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Soil engineering by ants facilitates plant compensation for large herbivore removal of aboveground biomass

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Abstract. The interplay between top-down and bottom-up processes determines ecosystem productivity. Yet, the factors that mediate the balance between these opposing forces remain poorly understood. Furthering this challenge, complex and often cryptic factors like ecosystem engineering and trait-mediated interactions may play major roles in mediating the outcomes of top-down and bottom-up interactions. In semiarid grasslands of northeastern China, we conducted a large-scale, three-year experiment to evaluate how soil engineering by ants and plasticity in plants independently and jointly influenced the top-down effects of grazing by a ubiquitous herbivore (cattle) on aboveground standing biomass of the dominant perennial grass, *Leymus chinensis*. Herbivory had strong top-down effects, reducing *L. chinensis* AB by 25% relative to baseline levels without cattle or ants. In contrast, soil engineering by ants facilitated weak bottom-up effects in the absence of herbivory. However, in the presence of herbivory, soil engineering effects were strong enough to fully offset herbivore removal of aboveground biomass. This outcome was mediated by *L. chinensis*'s plasticity in reallocating growth from below- to aboveground biomass, a result linked to additive effects of engineers and herbivores increasing soil N availability and engineering effects improving soil structure. Soil engineering increased soil N by 12%, promoting aboveground biomass. Herbivores increased soil N by 13% via defecation, but this increase failed to offset their reductions in aboveground biomass in isolation. However, when combined, engineers and herbivores increased soil N by 26% and engineers improved soil bulk density, facilitating *L. chinensis* to shift resource allocations from below- to aboveground biomass sufficiently to fully offset herbivore suppression of aboveground biomass. Our results demonstrate that soil engineering and trait-mediated effects of plant plasticity can strongly mediate the outcome of top-down and bottom-up interactions. These cryptic but perhaps ubiquitous processes may help to explain the long-debated phenomenon of plant compensatory responses to large grazers.

Key words: ants; bottom-up effects; ecosystem engineering; herbivory; indirect effects; plant compensatory response; plasticity; resource allocation; root-to-shoot ratios; top-down effects; trait-mediated interactions.

Introduction

Primary productivity is a fundamental currency of ecological systems (Odum 1953). Elucidating the processes regulating primary productivity is central to understanding how natural systems are structured, as well as how to conserve biodiversity and ecological function in the face of anthropogenic change (Stevens et al. 2004, Dirzo et al. 2014, Ripple et al. 2015). It has long been recognized that net primary productivity (NPP) derives from the interplay between bottom-up processes such as resource availability/inputs and top-down processes such as consumption (McNaughton 1979, Paine 1980, Strong 1992, Frank et al. 2018). Yet, despite decades of effort and debate, our grasp of the factors regulating the interplay between top-down and bottom-up forces remains limited (Power 1992, Hunter and Price 1992, Gruner et al. 2008, Gough et al. 2012). A variety of factors can influence the interplay between

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primary producers and consumers, but two ubiquitous and influential processes that are particularly challenging to decipher are ecosystem engineering, due to the sheer complexity of engineering interactions (Hastings et al. 2007), and trait-mediated interactions, because they are inherently cryptic and difficult to quantify (Werner and Peacor 2003). Understanding how these widespread processes influence productivity and standing biomass is essential for advancing ecology and for predicting how anthropogenically driven changes in resource inputs (Stevens et al. 2004) and consumer composition and abundance (Dirzo et al. 2014, Ripple et al. 2015) may affect future NPP.

Ecosystem engineering is a widespread ecological phenomenon generating complex effects on top-down and bottom-up interactions that can powerfully influence primary productivity (Hastings et al. 2007). Ecosystem engineers are defined as "organisms that modulate the availability of resources to other species by causing physical state changes in biotic or abiotic materials" (Jones et al. 1994, 1997). Hence, engineering directly regulates the type and amount of resources to which certain organisms, in some cases many organisms within a system, have access. Ecosystem engineers can exert profound mediating effects on the strength and direction of trophic interactions (Pearson 2010, Zhong et al. 2017), and they can initiate trophic cascades via both bottom-up and top-down effects (Daleo et al. 2007, DeVore and Maerz 2014, Sanders et al. 2014).

Soil engineers such as ants, termites, and earthworms can generate pervasive effects on primary productivity by improving soil physiochemical properties in both natural and managed systems (Edwards and Bohlen 1996, Bignell 2006, Jouquet et al. 2006, 2014, Veen et al. 2012). In many semiarid grasslands, ants alter soil structure by increasing porosity and decreasing bulk density, both of which lead to greater water infiltration (Evans et al. 2011). Ants can also increase the supply of key limiting resources such as nitrogen by transporting nutrients across soil horizons and by nest-building activities that accelerate plant debris decomposition (Wardle et al. 2011, Farji-Brener and Werenkraut 2017). Through similar processes, ants, termites, earthworms, and other soil engineers may generate strong bottom-up effects that increase primary productivity by improving soil physiochemical properties across many ecosystems (Edwards and Bohlen 1996, Bignell 2006, Jouquet et al. 2006, 2014, Farji-Brener and Werenkraut 2017).

Trait-mediated interactions arising from behavioral, morphological, or physiological plasticity can also strongly influence a range of ecological processes, including NPP (Schmitz et al. 2004). While trait-mediated interactions between predators and consumers have received much attention (Werner and Peacor 2003), plants exhibit plasticity in response to resource availability and herbivory that is more closely linked to NPP (Silvertown and Gordon 1989, Callaway et al. 2003, Koffel et al. 2018). Perhaps the most direct linkage between plant plasticity and NPP is the ability of plants to maximize their total biomass by allocating growth between shoot (aboveground) and root (belowground) biomass in a manner that maximizes uptake of the most limiting resources and hence growth (Ågren and Ingestad 1987, Crick and Grime 1987, Koffel et al. 2018). Generally, when belowground nutrients or water are limiting, plants allocate more biomass toward root production, and when light is limiting, they allocate more toward shoot production (Bloom et al. 1985). Conversely, grazing by large herbivores can directly reduce aboveground standing biomass (AB hereafter). More interesting, grazing can influence plant resource allocation processes in a variety of ways, including causing plants to increase allocations to AB to compensate or even overcompensate for biomass losses to grazing (McNaughton 1979, Verkaar 1986, Daleo et al. 2007, Charles et al. 2017), although the mechanisms underlying such compensatory responses are complex and controversial (McNaughton 1979, Bergelson and Crawley 1992, Ramula et al. 2019). Meanwhile, soil engineering activities can alter plant resource allocations in potentially complementary ways by enhancing nutrient and soil conditions in ways that allow plants to shift allocations from below- toward aboveground biomass (Farji-Brener and Werenkraut 2017). Despite the widespread prevalence of these processes, little is known about how herbivory by large grazers and soil engineering interact to affect aboveground productivity and biomass, and how plant plasticity might mediate overall outcomes.

To better understand how soil engineering and trait-mediated effects of plant plasticity may mediate the effects of large herbivores on AB, we manipulated the presence of large herbivores (cattle) and soil ecosystem engineers (ants) to evaluate their independent and joint effects on AB of the dominant grass, *Leymus chinensis*, in semiarid grasslands of northeastern China over 3 yr. We also monitored changes in soil physicochemical properties and resource allocation by the plants between above- and belowground biomass to identify underlying mechanisms.

Methods

Study system and overall experimental design

We conducted our study at the Grassland Ecological Research Station of Northeast Normal University, Jilin Province, China (44°35.5′ N, 123°30.5′ E). This site is characterized by a semiarid continental monsoon, where annual precipitation is 280–400 mm and annual mean temperature is 4.6°–6.4°C. The perennial grass *Leymus chinensis* is the dominant plant species, accounting for > 60% of total plant biomass (Liu et al. 2015). Other plants include the forbs *Kalimeris integrifolia* and *Artemisia scoparia*, and the grass *Phragmites australis*. The soil is a nutrient-poor salt-alkali meadow steppe (Salid Aridisol, U.S. Soil Taxonomy) with available N content ranging from 15.4 to 27.6 mg/kg, and available P

content ranging from 2.6 to 5.9 mg/kg. The area hosts a high density (~0.5–4 mounds/m^2) of ant nests, mainly *Formica* spp. (e.g., *F. sinae*, *F. gagatoides*, and *F. glauca*), *Tetramorium* spp. (e.g., *T. caespitum*), and *Lasius* spp. (e.g., *L. flavus* and *L. alienus*). Invertebrate herbivores such as grasshoppers are common, but their densities are relatively low (~1–3 individuals/m^2) in the cattle grazed areas where they have limited effects on plant biomass (Zhu et al. 2019). Vertebrate herbivores such as geese and rodents are present but rare. The area has a long history of low-intensity livestock grazing and mowing; however, it was fenced in 2005 when it became a research site (see more details of the research ecosystem in Appendix S1: Fig. S1).

In June 2009, we established six experimental blocks (150–300 m spacing), each containing a pair of 50 × 50 m enclosure plots (30 m spacing; Li et al. 2018). One enclosure plot per block was randomly assigned to cattle grazing and the other to no grazing. Within each plot, we established eight 3 × 3 m subplots (7-m spacing), one-half of which were randomly assigned to ant presence and the other half to ant removal. Thus, we had six replicates of four experimental treatments in a fully crossed 2 × 2 split-plot design (i.e. cattle only, cattle and ants, ants only, and no cattle and no ants).

Cattle grazing and ant engineering treatments

From 2010 to 2012, the grazed plots were grazed by cattle (mass 300 ± 8 kg [mean ± SE]) at light to moderate intensity (less than 50% of aboveground plant biomass consumed by cattle), as recommended by local governments. A total of 48 mature cows were assigned to the six grazed plots, with eight cows enclosed in each plot. Grazing occurred each year from June to September during the first two weeks of each month, with daily grazing occurring between 06:00–08:00 and 16:00–18:00, creating grazing intensities that simulated local grazing habits. From 2010 to 2012, we applied 10 g of poison ant baits (Jingkang Ant Bait Granules, Lekang Technology, Beijing, China) around the entrance of active ant nests to suppress ants in the ant removal subplots from June to August, the active period for ants each year. The main active ingredients of the ant bait are 0.45% Tetramethrin and 0.02% Alpha-cypermethrin. The ant bait is specifically designed to appeal to ants and kill their colonies, has been used successfully in reducing ant populations in the region, and reduced active act nests by 95% in treated subplots. Additional experiments indicate that, except for ants and crickets, the ant bait has limited impacts on other arthropods, plant growth, soil nutrients, and cattle behaviors in our system (Li et al. 2018).

Effects of cattle and ants on L. chinensis *biomass, soil properties, and microclimate*

To understand the effects of cattle grazing and ant removal treatments on plant productivity and system processes, we quantified *L. chinensis* biomass, soil properties, and microclimate within each of the eight 3 × 3 m subplots per plot in August (the peak of the growing season) of 2009, the year before treatments began, and again in August of 2012, after 3 yr of treatment. For *L. chinensis*, we measured above- (AB) and belowground standing biomass (BB hereafter). We estimated AB by clipping *L. chinensis* plants to ground level in 1 × 0.2 m area in two random locations within each of the eight subplots within each plot. We then took two, 7.5 cm diameter, 35 cm deep cores within the 1 × 0.2 m area of each subplot to determine BB (*L. chinensis* roots and rhizomes are typically concentrated within the top 30 cm of soils). Cores were pooled and sieved (2-mm mesh), then sorted into *L. chinensis* roots or other plant material. We identified *L. chinensis* live roots visually as they have a unique color and texture compared to other plant species. AB and BB were then dried for 48 h at 70°C and weighed.

For soil properties, soil moisture and bulk density were determined using two portable soil meters (OSA-1; OUSU Electronic Technology, Hebei, China; and YDRZ-4L; Tuopu Yunnong Technology, Zhejiang, China), taking readings from five random locations within each of the eight subplots. Soil nutrients were measured by using a 4 cm diameter soil auger to randomly collect five replicate 0–20 cm soil samples from each subplot, which were pooled to homogenize the samples. For each soil sample, a 10-g subsample was extracted with 70 mL 2 mol/L KCl. Extracts were frozen at 20°C for analysis of NH_4^+ and NO_3^- content by continuous flow analyzer (Futura; AMS-Alliance Instruments, Frépillon, France). Total available N was the sum of $NH_4^+$and NO_3^- concentrations. For total available P, another 10-g subsample of soil was extracted using acidified NH_4OAc-EDTA and analyzed by the inductively coupled plasma-atomic emission spectrometer (ICP-AES; Spectroflame, Spectro Analytical Instruments, Kleve, Germany).

We measured light penetration and air temperature at the soil surface by taking readings from two random locations within each subplot. Light penetration was measured using a photosynthetically active radiation point sensor (GLZ-C-G PAR; Top Instrument, Zhejiang, China), taking light intensity readings from above the vegetation canopy and from the ground surface. We measured ambient air temperature using a digital thermo-hygrometer (AR-847; Smart Sensor Instruments, Dongguan, China).

Because herbivores not only remove plant biomass but also alter system inputs through defecation, we quantified these inputs and their effects on soil properties across the treatments by counting the total number of cattle dungs in each subplot in August 2012. We also visually assessed the total number of active ant nests in each subplot in August 2012 (see Li et al. 2018). Urine inputs were not estimated.

Data analyses

We used generalized linear mixed effects models (GLMMs) in SAS version 9.4 (PROC GLIMMIX; SAS Institute 2013) to test for effects of cattle grazing and ant removal treatments on response variables representing *L. chinensis* biomass, soil properties, and microclimate. We first used the model structure described below to test each response variable for differences among treatments as measured in the pretreatment year (2009). In no case were pretreatment differences significant ($P > 0.2$; Appendix S1: Table S1-S2). We therefore focus presentation on analysis of data from the final treatment year (2012). In all models, cattle (grazed or ungrazed), ants (present or removed), and their interaction were treated as fixed factors, with block and plot within block included as random factors to account for the split-plot design. For each response variable, we specified the error distribution that best fit the data, using a lognormal distribution for variables with positive skewness (AB, BB, available N), a beta distribution for variables representing proportions (soil moisture, light penetration), and a Gaussian distribution for those approximating normality (available P, soil bulk density, air temperature), as checked using plots of predicted vs. residual values. To assess plant resource allocation between above- and belowground biomass, we constructed an additional response variable, the root mass ratio (RWR), a standardized metric calculated as BB divided by total plant biomass (Reynolds and D'Antonio 1996). We tested RWR with a beta distribution, given that it is a proportion. For all response variables, we were interested in comparing the individual and combined effects of cattle grazing and ant removal treatments. Therefore, in cases where the cattle × ant interaction was at least marginally significant ($P < 0.1$), we tested for post hoc differences among treatment means using multiple comparisons wherein the P value was adjusted for the number of comparisons via the Bonferroni method (note: where P unadjusted ≥ 0.17, the P adjusted = 1.0).

We conducted additional analyses to consider counts of cattle dungs and ant nests - variables that might explain the intensity of treatment effects, particularly for soil properties. First, we examined the effect of each treatment on the intensity of the other, as measured by these variables. Specifically, for grazed subplots, we tested for differences in the number of cattle dungs by ant treatment using a GLMM with ants (present or removed) included as a fixed factor, block and plot within block as random factors, and a negative binomial distribution given that these data were counts. Conversely, for subplots with ants present, we used a parallel model structure to test for differences in ant nest density by grazing treatment, treating cattle (grazed or ungrazed) as the fixed factor and specifying a lognormal distribution. Next, we examined the relationship between treatment intensity variables and the four soil variables, with the latter treated as response variables in separate models (using the same error distributions as above). Fixed factors were number of cattle dung, density of ant nests, and their interaction. We note that relationships between these treatment factors and soil response variables were equivalent when we constructed separate, single-factor models using only data from the relevant treatment type, i.e., when we tested for (1) the influence of cattle dung on soil properties in grazed subplots and (2) the influence of ant nests on soil properties in ant-present subplots.

Results

Effects of cattle and ants on L. chinensis *biomass and RWR*

Cattle grazing reduced aboveground biomass (AB) of *L. chinensis* across ant treatments (cattle: $F_{1,5} = 11.95$, $P = 0.018$), while ants had the opposite effect across grazing treatments (ants: $F_{1,82} = 10.1$, $P = 0.002$). However, there was a marginally significant interaction between these factors (cattle × ants: $F_{1,82} = 3.26$, $P = 0.075$), and post hoc comparisons showed that the strength of the grazing effect shifted as a function of ant removal (Fig. 1a). More specifically, grazing significantly reduced AB when ants were removed (25% loss in grazed vs. ungrazed subplots; $t_{1,82} = -3.72$, $P = 0.002$), but when ants were present, AB was similar between grazing treatments ($t_{1,82} = -1.17$, $P = 1.0$). Conversely, the positive effect of ants on AB was significant when cattle were present (31% gain in subplots with ants present vs. removed; $t_{1,82} = 3.52$, $P = 0.004$), and not when cattle were absent ($t_{1,82} = 0.97$, $P = 1.0$). Given these combined effects, mean AB in subplots with both grazing and ants was comparable to levels in ungrazed subplots, with ants compensating for losses seen with grazing alone (Fig. 1a).

Belowground biomass of *L. chinensis* (BB) was also affected by treatments. As seen for AB, cattle grazing tended to reduce BB across ant treatments (cattle: $F_{1,5} = 4.85$, $P = 0.08$). While ants generally increased AB, ants had the opposite effect on BB across grazing treatments (ants: $F_{1,82} = 12.01$, $P = 0.0008$). In addition, there was a marginally significant difference in grazing effects by ant treatment (cattle × ants: $F_{1,82} = 3.33$, $P = 0.07$), and post-hoc comparisons once again showed that the strength of the grazing effect shifted as a function of ant removal (Fig. 1b). However, for BB, the negative effect of grazing was significant when ants were present (26% loss in grazed vs. ungrazed subplots; $t_{1,82} = -2.85$, $P = 0.033$), but not when they were absent ($t_{1,82} = -0.72$, $P = 1.0$). Similarly, the negative effect of ants on BB was significant when cattle were present (27% loss in subplots with ants present vs. removed; $t_{1,82} = -3.74$, $P = 0.002$) and not when they were absent ($t_{1,82} = -0.72$, $P = 1.0$). Hence, mean BB was lowest in subplots with both grazing and ants and comparable in remaining treatments (Fig. 1b).

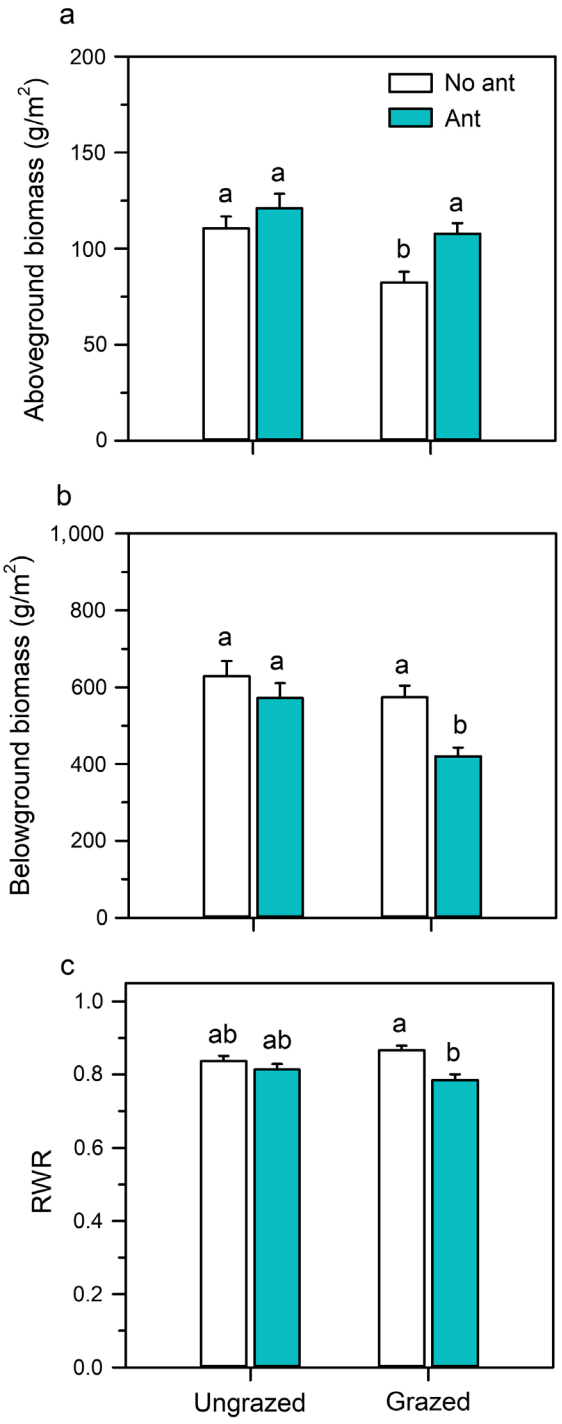

FIG. 1. Combined effects of 3-yr cattle grazing and ant removal treatments on least squares mean (+SE) (a) aboveground standing biomass, (b) belowground biomass, and (c) RWR (root mass ratio) of the grass, *Leymus chinensis*, measured in 3 × 3 m subplots in 2012. Different letters above bars indicate significant differences among treatments, as assessed via post hoc comparisons used to evaluate the interaction between grazing and ants.

Treatment induced effects on AB and BB translated to shifts in RWR (Fig. 1c). Cattle grazing did not significantly affect RWR overall (cattle: $F_{1,5} = 0.03$, $P = 0.87$), while ants reduced allocation to roots when tested across grazing treatments (ants: $F_{1,82} = 16.35$, $P = 0.0001$). However, grazing interacted significantly with ant removal (cattle × ants: $F_{1,82} = 5.28$, $P = 0.024$). According to post-hoc comparisons, grazed vs. ungrazed subplots did not differ significantly whether ants were present ($t_{1,82} = -1.16$, $P = 1.0$) or removed ($t_{1,82} = 1.36$, $P = 1.0$), although there were weak trends towards negative vs. positive effects, respectively. More importantly, ants had strong conditional effects on root allocation, significantly reducing RWR in grazed subplots (9% decline with ants present vs. removed; $t_{1,82} = -4.44$, $P = 0.0006$) and not in ungrazed subplots ($t_{1,82} = -1.25$, $P = 1.0$).

Effects of cattle and ants on soil properties and microclimate

Cattle positively affected total available N, with an increase of 13% in grazed relative to ungrazed plots ($F_{1,5} = 10.47$, $P = 0.023$). Ants had a similar effect on total available N, with an increase of 12% in subplots where ants were present vs. removed ($F_{1,82} = 7.59$, $P = 0.007$). Grazing and ant removal did not have significant interactive effects on this nutrient (cattle × ants: $F_{1,82} = 0.31$, $P = 0.58$). However, given the additive effects of treatments, total available N was 26% higher in subplots with cattle and ants relative to subplots lacking both factors (Fig. 2a).

Cattle grazing did not significantly affect soil bulk density when assessed across ant treatments ($F_{1,5} = 1.72$, $P = 0.25$). Ants reduced soil bulk density overall ($F_{1,82} = 30.05$, $P < 0.0001$), but this factor significantly interacted with grazing (cattle × ants: $F_{1,82} = 9.15$, $P = 0.003$; Fig. 2b). According to post-hoc comparisons, although differences between grazing treatments were not significant when ants were present in subplots ($t_{1,82} = -0.49$, $P = 1.0$), grazing significantly increased soil bulk density when ants were removed (6% gain in subplots with grazing vs. not; $t_{1,82} = 2.71$, $P = 0.049$). Conversely, ants significantly reduced soil bulk density in the grazed (10% loss in subplots with ants present vs. removed; $t_{1,82} = -6.01$, $P < 0.0001$), but not the ungrazed treatment ($t_{1,82} = -1.74$, $P = 0.52$). Given these combined effects, mean soil bulk density was higher in subplots with grazing and ant removal compared to other treatments (Fig. 2b). Neither total available P nor soil moisture were significantly affected by grazing ($F_{1,5} = 0.15$, $P = 0.72$; $F_{1,5} = 0.03$, $P = 0.86$, respectively) or ants ($F_{1,82} = 1.11$, $P = 0.3$; $F_{1,82} = 2.2$, $P = 0.14$, respectively), and the interaction between treatments was not significant for either response ($F_{1,82} = 0.05$, $P = 0.83$; $F_{1,82} = 0.05$, $P = 0.82$, respectively).

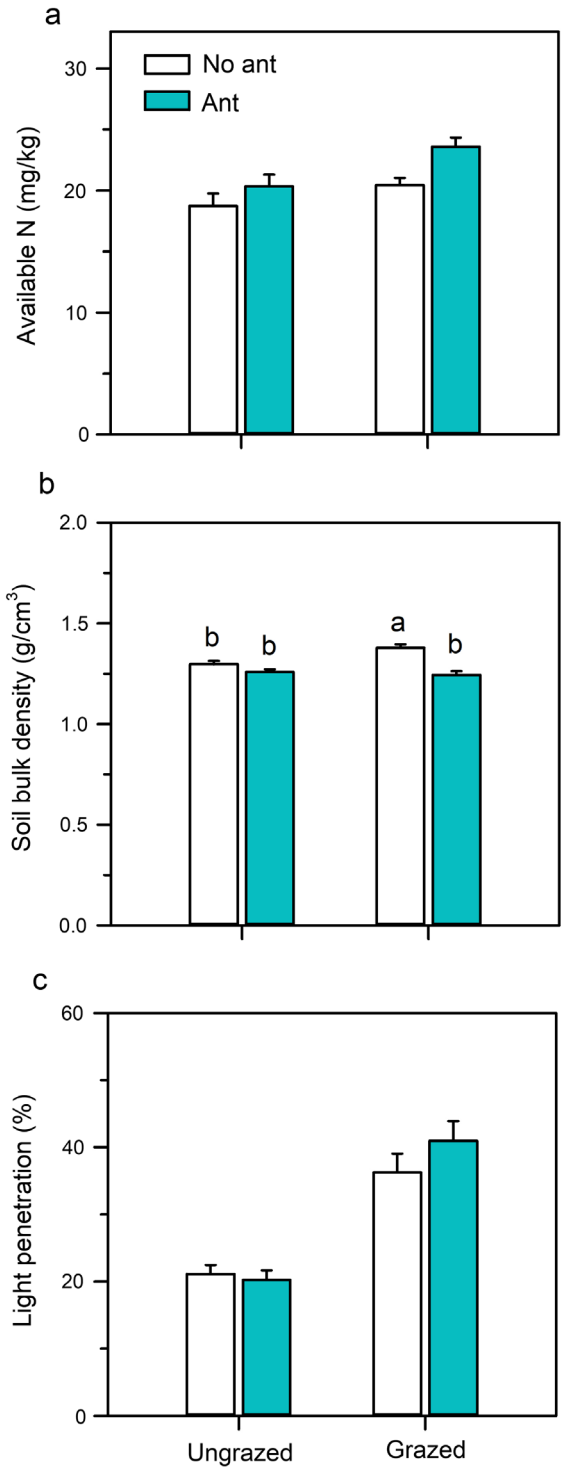

FIG. 2. Combined effects of 3-yr cattle grazing and ant removal treatments on least squares mean (+SE) (a) total available N, (b) soil bulk density, and (c) light penetration, measured in 3 × 3 m subplots in 2012. Different letters above the bars indicate significant differences among treatments, as assessed via post hoc comparisons used to evaluate the interaction between grazing and ants; for panels a and c, only main effects were significant (see *Effects of cattle and ants on soil properties and microclimate*).

Cattle grazing significantly increased the percentage of light penetration to the soil surface ($F_{1,5} = 64.51$, $P = 0.0005$) by nearly twofold, but ants and the interaction with grazing were not significant (both $F_{1,82} < 1.34$, $P > 0.25$). Air temperature at the soil surface did not vary significantly by grazing ($F_{1,5} = 1.37$, $P = 0.29$) or ant treatment ($F_{1,82} = 0.18$, $P = 0.67$), nor did these factors interact ($F_{1,82} = 0.26$, $P = 0.61$).

Ant removal did not affect the number of cattle dungs in grazed subplots ($F_{1,41} = 0.28$, $P = 0.6$), and there were no dungs in ungrazed subplots. However, where ants were left untreated, the density of ant nests was significantly higher in the grazed (3.9 ± 0.8 nests/m^2 [mean $\pm$ SE]) vs. ungrazed treatment (1.2 ± 0.2 nests/m^2; $F_{1,5} = 19.3$, $P = 0.007$). Across all subplots, total available N varied positively with both the number of cattle dungs ($F_{1,81} = 6.36$, $P = 0.014$; Fig. 3a) and the density of ant nests ($F_{1,81} = 6.48$, $P = 0.012$; Fig. 3b), with no significant interaction between these variables ($F_{1,81} = 0.32$, $P = 0.58$). Soil bulk density across all subplots had a positive, albeit marginally significant, relationship with the number of cattle dungs ($F_{1,81} = 3.73$, $P = 0.057$) and a negative relationship with the number of ant nests ($F_{1,81} = 12.16$, $P = 0.0008$); while the interaction between variables was not significant ($F_{1,81} = 2.48$, $P = 0.12$). Neither total available P nor soil moisture were significantly correlated with number of cattle dungs ($F_{1,81} = 0.29$, $P = 0.59$; $F_{1,81} = 0.27$, $P = 0.6$) or ant nests ($F_{1,81} = 0.93$, $P = 0.34$; $F_{1,81} = 0.03$, $P = 0.87$), and the interaction between variables was not significant for either response ($F_{1,81} = 0.44$, $P = 0.51$; $F_{1,81} = 0.47$, $P = 0.5$).

Discussion

Primary productivity is a fundamental ecosystem attribute reflecting the dynamic between the inherent productivity of a system derived from available resources and the top-down effects of consumers that can both reduce standing biomass and increase productivity (Odum 1953, McNaughton 1979, Milchunas and Lauenroth 1993). While it is well-recognized that a variety of processes can influence this dynamic, the explicit roles that different processes play and their underlying mechanisms are not well understood. In our system, large herbivores, not surprisingly, reduced aboveground standing biomass (AB) by 25% compared to baseline levels when large herbivores and soil engineers were absent (Fig. 1). Also not surprising was the finding that soil engineering by ants promoted AB, given that engineering increased available soil N and improved soil structure for plant growth (Fig. 2). Soil engineering in the presence of herbivory was powerful enough to fully offset herbivore reductions in AB. As we discuss below, this result appeared to derive primarily from the combination of herbivory and soil engineering elevating available N enough to allow the plants to reallocate resources from below- to aboveground biomass. Importantly, these

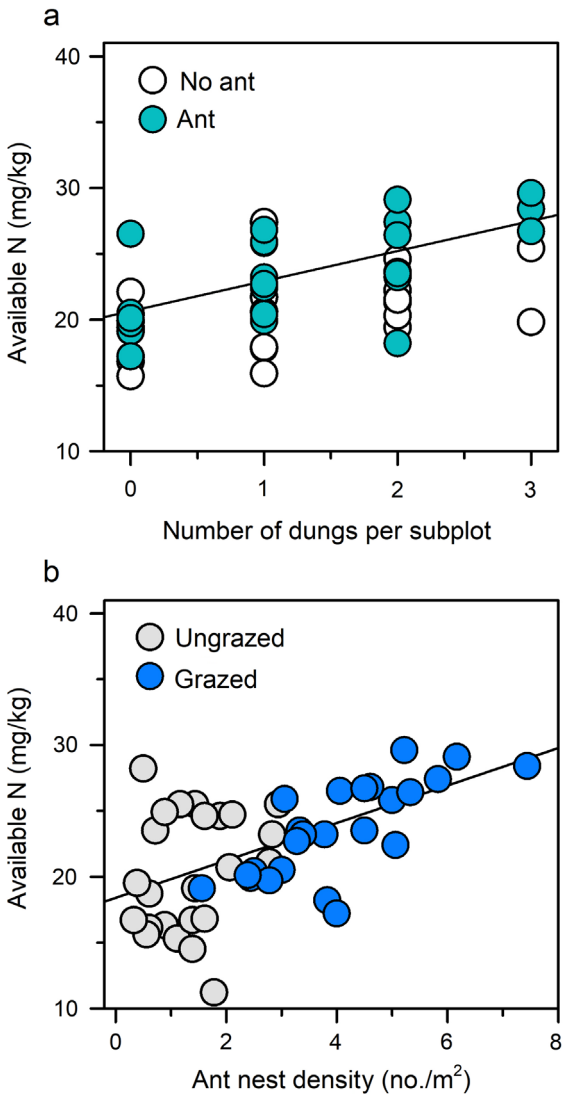

FIG. 3. Relationships between total available N and the predictors (a) number of cattle dungs and (b) ant nest density, measured in 3 × 3 m subplots in 2012 after 3 yr of cattle grazing and ant removal treatments. Both predictors were tested in the same model. In the panel for each predictor: (1) the dotted line shows the predicted value for N when the other predictor was set to its median value, and (2) data points were adjusted to control for background variation in the other predictor, as set to the median value.

outcomes were consistent with predictions from plant resource allocation theory (Bloom et al. 1985), suggesting that integrating such theory may help to inform understandings and management of grassland productivity.

Our finding that strong top-down effects of large herbivores on AB were offset by bottom-up engineering effects of ants suggests an important interplay between these processes. The mechanisms underlying this result appear to derive from (1) additive effects of cattle and ants on total available soil N, (2) compensatory effects of engineering on soil bulk density, and (3) cattle-driven increases in ant densities, processes that combined to facilitate plant resource reallocations that offset grazer impacts on AB. The finding that dung counts were positively correlated with available N suggests that defecation was a mechanism by which cattle increased available N, a pattern documented for large herbivores elsewhere (Hamilton et al. 1998, van der Wal et al. 2004, Frank et al. 2018, Liu et al. 2018). Similarly, higher ant nest densities correlated with higher available N, regardless of dung prevalence, directly linking ant engineering to increased N levels (Farji-Brener and Werenkraut 2017). Cattle grazing in the absence of ants increased soil bulk density, presumably via soil compaction (Fig. 2b); whereas ant engineering reduced soil bulk density in grazed plots to levels similar to those in ungrazed plots. Reduced soil bulk density can facilitate water infiltration (Evans et al. 2011), and elevate soil microbial activities (Z. Zhong et al.,*unpublished data*) linked to higher N availability (Dauber et al. 2001). Importantly, ant nest densities were elevated nearly fourfold in grazed relative to ungrazed subplots. Our prior work in this system indicates that grazing benefits ants by reducing litter cover and creating more favorable micro-environments for ants (Li et al. 2018). Inclusion of ant nest density in models testing for treatment effects on *L. chinensis* standing biomass as well as soil properties indicated that ant densities drove the overall strength of engineering effects in this system (Appendix S1: Table S3). Collectively, these results suggest that the enhanced effects of ant soil engineering seen in the presence of grazers were driven largely by strong positive effects of grazers on ant abundance (Fig. 4), establishing an important feedback between large herbivores and soil engineers in this system.

The resulting changes in soil conditions corresponded with shifts in plant resource allocation strategies, suggesting that soil engineering combined with plant plasticity to enhance bottom-up effects. The baseline RWR in this system was low relative to other grasslands (Reynolds and D'Antonio 1996), a pattern suggestive of strong belowground resource limitation and consistent with the low baseline nutrient levels in this system. In the absence of ants, cattle grazing reduced AB but did not alter belowground biomass (BB) or RWR, suggesting that N inputs from cattle defecation were insufficient to offset aboveground losses in shoot biomass. However, these inputs may have offset belowground reductions in root biomass, which were not evident here, but are sometimes linked to losses in shoot biomass from grazing (e.g., Verkaar 1986, McNaughton et al. 1998, Gao et al. 2008, Charles et al. 2017). In contrast, ant engineering in the presence of cattle grazing was associated with increased AB and decreased BB as well as a shift in RWR toward higher allocations to AB, suggesting that engineering effects mitigated belowground resource limitations sufficiently to allow plants to redirect allocations

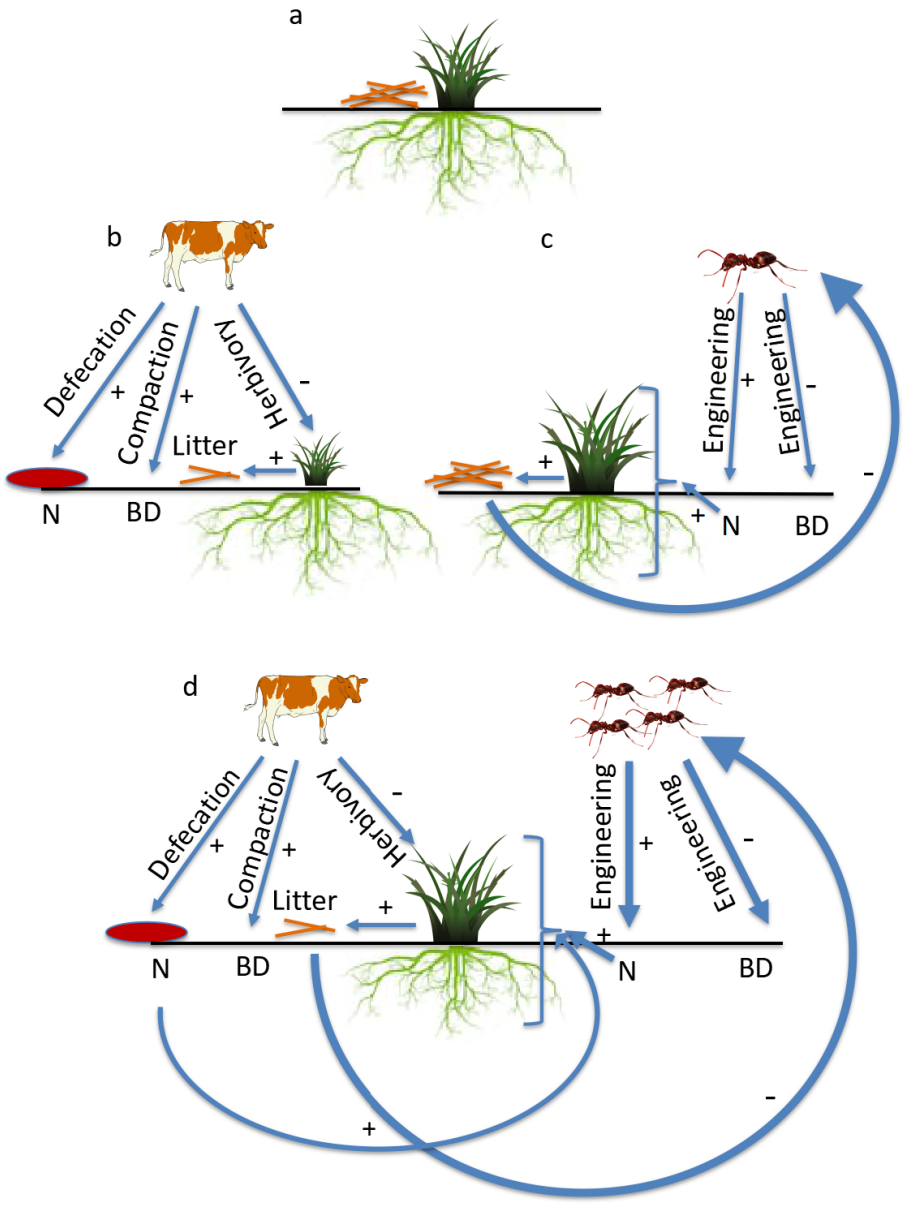

FIG. 4. In the absence of cattle herbivores and ant engineers (e.g., *Formica* spp., *Tetramorium* spp., and *Lasius* spp.) (a), *Leymus chinensis*, the dominant grass in semiarid steps of Northeastern China, exhibits high root to shoot ratios (RWR) reflective of the low soil nutrient levels and belowground resource limitation in this system. When cattle are present without ants (b), they reduce aboveground biomass (AB) by 25% through herbivory (which also reduces litter production), increase soil bulk density (BD) through compaction, and increase soil nitrogen availability (N) through defecation. Collectively, these interactions cause reduced AB with no change in belowground biomass (BB) or RWR. When ants are present and cattle are absent (c), ant soil engineering elevates N and reduces BD, which tends to increase AB and reduce BB and RWR, albeit by small amounts. When cattle and ants are present (d), they generate additive effects on N and ant engineering restores soil BD levels. Importantly, cattle reductions in litter (which occurs even when plants compensate for AB losses) create favorable microhabitats that elevate ant densities nearly fourfold; this elevated ant density greatly enhances ant engineering effects. Collectively, the 26% increase in N and improved soil conditions allow plants to reallocated resources from root toward shoot biomass, reducing RWR and restoring AB to baseline levels without herbivores. Line weights indicate interaction strength. Plus signs indicate positive direct effects, while minus signs indicate negative direct effects.

toward aboveground resources and productivity, returning AB to baseline levels. These results suggest that only when combined did herbivores and engineers increase N and improve soil conditions enough for plants to reallocate resources sufficiently to fully offset herbivore impacts on AB. The linkage between higher N availability and greater allocation of resources toward aboveground biomass is well-documented across many plant

species (Reynolds and D'Antonio 1996). Since aboveground resources did not appear to be initially limited in this system, observed increases in light linked to herbivore reductions in shoot biomass were unlikely to affect RWRs (Fig. 2c). Moreover, tissue losses to herbivores may have constrained carbon uptake, creating an aboveground resource limitation even though light levels increased (Gao et al. 2008). While plant developmental stages can strongly influence resource allocation patterns, thereby confounding interpretation of RWR comparisons across divergent age structures (Weiner 2004), our system represents a long-established grassland comprised of long-lived plants where mature plants dominate demographics.

Our results also offer insights into classical understandings of plant compensatory responses to grazing. While is it generally the case that large herbivores suppress AB across many systems (Milchunas and Lauenroth 1993, Chase et al. 2000), plants can compensate to varying degrees for herbivore losses (McNaughton 1979, Bagchi and Ritchie 2010, Knapp et al. 2012, Ramula et al. 2019). Several mechanisms have been identified to explain this phenomenon, including internal plant feedbacks linked to senescence and growth and coevolutionary responses to herbivory (McNaughton 1979, McNaughton et al. 1983), and external processes linked to litter production, nutrient cycling, and microbial feedbacks (Bardgett et al. 1998, Hamilton and Frank 2001, Charles et al. 2017), with environmental context regulating outcomes (Hawkes and Sullivan 2001). In our system, plant feedbacks could not explain the recovery of AB from herbivory. By removing both large herbivore and ant engineering effects, we were able to establish "baseline" levels of AB and BB for the dominant grass in this system (although invertebrate herbivores and belowground interactions were not controlled for) to quantify the independent and interactive effects of these biotic processes on AB. In isolation, large grazers substantially reduced AB. If plant feedback-related compensatory responses occurred, they were insufficient to offset the 25% reductions in AB from baseline levels. It was only in the presence of ant engineering that we observed system-level compensation to these responses. Hence, the overall compensatory response to grazing was driven by engineering effects and their interplay with grazing, with plant plasticity in resource allocations playing a defining role as detailed above. These findings indicate the value of establishing baseline levels of productivity to better understand how different biotic interactions affect AB, BB, and plant resource allocations. They also raise the question: to what extent might cryptic processes like ecosystem engineering and other biotic interactions (e.g., Belovsky and Slade 2018) underlie grassland NPP and compensatory responses in AB to large herbivores?

In this study, AB losses to large grazers were restored by ant soil engineering. This result demonstrates how powerful soil engineering effects can be in maintaining grassland resiliency to top-down effects of consumers. They also highlight the pivotal role that plasticity in plant resource allocation strategies may play in determining the overall outcome of top-down and bottom-up interactions on AB. Given burgeoning human populations and associated increasing demands on grazing systems around the world (Ripple et al. 2015, Wang et al. 2019), advancing our understandings of the processes that mitigate losses in AB or potentially increase NPP in the context of the ecosystem services provided by grasslands such as livestock production for food is essential. In our system, grazing was set at moderate levels consistent with local government recommendations, which as we demonstrate generated cattle production with essentially no cost to AB. Our results suggest that greater understandings of ecosystem engineering and other drivers of grassland NPP may allow us to better balance competing demands on grazing systems or even increase production while minimizing negative impacts. Given the ubiquity of ants and other soil engineers such as termites and earthworms (Edwards and Bohlen 1996, Bignell 2006, Jouquet et al. 2006, 2014, Evans et al. 2011), the interplay that we observed between soil engineering and plant resource allocation plasticity in determining AB may reflect a widespread phenomenon regulating primary productivity globally.

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Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.3312/suppinfo

Data Availability

Data are available (Zhong 2021) in Dryad: https://doi.org/10.5061/dryad.2rbnzs7kc