



# Leaf traits mediate herbivory across a nitrogen gradient differently in extirpated vs. extant prairie species

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## Abstract

Increasing nitrogen deposition threatens many grassland species with local extinction. In addition to the direct effects of nitrogen deposition, nitrogen can indirectly affect plant populations via phenotypic shifts in plant traits that influence plant susceptibility to herbivory. Here, I test how herbivory varies across an experimental nitrogen gradient and whether differences in susceptibility to herbivory might explain patterns of local species loss. Specifically, I examine how increasing nitrogen availability in a restored prairie influences leaf traits and subsequent herbivory (by leaf-chewers like insects/small mammals versus deer) and the severity of herbivore damage on confamilial pairs of extirpated versus extant species from Michigan prairies. Nitrogen increased herbivory by both leaf-chewers and deer as well as herbivore damage (proportion of leaves damaged). Leaf hairiness and specific leaf area affected patterns of herbivory following nitrogen addition, although patterns varied between extirpated vs. extant taxa and herbivory type. Nitrogen increased leaf hairiness. At high levels of nitrogen addition, hairy extant plants experienced less herbivory and damage than smooth-leaved plants. In contrast, hairy extirpated plants were more likely to experience leaf-chewer herbivory. Extirpated plants with thin leaves (high specific leaf area) were less likely to experience leaf-chewer herbivory; the opposite was true for extant species. Generally, extant species experienced more herbivory than locally extirpated species, particularly at high levels of nitrogen addition, suggesting that increasing herbivory under nutrient addition likely does not influence extirpation in this system. This study suggests that trait-mediated responses to nitrogen addition and herbivory differ between extant and extirpated species.

**Keywords** Extirpation · Leaf traits · Herbivory · Nitrogen · Prairie

## Introduction

Human activities have vastly increased the amount of nitrogen entering natural ecosystems over the past century (Galloway et al. 2004). Nitrogen deposition represents a major threat to plant diversity in grasslands (Borer et al. 2015; Hodapp et al. 2018), even at low levels of nitrogen addition (Clark and Tilman 2008; Simkin et al. 2016). The negative effects of increasing nitrogen enrichment can occur via several potential mechanisms. First, increased productivity

and increased competition for light can result in the loss of inferior, often short-statured competitors (Hautier et al. 2009). Alternatively, nitrogen enrichment's negative effects on plant populations can be via altered ecological interactions (Hernández et al. 2016).

One such interaction likely to be affected by nitrogen enrichment is plant–herbivore interactions. The effects of herbivores on plant communities are often driven by differences in productivity (Eskelinen et al. 2012). For example, herbivores can alleviate some effects of nitrogen by consuming plant biomass, thereby decreasing plant competition for light in nutrient-enriched conditions and preventing competitive exclusion of inferior competitors (Hautier et al. 2009; Borer et al. 2014). Alternatively, increasing resource availability can increase plant nutrient levels or photosynthetic rates (Throop et al. 2004). These more productive plant populations can then support larger herbivore populations (Haddad et al. 2000). Under these high-nitrogen conditions, then, plants might experience increased herbivory

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(Throop et al. 2004; Blue et al. 2011), which can have negative effects on plant population dynamics. Increased herbivory can negatively affect plant performance (Crawley 1997; Strauss and Zangrel 2002; Côté et al. 2004; Halpern and Underwood 2006; Maron and Crone 2006; Morris et al. 2007; Carson et al. 2014; Pruszenski and Hernández 2020) and reduce plant population growth rates (Louda et al. 2005; Rose et al. 2005; Maron and Kauffman 2006; Knight et al. 2009; Miller et al. 2009; Eckberg et al. 2014; Bialic-Murphy et al. 2019), potentially affecting local extinctions (Olf and Ritchie 1998). Altogether, understanding how patterns of herbivory shift under increasing nitrogen deposition may help us understand plant population declines (and subsequent local species losses) under global change (Borgström et al. 2016; Stevens et al. 2018).

If increasing nitrogen deposition increases herbivory and the resulting herbivory negatively affects individual plants and plant populations, then local species losses under nitrogen deposition might be driven in part by greater herbivory. In lieu of quantifying population declines, one method to test this hypothesis is to compare herbivory on closely related congeners in which one species is locally extinct, or extirpated (defined here as a species that has disappeared from a particular county), while the other persists (Murray et al. 2014; Zettlemoyer et al. 2021; Johnson and Zettlemoyer 2022). Like comparisons of invasive vs. native species (van Kleunen and Richardson 2007), this approach can help reveal whether extirpated species respond differently to anthropogenic changes (here, increasing nitrogen deposition and subsequent herbivory) than their more successful (i.e., still extant) counterparts and test potential mechanisms underlying species persistence versus extirpation (Bevill and Louda 2001). Specifically, if increasing herbivory under nitrogen addition played a role in local species declines, we might expect that extirpated species will experience more herbivory than extant species, especially in nitrogen-enriched conditions.

Furthermore, extirpated and extant species might differ in traits related to herbivore susceptibility, which could help us understand the potential role of herbivory in species declines (Hillebrand and Matthiessen 2009). A suite of traits such as high leaf nitrogen content, low investment in carbohydrates and carbon-based defenses, and thin leaves (high specific leaf area, or SLA) are commonly associated with herbivore susceptibility (Agrawal and Fishbein 2006; Hanley et al. 2007; Rasmann and Agrawal 2009; Carmona et al. 2011; Eskelinen et al. 2012), as well as with rapid nutrient acquisition and high growth rates (Reich et al. 1997; Wright et al. 2004). In contrast, plant defenses such as trichomes can decrease herbivory (Ågren and Schemske 1994; Handley et al. 2005). Interspecific variation in these plant traits can affect patterns of herbivore attack (Kudo 2003; Loranger et al. 2012; Burghardt 2016). Since plant traits

related to herbivore defense and consumption vary across species, locally extinct and extant species' traits may differ in ways that could impact their susceptibility to herbivory. For example, extant species in Australia tend to have high SLA and soft leaves while extinct species had low SLA and tough leaves (Kyle and Leishman 2009). Differences in toughness or SLA could correlate with differences in herbivore susceptibility between these species (Lucas et al. 2000; Eskelinen et al. 2012).

Nitrogen addition could affect plant traits that might in turn influence a plant's chances of experiencing herbivory (Navas and Garnier 2002; Abdala-Roberts et al. 2016), but fewer studies account for the indirect effects of soil abiotic factors on plant traits and herbivory (Eskelinen et al. 2012; Larios and Maron 2020; Moreira et al. 2018, 2021). For instance, nitrogen fertilization can increase plant pubescence (Bilkova et al. 2016), which should decrease herbivory, and increase SLA (Knops and Reinhart 2000), which may subsequently increase herbivory. Altogether, this suggests that nitrogen deposition might influence herbivore damage to plants via larger herbivore populations or via changes in plant traits that affect herbivore susceptibility. Given that herbivory can decrease plant performance (see above), examining (i) how individual plants respond to nutrient enrichment and subsequent herbivory and (ii) how traits mediate those responses may help link community-level patterns (e.g., decreasing biodiversity under nitrogen enrichment) to individual-level mechanisms (e.g., amount of herbivore damage received) (Maron and Crone 2006).

Here, I use an experimental nitrogen addition gradient to test how nitrogen availability affects two leaf traits (specific leaf area and leaf hairiness) and subsequent herbivory on native prairie species and how these patterns differ between locally extinct versus extant prairie species from Kalamazoo County, Michigan (MI), USA. Nitrogen influences extirpation in this system (Zettlemoyer 2022a; see below), but the potential mechanisms underlying population decline (e.g., competition for light versus increased susceptibility to herbivory under high nitrogen conditions) remain unknown. Here I ask: does nitrogen addition affect leaf traits and subsequent herbivory, and do these patterns differ between locally extinct and extant species?

## Materials and methods

I set up a nitrogen gradient experiment at the Boudeman Conservation Farm in Richland, Michigan, in May 2018. The farm hosts a 20-year-old restored prairie, last burned in fall 2015. I applied nitrogen (N; 44% time-release urea) to 3 m<sup>2</sup> plots (all separated by 3 m) at six levels, assigned randomly to each plot: 0, 1, 2, 4, 8, and 12 g m<sup>-2</sup> yr<sup>-1</sup> ( $N=6$  levels  $\times$  3 plots/level = 18 plots), designed to test a range of

nitrogen levels from natural deposition to agricultural fertilization (agricultural nitrogen fertilization recommendations range from 0.67 to 20 g N m<sup>-2</sup>; Warncke et al. 2009).

To test how locally extinct versus extant species respond to herbivory across this gradient, I selected two confamilial pairs and one triplet (Table S1) of prairie forbs in which one species is locally extinct (these species disappeared from Kalamazoo County between 1890 and 2004) while the other persists (i.e., extant) (Hanes and Hanes 1947; McKenna 2004). The selected pairs represent locally extinct species that are perennial, native, prairie specialists. Species with these three characteristics are at high risk of extirpation in Kalamazoo County (Zettlemoyer et al. 2019). Each locally extinct species was then paired with the most closely related extant species that was also a native, perennial, prairie specialist forb. Although habitat loss is certainly a significant driver of species loss in the area, more species have been lost than predicted based on the species–area relationship (Zettlemoyer and Srodes 2019). This suggests that other anthropogenic factors, such as nitrogen enrichment, may play a role in plant extinctions in this area. Indeed, nitrogen enrichment decreases population growth rates in this system and demographic responses to nitrogen differ between locally extinct and extant species (Zettlemoyer 2022a). Specifically, locally extinct species experience decreased survival and less benefit to growth from fertilization than their extant congeners (Supplementary Information Fig. S1), suggesting that increasing nitrogen enrichment influences extirpations in this area.

Seeds were sourced as locally to Michigan as possible and always from a Midwestern seed source (nurseries in order of selection: Michigan Wildflower Farm [Portland, Michigan], Naturally Native Nursey [South Bend, Indiana], Agrecol [Edgerton, Wisconsin], Prairie Moon [Winona, Minnesota]). I chose to use nursery-grown seeds to avoid any local adaptation to collection sites. I germinated the seven species in low-nutrient potting media (Sunshine Mix LP5, BFG Supply, Kalamazoo, Michigan) in the greenhouse before transplanting seedlings (all 20 cm apart) into randomly selected field locations within each plot ( $N = 10$  seedlings/species/plot  $\times$  18 plots  $\times$  7 species = 1260 seedlings) in 2018.

In July 2019, a team monitored presence/absence of herbivory by insects and small mammals (i.e., leaf-chewers), presence/absence of deer herbivory, and the total amount of herbivore damage on individual plants (Weissflog et al. 2018). Each metric addresses a different biological process. Presence/absence of herbivory reflects the probability of encountering an herbivore (Endara and Coley 2011) while amount of herbivore damage reflects plant quality. Presence/absence of leaf-chewer herbivory was indicated by chewed leaves while presence/absence of deer herbivory was indicated by removal of the top of plants. Amount of (leaf-chewer) herbivore damage was

estimated by two people in the field as the percentage of total leaves damaged. We did not estimate amount of deer damage because we could not estimate how much of a plant was removed. However, percentage of leaves damaged likely underestimates total herbivory (Ansett et al. 2016) for several reasons. First, we excluded stem damage because we could not clearly distinguish between deer, small mammal, and insect herbivore damage to plant stems. Second, if mammals removed substantial amounts of a plant, we may have limited estimates of folivory on the entire plant. However, intensive deer herbivory often occurs later in the growing season (ca. September) in this system (pers. obs.), so most herbivore damage recorded in July is likely due to insects and small mammals. Third, we estimated only folivory; other forms of herbivory like sap-feeding or gall-forming can also affect plant performance (Zvereva et al. 2010) but these were not measured here because we saw no evidence of sap-feeding or galls. Finally, herbivores could consume a large fraction of a single leaf versus a small fraction of many leaves, resulting in similar estimates of total percent damage to the entire plant; this study does not distinguish between such feeding patterns but rather focuses on total damage.

I measured two leaf traits, hairiness and specific leaf area (SLA), due to their potential responses to nitrogen addition (Laliberté et al. 2012; Bilkova et al. 2016) and effects on plant nutrient acquisition and growth rate (Reich et al. 1997; Wright et al. 2004) and susceptibility to herbivory (Eskelinen et al. 2012; Eaton and Karban 2014). I predicted that both leaf hairiness and SLA would increase under increasing nitrogen addition and hairier plants would experience less herbivory and plants with thin leaves (high SLA) would experience more herbivory. Hairiness was ranked on a scale of 0–3, with 0 meaning no hair on the leaves or stems and 3 meaning the plant had very hairy leaves and stems (following Lau et al. 2008). I estimated SLA on individual plants by hole-punching one fully expanded leaf collected approximately 1 cm from the top of the plant, drying leaf material at 70 °C for 48 h, weighing leaf material, and calculating leaf area/mass (cm<sup>2</sup> g<sup>-1</sup>). Hairiness and SLA measurements were taken simultaneously with herbivory estimates; however, measurements were taken before there was substantial deer browse (see above). Data are deposited in Figshare: <http://doi.org/10.6084/m9.figshare.15494643> (Zettlemoyer 2022b). This study tests the effects of two years of nitrogen addition on two functional traits and subsequent patterns of herbivory. However, a myriad of other unmeasured traits (e.g., leaf nitrogen content; chemical defenses) could influence herbivory. It is also possible that shifts in plant traits could be a phenotypically plastic consequence of herbivory in current (Burghardt 2016) or previous years (i.e., transgenerational plasticity; Sobral et al. 2021).

## Data analysis

### Does nitrogen addition affect leaf traits, and do its effects differ between locally extinct and extant species?

I used generalized linear mixed models (GLMMs) in the lme4 package in R v.3.3.1 (Bates et al. 2015; R Core Team 2016) to test whether nitrogen addition affects leaf traits differently in locally extinct and extant species. I conducted two GLMMs, with (1) leaf hairiness and (2) SLA as separate response variables. I included nitrogen treatment (continuous, 0–12 g N m<sup>-2</sup> yr<sup>-1</sup>), local extinction status (locally extinct vs. extant) and their interaction as predictor variables and plot (nested in nitrogen treatment) and species (nested in plant family [included to ensure comparisons are within each phylogenetic contrast], nested in status) as random factors.

### Do relationships between herbivory, nitrogen, and leaf traits differ between locally extinct and extant species?

I then tested whether relationships between nitrogen addition, plant traits, and herbivory differ between locally extinct and extant species. I conducted three GLMMs with probability of leaf-chewer herbivory (1 = yes, 0 = no; binomial distribution), probability of deer herbivory (1 = yes, 0 = no; binomial distribution), and amount of herbivore damage (given that a plant experienced herbivory; Gaussian distribution) as separate response variables. I included nitrogen treatment (continuous, 0–12 g N m<sup>-2</sup> yr<sup>-1</sup>), status, leaf traits (SLA and hairiness), two-way interactions between nitrogen × traits, and three-way interactions between nitrogen × status × traits as predictor variables. I included plot (nested in nitrogen treatment) and species (nested in family, nested in status) as random factors.

To examine species-specific patterns of herbivory, I again used three GLMMs with Pr(leaf-chewer herbivory), Pr(deer herbivory), or herbivore damage as separate response variables. I included nitrogen treatment, species, leaf traits

(hairiness and SLA), and interactions between nitrogen × traits, species × traits, and nitrogen × species × traits. Because several significant interactions between species and nitrogen treatment or plant traits were detected, I then conducted separate models for each of the seven species. Models included either Pr(leaf-chewer herbivory), Pr(deer herbivory), or herbivore damage as separate response variables, nitrogen treatment, plant traits, and interactions of nitrogen × traits as predictor variables, and plot (nested in nitrogen treatment) as a random factor.

## Results

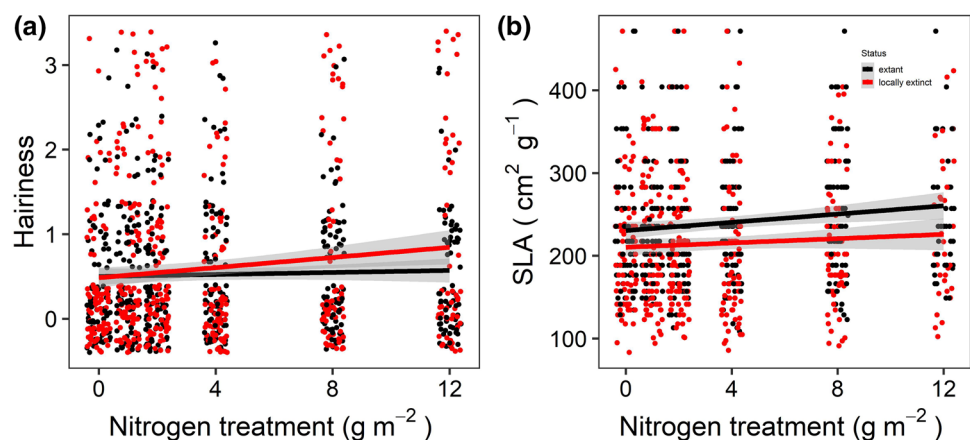
### Does nitrogen addition affect leaf traits, and do its effects differ between locally extinct and extant species?

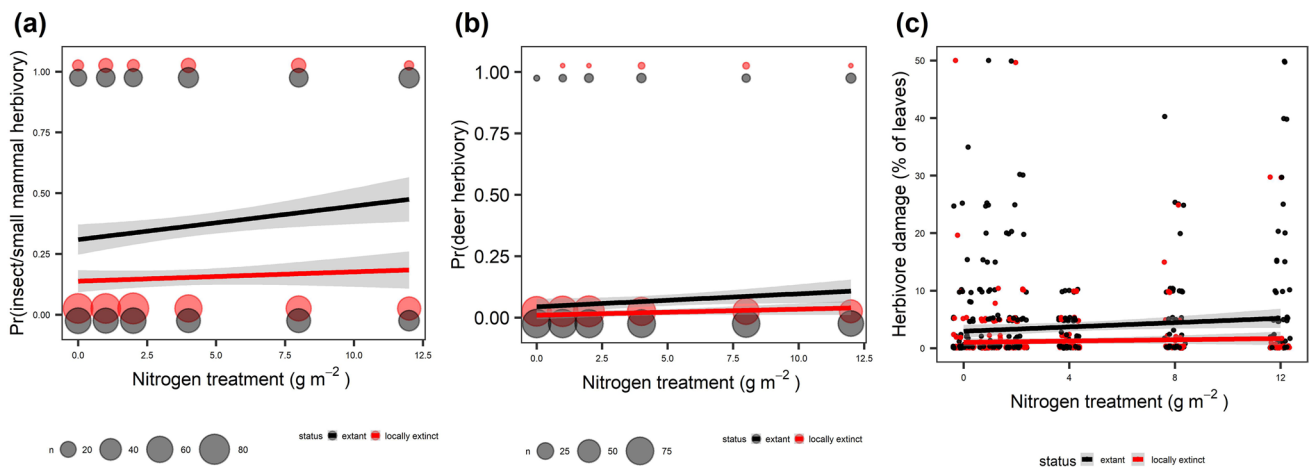
Nitrogen addition increased specific leaf area, although this effect was stronger in extant species (N × status  $\chi^2_{1,877.4} = 3.61$ ,  $p = 0.05$ ). Nitrogen also increased leaf hairiness, but only in extant species (N × status  $\chi^2_{1,876.4} = 4.29$ ,  $p = 0.03$ ; Table S2; Fig. 1). Species varied in their leaf traits and responses to nitrogen addition (N × species: SLA  $\chi^2_{1,860.01} = 22.95$ ; hairiness  $\chi^2_{1,857.66} = 43.87$ ; both  $p < 0.0001$ ; Table S2; Fig. S2), but locally extinct and extant species did not differ in their mean leaf traits (Table S2).

### Do relationships between nitrogen, leaf traits, and herbivory differ between locally extinct and extant species?

Nitrogen addition increased the likelihood of experiencing leaf-chewer and deer herbivory and the amount of herbivore damage received (N: Pr(leaf-chewer herbivory)  $\chi^2_{1,34.97} = 3.93$ ,  $p = 0.04$ ; Pr(deer herbivory)  $\chi^2_{1,60.84} = 11.03$ ,  $p = 0.0009$ ; damage  $\chi^2_{1,44.28} = 9.78$ ,  $p = 0.002$ ; Table S3; Fig. 2). Nitrogen addition increased

**Fig. 1** Effect of a nitrogen gradient (0–12 g N m<sup>-2</sup> yr<sup>-1</sup>) on **a** leaf hairiness (0–3; 0 = no hair to 3 = hairy) and **b** specific leaf area (SLA; cm<sup>2</sup> g<sup>-1</sup>) in locally extinct (red) vs. extant (black) species. Grey areas represent 95% confidence intervals. Values are jittered to show sample size





**Fig. 2** **a** Probability of insect/small mammal (leaf-chewer) herbivory, **b** probability of deer herbivory, and **c** herbivore damage (% of leaves damaged) across a nitrogen gradient (0–12 g N m<sup>-2</sup> yr<sup>-1</sup>) in locally

extinct (red) and extant (black) prairie species. Dot sizes in **a–b** are scaled by the number of individuals (*n*)

herbivory (all metrics) in extant *Ratibida pinnata* and increased herbivore damage on extant *Penstemon digitalis* and locally extinct *P. hirsutus* (Table S4). However, the effects of nitrogen on herbivory depended on leaf traits and local extinction status.

Hairier extant plants were more likely to experience deer herbivory and received more herbivore damage, while hairiness did not affect deer herbivory in locally extinct species (status × hairiness: Pr(deer herbivory)  $\chi^2_{1,865.9} = 3.77$ ; damage  $\chi^2_{1,213.6} = 3.36$ ; both  $p = 0.05$ ; Table S3). This pattern depended somewhat on nitrogen addition. Hairier extant plants were more likely to experience leaf-chewer herbivory than smoother-leaved extant plants at low levels of nitrogen addition (1–2 g N m<sup>-2</sup> yr<sup>-1</sup>). In contrast, hairier extant plants experienced less leaf-chewer herbivory at high levels of nitrogen addition and under natural field conditions (no nitrogen added) (N × status × hairiness  $\chi^2_{1,868.3} = 2.84$ ,  $p = 0.09$ ; Table S3; Fig. 3a). Patterns for deer herbivory and herbivore damage on extant plants were similar to those for leaf-chewer herbivory: hairier extant individuals were more likely to experience deer herbivory (and received more damage) at low levels of nitrogen addition (0–2 g N m<sup>-2</sup> yr<sup>-1</sup>). However, hairy extant individuals were less likely to experience deer herbivory (and received less damage) at higher levels of nitrogen addition (N × status × hairiness: Pr(deer herbivory)  $\chi^2_{1,871.8} = 5.30$ ,  $p = 0.02$ ; damage  $\chi^2_{1,210.8} = 3.01$ ,  $p = 0.08$ ; Table S3; Fig. 3b). These patterns for extant species were largely driven by extant *Ratibida pinnata* (N × hairiness: Pr(leaf-chewer herbivory)  $\chi^2_{1,142.67} = 6.10$ ,  $p = 0.01$ ; Pr(deer herbivory)  $\chi^2_{1,143} = 13.01$ ,  $p = 0.003$ ; damage  $\chi^2_{1,42.31} = 6.62$ ,  $p = 0.01$ ; Table S4; Figs. S3–5). Meanwhile, hairy individuals of locally extinct *Penstemon hirsutus* and *P. pallidus*, as well as extant *Monarda fistulosa*, tended to experience less damage (hairiness: PH  $\chi^2_{1,20.18} = 2.92$ ,

$p = 0.1$ ; PP  $\chi^2_{1,6.25} = 2.79$ ,  $p = 0.09$ ; MF  $\chi^2_{1,48.52} = 3.41$ ,  $p = 0.06$ ; Table S4; Fig. S5).

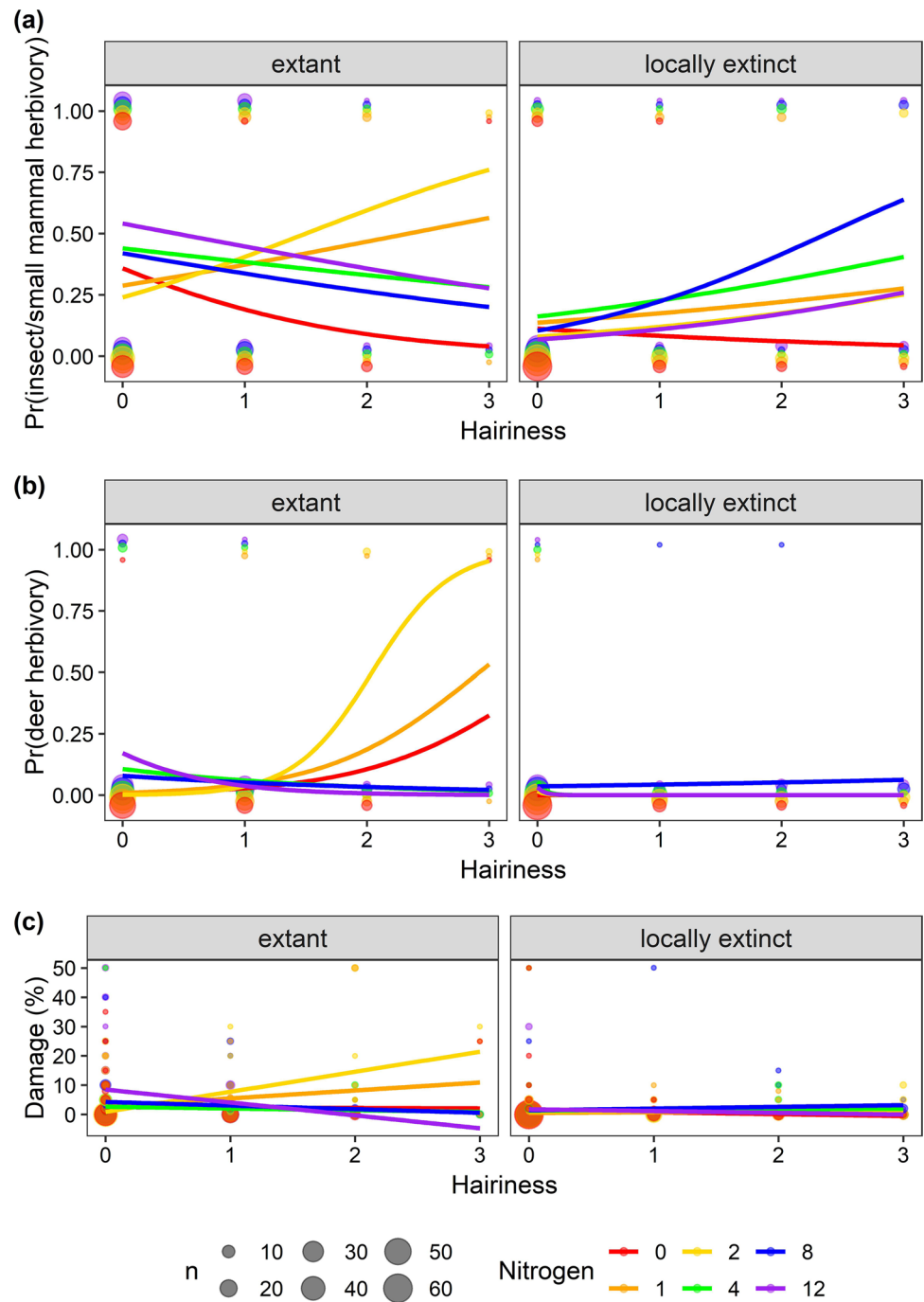
Specific leaf area affected leaf-chewer herbivory differently in locally extinct vs. extant species (Table S3; Fig. 4). Thin-leaved extant individuals were more likely while thin-leaved locally extinct species were less likely to experience leaf-chewer herbivory than thicker-leaved individuals (status × SLA  $\chi^2_{1,862.5} = 4.84$ ,  $p = 0.02$ ; Table S3; Fig. 4a). Thin-leaved individuals of locally extinct *Ratibida columnifera* tended to experience less leaf-chewer herbivory and less herbivore damage (SLA: Pr(herbivory)  $\chi^2_{1,85} = 2.39$ ,  $p = 0.1$ ; damage  $\chi^2_{1,5.58} = 31.14$ ,  $p < 0.0001$ ; Table S4; Figs. S3, 5). Otherwise, SLA did not affect the likelihood of deer herbivory or amount of herbivore damage (Table S3).

Finally, extant species tended to experience more leaf-chewer herbivory than locally extinct species, particularly as nitrogen addition increased (N × status  $\chi^2_{1,895.05} = 2.17$ ,  $p = 0.09$ ; Fig. 2a). On average, locally extinct and extant species experienced similar deer herbivory and herbivore damage (Table S3). These data suggest that increased herbivory following nitrogen enrichment likely did not influence local species declines in Kalamazoo County.

## Discussion

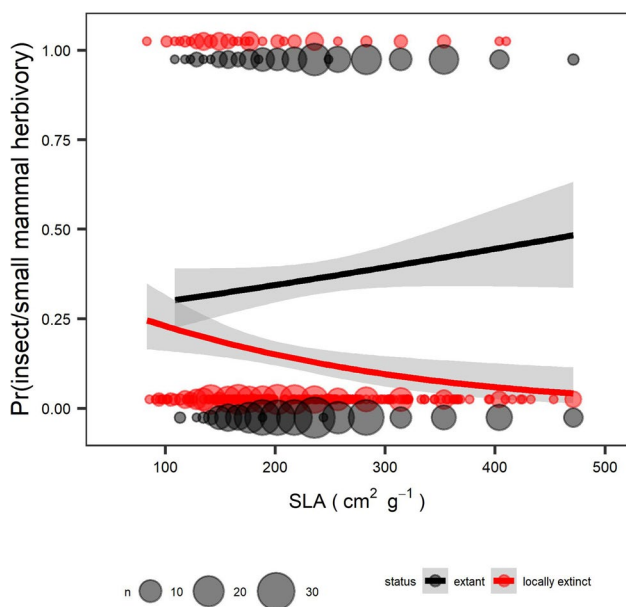
This study investigates how plant traits affect herbivore consumption in response to nitrogen enrichment. Increasing levels of nitrogen addition increased the likelihood of herbivory by leaf-chewers (i.e., insects and small mammals) and deer as well as the amount of herbivore damage plants received. However, nitrogen enrichment affected herbivory differently depending on two leaf traits, leaf hairiness and specific leaf area, and patterns

**Fig. 3** Effect of leaf hairiness (0=no hair to 3=very hairy) on **a** probability of insect/small mammal (leaf-chewer) herbivory, **b** probability of deer herbivory, and **c** herbivore damage (% of leaves damaged) across a nitrogen gradient (0–12 g N m<sup>-2</sup> yr<sup>-1</sup>) in extant (left) vs. locally extinct (right) species. Dot sizes are scaled by the number of individuals (*n*)



differed between locally extinct and extant taxa. Nitrogen increased leaf hairiness, especially in extant species, and hairy extant plants were less likely to experience herbivory at high levels of nitrogen addition. At low levels of nitrogen addition, hairy extant plants instead experienced more herbivory. Nitrogen also increased specific leaf area (i.e., thinner leaves) in extant species, and thin-leaved extant plants were more likely to experience leaf-chewer herbivory (the opposite was true for locally extinct species). Additionally, extant species experienced more leaf-chewer

herbivory than locally extinct species under high levels of nitrogen addition (and similar levels of deer herbivory and herbivore damage), suggesting that increasing susceptibility to herbivory under nitrogen enrichment did not influence extirpation events in these species. Altogether, this study suggests that a major global change driver, nitrogen deposition, can affect plant–herbivore interactions via shifts in leaf traits and that plant traits mediate susceptibility to herbivory differently in locally extinct versus extant taxa.



**Fig. 4** Effect of specific leaf area (SLA;  $\text{cm}^2 \text{g}^{-1}$ ) on the probability of insect/small mammal (leaf-chewer) herbivory in locally extinct (red) vs. extant (black) species. Dot sizes are scaled by the number of individuals ( $n$ ). Grey areas represent 95% confidence intervals

### Nitrogen increases frequency and severity of herbivore damage

Herbivory by leaf-chewers and deer increased across a nitrogen gradient, corresponding with predictions of greater herbivory in areas with higher nitrogen deposition (Haddad et al. 2000; Throop and Lerdaun 2004; Majetic et al. 2017; Chen et al. 2019). The direct effect of nitrogen on herbivory detected here could be due to greater plant productivity, which could support larger herbivore populations (Pöyry et al. 2017). Although we did not collect biomass on the plants measured in this experiment to avoid destructive sampling, light measurements at the plot level indicate decreasing photosynthetic active radiation at higher levels of nitrogen addition (Fig. S6). Nitrogen also increases plant height ( $\chi^2_{1,22,7} = 8.36$ ,  $p = 0.004$ ; Fig. S7), suggesting that increasing nitrogen addition correlates with increasing biomass in this system. Future work should quantify changes in plant biomass (and how much herbivore damage increases relative to those changes in biomass) as well as local herbivore population sizes across the nitrogen gradient. Overall, this result supports the hypothesis that herbivory should be higher in resource-rich environments.

### Nitrogen indirectly increases insect herbivory via shifts in plant traits

Nitrogen addition indirectly affected herbivory via its effects on two leaf traits: leaf hairiness and specific leaf

area. Increasing nitrogen levels produced thinner plant leaves (high SLA), as found in other grassland ecosystems (McIntyre 2008; Laliberté et al. 2012). High SLA typically correlates with fast growth and less investment in herbivore defense (Endara and Coley 2011; Reese et al. 2016). Thinner-leaved extant individuals were both more likely to receive herbivory from insects and small mammals and experienced more herbivore damage, similar to other studies associating high SLA with high levels of herbivory (Cingolani et al. 2005; Eskelinen et al. 2012). However, locally extinct plants with thinner leaves instead experienced less herbivory. This effect is partially driven by locally extinct *Pycnanthemum tenuifolium*, which has thin leaves but experienced almost no herbivory (Figs. S3–5; model without *P. tenuifolium* status  $\times$  SLA  $\chi^2_{1,712,72} = 3.42$ ,  $p = 0.06$ ). Alternatively, nitrogen could affect herbivory through other unmeasured traits (e.g., shifts in leaf nitrogen content under nitrogen addition or chemical defenses).

Increasing nitrogen levels produced hairier plants. Abiotic stressors, such as nitrogen, drought, and temperature, can increase trichome densities (i.e., hairiness), as seen in *Artemisia annua* (Bilkova et al. 2016), *Tillandsia* spp. (Benz and Martin 2006), and *Raphanus raphanistrum* (Agrawal et al. 2004). Increasing hairiness subsequently affected patterns of herbivory under nitrogen enrichment in this system, although patterns differed depending on local extinction status. Hairiness did not affect herbivory on locally extinct species overall. However, the two hairiest locally extinct species (*Penstemon hirsutus* and *P. pallidus*) tended to experience less herbivore damage as leaf hairiness increased, regardless of nitrogen addition. In extant species, hairier plants were less likely than smooth-leaved plants to experience herbivory at high levels of nitrogen addition. Herbivore damage also followed this pattern, with decreased damage on the hairiest plants at high levels of nitrogen addition. This finding is consistent with trichomes' role as a defense mechanism (Levin 1973; Agrawal and Fishbein 2006; Eaton and Karban 2014). However, at low levels of nitrogen addition, hairy extant individuals instead experienced greater herbivory. Given that the hairiest individuals were found at high nitrogen levels (such that individuals at low levels of nitrogen have fewer leaf trichomes), this may be due to differential defense production across nutrient gradients. At low levels of nitrogen addition, it might be less costly to grow fast than to produce more structural tissue (Burghardt and Schmitz 2015). Alternatively, large herbivores such as grasshoppers, which are abundant in this prairie, can be less affected by trichomes, as found in *Arctostaphylos* species (Andres and Connor 2003).

## Conclusions and implications for management

This study indicates that intraspecific variation in plant traits can mediate plant–herbivore interactions following nitrogen enrichment. Increasing nitrogen addition correlated with increased herbivory and herbivore damage. Herbivory was similar between locally extinct and extant species, likely due to similarity in their mean leaf traits. Similarly, these species do not differ in seed traits or rates of seed predation (Johnson and Zettlemoyer 2022). This finding suggests that herbivory following nitrogen enrichment likely did not influence species losses among the forbs studied here. Future work in this system should investigate whether observed lower population growth rates and demographic differences between locally extinct and extant species are instead due to competitive differences under nitrogen addition. However, leaf traits mediated herbivory differently between locally extinct and extant species, suggesting that locally extinct and extant taxa differ somewhat in their vulnerability to enemies. These results have several implications for using plant traits in conservation and management (Clark et al. 2012). First, relationships between leaf traits and the frequency and severity of herbivory varied with nitrogen fertilization, similar to studies finding that soil histories affect herbivore feeding patterns (Heinen et al. 2018), suggesting that plant–soil–herbivore interactions should be considered in restorations (Kaplan et al. 2018). Second, land managers may need to consider how nitrogen enrichment might increase or decrease plant susceptibility to herbivores and the need for pesticides or fences. For instance, leaf hairiness only correlated with lower herbivory (i.e., as a defense mechanism) at high levels of nitrogen. Third, relationships between plant traits, nitrogen, and herbivory differed between more “successful” (extant) vs. threatened species. Extant species demonstrated greater plasticity in leaf hairiness and SLA than locally extinct species in response to nitrogen addition, potentially benefiting these species in changing environments (Liancourt et al. 2015). Additionally, thicker leaved locally extinct individuals experienced more herbivory from leaf-chewers, in contrast to predictions that thinner leaves should correlate with more herbivory. This suggests that prairie restorations trying to reintroduce rare or threatened species might need to consider how plant traits influence herbivory differently in rare species relative to more common ones. As nitrogen deposition is one of the most dominant environmental changes occurring globally, these results provide insight into the effects of global change on plant–herbivore interactions and local species losses.

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**Data availability** All data for this study are available in the FigShare Data Repository, <http://doi.org/0.6084/m9.figshare.15494643>.

## Declarations

**Conflict of interest** The author has no conflicts of interest to report.

**Ethics approval** Ethics approval was not required for this study.

**Consent to participate** Not applicable.

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