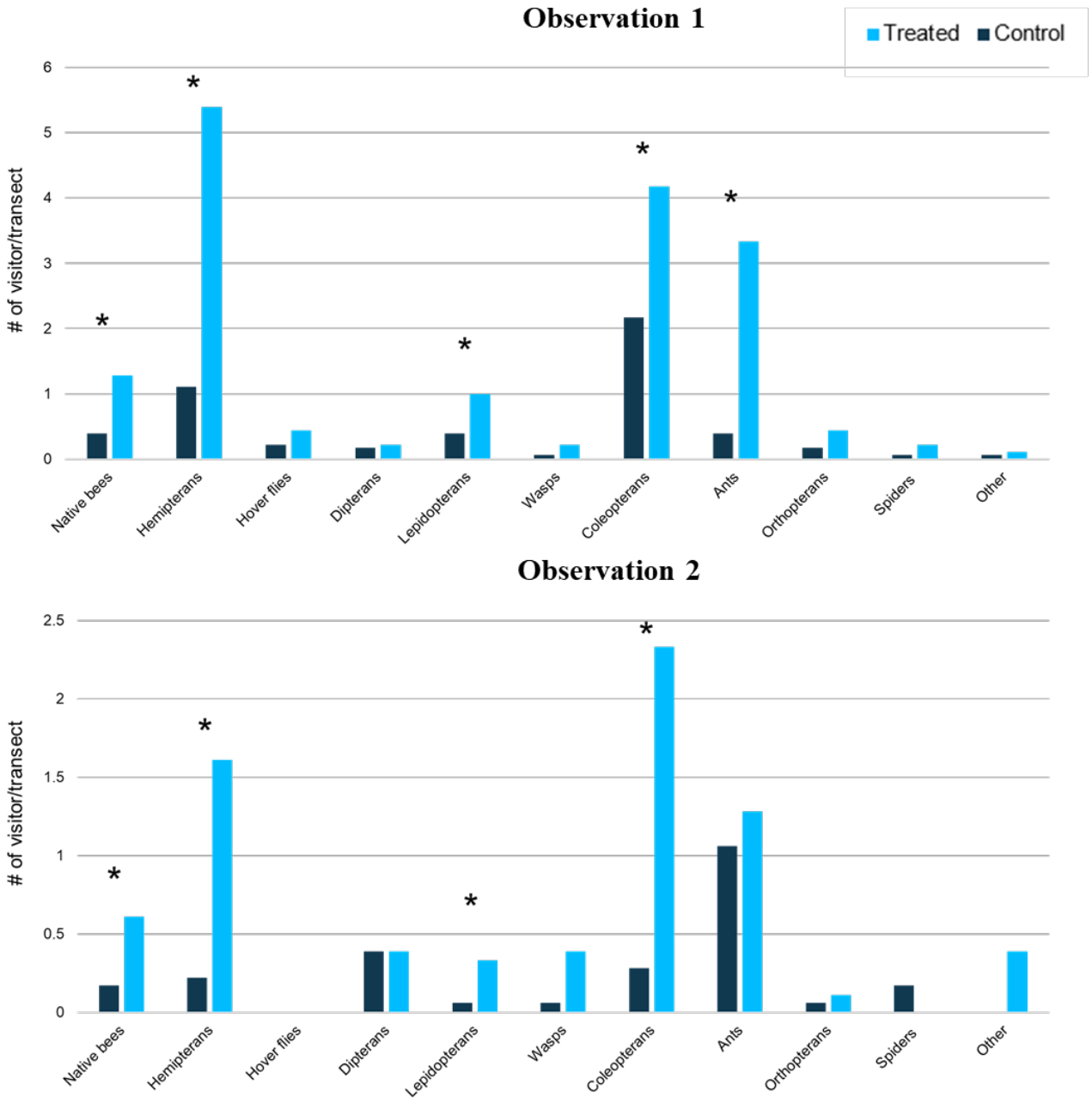
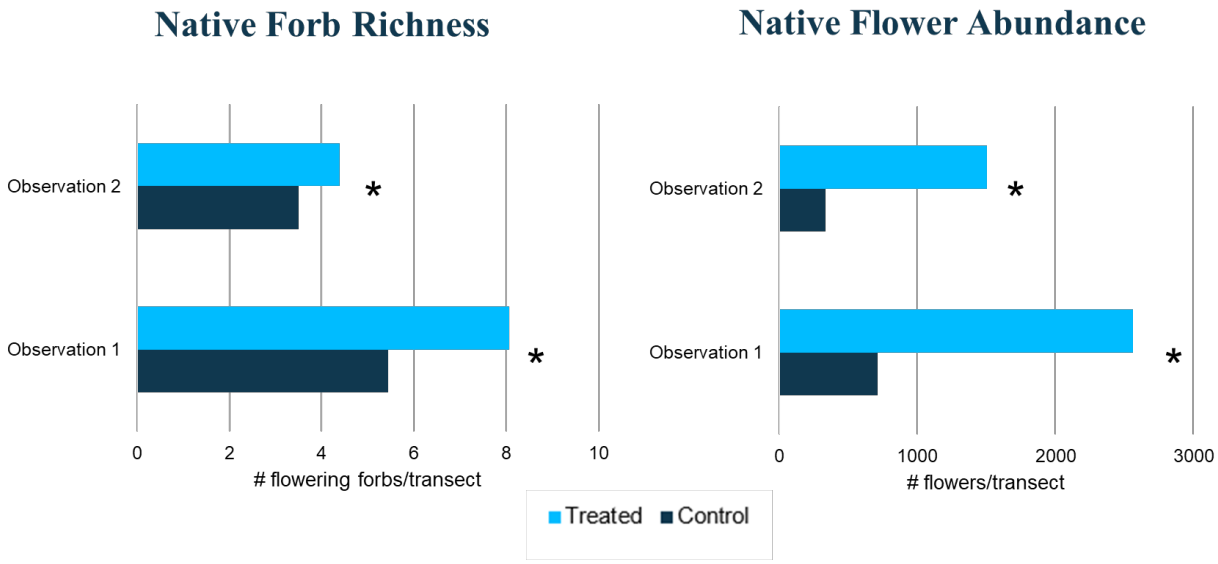


Figure 2. Response of floral visitor abundance to downy brome treatments separated by floral visitor category at Observations 1 and 2. Means averaged over all 50 m transects. Asterisks indicate means differ significantly between treatment and control ($P<0.05$) by floral visitor category.



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356 Figure 3: Native flowering plant community response to downy brome treatments compared to

357 control plots averaged over the six sites at Observations 1 and 2. Means averaged over all 50 m

358 transects. Asterisks indicate means differ significantly between treatment and control ($P<0.05$).

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