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Nitrogen inputs suppress plant diversity by overriding consumer control

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Anthropogenic nitrogen (N) deposition presents a global threat to ecosystem functions. In terrestrial ecosystems, N-deposition is predicted to increase plant productivity but reduce diversity by bolstering dominant plants that suppress subordinate species. However, herbivores are predicted to offset these effects by consuming excess biomass produced by N-deposition. Here, we use a multifactorial field experiment in a grassland ecosystem to investigate the effects of N-addition on top-down control by herbivores and plant diversity. We show that at ambient N, grasshoppers suppress total plant biomass and community dominance sufficiently to increase plant Shannon diversity, indicating top-down control. Without grasshoppers, N-addition increases total plant biomass by promoting the community dominant and suppressing some subordinates as predicted, but it does not affect plant Shannon diversity relative to ambient-N levels. However, with grasshoppers, N-addition eliminates herbivore controls while simultaneously increasing total plant biomass and community dominance, triggering a 21% plant Shannon diversity loss compared to ambient-N conditions. Mechanistically, we find that N-addition disrupts top-down control by reducing herbivore abundance via effects on (1) plant chemistry, which diminishes food quality, and (2) plant architecture, which elevates predatory spider abundance and lethality. Therefore, we show that N-deposition can toggle system controls from top-down to bottom-up, to the detriment of plant diversity.

Anthropogenic nitrogen (N) deposition can profoundly alter ecosystem functions[1–3]. N deposition is generally expected to increase plant productivity but decrease plant diversity in terrestrial ecosystems[2–4]. These outcomes arise from enhanced community dominance, wherein N deposition increases the biomass of one or a few dominant plant species, exacerbating competitive exclusion of subordinate species[5–8]. However, herbivores could potentially counteract these bottom-up effects by consuming the excess plant biomass stimulated by N deposition[9–11]. Several processes could facilitate such trophic feedbacks. First, herbivores can exert top-down effects by suppressing

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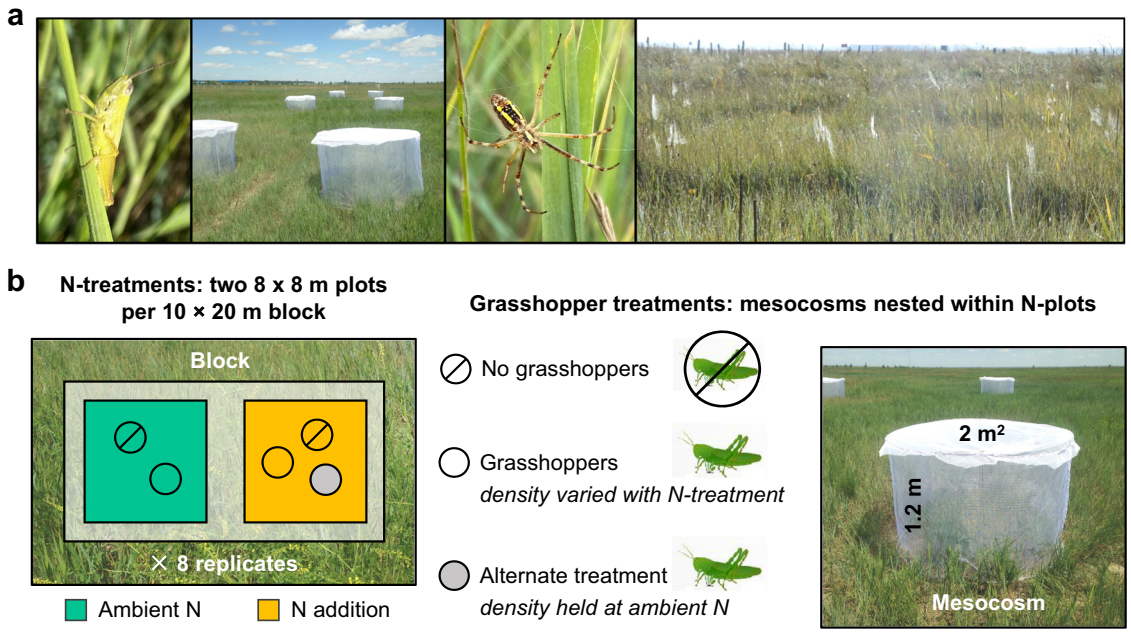

Fig. 1 | Study system and experimental design. a Our study system (from left to right) includes *Euchorthippus* grasshoppers, which feed primarily on the dominant grass, *Leymus chinensis*, and were manipulated using mesocosms (cages covered with mesh). The dominant spider, *Argiope bruennichi*, preys upon *Euchorthippus* via webs built on *L. chinensis*. **b** Grasshopper mesocosms were nested within paired, nitrogen (N)-treatment plots representing either ambient N or N addition. In the main experiment, grasshoppers were either removed (no grasshoppers) or stocked to match density in the surrounding N-treatment plot (grasshoppers). We also included an alternate grasshopper treatment in N-addition plots that was stocked to match the density in adjacent, ambient-N plots. For details, see "Methods" section. Credits: Xiaofei Li (illustrations) and Zhiwei Zhong (all photographs).

biomass of dominant plant species, thereby releasing subordinate species from competition and enhancing diversity[12–15]. Second, herbivores are presumed to be limited by food availability, and hence they are expected to increase consumption in response to N-driven increases in plant production[9–12,14]. Finally, N deposition can alter plant chemistry by elevating N content, which can in turn increase food quality, herbivore performance, and herbivore abundance[3,9,16]. Theoretically, these processes should generate trophic feedbacks sufficient to offset N-deposition effects on plant communities.

While these ideas are intuitively appealing, broader theory suggests that the net effect of bottom-up and top-down processes on plant productivity and diversity is determined by a variety of context-dependent factors[17,18]. For example, herbivores can suppress or increase plant biomass and diversity depending on dietary preferences, i.e., whether herbivores selectively consume dominant or subordinate species[13,19–21]. Moreover, while increased plant N content may benefit many generalist herbivores as commonly assumed, recent global syntheses indicate that increased N can shift overall plant chemistry and create nutrient imbalances (shifts in stoichiometry) that reduce fitness for many other consumers, particularly specialist herbivores[22–24]. At higher trophic levels, predator preferences and hunting modes can determine whether top-down effects of predators impact herbivores enough to generate trophic cascades that alter plant communities[25,26] or whether top-down effects attenuate at lower trophic levels[27]. Finally, ecosystem engineering initiated by or mediated through plants can influence a variety of processes, including plant-herbivore and predator-herbivore interactions in ways that enhance or weaken top-down effects of predators and herbivores[28–31]. Hence, system-level outcomes of N deposition are likely to be context dependent, contingent upon a variety of complex interactions among plants, herbivores, and predators. That said, current literature emphasizes the simplified idea that N inputs should, if anything, increase top-down effects of herbivory to offset bottom-up effects of N deposition on terrestrial plant productivity and diversity[3,5,11,32].

We test this prediction by conducting a 3-year field experiment quantifying the effects of N-addition and trophic feedbacks on plant biomass and diversity in a semi-arid grassland of northeast China. This system is dominated by the perennial grass, *Leymus chinensis*, which strongly suppresses other grasses and forbs[33]. The study system hosts high densities of grasshoppers (*Euchorthippus* spp.) that feed predominantly on *L. chinensis*. Like grasshopper species in other ecosystems[34–37], *Euchorthippus* grasshoppers can exert strong top-down effects on plant diversity and composition[38]. The orb-weaving spider *Argiope bruennichi* (Araneae: Araneidae) is a dominant predator that builds its webs on *L. chinensis* and preys upon *Euchorthippus* spp[31]. We experimentally manipulate this system to evaluate the interactive effects of N-addition and grasshopper herbivory on plant biomass and diversity while accounting for N effects on plant traits, abiotic conditions, herbivore and predator abundance, and predator-herbivore interactions (Fig. 1).

Results and discussion

Interactive effects of N and herbivores on plant communities

Our main field experiment showed that N-addition can disrupt the top-down effects commonly predicted to offset N inputs on plant biomass and diversity. At ambient N, top-down effects of herbivores were strong: grasshoppers suppressed biomass of the community dominant, the grass *L. chinensis*, sufficiently to reduce total plant biomass and increase forb biomass and Shannon diversity (Fig. 2). Theoretically, such a baseline system driven by strong consumer control, wherein specialist herbivores are capable of suppressing an N-limited dominant plant, is an ideal setup for consumer feedbacks to offset effects of N inputs. However, instead of enhancing top-down control, N-addition eliminated top-down control by herbivores (Fig. 2: compare ±grasshopper treatments, solid orange vs blue symbols, at each N-level) while simultaneously generating bottom-up effects on plant biomass and diversity (Fig. 2: compare ±N treatments, represented by solid lines, at each grasshopper level). As a result, N-induced changes in communities were greater in the presence of herbivores than in their

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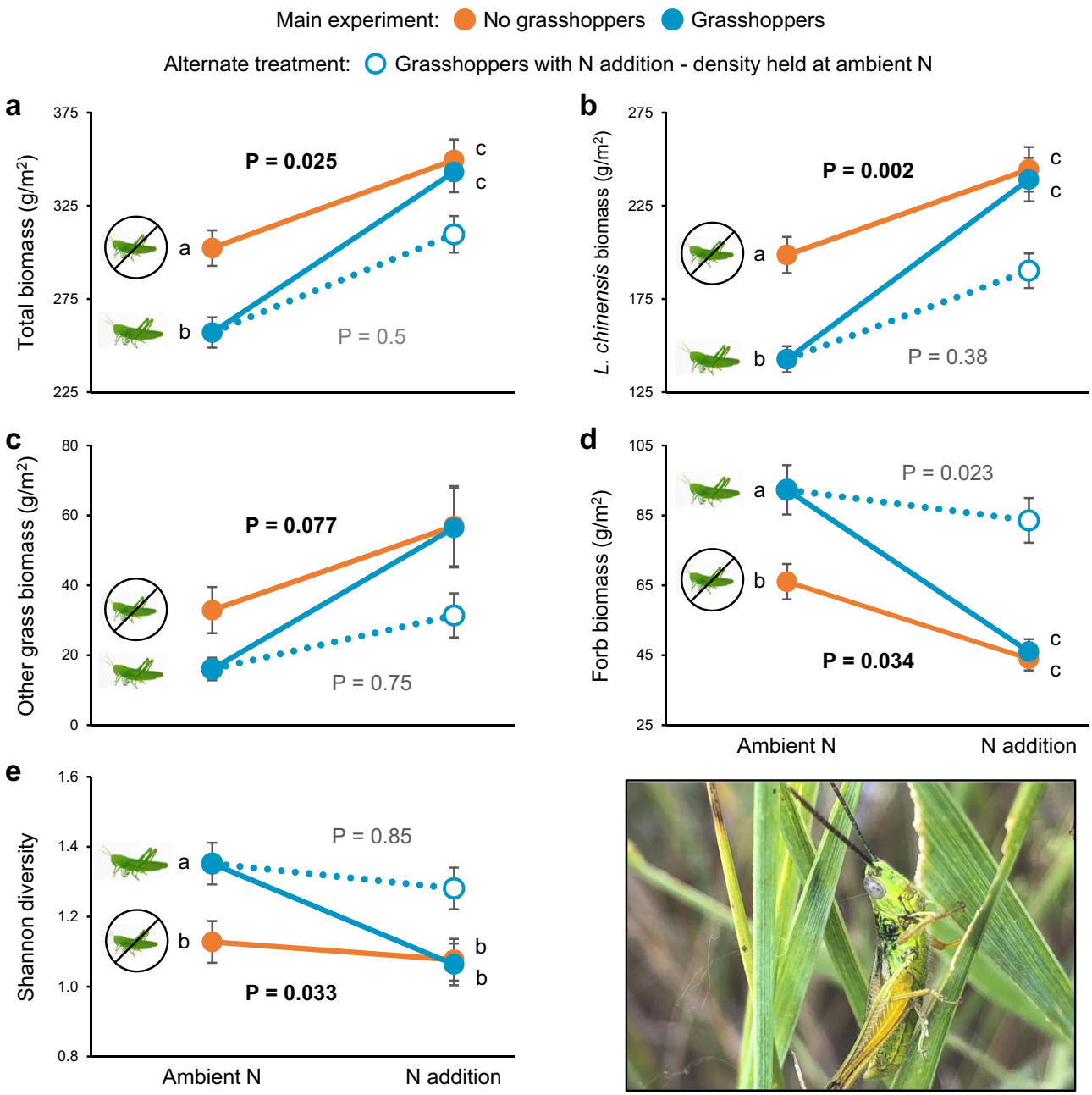

Fig. 2 | Effects of nitrogen (N) addition and grasshoppers on plant communities. Mean ± 1 SE **a** total aboveground biomass; aboveground biomass of **b** the dominant grass (*Leymus chinensis*), **c** other grasses, and **d** forbs; and **e** plant species diversity (Shannon index); as measured in mesocosms ($n = 8$ per treatment) in 2018 after three years of treatment. For each response, we used a single generalized linear mixed model (log-normal distribution) to set up contrasts, with the primary goal of testing for interactive effects of N (ambient or N addition) and grasshoppers (±) under two experimental scenarios. In the main experiment, grasshopper densities in +grasshopper treatments were stocked to match levels in surrounding N-treatment plots and therefore varied with N (solid blue symbols). In our alternate experiment, grasshopper densities in the +grasshopper, N-addition treatment were held at ambient-N levels to control for N-induced effects (open blue symbols). Lines depict the N-addition x grasshopper interaction, signifying the change in plant metrics caused by N addition as compared between no-grasshopper treatments (orange lines) and the relevant +grasshopper treatments corresponding to either the main or alternate experiment (solid or dotted blue lines, respectively), with two-sided P values given for the interaction in each scenario (bold or gray text, respectively). The interaction was further evaluated with two-sided Bonferroni post-hoc comparisons when significant ($P < 0.05$), with differences among means in the main experiment denoted with letters (means that do not share letters are different). Note that other grasses followed a similar pattern as seen for *L. chinensis*, but only the main N-addition effect was significant ($P < 0.001$). See Supplementary Tables 1 and 2 for full test statistics, including analysis of species richness, which did not differ among treatments ($P > 0.5$). Source data are provided as a Source Data file. Credits: Xiaofei Li (illustrations) and Zhiwei Zhong (photograph).

absence. Specifically, in the absence of grasshoppers, N-addition elevated total plant biomass by 16% relative to ambient-N levels by bolstering biomass of the dominant species, *L. chinensis*, which suppressed forb biomass but did not significantly reduce plant diversity (Fig. 2: solid orange lines). In contrast, in the presence of grasshoppers—the condition reflecting the natural system—N-addition elevated total biomass by 34% and reduced Shannon diversity by 21% compared to ambient N-levels (Fig. 2: solid blue lines), as increased *L. chinensis* dominance drove declines in forb abundance (species richness did not change significantly; Supplementary Table 1). Importantly, total plant biomass and diversity attained the same levels in N-addition treatments, whether grasshoppers were present or not in our main

experiment, which might suggest that herbivores did not influence N-addition outcomes. However, our experiments revealed that grasshoppers strongly controlled plant community metrics under ambient N, and it was the loss of this top-down control that drove N-addition outcomes. Given these counterintuitive results, identifying the mechanisms by which N addition can suppress top-down control from herbivores is essential for predicting outcomes across systems.

Herbivore density effects

Mechanistically, N addition negated top-down controls in our system by reducing grasshopper abundance. By measuring system responses to N treatments in large open plots, we revealed that grasshopper abundance declined by 53% in response to N-addition (Fig. 3a and Supplementary Table 3), while grasshopper behaviors (e.g., feeding and jumping) did not differ significantly between N-treatments (Supplementary Table 4). Using this information to guide manipulations of grasshopper abundance within embedded mesocosms, we found that that top-down effects of grasshoppers on plant communities were 1) lost in N-addition plots when we experimentally simulated grasshopper declines found in open field plots of our main experiment but 2) retained when we held grasshopper abundance at ambient-N levels, i.e., controlled for N-induced herbivore declines (Fig. 2 and Supplementary Tables 1, 2). These supplementary experiments provide direct evidence that N-addition disrupted top-down controls by reducing grasshopper abundance.

Potential causal pathways

We explored three plausible pathways that might explain N-induced grasshopper declines. First, N-addition commonly elevates N concentrations in plant tissues, which can alter plant chemistry and shift nutrient balances (stoichiometry) in ways that influence herbivore fitness and foraging behavior[9,16,23,24,39]. We found that *L. chinensis* leaves in N-addition plots had nearly twice the N content than that found in ambient-N plots (Fig. 4a). This large change in N was accompanied by small increases in plant secondary compounds, specifically phenols (3% increase) and tannins (2% increase, albeit marginally significant), with no change in leaf carbon, the latter of which translates to lower C:N ratios (Fig. 4b–d). Hence, N-addition clearly altered the stoichiometry of *L. chinensis* leaves, the primary food for our grasshoppers. This result is consistent with previous experimental work showing that N-addition not only alters C:N ratios and concentrations of plant secondary compounds in *L. chinensis* but can also elevate protein and cellulose content[40]. In the present study, leaf N correlated negatively with overall grasshopper abundance across N-treatment plots ($R^2 = 50\%$, df = 14, $P = 0.002$; Supplementary Fig. 1a), suggesting a direct link between elevated leaf N and grasshopper population declines. Importantly, increased leaf N may be a marker for a multitude of chemical shifts[40], which we did not isolate. Interestingly, responses to N-addition differed for the two grasshopper species in our experiment, in alignment with their documented dietary preferences and associated links to fitness[40–42]. The dominant species, *E. unicolor*, which prefers *L. chinensis* leaves with lower leaf N, declined dramatically in response to N-addition, whereas the subordinate grasshopper, *E. cheui*, which prefers *L. chinensis* with higher leaf N, increased marginally (Supplementary Fig. 2). Importantly, the greater abundance of the dominant species and its stronger response to N-addition drove the overall decline in grasshopper abundance. These findings are consistent with recent stoichiometry frameworks predicting that N deposition should alter herbivore abundance and/or behavior according to each species' coevolved relationship with plant chemistry[23,24,43]. Accordingly, prior large-scale experiments in our system found that *E. unicolor* populations decreased and *E. cheui* populations increased in response to elevated plant N even when abundance of plant functional groups and abiotic factors remained constant[41]. Collectively, these findings suggest that N-addition impacted grasshopper abundance in our study via changes in food quality, presumably linked to N effects on plant stoichiometry.

Second, vegetation changes driven by N-addition could alter habitat conditions for predators or predator-prey interactions, thereby altering grasshopper abundance through ecosystem engineering effects[44]. For example, increasing the amount or altering the configuration of plant material used as scaffolding by web-building spiders can elevate spider abundance and favor construction of larger webs that increase prey capture rates[29,30,45,46]. Accordingly, we found that N-driven changes in plant architecture were linked to a doubling of spider densities and greatly elevated predation rates (Fig. 3). N-addition plots produced more abundant and larger *L. chinensis* plants (Fig. 3b, c), the primary web substrate for *A. bruennichi* spiders, which allowed the spiders to build more and larger webs (Fig. 3a, d–f) and thereby exert higher per capita prey capture rates (Fig. 3f). The dramatic increase in spider densities was evident in the first treatment year (Supplementary Table 3), suggesting this response was driven by substrate limitation (spiders lacking substrates took up residence), rather than demographic increases linked to higher per capita prey captures (which would likely unfold more slowly in a univoltine spider). While higher prey capture rates may have contributed to increased spider densities over time, even these increases would appear to be caused by engineering effects linked to larger web sizes and higher capture rates since spider prey generally declined in N-addition plots (grasshoppers declined and other prey did not increase significantly; Fig. 3a). The combined, positive effects of N addition on spider density (linked to more *L. chinensis* web substrates) and traits (larger, more lethal webs linked to larger *L. chinensis* web substrates) produced total capture rates that were more than triple those in ambient-N plots (Fig. 3g). Moreover, total prey capture rates correlated negatively with grasshopper abundance across N-treatment plots ($R^2 = 36\%$, df = 14, $P = 0.014$; Supplementary Fig. 1b), and we found no evidence for grasshopper behavioral shifts across treatments (Supplementary Table 4). While our experiment was not designed to quantitatively parse the relevance of potential pathways underlying N-induced grasshopper declines, bivariate correlations (Supplementary Fig. 1) suggest that shifts in both plant nutritional content and predation pressure were important.

Finally, changes in vegetation driven by N-addition could alter abiotic conditions important for grasshopper fitness—another form of ecosystem engineering[44]. For example, grasshoppers require moist conditions for oviposition, and grasshopper development is sensitive to ambient temperatures[47,48], factors potentially responsive to increases in plant biomass like those caused by N-addition in our study. Indeed, we found that N-addition plots had lower temperatures than ambient-N plots as measured 45 cm above, 5 cm above, and 5 cm below the soil surface (Supplementary Table 5). However, temperature differences were quite small (<0.5 °C), and none of these temperature measures correlated significantly with grasshopper abundance across plots ($R^2 < 13\%$, df = 14, $P > 0.18$). Soil moisture content was marginally higher in N-addition plots, though again differences were extremely small (Supplementary Table 5). Moreover, increased soil moisture is predicted to benefit grasshoppers, including *Euchorthippus* spp[48]. Hence, while N-addition did alter temperature and moisture conditions, these effects were minor and did not appear to explain the dramatic grasshopper declines observed.

Current theory and empirical reviews emphasize that N deposition can increase herbivory to offset N effects on plant communities[3,5,11,32]. However, our results illustrate that N-addition can suppress or even eliminate top-down controls by herbivores, with significant consequences for plant biomass and diversity. Importantly, by quantifying in situ effects of N-addition on herbivore abundance and experimentally manipulating this factor, we show that herbivore abundance can play a powerful role in driving system-level outcomes. Furthermore, by tracking feedback pathways, we provide evidence

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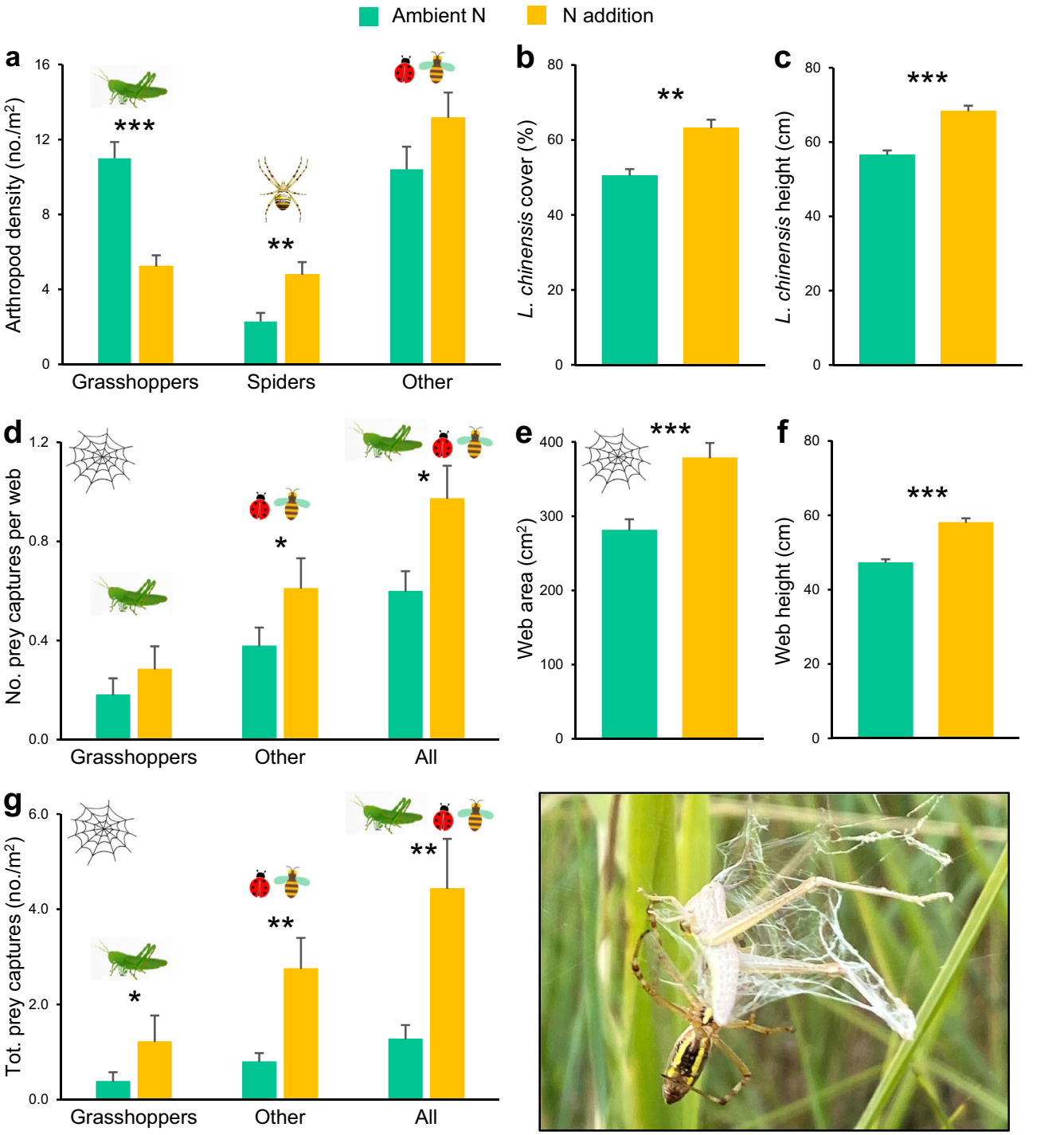

Fig. 3 | Effects of nitrogen (N) addition on plant and arthropod abundance, traits, and trophic interactions. Mean + 1 SE **a** abundance of *Euchorthippus* grasshoppers, *Argiope bruennichi* spiders, and other arthropods representing potential spider prey; **b** cover and **c** height of the dominant grass, *Leymus chinensis*; **d** captures per *A. bruennichi* web by prey category; **e** area and **f** height of *A. bruennichi* spider webs; and (**g**) total prey captures across *A. bruennichi* webs; as measured in ambient-N and N addition plots ($n = 8$ per treatment) in 2018 after three years of treatment. N-addition effects were tested with generalized linear mixed models (poisson distribution for arthropod abundance, lognormal for remaining responses), and asterisks denote significant differences between means (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$; two-sided tests). See Supplementary Table 3 (arthropod abundance by year) and Supplementary Table 4 (remaining responses) for full test statistics, including analysis of grasshopper behavior metrics, which did not differ among treatments ($P > 0.3$). Source data are provided as a Source Data file. Credits: Xiaofei Li (illustrations) and Zhiwei Zhong (photograph).

that N-driven effects on food quality and predation pressure can greatly influence herbivore abundance and top-down control of plant communities. Our findings challenge the dominant paradigm that herbivores will offset N-deposition effects through increased consumption[5,9–11,36]. While intuitively appealing, this traditional perspective is overly simplistic when considering modern stoichiometry frameworks that evaluate the linkage between plant chemistry and herbivore fitness[22–24]. For example, because herbivores must match the ratios of C:N:P uptake to taxa-specific physiological requirements, numerous species, particularly specialist invertebrates,

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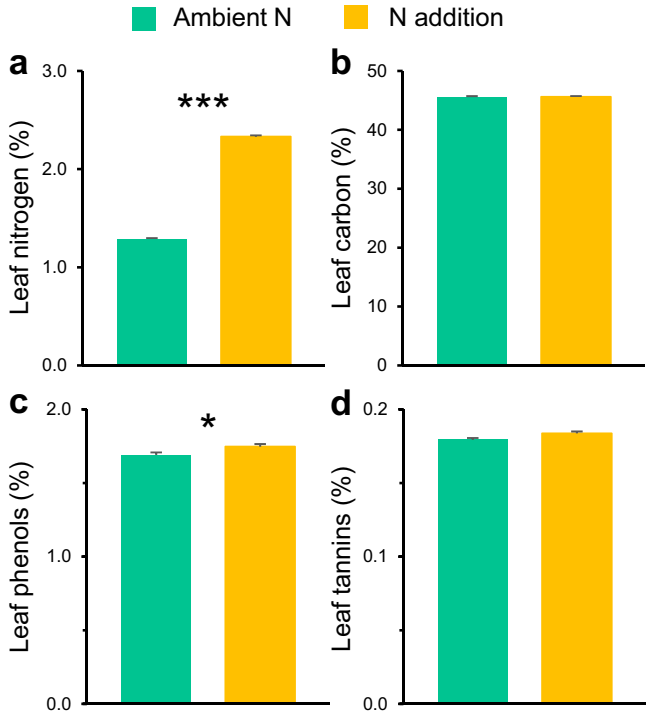

Fig. 4 | Effects of nitrogen (N) addition on leaf chemistry. Mean + 1 SE content of (**a**) nitrogen, (**b**) carbon, (**c**) total phenols, and (**d**) tannins in leaves of the dominant grass, *Leymus chinensis*, measured in ambient-N and N addition plots ($n = 8$ per treatment) in 2018 after three years of treatment. N-addition effects were tested with generalized linear mixed models (beta distribution), and asterisks denote significant differences between means (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$; two-sided tests). Note that this effect was marginal for tannins ($P = 0.051$). See Supplementary Table 4 for full test statistics. Source data are provided as a Source Data file.

are expected to respond negatively to N deposition[24]. In fact, one-third of 148 cases documenting grasshopper responses to elevated N across six continents have reported negative demographic effects[23]. Our study takes a further step to experimentally document how negative impacts of elevated N on herbivore abundance can feedback to affect plant biomass and diversity. In addition, we show that vegetation changes commonly associated with N-deposition, such as increased plant biomass and size, can generate ecosystem engineering effects that further affect herbivore abundance and plant communities by altering predator abundance and predator-prey interactions. This latter pathway is rarely considered in N-deposition research.

Whereas our case study illustrates how N-inputs can suppress or eliminate top-down control by herbivores, we emphasize that the pathways we identified could produce divergent outcomes across systems due to context dependency, consistent with broader trophic theory[17,18,49]. For example, while N-driven increases in plant biomass had weak engineering effects on abiotic factors in our system, these effects could be more powerful in less productive systems, potentially altering herbivore abundance or consumption rates[3–5,9–11]. Regarding N effects on food quality, stoichiometric frameworks predict that whether invertebrate herbivores should increase or decrease in response to elevated plant N will depend on taxa-specific physiology and ecology[22–24]. In terms of higher trophic levels, N-driven changes in plant architecture like those shown here to increase web-spider abundance and lethality could have the opposite effect on active hunting spiders, which can be inhibited by heavy vegetative cover, thereby reducing predation on herbivores[50,51]. Finally, scale (both ecological and experimental) may strongly influence outcomes, particularly relevant to herbivore abundance. While densities of large vertebrate herbivores may be constrained more by landscape factors than food quality, as some have argued[11], in most terrestrial studies, N-additions and herbivore manipulations have been too small in space and/or short in time to effectively measure herbivore density responses[9–11,36]. Larger-scale experiments may further elucidate how N additions affect abundance of herbivores and their interactions with both plants and predators. While it is widely assumed that herbivory will offset the impacts of N inputs on plant communities, our findings illustrate that N inputs can actually disrupt top-down controls by altering plant chemistry and architecture, thereby allowing bottom-up impacts on plant biomass and diversity to prevail.

Methods

Study system and background

All experimental procedures were carried out in accordance with the Law of the People's Republic of China on the Protection of Wildlife (1988). The study was conducted at the Grassland Ecosystem National Observation and Research Station of Northeast Normal University (Changling County; 44.58° N, 123.50° E) in Jilin Province, China. The study area is low elevation (150–200 m) semi-arid grassland, with annual precipitation averaging 280–400 mm. The experimental site is dominated by the perennial grasses, *Leymus chinensis*, accounting for >60% total plant aboveground biomass[38]. Other grass species include *Phragmites australis* and *Calamagrostis epigeios*, and forb species include *Kalimeris integrifolia, Artemisia scoparia*, and *A. mongolica*. The study site has a long history (>15 y) of mowing with occasional sheep grazing, but the site was fenced to exclude these practices in 2014, 2 years prior to the study.

Two grasshopper species, *Euchorthippus unicolor* and *E. cheui* (Orthoptera, Acrididae) dominate the insect herbivore community (annually accounting > 70% of all insect individuals[52]), with *E. unicolor* the more abundant species of the two (Supplementary Fig. 2). These grasshoppers have similar body sizes and both are grass specialists, feeding predominately on *L. chinensis* and never on forbs[38]. However, prior studies offering each species *L. chinensis* leaves with either high or low N indicate that *E. unicolor* prefers low N and *E. cheui* prefers high N leaves[40–42]. *Euchorthippus* grasshoppers are preyed upon predominantly by spiders, and occasionally by insectivorous birds[52]. The orb-weaver *Argiope bruennichi* (Araneae: Araneidae) is a dominant spider species in our system and preys upon *Euchorthippus*[31]. This species appears from June to September, attaining peak density in August. Female *A. bruennichi* make vertical spiral orb webs, usually among *L. chinensis* plants, and capture and consume a variety of arthropod taxa, including grasshoppers[31]. Sample sizes were determined as the maximum of our logistical capacity for all experiments, as detailed below.

Experimental design and pre-treatment conditions

In early May 2016, we established eight 10 × 20 m blocks at 20–30 m intervals, and a pair of 8 × 8 m experimental plots within each block spaced about 4 m apart (delineated by plastic edging) for a total of 16 plots (Fig. 1b). Nitrogen treatments (ambient N or N-addition) were randomly assigned to the plots in each block. These N-treatment plots were large enough to allow changes in abundance of grasshoppers and their spider predators, although we cannot rule out that some movement might occur between treatments. Within each N-treatment plot, a pair of randomly located 2 m^2 circular subplots was assigned to grasshopper mesocosm treatments. In each mesocosm, grasshoppers were either removed or stocked at the same density measured in the surrounding N-treatment plot (Fig. 1b). We also added an alternate, third grasshopper treatment into each of the N-addition plots wherein the mesocosm was stocked to match the grasshopper density in the adjacent ambient-N plot. These treatments allowed us to evaluate N-addition effects on plant communities (1) in the absence of herbivores, (2) in the presence of herbivores at densities that varied in

response to N-addition (i.e., reflecting overall effects of N addition on herbivore density when trophic interactions, including those with spiders, were accounted for), and (3) in the presence of herbivores at densities held at ambient-N levels (i.e., to isolate the mechanistic role of herbivore abundance in mediating N-addition effects).

In mid-May 2016, before treatments were applied, we measured plant properties in each N-treatment plot by centrally placing a 1 m^2 quadrat at the location designated for each grasshopper-treatment subplot. Within each quadrat, we visually estimated cover per species and for each of the three key plant groups: *L. chinensis*, other grasses, and forbs. Species richness was represented by the number of species in each quadrat. In addition, we calculated plant diversity using the standard Shannon index[53]: $H' = -\Sigma\ p_i\ \ln\ p_i$, where p_i is the relative abundance of species i in the quadrat, represented by relative cover. We note that all pretreatment measures of plant abundance relied on cover rather than biomass to minimize disturbance.

After initial sampling, N treatments were applied to the 8 × 8 m plots. For the N-addition treatment, we sprayed an aqueous solution of ammonium nitrate at a rate of 10 g m^{-2} $year^{-1}$, which lies at the upper end of airborne nutrient deposition observed in Northern China[54]. For the ambient-N treatment, we sprayed an equivalent amount of water to account for potential irrigation effects. N treatments were repeated in mid-May of 2017 and 2018. Two days after initial treatment, the embedded 2-m^2 circular subplots were cleared of invertebrates (in vegetation and on ground surfaces) using a Univac Portable Suction Sampler (Burkard Co. Ltd, Rickmansworth, Herts, UK). We then created the grasshopper mesocosms by placing a cylindrical cage (1.2 m high frame with a 2 m^2 circular top covered with a 5 × 5 mm plastic mesh insect screen; Fig. 1) over each subplot. *Euchorthippus* grasshoppers were stocked into + grasshopper mesocosms according to densities and developmental stages found in the relevant N-treatment plot (see above), as determined by biweekly surveys (see below). Grasshopper treatments were maintained during the growing season (from June to September) from 2016 to 2018 by counting grasshoppers in each caged mesocosm every two weeks and adding or removing individuals to match target levels. We did not attempt to distinguish between the two *Euchorthippus* species when stocking mesocosms, given that they are difficult to separate in the field. However, as detailed below, we included extra sampling in our grasshopper surveys to consider species-specific responses to N addition. All experiments were executed successfully on the first trial. No experiments were blind because treatment responses were evident.

N-addition and grasshopper herbivory effects on plant biomass and diversity

In August 2018, at peak biomass for the growing season, we investigated how three years of N and grasshopper treatments affected plant aboveground biomass and diversity by sampling one 1 m^2 quadrat in the center of each mesocosm. Living biomass was harvested from the quadrat and sorted to species to determine species richness. Samples were then dried at 75 °C for 48 h to constant mass; and weighed to determine aboveground biomass per species, per plant group (*L. chinensis*, other grasses, and forbs), and total biomass across all taxa. We calculated Shannon diversity using the standard index (see above), with relative abundance (p_i) measured by the relative biomass of each species in the quadrat.

N-addition effects on abundance of spiders, grasshoppers, and other prey

From 2016 to 2018, during the growing season (June to September), we estimated the abundance of *A. bruennichi* spiders, *Euchorthippus* grasshoppers, and other arthropods representing potential *A. bruennichi* prey every two weeks in the N-treatment plots (outside the grasshopper mesocosms). To do so, we randomly placed three 0.10 m^2 rings within each plot and left the rings undisturbed for two days. Surveys were conducted from 10:00 to 16:00 on days with minimal cloud cover and calm conditions by slowly approaching each ring and then counting the number of *A. bruennichi* webs, *Euchorthippus* grasshoppers, and other arthropod prey within[55]. In the field, one *A. bruennichi* web is typically occupied by one *A. bruennichi* individual, so the number of webs and spiders is equivalent. For *Euchorthippus* grasshoppers, this survey method, which relied on visual observation with minimal disturbance, was ideal for estimating overall densities across developmental stages and for determining the ratio of adults vs nymphs to stock into experimental mesocosms. However, it was not feasible to also distinguish *E. unicolor* from *E. cheui* using this method, as these species can only be separated when inspected in the hand as adults. For this reason, we conducted additional surveys (mid–June, July, and August, 2016–2018) that relied on standard sweep netting to capture and count adult grasshoppers by species. Each of these surveys was conducted by sweeping a light muslin net through the vegetation 30 cm above the ground at each of four random locations per N-treatment plot. This extra sampling effort allowed us to consider species-specific responses to N addition, albeit based on adult grasshoppers only.

N-addition effects on traits and trophic interactions

In late July and mid-August of 2018 (two sampling periods encapsulating peak growing season), we quantified traits of the dominant *L. chinensis* grass, *Euchorthippus* grasshoppers, and *A. bruennichi* spiders, along with relevant interactions, within the N-treatment plots (outside the grasshopper mesocosms). For *L. chinensis*, we measured plant cover and height, which can affect interactions between *Euchorthippus* grasshoppers and *A. bruennichi* spiders, within three randomly located 1 m^2 quadrats per plot in each sampling period. Cover was visually estimated, and height was recorded for three haphazardly chosen *L. chinensis* stems per quadrat. We also measured chemical attributes of *L. chinensis* leaves that might influence *Euchorthippus* herbivory: N and carbon C content, which can affect nutritional quality, and phenols and tannins, which can act as secondary defense compounds[23,24]. Within each of the 1 m^2 quadrats, we haphazardly selected twenty *L. chinensis* plants for tissue analysis. From each plant, we selected two of the uppermost, fully expanded leaves that had not been damaged by herbivores. Leaves were oven-dried for 48 h at 70 °C, ground to fine powder using a ball mill, and analyzed for total N and C content using a Vario MICRO cube Elemental Analyzer (Elementar GmbH, Hanau, Germany). Content of total phenols and tannins, respectively, was determined using assay kits (Suzhou Keming Science & Technology Inc., Suzhou, China), and all procedures followed the manufacturers' instructions.

For *Euchorthippus* grasshoppers, we quantified feeding and jumping behaviors, which can affect their interactions with their host plants and spiders[31]. These behaviors were defined as follows: (i) feeding: grasshopper observed eating foliage without retracting the head from *L. chinensis* plants, (ii) jumping: grasshopper observed jumping over the ground or among plants. To measure these behaviors, we randomly collected three adult *Euchorthippus* grasshoppers within each N-treatment plot per sampling period. Grasshoppers were given an identifying paint mark on their thoraxes to facilitate tracking. During each sampling period, the three grasshoppers were observed simultaneously by separate observers who recorded the frequency of each behavior during four peak hours of grasshopper activity, from 10:00 to 12:00 and from 15:00 to 17:00 on the same day.

For *A. bruennichi* spiders, we quantified web area and height, which can influence spider foraging success[29], and estimated per capita prey capture rates. Three spider webs within each N-treatment plot were randomly selected for measurement in each of the two sampling periods. We recorded web diameter and used this to estimate area, given that webs of this species are circular. To obtain web height, we measured the vertical distance from the ground to web

center. Finally, we recorded how many individuals of *Euchorthippus* grasshoppers and other arthropods were captured in each web. Per capita prey capture rates were multiplied by mean spider density from surveys to calculate total captures per N-treatment plot.

N-addition effects on abiotic conditions

In late July and mid-August of 2018, we also quantified abiotic conditions at three random locations per N-treatment plot (outside the grasshopper mesocosms). Temperature was measured with an AR-847 digital thermo-hygrometer (Jinzhan Inc., Shenzhen, China) 45 cm above, 5 cm above, and 5 cm below the soil surface at each sampling location. Soil moisture content was measured 5 cm below the soil surface using a handheld soil moisture reader (OSA-1, OUSU Technology, Hebei, China).

Data analyses

We used generalized linear mixed effects models (GLMMs) in SAS version 9.4[56] for all analyses of treatment effects. To evaluate effects of N-addition and grasshoppers on plant aboveground biomass (total and by plant group) and diversity measured in mesocosms after 3 years of treatment (2018), we ran a separate model for each response that included data from all mesocosm treatments. These models included a fixed factor distinguishing each of the five treatment combinations and used contrasts to test for independent and interactive effects of N addition (ambient or added) and grasshoppers (±) under each of two experimental scenarios that varied which +grasshopper, N-addition treatment was considered (see experimental design section above for treatment details). For our main set of contrasts, we used the +grasshopper, N-addition treatment wherein grasshoppers were maintained at densities that varied with N-addition according to surveys conducted in N-treatment plots outside the mesocosms. For our second set of contrasts, we used the alternate +grasshopper, N-addition treatment wherein grasshoppers were maintained at ambient-N densities, allowing us to control for effects of N addition on grasshopper abundance. Random factors were block and plot within block to account for the split-plot design (i.e., nesting of grasshopper mesocosms within each N-treatment plot). In cases where the N-addition x grasshopper interaction was significant ($P < 0.05$), we tested for post-hoc differences among treatment means using multiple comparisons wherein P values were adjusted for the number of comparisons via the Bonferroni method. We also tested for pre-treatment differences in plant communities, as measured by plant cover (total and by plant group) and diversity in mesocosm locations prior to initiation of treatments (2016). In no case were pre-treatment differences significant ($P > 0.09$; Supplementary Table 6). We therefore focus presentation on post-treatment patterns. All data were included as relevant to each analysis.

Remaining analyses of experimental outcomes examined the effects of N addition on arthropod abundance, traits, trophic interactions, and abiotic conditions, as measured in N-treatment plots. N treatment was a fixed factor in all models, with the structure of random effects varying to accommodate the response variables as detailed below. For analysis of arthropod abundance, each taxon/group (*Euchorthippus* grasshoppers, *A. bruennichi* spiders, and other arthropods) was considered in a separate model for each post-treatment sampling year (2016–2018). Arthropod counts per group from detailed visual surveys were summed across subsampled rings per plot and survey ($n = 6$ spanning the growing season), and this response was treated as a repeated measure in a model that also included block as a random factor. For analysis of grasshopper counts by species from sweep net sampling, we used a simpler approach given the relatively crude nature of this effort. Counts per grasshopper species were summed across subsamples per plot and survey, and the mean across surveys ($n = 3$ per growing season) was evaluated in a model that included block as a random factor. Arthropod abundance patterns were parallel among years (Supplementary Table 3), so we focus presentation on the final year. For analysis of traits, trophic interactions, and abiotic factors, all of which were sampled twice at peak growing season in the final year only, each metric was averaged over all subsamples per plot and evaluated with a model that included block as a random factor.

For each response variable, we specified the error distribution that best fit the data as follows: lognormal for variables with positive skewness (plant biomass, cover, and height; grasshopper behavior measures; spider web/prey capture measures), beta for variables representing proportions (leaf chemical concentrations, soil moisture), Poisson for count data (species richness, arthropod abundance by group), and Gaussian when approximating normality (remaining variables), all checked using plots of predicted vs. residual values. Reported P values reflect two-tailed tests in all cases. Means in figures are presented as least squares means with associated standard errors as obtained from GLMMs to properly account for error distributions and the experimental design. Spearman's rank correlation coefficient was used to examine bivariate correlations between grasshopper abundance per N-treatment plot and potential explanatory variables measured therein (mean values measured in 2018).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All data used in this study have been deposited in the Dryad database and are available at (https://doi.org/10.5061/dryad.kkwh70sb8). Source data are provided with this paper.

Code availability

No unique code was created for analyses. All analyses were conducted using SAS as stated in the Methods.

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Author contributions

Z.Z. and X.L. conceived the ideas, designed, and conducted the experiments. D.E.P. developed and framed the research questions in this paper. D.E.P., Z.Z. and Y.K.O. wrote the paper with input from all authors. Y.K.O. analyzed the data. X.L., L.J., D.W., Q.G., S.W. and Y.H. revised the manuscript. All authors edited the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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