



Behaviorally-mediated trophic cascade attenuated by prey use of risky places at safe times

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Abstract

The mere threat of predation may incite behavioral changes in prey that lead to community-wide impacts on productivity, biodiversity, and nutrient cycling. The paucity of experimental manipulations, however, has contributed to controversy over the strength of this pathway in wide-ranging vertebrate systems. We investigated whether simulated gray wolf (*Canis lupus*) presence can induce behaviorally-mediated trophic cascades, specifically, whether the ‘fear’ of wolf olfactory cues alone can change deer foraging behavior in ways that affect plants and soils. Wolves were recently removed from the Cedar Creek Ecosystem Science Reserve (Minnesota, USA), such that consumptively mediated predator effects were negligible. At 32 experimental plots, we crossed two nested treatments: wolf urine application and herbivore exclosures. We deployed camera traps to quantify how white-tailed deer (*Odocoileus virginianus*) adjusted their spatiotemporal habitat use, foraging, and vigilance in response to wolf cues and how these behavioral changes affected plant productivity, plant communities, and soil nutrients. Weekly applications of wolf urine significantly altered deer behavior, but deer responses did not cascade to affect plant or soil properties. Deer substantially reduced crepuscular activity at wolf-simulated sites compared to control locations. As wolves in this area predominantly hunted during mornings and evenings, this response potentially allows deer to maximize landscape use by accessing dangerous areas when temporal threat is low. Our experiment suggests that prey may be sensitive to ‘dynamic’ predation risk that is structured across both space and time and, consequentially, prey use of risky areas during safe times may attenuate behaviorally-mediated trophic cascades at the predator–prey interface.

Keywords Behaviorally-mediated trophic cascade · Gray wolf · Top-down effect · Non-consumptive effect · Predation risk

Introduction

Trophic cascades occur when impacts of higher trophic levels are transmitted through a community to indirectly affect lower levels (Hairston et al. 1960; Paine 1980; Carpenter et al. 1985). The classic cascade of carnivores affecting plants by altering herbivore dynamics has been detected in a variety of systems, although the strength of these effects can vary substantially within and across communities (Shurin et al. 2002; Borer et al. 2005; Estes et al. 2011). Ecologists still struggle to predict the conditions under which trophic cascades may occur, due in large part to our current lack of understanding regarding the mechanisms by which predator effects propagate (Schmitz et al. 2004).

Predators can modify their ecological communities by consuming prey (‘consumptive predator effects’), and by inciting behavioral changes in prey (‘non-consumptive predator effects’) (Lima and Dill 1990; Werner and Peacor 2003; Preisser et al. 2005). The first pathway can generate

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density-mediated trophic cascades (Paine 1966, 1980; Polis and Strong 1996), which have been the historical focus of much predator–prey ecology (e.g., Estes and Palmisano 1974; Carpenter and Kitchell 1996; Pace et al. 1999). The second pathway can lead to behaviorally-mediated trophic cascades (Abrams 1984; Beckerman et al. 1997; Schmitz et al. 2004), which develop when prey attempt to manage predation risk by engaging in anti-predator behaviors (Lima and Dill 1990; Werner and Peacor 2003). These behaviors, such as spatial or temporal avoidance or increased vigilance, often come at the cost of foraging opportunities and consequentially positively impact certain lower trophic levels (Schmitz et al. 1997, 2004). The degree to which natural communities are shaped by either pathway and the relative strengths of each mechanism are topics of great interest and debate (Werner and Peacor 2003; Preisser et al. 2005).

Behaviorally-mediated effects can hypothetically produce stronger trophic cascades than density-mediated effects, as the ‘fear’ of predation may alter behavior of an entire prey population, whereas direct predation only affects individuals that are killed (Schmitz et al. 2004; Schmitz 2005; Preisser et al. 2005). This notion is supported in small-scale experimental manipulations of invertebrates, amphibians, fish, and birds (Schmitz 1998; Peacor and Werner 2001; Preisser et al. 2005; Cresswell 2008; and references therein). Evidence for either of these effects in natural ecosystems, particularly terrestrial mammal communities, is more ambiguous. In situations where cascades purportedly occur, the driver was primarily identified as prey density-mediation, with changes in prey movement and foraging behaviors deemed too weak to alter plant biomass or community composition (McLaren and Peterson 1994; Hebblewhite and Smith 2010; Kauffman et al. 2010; Painter et al. 2015). Contention has arisen, however, around the findings of many of these studies and new research highlighting the strength of behaviorally-mediated trophic cascades is now gaining traction (Fortin et al. 2005; Peckarsky et al. 2008; Ripple and Beschta 2012; Cherry et al. 2016; Flagel et al. 2016; Suraci et al. 2016; Donadio and Buskirk 2016). It remains difficult to disentangle the relative importance of the two pathways and the system-wide consequences of non-consumptive predator effects in particular are habitually understudied.

One reason for continued ambiguity regarding the importance of behaviorally-mediated pathways in trophic cascades is that predator effect studies in large, free-roaming animals tend to be observational rather than experimental. Due to the challenges of working with large vertebrates in natural systems, the majority of research has either examined a single site following predator (re)establishment or decline (Ripple and Beschta 2007, 2012; Frank 2008, 2013; Kimble et al. 2011) or compared locations where predators exist at different densities (McLaren and Peterson 1994; Berger et al. 2001; Terborgh et al. 2006).

These studies meticulously investigated correlations but struggled to establish causation because of their inability to isolate and manipulate trophic levels of interest. They often lacked replication, randomization, and controls (Eberhardt 1997; Schmitz et al. 2000; but see Donadio and Buskirk 2016; Atkins et al. 2019). While the ideal experiment would be to scale up the small-scale invertebrate studies (*sensu* Schmitz et al. 1997, Schmitz 1998), rendering free-ranging vertebrate predators non-lethal is logistically unfeasible. The scarcity of manipulative experiments involving large mammals has impeded our ability to predict the conditions under which behaviorally-mediated trophic cascades will occur (Schmitz et al. 2000; Shurin et al. 2002; Borer et al. 2005; Peers et al. 2018; but see Suraci et al. 2019).

Here, we report results from an experiment to rigorously test whether predators alter plant communities and ecosystem functioning by inciting behaviorally-mediated trophic cascades. By simulating gray wolf (*Canis lupus*) presence through repeated wolf urine applications across a replicated series of plots in currently wolf-free grasslands, we isolated the behaviorally-mediated (non-consumptive) pathway through which wolves might affect plants by altering the behavior of white-tailed deer (*Odocoileus virginianus*). The resident wolf population at our site was removed in 2016–2017 (Mech et al. 2019), eliminating consumptive effects in an area where prey retain ecological experience with these predators. Deer form a significant portion of the gray wolf diet (Mech et al. 2015) and shape grassland plant diversity, richness, and succession in our study system (Bakker et al. 2006; Olff and Ritchie 1998; Ritchie et al. 1998; Knapp et al. 1999). We focused on herbaceous grassland plant species that, due to annual winter die-off, exhibit detectable shifts in relative abundance and aboveground biomass production over a single growing season (Tilman 1987) and in response to fencing treatments that exclude ungulates (Isbell and Wilsey 2011) in our system. We used a paired plot design in which sites, randomly assigned to a wolf urine or water control treatment, contained both fenced and unfenced plots. Predator detection is a multi-modal process and our experiment examines prey responses to predator odor. Repeated application of odor was chosen to mimic chronic wolf use areas (i.e., create the perception of an underlying spatial landscape of long-term risk). Sustained anti-predator behaviors in deer in response to experimental application of wolf odor has been demonstrated in numerous wolf-deer systems (e.g., Chamaillé-Jammes et al. 2014; Kuijper et al. 2014; Osada et al. 2014; see Parsons et al. 2018 for overarching review of prey responses to predator odor cues). The paired plot approach allowed us to compare community-level responses to deer herbivory to ungrazed exclosures (Ford and Goheen 2015). Sites were continuously monitored using camera traps and soil nutrients and plant

communities were sampled during and after the growing season, respectively.

If threat from simulated chronic wolf presence alone could incite a trophic cascade, we anticipated that deer avoidance (fewer or shorter visits) of urine-treated sites would release plants from grazing pressure, increasing herbaceous biomass and decreasing soil nutrient availability relative to control sites (i.e., plant and soil communities would closely resemble the ungrazed communities inside exclosure plots; Loreau 2010; Thebault and Loreau 2003). As deer preferentially consume forbs and generally avoid grasses and sedges (Kohn and Mooty 1971; Russell et al. 2001), we predicted that plant community composition at predator-cue and control sites would differ at the end of the growing season, with the proportion of forbs being lower at ‘safe’ control sites and higher in the predator-simulated locations and herbivore-exclosure plots. Alternatively, behaviorally-mediated trophic cascades may be generated by increased vigilance at the expense of foraging behavior, reducing deer impacts on plants and soils as described above even if deer continued to visit ‘risky’ sites. However, we predicted that a trophic cascade would not materialize if deer responded to perceived predation risk by altering spatiotemporal patterns of habitat use, visiting risky sites at a rate comparable to safe locations but only during times of day perceived less dangerous based on experience with resident wolves (see Palmer et al. 2017; Kohl et al. 2018; Smith et al. 2019).

Materials and methods

Study site

We conducted this experiment at the Cedar Creek Ecosystem Science Reserve (CCESR; lat. 45°25'N, long. 93°10'W; 21 km²), a long-term ecological research station situated in a transitional vegetation zone encompassing prairies, evergreen forests, and leafy woodlands (Pierce 1954). Soils at CCESR originate from a 12,000–13,000-year-old glacial outwash deposit and are sandy and low in nitrogen and organic matter (Grigal et al. 1974; Inouye et al. 1994). Our experiment was conducted within herbaceous grassland patches, either old fields (abandoned from agriculture in the 1900s) or dry oak savannas. These plant communities contained a variety of grasses (e.g., *Andropogon gerardi*, *Poa pratensis*, *Sorghastrum nutans*, *Schizachyrium scoparium*, *Stipa spartea*, *Panicum praecocius*), non-leguminous forbs (e.g., *Asclepias tuberosa*, *Asclepias syriaca*, *Ambrosia coronopifolia*, *Lithospermum carolinense*, *Comandra richardsoniana*, *Artemisia ludoviciana*), and nitrogen-fixing legumes (e.g., *Lathyrus venosus*, *Amorpha canescens*, *Lespedeza capitata*). See Tilman (1987) and Inouye et al.

(1994) for a complete description of the history, biology, and geology of CCESR.

Deer occur in the area at estimated densities of 8.5 deer/km² (D'Angelo et al. 2016). Their foraging activity has demonstrable effects on the woody plant, legume, and non-leguminous forb communities and nitrogen cycling at our site (Inouye et al. 1994; Ritchie and Tilman 1995; Ritchie et al. 1998; Lawson et al. 1999). Other herbivores (e.g., plains pocket gopher *Geomys bursarius*, eastern cottontail rabbit *Sylvilagus floridanus*) were not captured in our experimental site camera traps and therefore not considered further.

Gray wolves are the natural top predator of white-tailed deer in Minnesota (Mech and Karns 1977; Mech and Peterson 2003), and while extensive extirpation efforts in the early 1900s eliminated gray wolves from many parts of the continental United States, residual populations persisted in northern Minnesota. A breeding pair naturally recolonized CCESR in 2015, establishing a den, and the pack increased to 19 wolves (estimated eight adults/yearlings and 11 juveniles) by 2016 (Mech et al. 2019). Wolf predation on domestic animals led to authorities removing all adult members by 2017. Scat, tracks, and data from an extensive monitoring project (continuously deployed camera trap grid covering CCESR at density of 4.76 cameras/km²) suggested that no wolves were resident at CCESR during this study. Many of the adult deer at CCESR would have been present during the wolves' recent tenure (Mech et al. 2019). While coyotes (*Canis latrans*) and foxes (*Vulpes vulpes*, *Urocyon cinereoargenteus*) have been detected in this system, only one coyote sighting was captured on camera traps at our experimental sites during the course of the experiment and, as such, we considered risk from other predators negligible. Human activity at CCESR is low, primarily restricted to researchers, staff, and students. Apart from vehicles on distant roads, no humans other than the project researchers were detected via camera traps at our experimental sites.

Field experiment

Sixteen grassland locations (mean $\pm$ SD distance apart = 0.712 $\pm$ 0.259 km) were randomly assigned as urine-treatment applications or control sites (“sites”; Appendix 1: Fig. S1). Half the sites were subjected to weekly applications of wolf urine to simulate marks left by wolves advertising their territory (Peters and Mech 1975), while the remaining sites received a water control treatment. From 05-Jun-2018 to 11-Aug-2018, the treatment or control fluid was sprayed on the ground or vegetation at eight points in a 20-m-radius circle (9 mL $\times$ 8 points for a total of 72 mL per week; Appendix 1: Fig. S1). Wolf urine was obtained from captive wolves (Murray's Lure [www.murrayslure.com]) twice during the study and refrigerated between applications. Urine from this

purveyor has evoked strong anti-predator responses in deer in other behavioral experiments (e.g., Chamaillé-Jammes et al. 2014).

Deer visitation rates and behavior at each site were monitored using camera traps (Reconyx HyperFire™ PC900 with IR flash) attached at a height of 1.00 m on wooden poles at the center of the treatment circle facing the experimental plots (Appendix 1: Fig. S1). The camera traps employ passive infrared sensors with a 30.5-m trigger distance, capturing three timestamped images (1-s delay) per trigger event. From the images, we counted adult male, adult female, and juvenile deer and quantified the presence of foraging and vigilance during each visitation bout (as per Lashley et al. 2014; Cherry et al. 2015). We distinguished age–sex class as foraging requirements and vulnerability to predation differ by life stage and sex, potentially resulting in differential evaluation of and response to risk–resource trade-offs (Cherry et al. 2015). Deer were considered foraging when their heads were down and/or vegetation was visible in their mouths and vigilant when their heads were up scanning the surrounding environment. We defined visitation bouts as lasting from the first time a deer was photographed until the last picture after which deer were not resighted for > 30 min.

To experimentally test top-down effects on plants and soils, we created paired 4×4-m plots at each site, one fenced against mammalian herbivores and the other open to grazing (“plots”; Ford and Goheen 2015). Fenced and unfenced enclosure treatments were randomly assigned to the two plots within each of the 16 sites (32 plots total; Appendix 1: Fig. S1). These plots were 2 m apart within the camera’s field of view (2 m from camera), such that habitat was consistent between plots and all herbivore activity could be monitored. Fences were 2-m high, constructed of 14-gauge galvanized wire and skirted with chicken wire. Plots were established immediately prior to the first treatment application (16–20–May-2018). At the end of the growing season (11-Aug-2018), we collected the aboveground live plant biomass, as well as woody debris and litter, within a 10 cm×2-m area of each plot. In these temperate grasslands, all aboveground plant biomass dies every winter, essentially all ramets come from vegetative growth (Benson and Hartnett 2006), and plants exhibit phenological niche partitioning (i.e., take turns growing at different times of the year; Fargione and Tilman 2005). Thus, shifts in top-down or bottom-up processes can and do rapidly shift relative abundances and aboveground biomass production of herbaceous plants in these grasslands within a single growing season, and any cascading effects would have manifested within the timeframe of the study (Tilman 1987; Isbell and Wilsey 2011). Vegetation was clipped to ground level for all plants rooted inside the sampling strip and sorted into graminoids, legumes, non-leguminous forbs, pterophytes, bryophytes, herbaceous litter, woody litter, current year’s woody growth,

and previous year’s woody growth. All biomass was dried at 60 °C for 2 weeks prior to weighing to the nearest 0.1 g.

We measured in situ availability of soil cations (NH_4^+ , K^+ , Ca^{2+} , Mg^{2+}) and anions (NO_3^- , H_2PO_4^- , HPO_4^{2-} , SO_4^{2-}) using Plant Root Simulator probes (PRM™; Western Ag Innovations Inc., Saskatoon, Canada). The probes were composed of an ion-exchange resin membrane within a plastic support and provided a measure of ion flux, capturing the nutrient supply available to plants during deployment. We inserted the probes vertically into the top soil layer, sampling 6–12 cm below the surface (absorbing surface area = 17.5 cm²). Probes were left from 05-Jun-2018 to 09-Jul-2018 to avoid saturating the probes and to capture the early season nutrient release as the soils warmed and prior-year plant litter mineralized. A pair of cation and anion probes was placed in each corner of every fenced or unfenced plot. All cation or anion probes from each plot were analyzed as a single sample, as per Western Ag (2010).

Statistical analyses

Analyses were conducted in R (v.3.5.0; R Core Team 2018) and results considered significant at $\alpha=0.05$. We used a combination of linear mixed models (LMMs) and generalized linear mixed models (GLMMs) to quantify simulated wolf effects on deer, plants, and soil responses. For the LMMs, each response was log-transformed (+0.01 added to 0 responses) to normalize variance based on residual spread. We first derived the optimal error and random effect structures (Appendix 1: Table S1) for the full model that included fixed effects for both main effects (urine and fence treatments) and their two-way interaction using REML estimation and ΔAIC_c (LMM: function *lme*, package *nlme*, Pinheiro et al. 2018; GLMM: function *glmmTMB*, package *glmmTMB*, Brooks et al. 2017). Best-fit models included site as a random intercept but did not contain auto-correlation structures (Appendix 1: Table S1). We then performed model selection and multimodel inference (function *dredge*, package *MuMIn*; Barton and Barton 2018) to identify the most parsimonious fixed-effects structure using ML estimation (LMM: function *lme*, package *nlme*, Pinheiro et al. 2018; GLMM: function *glmer*, package *lme4*, Bates et al. 2015). We applied conditional model averaging of the top subset of models with $\Delta\text{AIC}_c < 2$ (function *model.avg*, package *MuMIn*; Barton and Barton 2018) to derive our final parameter estimates (Table 1).

Deer behavior

We quantified four aspects of deer behavior: number of deer visiting each site (visitation rates), presence of foraging and vigilance during a visitation bout, and duration of each visitation bout. Visitation, foraging, and duration were

Table 1 Block diagram representing the top models ($\Delta AIC_c < 2$) for (A) deer behavioral response, (B) plant community composition, and (C) soil ion concentrations that were averaged to produce conditional

model estimate covariates. Each coefficient included in the top models is indicated with an asterisk

(A) Deer response	UT	DM	DF	DJ	DM:UT	DF:UT	DJ:UT	ΔAIC_c
Visitation rate		*	*	*				0.000
Visitation rate	*	*	*	*				1.905
Feeding behavior		*						0.000
Feeding behavior		*	*					0.036
Feeding behavior		*		*				0.758
Feeding behavior	*	*	*			*		1.412
Feeding behavior	*	*	*					1.471
Feeding behavior	*	*						1.489
Feeding behavior		*	*	*				1.680
Vigilance behavior	*	*	*	*			*	0.000
Vigilance behavior	*	*	*	*	*		*	0.215
Vigilance behavior	*	*		*			*	0.222
Vigilance behavior	*	*		*	*		*	0.320
Vigilance behavior		*	*	*				0.434
Vigilance behavior	*	*	*	*				0.445
Vigilance behavior		*		*				0.523
Vigilance behavior	*	*		*				0.654
Vigilance behavior	*	*	*	*		*	*	1.039
Vigilance behavior	*	*	*	*		*		1.400
Vigilance behavior	*	*	*	*	*			1.582
Vigilance behavior	*	*		*	*			1.702
Vigilance duration		*		*				0.000
Vigilance duration		*	*	*				0.924
Vigilance duration	*	*	*	*		*		1.477
(B) Biomass response	UT		UT		UT:FT			ΔAIC_c
Total Biomass		-		-		-		0.000
Total Biomass		*						0.643
Graminoids		-		-		-		0.000
Graminoids				*				0.430
Legumes		*						0.000
Legumes		-		-		-		1.856
Non-leguminous forbs		*		*		*		0.000
(C) Soil response	UT		FT		UT:FT			ΔAIC
[Ca ²⁺]		-		-		-		0.000
[Mg ²⁺]		-		-		-		0.000
[K ⁺]		*		*		*		0.000
[P]		*						0.000

Coefficients include urine treatment (UT), fencing treatment (FT), deer age–sex class (DM adult male, DF adult female, DJ juvenile) and interactions between these variables

anticipated to decrease in the presence of wolf urine, while vigilance was expected to increase. For each response, we ran models which included main effects of and the two-way interaction between urine treatment and age–sex class. We ran a GLMM with a Poisson error distribution and a log-link function to analyze deer counts, GLMMs with a binomial

error distribution and a logit-link function to analyze the presence of foraging or vigilance, and an LMM to analyze visit duration.

We further examined whether the temporal pattern of deer activity at urine-treated sites differed from that at control sites by fitting kernel-density functions to the distribution

of independent deer sightings across the 24-h day and calculating overlap between the two densities (function *overlapEst*, package *overlap*; Meredith and Ridout 2014). We used the general nonparametric estimator Δ_4^* for sample sizes > 75 (Ridout and Linkie 2009) and bootstrapped the data $1000\times$ to generate mean and 95% confidence intervals for the amount of temporal overlap (function *bootEst*, package *overlap*; Meredith and Ridout 2014). Additionally, we evaluated how the duration of visits and proportion visits in which vigilance and foraging were detected changed across the diel cycle. We divided the day into four periods: dawn (1 h before and after sunrise), day (1 h after sunrise to 1 h before sunset), dusk (1 h before and after sunset), and night (1 h after sunset to 1 h before sunrise). For each camera, we calculated the relative visit duration and relative vigilance or foraging activity by dividing the total duration of visits or total number of observations in behaviors were detected by the number of camera trap-hours in each period (dawn and dusk: 2 h, day: 13.5 h, night: 6.5 h) (sensu "Relative Activity Index"; Carbone et al. 2001). We used LMMs to analyze how these values were affected by the interaction between treatment and period, with camera trap site included as a random intercept. We performed post hoc comparisons on interacting factors using the function 'lsmeans' (package 'lsmeans', Lenth 2016).

Plant biomass production

We tested whether deer responses to wolf urine decreased the difference in peak aboveground biomass of herbaceous plants observed in the unfenced and fenced enclosure plots. We expected less plant biomass in the unfenced plots than inside the fenced enclosures, due to plant consumption by deer; however, if wolf cues reduced local deer abundance, frequency or duration of their visits, or time spent grazing, then the effects of enclosures on plant biomass would be reduced. Peak aboveground plant biomass inside enclosures provides a reasonable approximation of annual aboveground net primary productivity in grasslands at our site, where all aboveground herbaceous plant tissues die each winter. We tested the effects of interaction between the urine and plot (fenced/unfenced) treatments on biomass (g/m^2) using an LMM that accounted for the split-plot treatment design, where urine treatments were randomly assigned to sites and fencing treatments randomized to plots. The model included main effects of, and the two-way interaction between the urine (site) and fencing (plot) treatment.

Plant community composition

To examine if deer preference for legumes and other forbs over graminoids was reduced in urine-treatment sites, we tested whether graminoid, legume, and non-leguminous forb

biomass differed by treatment. We constructed LMMs for each plant functional group, with plant biomass (g/m^2) as the response. Main effects included urine and fencing treatments and their interactions.

Soil responses

If deer avoid or forage less in the presence of wolf urine, we anticipated that increased plant growth in these sites would deplete the soils of nutrients. Ca^{2+} , Mg^{2+} , K^+ , and phosphate (H_2PO_4^- , HPO_4^{2-}) were present in high enough concentrations ($\text{mg}/10\text{ cm}^2/\text{burial length}$) to be detected by the probes. We ran LMMs to examine the main and interacted effects of urine and fencing treatments on each ion concentration.

Results

Deer behavior

From 05-June-2018 to 12-Aug-2018, the cameras captured 14,516 deer pictures, representing 756 unique visitation bouts. Urine treatment had no effect on deer visitation ($p_{\text{urine}} = 0.727$, $z\text{-value} = 0.349$; Fig. 1a), feeding activity ($p_{\text{urine}} = 0.358$, $z\text{-value} = 0.920$; Fig. 1b), or vigilance ($p_{\text{urine}} = 0.346$, $z\text{-value} = 0.943$; Fig. 1c) at each site (Table 1a). Independent of urine treatment, males fed during a higher percentage of visits than females or juveniles ($\beta_{\text{male}} = 0.659$, $\text{SE}_{\text{male}} = 0.293$, $p_{\text{male}} = 0.025$, $z\text{-value} = 2.247$), and males and juveniles were more often vigilant than females ($\beta_{\text{male}} = 0.916$, $\text{SE}_{\text{male}} = 0.368$, $p_{\text{male}} = 0.013$, $z\text{-value} = 2.488$; $\beta_{\text{juvenile}} = 1.201$, $\text{SE}_{\text{juvenile}} = 0.559$, $p_{\text{juvenile}} = 0.032$, $z\text{-value} = 2.147$). Wolf cues did not affect visit duration ($p_{\text{urine}} = 0.228$, $z\text{-value} = 1.207$; Fig. 1d), and while visits were longer when juveniles were present ($\beta_{\text{juvenile}} = 0.401$, $\text{SE}_{\text{juvenile}} = 0.172$, $p_{\text{juvenile}} = 0.020$, $z\text{-value} = 2.330$), no other variables influenced visitation length. Supplemental analyses suggest that there is no interaction between how females with and without juveniles present respond to the predator treatments (all $p > 0.05$; Appendix 1: Analysis S1), although females with juveniles present were vigilant in a greater proportion of visits than females without juveniles ($\beta = 1.580$, $\text{SE} = 0.669$, $z\text{-value} = 2.362$, $p = 0.018$).

Temporal patterns of deer activity at urine sites differed significantly from those at control sites. Deer at control sites were primarily active at dawn and dusk whereas those at urine sites were increasingly active during daylight (Fig. 2a). Activity patterns between treatments differed by over 20%: the bootstrapped mean overlap between the temporal activity patterns of deer in each treatment was 0.799 (95% CI 0.744–0.843). When diel activity patterns were disaggregated by treatment site (restricting analyses

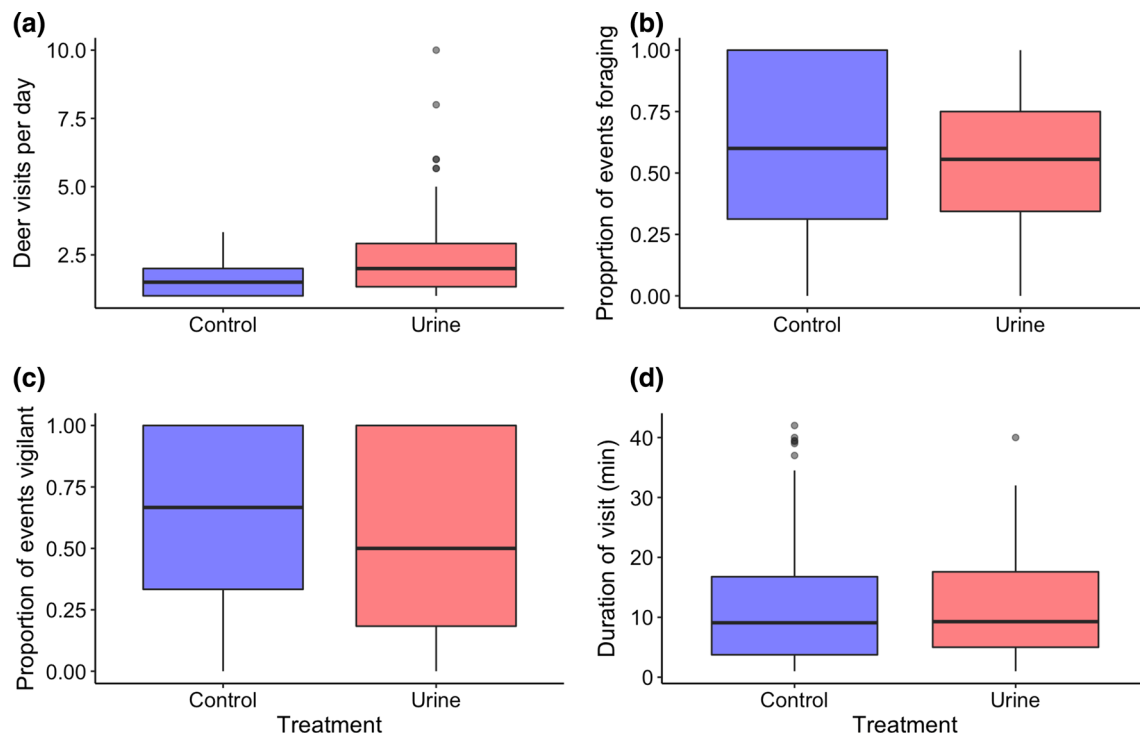


Fig. 1 Urine treatment had no significant effect on the average number of deer visits to each site per day (**a**), the proportion of visits in which foraging behavior occurred (**b**), the proportion of visits in which vigilance behavior occurred (**c**), nor the duration of these vis-

its (**d**). The black bars indicate the median, with the upper and lower ends of the boxes representing the upper and lower quartiles, respectively. Whiskers extend to the minima and maxima (excluding outliers, which are depicted as points)

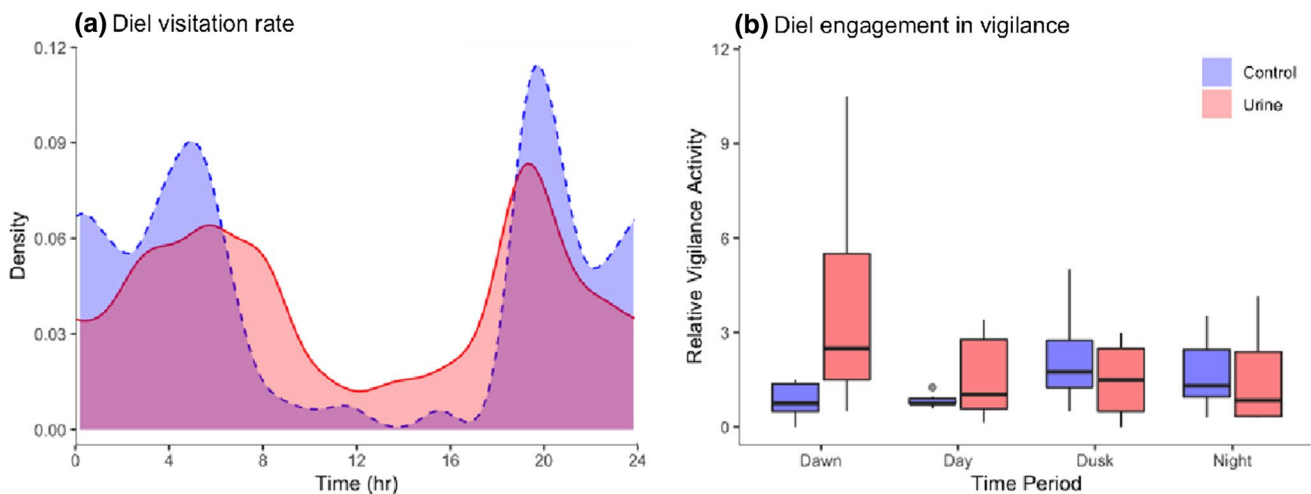


Fig. 2 a Deer were more diurnal and less crepuscular at wolf urine-treated relative to control sites. 24-h activity patterns of deer are shown at urine-treated sites (solid line) and control sites (dashed line). The degree to which these diel patterns overlap (80%) is rep-

resented by the shaded area. **b** Relative engagement in vigilance also changed across the diel cycle, with a greater proportion of visits containing vigilant deer during dawn at urine-treated sites relative to dawn at control sites or urine-treated sites during other diel periods

to the four urine-treatment and four control sites with ≥ 50 deer sightings to minimize bias), we found increasing use of urine-treated sites during the day with concomitant decrease in activity during crepuscular periods for three

of the four locations (range individual bootstrapped mean overlap: 0.595–0.812; Appendix 1: Fig. S2). We further detected shifting periods of peak activity towards the middle of the day for two locations and earlier in the mornings

and evenings for two locations relative to activity patterns at the control sites (Appendix 1: Fig. S2). The magnitude of response remained consistent throughout the study, suggesting lack of habituation to the urine cue through time (Appendix 1: Analysis S2). The duration of deer visits and relative feeding activity did not vary across the four diel time periods nor differed between treatment or control sites (duration: $p=0.160$; f -value = 1.865; feeding: $p=0.241$; f -value = 1.488). Relative vigilance, however, was elevated at urine-treatment sites relative to control sites at dawn (contrast = 3.198, SE = 1.074, df = 10, t ratio = 2.977, $p=0.014$) and higher than vigilance levels at urine sites during other diel periods (dawn–day contrast = 2.46, SE = 0.912, df = 26, t ratio = 2.702, $p=0.012$; dawn–dusk contrast = 2.764, SE = 0.963, df = 26, t ratio = 2.872, $p=0.008$; dawn–night contrast = 2.493, SE = 0.912, df = 26, t ratio = 2.734, $p=0.011$) (Fig. 2b).

Plant biomass

Urine treatment did not significantly affect peak above ground biomass across sites ($p=0.416$; z -value = 0.813) and did not interact with fencing treatment within plots in the conditional averaged top model (Table 1b). Fencing treatment did not affect primary productivity ($p=0.512$,

z -value = 0.656), although the non-significant differences were in the expected directions.

Plant community composition

We found no effects of urine or fencing treatments on the biomass of any plant functional group (Table 1b; graminoids: $p_{\text{enclosure}}=0.479$, z -value = 0.707 [urine treatment not included in top models]; legumes: $p_{\text{urine}}=0.164$, z -value = 1.392 [fencing treatment not included in top models]; non-leguminous forbs: $p_{\text{urine}}=0.1574$, z -value = - 1.494; $p_{\text{outside}}=0.420$, z -value = - 0.831; Fig. 3b).

Soil nutrients

We detected no effect of urine or fencing treatment on soil ion concentration (Table 1c; K^+ : $p_{\text{urine}}=0.360$, z -value = 0.947 [fencing treatment not included in top models]; P : $p_{\text{outside}}=0.781$, z -value = 0.284; $p_{\text{urine}}=0.220$, z -value = - 1.284; null models ranked highest for Ca^{2+} and Mg^{2+} ; Fig. 3c).

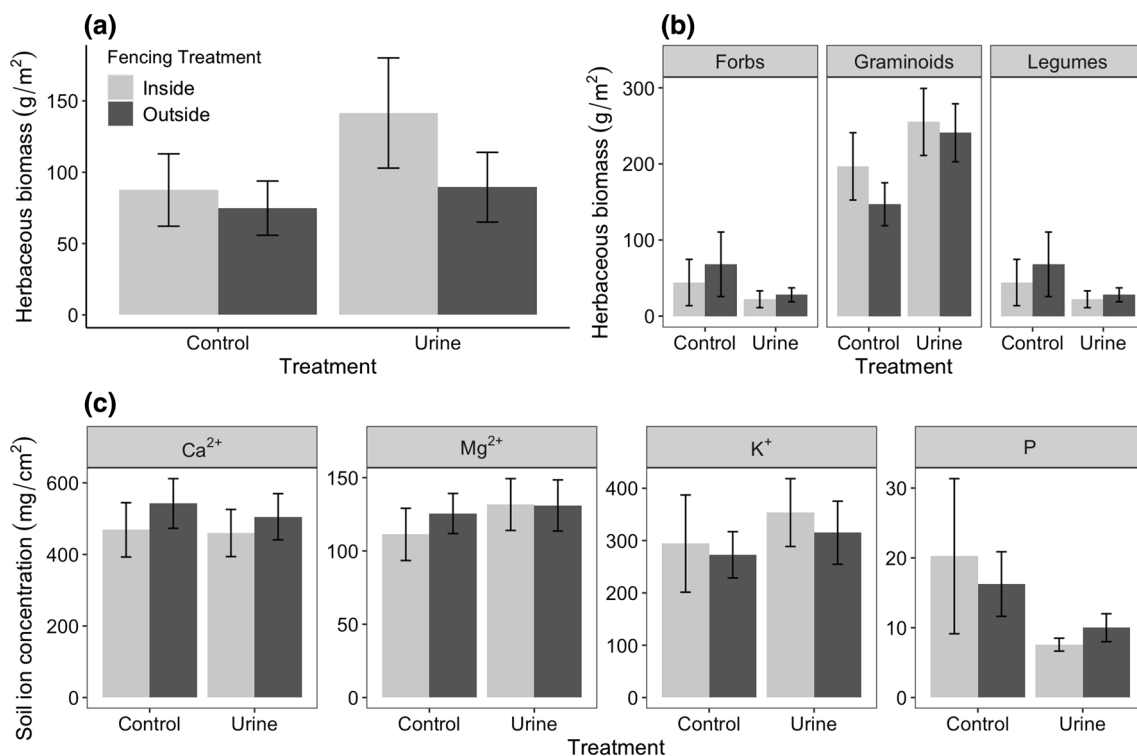


Fig. 3 Urine treatment had no significant cascading effect on the amount of herbaceous biomass produced (a), plant community composition (b), or soil ion concentrations in the fenced relative to the unfenced plots. “P” refers to phosphate ($H_2PO_4^-$, HPO_4^{2-}) anions. Error bars depict ± 1 SE

Discussion

This work contributes to the small but critical body of literature experimentally examining large predator-generated trophic cascades and provides a valuable contribution to our knowledge of the strength of behaviorally-mediated top-down pathways ('non-consumptive' or 'fear' effects) in natural systems (Callan et al. 2013; Flagel et al. 2016; Suraci et al. 2016). We did not find evidence for behaviorally-mediated simulated wolf effects on plants or soil nutrients in this temperate grassland ecosystem. Overall deer visitation rates and foraging activity remained the same in wolf urine treatment and control sites, and neither the biomass nor species compositions of plant communities nor soil nutrients differed at urine vs. control sites or between areas exposed to deer grazing and areas protected from herbivory (fenced vs. unfenced plots). However, wolf urine treatment did cause deer to change the timing of their visitation at 'risky' urine-treated sites from primarily crepuscular to increasingly diurnal, shifting activity away from what would have been wolf hunting periods to safer times of day. Such a response allows the deer to access resources across the entire spatial landscape via sensitivity to temporal fluctuations in predation risk. This alteration in spatiotemporal utilization of habitat patches may be the mechanism attenuating any top-down predator effects that might have otherwise occurred.

For predators to incite behaviorally-mediated trophic cascades, predators must change prey behavior such that prey consistently avoid 'risky' portions of the landscape and concentrate their activity in 'safe' locations (Schmitz et al. 2004; Gude et al. 2006; Cherry et al. 2016). Areas where predators occur in low densities or have poor hunting success should, therefore, experience relatively more browsing, grazing, and trampling, resulting in differing community dynamics (Schmitz et al. 2004). This heterogeneous spatial distribution of perceived predation risk is often referred to as a 'landscape of fear' (Brown et al. 1999; Laundré et al. 2001), and there exist numerous examples of prey from fish to ungulates responding to spatially structured perceived risk in an adaptive manner (Kotler et al. 1991; Makin et al. 2012; Hintz and Relyea 2017; Atkins et al. 2019). We, however, did not detect consistent avoidance of predator-cue areas, despite evidence from other systems that wolf-generated landscapes of fear produce strong avoidance responses in other large ungulate prey (Laundré et al. 2001; Fortin et al. 2005; Creel and Winnie 2005; Lashley et al. 2014). Overall deer utilization of 'safe' and 'risky' areas remained the same, and consequentially, we observed no trickle-down impact on plant or soil dynamics.

This spatial focus, however, ignores a key behavioral modification that deer made to minimize risk of predation.

Deer demonstrated fine-scale sensitivity to how predation threat would have varied across time as well as space by changing their diel activity patterns where wolf presence was simulated. Wolves are generally most active during dusk, night, and dawn (Packard 2003), and one of the best documented investigations of wolf hunting activity found that wolves primarily hunted in the mornings and evenings (Kohl et al. 2018). Assuming the wolves previously present in our area were similarly crepuscular, the deer behavior shift we observed would have allowed those potential prey to maximize their landscape use by avoiding risky areas during the dangerous times and utilizing them when temporal risk was low.

We additionally detected that deer using urine-treated areas were more vigilant during the 'risky' dawn hours than they were at control sites or at urine-treatment sites during the rest of the day. Increasing vigilance levels is a well-documented response of ungulates to mitigate heightened levels of perceived predation risk (reviewed in Quenette 1990), supporting our conclusion that deer perceived the wolf-simulated locations during wolf activity times to be particularly dangerous.

Behavioral responses to temporal fluctuations in predation risk have been studied for decades (reviewed in Kronfeld-Shor and Dayan 2003), but only recently have we begun examining prey temporal risk-sensitivity within a spatially structured risk framework (i.e., exploring the idea that spatially structured predation risk that is perceived as more or less threatening depending on temporal fluctuations in risk from the same predator; Palmer et al. 2017; Kohl et al. 2018; Smith et al. 2019). Adaptive allocation of foraging across the landscape during periods of high and low predator activity is proposed to be a mechanism by which prey mitigate fitness trade-offs, reducing the ecological impact of the landscape of fear (Kohl et al. 2018). Kohl et al. (2018) examined elk responses to 'dynamic' or temporally changing fear landscapes generated by wolves, concluding that spatiotemporal sensitivity to predation risk "may help explain evidence that wolf predation risk has no effect on elk stress levels, body condition, pregnancy, or herbivory". Our work provides the first demonstration of spatiotemporal sensitivity to wolf predation risk in white-tailed deer and is one of the first studies to examine how this spatiotemporal sensitivity may impact community dynamics in a trophic cascade context. Work in other carnivore-ungulate systems suggests these types of prey may be sensitive to risk structured across both time and space (Palmer et al. 2017; Kohl et al. 2018; Smith et al. 2019). In our system, deer navigation of this dynamic landscape of fear appears to have reduced the cascading impact of non-consumptive predator effects; the weakness of this pathway may provide a potential explanation for why trophic cascades often fail to materialize in terrestrial mammalian systems (Borer et al. 2005).

This mechanism may not be universal: a few studies which have been able to tease apart the consumptive vs. non-consumptive pathways have suggested that wolves can generate behaviorally-mediated cascades in other systems (Flagel et al. 2016). Variability in the relative strengths of consumptive vs. non-consumptive predator effects in different contexts suggests that biotic and abiotic differences between sites are important for determining the cascading consequences of predator–prey interactions (reviewed in Flagel et al. 2016). Factors such as habitat complexity (e.g., visibility, impediments, slope), prey size, and prey social structure can influence wolf hunting strategy (Kunkel and Pletscher 2001; Peterson and Cucci 2003), which may shift relative magnitude of consumptive vs. non-consumptive effect strength (Preisser et al. 2007). Overall resource availability, inter- and intra-specific resource competition, presence of additional prey or predators, habitat type, and individual state can alter strength and type of prey reactions to predation risk with consequences for cascading community-level impacts (Trussell et al. 2006; Schmitz 2005; Preisser et al. 2007). For example, we detected a signal that deer age–sex class altered their consideration of risk–resource trade-offs. Females with juveniles, which are vulnerable to additional predators such as coyote (Grovenburg et al. 2011), were more vigilant than those without. Juveniles and males also displayed heightened vigilance, possibly social vigilance directed at their mothers (Lashley et al. 2014; Percorella et al. 2018) or increased wariness from being alone (90.16% male detections of single individual), respectively (Quenette 1990; Lashley et al. 2014). Although we could not measure duration of vigilance or how this detracted from duration of foraging, heightened vigilance is well documented to have consequences for resource acquisition (Brown 1999).

It is also important to be conscientious of the trophic response variables under consideration. Studies of cascading wolf effects primarily focus on ungulate impacts on forest plants (saplings, forbs; e.g., McLaren and Peterson 1994; Ripple et al. 2001; Hebblewhite et al. 2005; Callan et al. 2013), and while deer are known to be key determinants of grassland plant diversity (Bakker et al. 2006), species richness (McNaughton et al. 1989; Collins et al. 1998; Olff & Ritchie 1998; Knapp et al. 1999), and succession (Ritchie et al. 1998), neither patterns of herbaceous biomass nor community composition were impacted by wolf cue-induced changes in deer behavior in our system. Even if this pathway is weak in temperate grasslands, wolf predation may still play a key role in structuring these types of ecological communities through density-mediated impacts on deer populations (McLaren and Peterson 1994; Callan et al. 2013).

We note, however, that our effect sizes, while non-significant, were large. While the duration and timing of our experiment were appropriate for detecting community changes in

herbaceous plant species (Tilman 1987; Isbell and Wilsey 2011), strength of the proposed mechanism for attenuating predator effects should be interpreted with these results in mind. We found substantially, but not statistically significantly, less plant biomass outside fences. Previous results from our study location (Ritchie et al. 1998; Tilman et al. 2012) suggest that had there been more observations, less observation error, or more years of data, these effects would have likely been statistically significant. Thus, our results do not suggest that there were no effects of herbivores on plants, but rather that greater statistical power would be needed to detect these effects. Similarly, we found substantially, though not statistically significantly, more plant biomass at sites where wolf urine was applied. Thus, it is also possible that with additional data, we would have found that wolf urine treatment could increase plant biomass. Note, however, that our camera-trap data do not support the hypothesis that this would be due to reduced herbivory by deer at sites where wolf urine was applied.

A key assumption of our approach was that the choice, strength, and application frequency of wolf urine effectively simulated consistent wolf activity (Peers et al. 2018). As our goal was to create areas suggestive of long-term predator occupancy rather than immediate predator presence, we chose the regular application of wolf urine in the amount and on the timescale deposited by a pack patrolling its territory (Peters and Mech 1975). The type and amount of cue was chosen based both on the extensive experience of author LDM (60+ years studying wolves and their prey) and the large body of literature demonstrating that ungulates significantly change their anti-predator behavior in response to the scent of wolf urine (e.g., Berger et al. 2001; Chamaillé-Jammes et al. 2014; Osada et al. 2014; and references therein). Lacking predators to reinforce the consumptive consequences of the cue, fear effects may have been less than those expressed in the presence of actual wolves (Parsons et al. 2018; Peers et al. 2018); however, our supplemental analyses demonstrate that deer's magnitude of response to urine did not attenuate over time. We additionally acknowledge that risk assessment can be a multi-modal process. Nevertheless, we find it compelling that wolf urine, without scats, howls, tracks, or actual wolves, affected deer temporal anti-predator behavior in a seemingly risk-sensitive manner. We further note that the use of a single predator cue alone has been demonstrated sufficient to generate behaviorally-mediated trophic cascades (Suraci et al. 2019) and multi-generational fitness effects (reviewed in Zanette et al. 2020) in other vertebrate systems. While our findings indicate that deer did recognize this cue as indicative of predation threat, we do encourage future studies to consider other application regimes.

In summary, our results suggest that in this community, the non-consumptive predator effect we found attenuates at

the predator–prey interface and does not cascade down to affect lower trophic levels. While simulated wolf presence did alter deer behavior, deer mitigated this ‘threat’ by altering their temporal use of spatial locations treated with wolf urine. The loss of top predators from ecosystems worldwide is one of the most pervasive impacts of humans on nature, but it remains unclear whether recovery of predator populations would reverse these impacts or not (Schmitz 2004; Estes et al. 2011; Berger et al. 2001; Atkins et al. 2019). This unique experimental design, including all combinations of simulated predator (urine) and herbivore (fencing) treatments, while measuring herbivore behavior, plant productivity, and soil nutrients, enables us to begin understanding the mechanisms that underpin how and how strongly predators may impact ecosystem dynamics.

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Author contribution statement MSP conceived of the ideas; MSP, FI, and LDM designed the methodology. MSP, CP, and CP collected the data. MSP analyzed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval.

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