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Functional and taxonomic diversity of grasshoppers differentially shape above- and below-ground communities and their function

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Abstract

1. Herbivores are important drivers in both above- and below-ground ecosystems. Despite their importance, studies of herbivores often focus on the effects of individual species, potentially missing the effects of herbivore diversity.
2. Here we examine how orthopteran herbivore species and functional (i.e. diet) diversity interact with nutrient availability to shape above- and below-ground ecosystems. We stocked six species of grasshoppers (Orthoptera) with two different feeding modes (i.e. grass-only or grass- and forb-feeders) at varying taxonomic compositions (0, 1 or 6 species) in field enclosures and measured their effects on above-ground plant biomass, chemistry and richness, as well as below-ground microbial community composition and function. We treated half the cages with nitrogen fertilizer to examine how herbivore effects may be mitigated by soil nutrient availability.
3. Above-ground, we predicted that grasshoppers would decrease plant biomass and richness, and increase the %N of the plant community. We also predicted that high diversity cages would have the largest decreases in plant biomass and richness, but these effects would be offset by fertilizer additions.
4. Below-ground, we predicted that herbivore taxonomic composition and diet would differentially shift soil microbial community composition and function. We also predicted that fertilization would decrease the soil pH and increase nutrient availability creating an interaction with herbivores that leads to shifts below-ground community composition.
5. We found that grasshoppers decreased plant biomass and richness, and this effect was not dependent on nutrient additions. Contrary to our predictions, we did not see the largest above-ground changes in high diversity cages.
6. Below-ground, herbivore diet and fertilization, but not their interaction, shifted bacterial communities. Grasshopper taxonomic composition did not influence bacterial communities. Grasshopper taxonomic composition and diet interacted

with fertilizer to increase below-ground levels of bioavailable C and microbial biomass. Fungal communities did not respond to any treatments.

7. The differing effects of herbivore diet versus taxonomic composition highlight the need for studies that examine multiple diversity metrics when exploring herbivore-meditated effects on above- and below-ground ecosystems. Combined, our results suggest that bottom-up and top-down controls are important factors to consider when studying the composition and function of grassland ecosystems.

KEYWORDS

functional diversity, grassland, herbivore, nutrient availability, Orthoptera

1 | INTRODUCTION

Herbivores are ecosystem drivers that create important connections between above- and below-ground ecosystems (Bardgett & Wardle, 2003). They cause direct effects on the producer subsystem through feeding or mechanical disruption (i.e. trampling of plants, breaking of soil surface; Hobbs, 2006; Wigley et al., 2020) while also directly influencing the decomposer subsystem through inputs of waste or carrion (Barton et al., 2019; Ritzenthaler et al., 2018). Additionally, herbivores can induce indirect effects on above-ground and below-ground ecosystems, via feeding effects on root exudates (Wardle & Bardgett, 2008) or changes in plant composition (Prather et al., 2018; Ritzenthaler et al., 2018). Most studies on herbivore-mediated linkages have focused on a single herbivore (Bardgett & Wardle, 2003; Zhou et al., 2017), despite most ecosystems containing several herbivore species. Therefore, studies that examine herbivore diversity are necessary for understanding above- and below-ground communities and their function.

A second limitation of existing research is that herbivore functional diversity is rarely explored (but see: Laws et al., 2018; Poe et al., 2019). Unlike species diversity, which accounts for the number of species, functional diversity represents the range of traits organisms have within an ecosystem (Tilman, 2001). For example, ecosystems can be highly speciose but functionally homogeneous (Fonseca & Ganade, 2001). Herbivore body size is one form of functional diversity that has received previous attention, with studies demonstrating that small invertebrate herbivores can have large effects compared to large mammalian herbivores (Risch et al., 2015, Vandegehuchte et al., 2015). Feeding preference effects (i.e. diets) are less explored but can create substantial diversity within a trophic level. For example, herbivores can range from host specific to feeding generalists. Accounting for functional trait differences alongside taxonomic composition is essential for understanding the range of effects herbivores can have on ecosystem processes.

While herbivores are important top-down regulators, their effects on an ecosystem are often influenced by underlying environmental nutrient availability. Previous studies have demonstrated that the above-ground influence of herbivores can be diminished or amplified with the addition of nutrients. For example, herbivore effects on plant biomass may be negligible if nutrient levels are high enough to sustain plant regrowth (Gough & Grace, 1998). Plants also can maintain more defensive compounds when nutrient levels are high (Hanssen et al., 2020), diminishing the overall effects of herbivores. In contrast, other studies show increased herbivory when fertilizer is added to an ecosystem due to an increase in plant quality (Rowen & Tooker, 2020). While studies have explored the above-ground effects of herbivores, we are lacking a thorough assessment of how the interacting effects of top-down herbivory and bottom-up nutrient availability influence above- and below-ground communities.

Orthopterans (i.e. grasshoppers, katydids) are model organisms for exploring the effects of herbivore taxonomic and functional diversity on ecosystems. In grasslands, orthopterans are common and important herbivores that drive ecosystem processes (Branson et al., 2006). Above-ground, orthopterans decrease plant biomass, change plant composition and provide a major food source for vertebrates (e.g. birds, rodents, bats; Jonas & Joern, 2008; Laws et al., 2018). Orthopteran herbivores also have diverse feeding modalities that create functional diversity in their communities. Some species feed almost exclusively on grasses, others on forbs, while others feed on a mixed diet of both grasses and forbs. Previous work suggests that orthopteran diet is more important than species richness or identity for predicting plant biomass (Laws et al., 2018). Specifically, grass-feeding orthopteran species decreased grass biomass and total plant biomass more than mixed-feeding species across three different ecosystems. Whether these above-ground effects are influenced by soil nutrient availability, and whether these changes cascade to shift below-ground communities and their function is unknown.

Below-ground, soil microbial communities regulate ecosystem processes, but microbial composition and function can be shaped by fertilization and herbivore presence. Specifically, fertilization can shift soil pH and nutrient stoichiometry, leading to shifts in microbial community composition (Fierer et al., 2012), increased enzymatic activity (Ajwa et al., 1999) and increased organic matter stabilization (Campbell et al., 1991). Invertebrate herbivores can stimulate below-ground activity by shifting plant allocation of nutrients, inducing root exudate production and via direct waste inputs (Fielding et al., 2013; Jonas & Joern, 2008; Wardle & Bardgett, 2008). Grasshoppers in particular have been shown to increase soil microbial respiration (Strickland et al., 2013), microbial biomass (Holland, 1995), enzymatic function (Prather et al., 2017) and soil fertility (Curry, 1987) via inputs of waste and their feeding behaviour. Grasshopper-induced changes in above-ground plant communities can also shift soil microbial community composition, as previous work has highlighted the interdependence of plant species and microbial communities (Zak et al., 2003). Additionally, the introduction of insect frass can introduce novel microbial groups, such as known endosymbionts from the phyla Proteobacteria, Actinobacter and Firmicutes (Muratore et al., 2020). However, most of this work has not examined the influence of herbivore diversity on below-ground communities, nor has it examined the combined influence of nutrient additions and herbivore presence.

Here, we examined the interactive effects of fertilizer and grasshopper functional and species diversity on above- and below-ground ecosystems in a Texas coastal tallgrass prairie. Above-ground, we predicted that grasshoppers would affect plant communities by decreasing overall plant biomass and increasing the %N of plants due to the promotion of secondary defensive compounds. We also predicted that the diet of the grasshoppers would cause shifts in plant richness, favouring grass or forb species in the presence of mixed-feeders or grass-feeders, respectively. Additionally, we predicted that cages containing a high diversity of species and functional groups would have the strongest declines in plant biomass and richness, as the largest amount of niche space would be occupied in these cages. However, we predicted that the decrease in plant biomass when grasshoppers are present would be offset in fertilized cages where nutrient availability was high.

Below-ground, we predicted that soil microbial community composition would shift in the presence of grasshoppers and that grasshopper diet would have a larger effect than species identity or richness, in accordance with Laws et al. (2018). In particular, we predicted that when grasshoppers were present, we would see an increase in endosymbiotic microbial taxa introduced through grasshopper frass and carrion (Muratore et al., 2020). These changes in microbial composition would also lead to changes in the function of soil communities. We predicted that cages with high taxonomic and functional diversity would have the largest shifts in below-ground communities and increased microbial activity because of the direct effect of diverse microbiomes being introduced through frass. We also predicted that herbivore-mediated changes in above-ground plant communities would shift soil properties through changes in root exudates and root structure, causing indirect effects on below-ground microbial communities. Finally, we predicted that fertilization would interact with herbivore effects to alter microbial community composition and function below-ground. While increased soil nutrient availability may offset herbivore effects above-ground, abiotic changes can lead to large shifts in below-ground microbial communities due to increase in nutrient availability and a decrease in soil pH (Fierer et al., 2012; Lauber et al., 2008). Therefore, we predicted that nutrient additions would create longer-lasting and more pronounced shifts below-ground as compared to above-ground.

2 | MATERIALS AND METHODS

2.1 | Treatments, study site and field experiment set-up

To examine how grasshopper taxonomic and functional diversity (hereafter referred to as diets), and their interaction with underlying nutrient availability, influence above- and below-ground communities, we conducted a field-based, herbivore enclosure experiment using six different orthopteran species representing two feeding functional groups (grass-feeders; mixed-feeders). Grass-feeders only consume grasses, whereas mixed-feeders consume both grasses and forbs. We used the six most common orthopteran species found at the site: *Melanoplus femurrubrum* (mixed-feeder), *Paroxya atlantica* (mixed-feeder), *Chortophaga viridifasciata* (grass-feeder), *Orphulella speciosa* (grass-feeder), *Dichromorpha viridis* (grass-feeder) and *Schistocerca americana* (mixed-feeder). As all orthopteran species used for this study are Acrididae, we refer to them as grasshoppers hereafter.

Grasshoppers (2nd–5th instars) were stocked into field cages in early June to create eight treatments: each of the six species alone, all six species together (hereafter the diverse treatment) and a control with no grasshoppers. We use the term taxonomic diversity to refer to our assessment of how cages with all species compare to each individual species cages and control cages. This allows us to examine the effect of species richness and species identity simultaneously. In this study, we also examine how grasshopper diet influences above- and below-ground communities. We use the term diet to refer to our comparison of cages with diverse feeders (i.e. grass- and mixed-feeders) to cages with only grass-feeders, mixed-feeders or control cages. Each grasshopper treatment was crossed with a fertilizer treatment (fertilized and not fertilized), and each treatment combination was replicated seven times (Figure S1a).

The study took place at the Indiangrass Preserve in Katy, TX (30.22, −97.83), which is part of the Katy Prairie Conservancy. This area was native coastal prairie vegetation, and plants consisted of a mixture of both perennial grasses and forbs and annual forbs. For a full species list of the plants at our field site, see online data at (Figshare DOI omitted for review). In early March, cage locations were delineated using PVC. Cages were laid out in rows of 12. In early June, to characterize the initial plant community, we identified each plant species in each cage. We also collected initial soil samples to get a starting assessment of the microbial community. After initial sampling, nitrogen fertilizer (granular urea, 10 g N/m^2) was added to half of the cages, selected haphazardly. After fertilization, we placed Lumite cages over the frames and secured them at the base with sandbags to prevent grasshoppers from escaping or entering cages (Laws et al., 2018). The square cages (0.5 m^2 basal area, 1 m high) were made of Lumite insect netting (amber, mesh size = 18 × 14 holes/inch^2) placed over a PVC frame. Cages had a zipper on one side to allow access to the interior. After initial soil analyses, we realized that the first three rows of cages had higher soil moisture (GVM) than the other rows, so we included row as a blocking factor to account for high moisture and low moisture differences throughout our experiment.

Once cages were constructed, we collected grasshoppers with sweep nets from a nearby field and kept them in terraria for 24 hr to prevent the use of injured individuals. After 24 hr, we stocked grasshoppers into field cages. Stocking rates differed among species to adjust for size disparities so that starting total grasshopper biomass from initial weights of grasshoppers was approximately the same for each cage and treatment (Table S1). For additional information regarding the stocking and survival rates of grasshoppers in each cage, see Supporting Information file 'GrasshopperSurvivalRates.xlsx', also available at Figshare (https://doi.org/10.6084/m9.figshare.11734374). Dead individuals were not replaced or removed throughout the experiment. Prior to stocking the grasshoppers, we checked and removed all other macroarthropods from each cage to limit interactions with stocked grasshoppers.

2.2 | Above-ground sampling: Plant biomass, richness and chemistry

When grasshopper numbers started to decline (about 8 weeks from the start of the experiment), we again measured plant richness and also measured plant biomass by destructively harvesting a 0.1 × 0.25 m strip from the centre third of each cage to 3 cm above the ground. Clipped vegetation was dried at 60°C for 48 hr, and weighed for total dry biomass. Dry biomass was then sorted into grasses and forbs and milled to allow for %N and %C nutrient content to be analysed. Total %C and %N was analysed using a CN elemental analyser by the Soil Nutrient Analysis Lab at the University of Connecticut (www.soiltest.uconn.edu).

2.3 | Below-ground sampling: Soil properties, function and microbial community composition

At the end of the experiment, soil samples were collected from each cage. Within each cage, two soil cores were taken to 10 cm depth on 9 August 2017. Soils were stored at 4°C and transported to the laboratory, where we sieved (4.75 mm) and homogenized each sample. Soil cores were pooled and then split. One set was stored at 4°C for determination of soil characteristics and the other was stored at −80°C for determination of microbial community composition.

For each soil sample, we determined active microbial biomass, mineralizable soil C (an estimate of labile, bio-available C), soil pH and soil moisture (GVM). Active microbial biomass was determined via substrate induced respiration (SIR) following Strickland et al. (2010), where soil slurries were incubated for 4 hr at 20°C, after 1 hr of shaking with excess substrate (i.e. autolysed yeast). After incubation, microbial biomass was estimated as CO_2 production using a static incubation technique (Fierer et al., 2005) and infra-red gas analysis of headspace CO_2 concentrations. Mineralizable C was determined using a 30-day C-mineralization assay (Fierer et al., 2005). Soil was maintained at 65% water holding capacity and 20°C during the time course of this assay and headspace CO_2 concentrations were periodically determined as per SIR. Mineralizable C was estimated as the total area under the curve derived by plotting CO_2 production against time. Soil pH (1:1, soil:water by volume) was determined on a benchtop pH meter, and soil moisture was determined gravimetrically (GVM) by determining the mass of field moist soil, drying soil at 105°C for 24 hr and then re-weighing.

Soil microbial communities were extracted, amplified and analysed using the same protocol outlined in Lucas et al. (2020). A more detailed description of the microbial community procedures is provided in the Supporting Information: Methods.

2.4 | Statistical analyses

We performed most analyses in the R statistical environment (R Core Team, 2017). The package MCTOOLSR (http://leffj.github.io/mctoolsr/) was used to facilitate microbial data manipulation and analyses. Due to a natural underlying moisture gradient, sample cages were blocked by sampling row (see Figure S1a).

We used linear mixed effect models to test how the treatments affected all measured aspects of above-ground and below-ground subsystems. We used two different linear model structures to test the effects of fertilization, grasshopper diet and grasshopper taxonomic composition. The first model was used to assess grasshopper functional diversity and its interaction with fertilizer. It included grasshopper diet, fertilizer addition and their interaction as the fixed effects, and row block and taxonomic composition (i.e. individual species identity in one species cages, all species in diverse cages or no species control cages) as random effects. The second model tested taxonomic diversity and fertilizer effects and included taxonomic composition, fertilizer addition and their interaction as fixed effects, but only included row block as a random effect. Additionally, we included average survival rate of grasshoppers in each cage as a predictor in each model to account for mortality effects on herbivory. We did not include a full model that contained the fixed effects of diet, taxonomic composition and fertilizer, as our study design did not allow us to separate the effects of high taxonomic diversity cages from high functional diversity cages. The response variables were plant biomass, plant chemistry (%C and %N), change in plant richness, bacterial and fungal Shannon's H diversity indices, microbial biomass, soil pH, mass-specific respiration of soils (SIR), gravimetric water content (GVM) and the amount of 'bioavailable' mineralized carbon (MinC). If there was a significant interaction between fertilizer and diet or taxonomic composition, we subsetted the data to assess how grasshopper diet and/or taxonomic composition affected above- and below-ground variables in fertilized and unfertilized environments, keeping block as a random variable. For all mixed effect models, we performed nested model reduction based on AIC values and *p* values from likelihood ratio tests using the LMER package (Bates et al., 2014). A decrease in dAIC >2 was considered significant. In all cases, we examined residuals to confirm appropriate model fits and used the Bonferroni correction to correct for multiplicity. We report alpha when different from 0.05.

We compared overall microbial community structure using the software Primer (ver. 7.0.13) and R (package VEGAN). We square-root transformed the microbial community data before calculating Bray–Curtis dissimilarity. We used community distance matrices to generate ordinations (non-metric multidimensional scaling or NMDS) for bacteria and fungi. Microbial samples we visually determined to be outliers or with low sequence depth were dropped from analyses and ordination plotting; bacterial samples from cage 3 (treatment *O. speciosa*), 21 (all species) and 77 (*D. viridis*) were dropped; no fungal samples were dropped. We used PERMANOVA to compare community composition among grasshopper taxonomic composition, diet, fertilizer additions and their interactions, with block as a random effect (Anderson et al., 2008). We also compared beta-diversity across our samples using PERMDISP tests (Anderson et al., 2006). PERMDISP tests calculate within group dissimilarity in community composition and then compares the magnitude of dissimilarity among each group (9,999 permutations). We performed follow-up analyses to determine which microbial orders might be driving any observed shifts in microbial community composition using the same LMER format described above. Only taxonomic orders of microbiota with >1.0% relative abundance were examined. To determine if changes in plant properties (biomass and chemistry) co-varied with shifts in below-ground microbial communities, and to determine if shifts in bacterial and fungal communities co-varied, we examined the correlations between community dissimilarity matrices using a permutation-based Mantel test with Spearman correlations (Legendre & Legendre, 2012).

3 | RESULTS

3.1 | Above-ground communities respond to grasshopper diets, taxonomic composition and fertilization

As predicted, grasshoppers decreased plant biomass while fertilization increased plant biomass (Table 1; Figure 1). All cages containing grasshoppers had lower biomass than control cages regardless of diet or species identity (Taxonomic Composition: dAIC = −11.1, $\chi^2_7 = 25.09$, $p < 0.001$, Figure 1A; Diet: dAIC = −12.1, $\chi^2_3 = 18.12$, $p < 0.001$, Figure 1B). However, contrary to our predictions, cages containing all species did not have lower biomass than cages with one species present (Figure 1A). Also contrary to our predictions, we did not observe significant interactions between fertilizer and grasshoppers on plant biomass (Taxonomic Composition × Fertilizer: dAIC = 12.8, $\chi^2_7 = 1.15$, $p = 0.99$; Diet × Fertilizer: dAIC = 5.6, $\chi^2_3 = 0.38$, $p = 0.94$, Table 1). As expected, fertilizer increased overall plant biomass (dAIC = −40.4, $\chi^2_1 = 42.4$, $p < 0.001$). Plant biomass decreased with higher grasshopper survivorship (dAIC = −4.2, $\chi^2_1 = 6.14$, $p = 0.01$, Table S2). Generally, the presence of herbivores decreased plant richness (Taxonomic composition: dAIC = −0.45, $\chi^2_7 = 14.88$, $p = 0.04$; Diet: dAIC = −3.9, $\chi^2_3 = 9.89$, $p = 0.02$), though this effect was primarily due to grass-feeders decreasing the overall richness of grasses (Table 1; Figure 2A,B). We saw no effect of fertilizer on plant richness (dAIC = −1.60, $\chi^2_1 = 0.33$, $p = 0.56$, Table 1). Plant richness was not influenced by grasshopper survivorship (dAIC = −7.5, $\chi^2_1 = 0.39$, $p = 0.53$, Table S2).

Grasshoppers and fertilization treatments also shifted the chemistry of forbs and grasses. The response of forb N content to grasshoppers depended on fertilization (Diet × fertilizer: dAIC = −5.79, $\chi^2_3 = 11.79$, $p = 0.008$; Taxonomic composition × Fertilizer: dAIC = −2.02, $\chi^2_7 = 16.02$, $p = 0.03$, Figure S2). In fertilized cages, cages with all species had lower forb N content than cages with *M. femurrubrum* and *C. viridifasciata* (Taxonomic composition in fertilized cages: dAIC = −9.25, $\chi^2_7 = 23.25$, $p = 0.002$, Figure S2). In unfertilized cages, forb N was unaffected by grasshopper species identity or richness. Though diet and fertilizer interacted to influence N, when we split our analyses, we no longer saw a significant effect of diet in either fertilized or unfertilized cages (Table 1). We also observed an interaction between grasshopper taxonomic composition and fertilizer on total forb C content (Figure S2), but did not observe an effect of diet (Table 1). Cages containing the species *M. femurrubrum* had higher forb C content than all other cages (except cages with *Schistocerca americana*) when fertilizer was added. In unfertilized cages, *Orpheulella speciosa* decreased forb C as compared to control cages and cages with all species, or cages with just *M. femurrubrum* or *Schistocerca americana*. Grasshoppers did not affect grass chemistry (Table 1), but fertilization increased grass total %N by 64.57% and total %C by 66.99% (grass total N: dAIC = −5.3, $\chi^2_1 = 7.3$, $p = 0.007$; grass total C: dAIC = −6.18, $\chi^2_1 = 8.18$, $p = 0.004$, Figure S3). Average grasshopper survival did not influence grass or forb chemistry (Table S2).

3.2 | Grasshopper diet and fertilization shifts bacterial but not fungal community composition

Bacterial community composition was affected by grasshopper diet, but beta-diversity (as determined via PERMDISP) was not, indicating that grasshopper diets shifted bacterial community composition rather than creating greater bacterial community dispersion (PERMANOVA: pseudo-$F_{3,95} = 1.19$, $p = 0.03$; PERMDISP: pseudo-$F_{3,105} = 2.26$, $p = 0.24$, Figure 3A). Specifically, bacterial communities from the grass-feeder treatment differed from bacterial communities in control cages and high diversity cages (pairwise comparisons: $p < 0.05$). Bacterial community composition also shifted with the addition of fertilizer (PERMANOVA: pseudo-$F_{1,97} = 3.27$, $p < 0.001$; PERMDISP: pseudo-$F_{2,109} = 0.24$, $p = 0.66$, Figure S4i), as did multiple orders of bacteria (Tables S3 and S4). Contrary to our predictions, there was no interactive effect of grasshopper diet and fertilizer on overall bacterial community composition (PERMANOVA: pseudo-$F_{3,91} = 1.01$, $p = 0.40$), but one order, Bacilli, decreased in grass-feeder cages only when fertilizer was added (diet by fertilizer: dAIC = −2.45, $\chi^2_2 = 8.45$, *df* = 3, $p = 0.04$, Table S3). Fertilization

TABLE 1 Statistical tests of all fixed effects in our linear models assessing the effects of diet, taxonomic composition and fertilization on above-ground variables. We performed nested model reduction, and the dAIC column represents the change in AIC value when the fixed effect term is removed from the model. We considered decreases in AIC values more than 2 to be significant (dAIC), as they indicate that the model containing that fixed effect is better than the model without it. We also provide the results of our likelihood ratio tests of each fixed effect and considered $p < 0.5$ to be significant and denote them in bold. When there was a significant interaction between diet or taxonomic composition and fertilization, we subsetted the data to examine how these variables influenced each variable in fertilized and unfertilized plots separately

	Full model	Subsetted models				
Response variable	Fixed effects	Fixed effects	dAIC	Chi-squared	*df*	*p* value
Diet						
Final plant biomass	Diet × fertilizer		5.6	0.38	3	0.94
	Diet		**−12.1**	**18.12**	**3**	**<0.001**
	Fertilizer		**−40.4**	**42.4**	**1**	**<0.001**
Change in plant richness	Diet × fertilizer		4.08	1.92	3	0.59
	Diet		**−3.9**	**9.89**	**3**	**0.02**
	Fertilizer		1.67	0.33	1	0.56
Forb N	**Diet × fertilizer**		**−5.79**	**11.79**	**3**	**0.008**
		Diet (unfertilized plots)	3.59	2.41	3	0.49
		Diet (fertilized plots)	0.06	5.94	3	0.11
Forb C	Diet × fertilizer		−0.25	6.25	3	0.1
	Diet		0.93	5.07	7	0.17
	Fertilizer		0.94	1.06	1	0.3
Grass N	Diet × fertilizer		2.06	3.94	3	0.27
	Diet		3.33	2.67	3	0.45
	Fertilizer		**−5.3**	**7.3**	**1**	**0.007**
Grass C	Diet × fertilizer		2.68	3.32	3	0.34
	Diet		4.17	1.83	3	0.61
	Fertilizer		**−6.18**	**8.18**	**1**	**0.004**
Taxonomic composition						
Final plant biomass	Tax. comp. × fertilizer		12.8	**1.15**	7	0.99
	Tax. comp.		**−11.1**	**25.09**	**7**	**<0.001**
	Fertilizer		**−40.2**	**42.4**	**1**	**<0.001**
Change in plant richness	Tax. composition × fertilizer		9.24	4.76	7	0.69
	Tax. comp.		**−0.45**	**14.88**	**7**	**0.04**
	Fertilizer		−1.60	0.33	1	0.56
Forb N	**Tax. comp. × fertilizer**		**−2.02**	**16.02**	**7**	**0.03**
		Tax. comp. (unfertilized plots)	9.5	4.5	7	0.72
		Tax. comp. (fertilized plots)	**−9.25**	**23.25**	**7**	**0.002**
Forb C	**Tax. comp. × fertilizer**		**−3.91**	**17.91**	**7**	**0.01**
		Tax. comp. (unfertilized plots)	**−7.88**	**21.88**	**7**	**0.003**
		Tax. comp. (fertilized plots)	**−5.43**	**19.43**	**7**	**0.007**
Grass N	Tax. comp. × fertilizer		8.19	5.81	7	0.56
	Tax. comp.		8.8	5.2	7	0.64
	Fertilizer		**−5.3**	**7.3**	**1**	**0.007**
Grass C	Tax. comp. × fertilizer		8.56	5.54	7	0.59
	Tax. comp.		9.71	4.29	7	0.75
	Fertilizer		**−6.18**	**8.18**	**1**	**0.004**

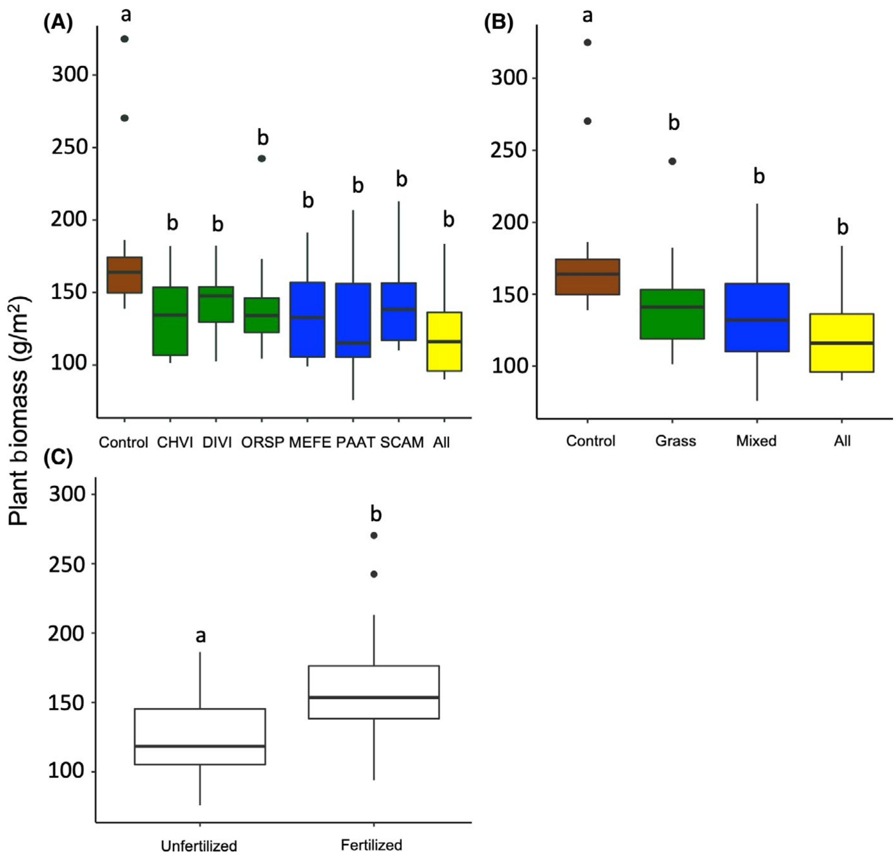

FIGURE 1 Boxplots of average plant biomass (g/m^2) across (A) grasshopper species, (B) functional groups and (C) fertilizer treatments. The boxes represent the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively) and the vertical line inside the box defines the median. Whiskers represent the lowest and highest values within 1.5 times the IQR from the first and third quartiles, respectively, and outliers are represented as points outside the whiskers. Letters denote significant differences across sampling locations. Green boxes represent grass-feeders, blue boxes represent mixed-feeders, yellow boxes represent cages with all diets and species, and control plots are in brown. Grasshopper species are abbreviated as follows *Chortophaga viridifasciata* (CHVI), *Dichromorpha viridis* (DIVI), *Paroxya atlantica* (PAAT), *Melanoplus femurrubrum* (MEFE), *Orphulella speciosa* (ORSP), *Schistocerca americana* (SCAM)

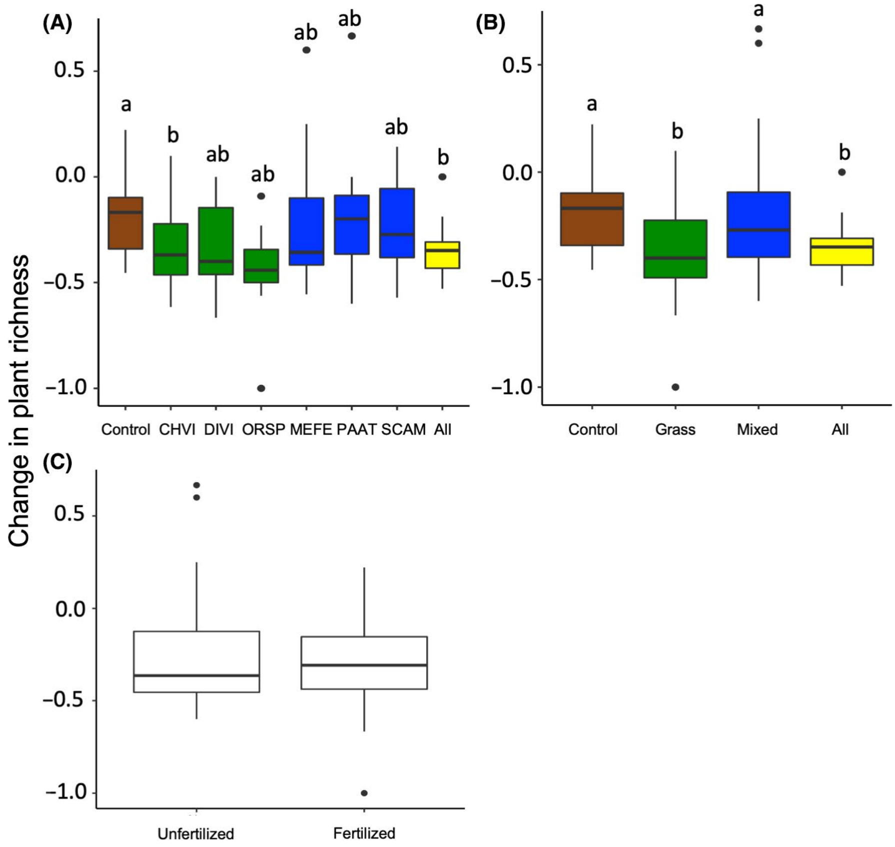

FIGURE 2 Boxplots of average change in plant richness across (A) grasshopper species, (B) diet and (C) fertilizer treatments. The boxes represent the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively) and the vertical line inside the box defines the median. Whiskers represent the lowest and highest values within 1.5 times the IQR from the first and third quartiles, respectively, and outliers are represented as points outside the whiskers. Letters denote significant differences across sampling locations. Species labels have been abbreviated in figure (A) as follows: *Chortophaga viridifasciata* (CHVI), *Dichromorpha viridis* (DIVI), *Paroxya atlantica* (PAAT), *Melanoplus femurrubrum* (MEFE), *Orphulella speciosa* (ORSP), *Schistocerca americana* (SCAM)

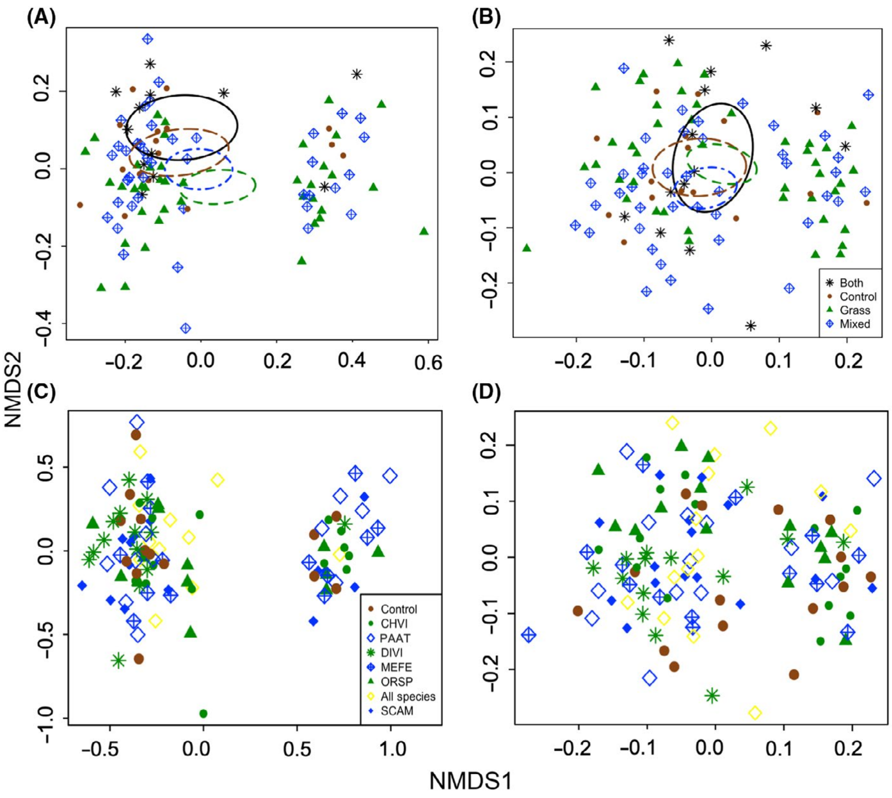

FIGURE 3 Non-metric dimensional scaling representation of (A, C) bacterial and (B, D) fungal communities associated with grasshopper diets (A, B) and species (C, D). Distances are based on dissimilarity matrices of sequence-based Bray–Curtis distances. Grasshopper functional groups did affect the bacterial communities (PERMANOVA Bacteria: pseudo-$F_{3,95} = 1.17$, $p = 0.03$; PERMDISP Bacteria: pseudo-$F_{3,105} = 2.26$, $p = 0.24$), but did not influence the fungal communities. Grasshopper species identity did not shift bacterial (PERMANOVA: pseudo-$F_{10,91} = 1.04$, $p = 0.22$; PERMDISP: pseudo-$F_{8,109} = 0.67$, $p = 0.77$) or fungal communities (PERMANOVA: pseudo-$F_{7,96} = 1.01$, $p = 0.45$; PERMDISP: pseudo-$F_{8,114} = 1.90$, $p = 0.10$). Species labels have been abbreviated in panels (C, D) as follows: *Chortophaga viridifasciata* (CHVI), *Dichromorpha viridis* (DIVI), *Paroxya atlantica* (PAAT), *Melanoplus femurrubrum* (MEFE), *Orphulella speciosa* (ORSP), *Schistocerca americana* (SCAM)

increased the relative abundance of Alphaproteobacteria, but decreased Deltaproteobacteria and Phycisphaerae (Tables S3 and S4; Figure S5i–iii). Grasshopper diet and fertilization, but not their interaction, had a marginally significant effect of increasing the relative abundance of Acidobacteria (Table S3; Figure S5iv,v).

As predicted, grasshopper taxonomic composition did not shift bacterial communities (PERMANOVA: pseudo-$F_{7,108} = 1.03$, $p = 0.29$; PERMDISP: pseudo-$F_{7,108} = 0.75$, $p = 0.70$, Table S4; Figure 3C), nor was there an interaction between orthopteran species and fertilization (PERMANOVA: pseudo-$F_{7,108} = 1.04$, $p = 0.20$). Grasshopper species richness and identity also did not influence the relative abundance of any bacterial order (Table S4). Bacterial Shannon diversity (H) was unaffected by grasshopper taxonomic composition, diet or fertilization (Table 2).

Unlike bacteria, fungal community composition did not respond to any of our experimental treatments (PERMANOVA diet: pseudo-$F_{3,100} = 0.95$, $p = 0.84$; PERMDISP diet: pseudo-$F_{4,114} = 5.91$, $p = 0.21$, PERMANOVA Fertilizer: pseudo-$F_{1,102} = 1.04$, $p = 0.31$; PERMDISP Fertilizer: pseudo-$F_{2,114} = 2.33$, $p = 0.88$, PERMANOVA Taxonomic composition: pseudo-$F_{7,96} = 1.01$, $p = 0.45$; PERMDISP Taxonomic composition: pseudo-$F_{8,114} = 1.90$, $p = 0.10$, Figures 3B,D and 4B). There was also no diet by fertilizer (PERMANOVA pseudo-$F_{3,97} = 1.03$, $p = 0.27$) or taxonomic composition by fertilizer interaction for fungi (PERMANOVA pseudo-$F_{7,113} = 0.99$, $p = 0.49$). Though community composition responses were not significant, fungal Shannon diversity (H) had a significant diet by fertilizer and grasshopper taxonomic composition by fertilizer interaction (Table 2). Specifically, in cages without fertilizer, cages with all species had lower Shannon diversity (H) than cages with *S. americana* (pairwise comparison: $p = 0.007$), and mixed-feeders had marginally higher Shannon diversity (H) as compared to control cages (pairwise comparison: $p = 0.07$).

Overall, bacterial and fungal communities co-varied in their shifts in community composition (Mantel test: $R = 0.52$, $p < 0.001$). Bacterial and fungal communities were also strongly correlated with gravimetric soil moisture (GVM; Mantel test bacteria: $R = 0.75$, $p < 0.001$; fungi: $R = 0.47$, $p < 0.001$), mineralizable C (Mantel test bacteria: $R = 0.12$, $p = 0.02$; fungi: $R = 0.05$, $p = 0.11$), substrate-induced respiration (Mantel test bacteria: $R = 0.08$, $p = 0.04$; fungi: $R = 0.08$, $p = 0.02$) and pH (Mantel test bacteria: $R = 0.23$, $p < 0.001$; fungi: $R = 0.17$, $p < 0.001$). A marginally significant correlation was observed between plant biomass and fungal communities (Mantel test plant biomass: $R = 0.07$, $p = 0.052$), but not the bacterial communities (Mantel test plant biomass: $R = -0.01$, $p = 0.63$). We did not see any significant correlations between either bacterial or fungal communities and the C and N content of the plant community (Mantel test plant chemistry Bacteria: $R = 0.01$, $p = 0.29$; Fungi: $R = 0.03$, $p = 0.22$).

3.3 | Grasshopper diet and taxonomic composition cause different below-ground shifts in functionality

Grasshoppers and nutrient levels also changed the function of below-ground communities. We saw a significant interaction between diet and fertilizer on mineralizable (i.e. bioavailable) C (dAIC = −3.00, $\chi^2_3 = 9.00$, $p = 0.03$, Table 2). Specifically, when fertilizer was added, grasshoppers increased below-ground bioavailable C by 6.59% and

TABLE 2 Statistical tests of all fixed effects in our linear models assessing the effects of diet, taxonomic composition and fertilization on microbial community and soil function variables. We performed nested model reduction, and the dAIC column represents the change in AIC value when the fixed effect term is removed from the model. We considered decreases in AIC values more than 2 to be significant (dAIC), as they indicate that the model containing that fixed effect is better than the model without it. We also provide the results of our likelihood ratio tests of each fixed effect and considered $p < 0.5$ to be significant and denote them in bold. When there was a significant interaction between diet or taxonomic composition and fertilization, we subsetted the data to examine how these variables influenced each variable in fertilized and unfertilized plots separately

	Full model	Subsetted model				
Response variable	Fixed factor	Fixed factor	dAIC	Chi-squared	*df*	*p* value
Diet						
Bacterial Shannon (H)	Diet × fertilizer		1.37	4.63	3	0.2
	Diet		3.77	2.24	3	0.52
	Fertilizer		−0.41	2.4	1	0.12
Fungal Shannon (H)	**Diet × fertilizer**		**−3.58**	**9.58**	**3**	**0.02**
		Diet (unfertilized plots)	−0.97	6.97	3	0.07
		Diet (fertilized plots)	2.65	3.35	3	0.34
Mineralizable C	**Diet × fertilizer**		**−3**	**9**	**3**	**0.03**
		Diet (unfertilized plots)	2.87	3.13	3	0.37
		Diet (fertilized plots)	**−2.84**	**8.84**	**3**	**0.03**
Microbial iomass (SIR)	Diet × fertilizer		0.81	6.81	3	0.08
	Diet		5.57	0.43	3	0.93
	Fertilizer		−1.48	3.59	1	0.06
pH	Diet × fertilizer		4.9	1.11	3	0.78
	Diet		5.06	0.94	3	0.82
	Fertilizer		−1.24	3.24	1	0.07
Gravimetric soil moisture (GVM)	Diet × fertilizer		5.07	0.92	3	0.82
	Diet		4.02	1.99	3	0.58
	Fertilizer		1.96	0.03	1	0.85
Taxonomic composition						
Bacterial Shannon (H)	Tax. comp. × fertilizer		2.513	11.49	7	0.12
	Tax. comp.		8.683	5.32	7	0.62
	Fertilizer		−0.41	2.4	1	0.12
Fungal Shannon (H)	**Tax. comp. × fertilizer**		**−1.75**	**15.75**	**7**	**0.03**
		Tax. comp. (unfertilized plots)	**−5.57**	**19.57**	**7**	**0.007**
		Tax. comp. (fertilized plots)	9.08	4.92	7	0.67
Mineralizable C	**Tax. comp. × fertilizer**		**−0.1**	**14.05**	**7**	**0.05**
		Tax. comp. (unfertilized plots)	5.56	8.44	7	0.29
		Tax. comp. (fertilized plots)	1.74	12.26	7	0.09
Microbial biomass (SIR)	**Tax. comp. × fertilizer**		**−5.69**	**19.69**	**7**	**0.006**
		Tax. comp. (unfertilized plots)	**−9.17**	**23.17**	**7**	**<0.002**
		Tax. comp. (fertilized plots)	5.68	8.32	7	0.31
pH	Tax. comp. × fertilizer		8.28	5.72	7	0.57
	Tax. comp.		7.54	6.46	7	0.49
	Fertilizer		−1.24	3.24	1	0.07
Gravimetric soil moisture (GVM)	Tax. comp. × fertilizer		10.7	3.3	7	0.86
	Tax. comp.		10.13	3.88	7	0.79
	Fertilizer		1.96	0.03	1	0.85

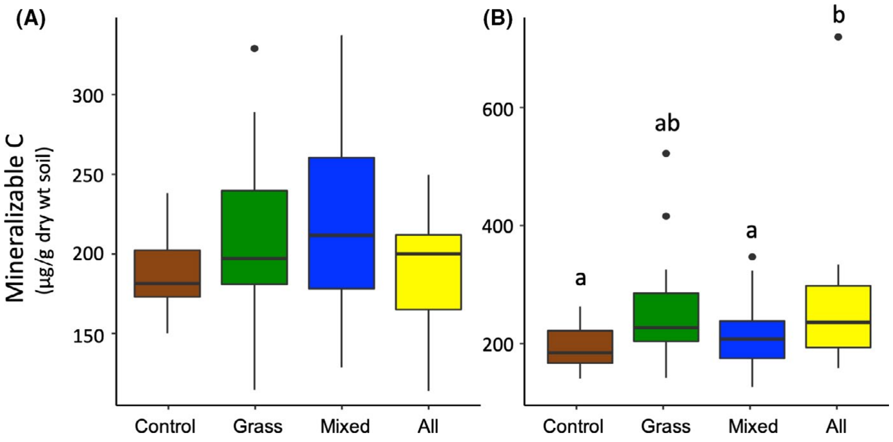

FIGURE 4 Boxplots of average cumulative mineralizable C in (A) unfertilized and (B) fertilized cages across functional groups after a 30-day incubation. Mineralizable carbon was increased in plots with both feeding types and high nutrient content, but unaffected in unfertilized plots. The boxes represent the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively) and the vertical line inside the box defines the median. Whiskers represent the lowest and highest values within 1.5 times the IQR from the first and third quartiles, respectively, and outliers are represented as points outside the whiskers. Letters denote significant differences across sampling locations

16.42% in cages with both feeding types as compared to cages with just mixed-feeder and control cages, respectively (dAIC = −2.84, $\chi^2_3 = 8.84$, $p = 0.03$, Figure 4B). Cages with just grass-feeders had a mildly significant increase in bioavailable C (Table 2; Figure 4B). However, when fertilizer was not present, there was no effect of diet on bioavailable C (dAIC = 2.87, $\chi^2_3 = 3.13$, $p = 0.37$; Figure 4A). Grasshopper taxonomic composition also had a significant interaction with fertilizer, but in conservative post-hoc we were unable to determine the specific differences across species (Table 2).

In contrast, taxonomic composition had a significant effect on microbial biomass (i.e. SIR), but grasshopper diet did not (Table 2). There was a significant interaction between taxonomic composition and fertilizer (dAIC = −5.69, $\chi^2_7 = 19.69$, $p = 0.006$), where one orthopteran species increased microbial biomass in unfertilized cages, but not in fertilized cages (Table 2; Figure S6). The response in unfertilized cages was due to *D. vidris* increasing microbial biomass as compared to cages with all species or cages with only *P. atlantica*, *C. viridifasciata*, or *S. americana* (Figure S6i). Soil pH and moisture (GVM) were not affected by grasshopper taxonomic composition, diet fertilization (Table 2). No below-ground variables were influenced by average grasshopper survival rates (Table S2).

4 | DISCUSSION

We found that grasshopper taxonomic composition (i.e. species identity and richness) and diet caused marked but often differing effects on above- and below-ground ecosystem properties. Above-ground, grasshoppers decreased plant biomass and richness. Below-ground, grasshopper diet shifted bacterial communities, but species-specific effects on community composition were not apparent. However, both grasshopper taxonomic composition and diet influenced the function of below-ground communities. Here, we discuss how documenting the effects of taxonomic composition versus diet (i.e. functional diversity) can result in different conclusions regarding how herbivores may shape prairie ecosystems. Additionally, we discuss how top-down herbivore effects and bottom-up nutrient availability interact to shape ecosystem function.

As predicted, the presence of grasshoppers decreased plant biomass and richness. The observed effect of grasshoppers on plant biomass was binary, with all species and diets of grasshoppers driving lower plant biomass compared to controls. This result challenges the previous finding that grass-feeding grasshoppers decrease plant biomass more than mixed-feeders (Laws et al., 2018). It also challenges our prediction that cages containing all species would have larger above-ground responses than cages containing only one species. Our observed binary effect of grasshopper presence on plant biomass could be due to the fact that all cages contained equivalent levels of grasshopper biomass. Studies of mammalian herbivores highlight that body size and grazing intensity (i.e. herbivore biomass) often regulate plant community responses (Chang et al., 2018). By maintaining equivalent herbivore biomass across treatments, we may have created equal feeding demands, and therefore masked diversity influences on plant biomass. Follow-up studies that explore above-ground effects of grasshoppers stocked at varying biomass and representative of location-specific field densities are needed to further understand how herbivore diversity influences plant biomass.

In contrast to our plant biomass results, we found that plant richness was strongly influenced by herbivore diversity. Specifically, we found that plant richness was lower in cages containing both functional groups and just grass-feeding grasshoppers. Cages with forb-feeding grasshoppers did not change in richness. One potential reason for this lack of change could be the high abundance of forbs in our study system. With high forb density, it is possible that forb-feeding grasshoppers were unable to fully eliminate forb species during the single growing season of our experiment. The decrease in richness when grass-feeding and both functional groups are present challenges previous findings of no effect of grasshopper species on plant richness (Laws et al., 2018). Interestingly, we did not see an interaction between grasshopper taxonomic composition or diet and fertilization treatments on plant richness. Previous studies frequently demonstrate that fertilization increases plant biomass and decreases plant richness, but this effect is mediated by the presence of vertebrate and invertebrate herbivores (Borer et al., 2014; Gough & Grace, 1998; Poe et al., 2019; Schädler et al., 2008). Additionally, most studies demonstrate that herbivores help maintain higher plant diversity (Borer et al., 2014; Richardson & Stiling, 2019). Our results challenge this sentiment and suggest that, regardless of the underlying nutrient levels, grass-feeding grasshoppers can provide enough herbivory to decrease plant richness in our grassland system. However, it is important to note that all observed changes in plant richness were due to changes in above-ground plant properties. It is possible that the roots of these plants were still present in our plots, and could allow for regrowth. Follow-up studies are necessary to determine how persistent these shifts in plant richness are.

In addition to decreasing plant biomass and richness, grasshoppers changed the chemical composition of forbs. When fertilizer was added, we found *M. femurrubrum* increased forb C and N levels. *Melanoplus femurrubrum* is a mixed-feeding grasshopper, capable of adjusting its diet depending on predator presence (Hawlena & Schmitz, 2010a). It is possible that because the grasshoppers in our study were kept in cages and were therefore protected from predation, *M. femurrubrum* preferentially consumed grasses over forbs (Hawlena & Schmitz, 2010b). It is also possible that forb species in fertilized cages were able to increase their investment in defensive compounds, decreasing *M. femurrubrum* feeding rates (Behmer & Joern, 2008). In unfertilized cages, this effect was no longer present suggesting that forb species may be nutrient limited in our grassland site. The nutrient-dependent response of plant chemistry to grazing underlines the importance of documenting bottom-up and top-down controls concurrently. We also highlight that C and N analyses were done on composite samples of forbs and grasses within our plots. It is possible that greater chemical composition changes may have occurred at the species level, but these were masked by our pooled design. Follow-up studies that can examine specific plant species responses may better illuminate the influence of grasshopper species composition and diet.

Below-ground, grasshopper diet but not taxonomic composition shifted bacterial community composition, and we observed no interaction between diet and fertilization. The changes in bacterial communities were seen between cages containing only grass-feeders and cages with both feeding groups or no grasshoppers. Contrary to our prediction, we did not find evidence of above-ground plant changes driving below-ground changes or that cages with all grasshopper species and functional groups present shifted the most. Instead, we suggest that frass inputs from grass-only feeding herbivores may be responsible for the observed changes in bacterial community composition. While multiple previous studies have highlighted the potential for orthopteran frass to influence soil nutrient cycling by shifting soil nutrient stoichiometry (Fielding et al., 2013; Zhang et al., 2014), few studies have directly examined the influence of microbial introductions in grasshopper frass (Snyder et al., 1999). Orthopteran microbiomes can contain diverse communities (Muratore et al., 2020). These microbiome communities inside invertebrates are often distinct from soil communities (Lucas et al., 2017), and therefore frass deposition may be an important source of novel bacteria. For example, Snyder et al. (1999) demonstrated that grasshopper frass can be an important dispersal mechanism for rhizobacterium. Additionally, orthopteran microbiomes vary according to host diet (Muratore et al., 2020). Interactions between the frass microbiomes of mixed-feeders and grass-feeder may explain why soil communities responded differently to the presence of grass-feeders alone versus cages with both mixed- and grass-feeders. Frass microbiome inputs could also explain why we observed an increase in microbial biomass when *D. viridis* was present. *D. viridis* may have a highly abundant microbiome capable of stimulating below-ground microbial biomass. How grasshopper microbiomes differ across species and diets, and the degree to which new bacteria are introduced through frass are important next steps in connecting herbivores and below-ground communities.

Fungal community composition did not respond to any treatment. We offer three possible explanations for the lack of response of fungal communities. The first is that fungal communities are often more resistant to disturbances than bacterial communities (De Vries et al., 2012; Shade et al., 2012). Because of their advanced hyphal network and ability to pull resources across space, slight changes in the local environment may not affect fungi as readily as bacteria. A second reason for why we did not see a response could be the short duration of this experiment. This study was conducted over one growing season, and fungi have been shown to take years to change in composition in other experiments (Hayden et al., 2012). It is possible that if grasshopper presence had been maintained over a longer period of time, we would have seen increased responses of fungal communities. Finally, a limitation of the DNA extraction process is that it is unable to differentiate between active and inactive fungal hyphae and spores. Therefore, it is possible that though the community of fungi did not shift, the active components of the community may have, and we were unable to capture it with our methodology.

Grasshoppers also interacted with nutrient additions to change the function of soils. We saw an increase in mineralizable C in fertilized cages with both diet types and cages with just grass-feeders. This could be because herbivore damage increases root exudates below-ground (Wardle & Bardgett, 2008). Plant C allocation is also influenced by herbivore pressures, often resulting in higher below-ground C investment (Wilson et al., 2018). This pulse of labile C could explain the observed changes in bacterial community composition and could lead to increases in long-term C storage in grasslands (Ritzenthaler et al., 2018). Herbivores are usually considered to be unimportant players in below-ground ecosystem C storage pathways. Our results challenge this interpretation, and emphasize that both top-down and bottom-up factors likely shape the potential for soil systems to sequester C.

Fertilization caused multiple shifts in above- and below-ground communities, underlining the control that nutrient availability can have on prairie ecosystems. Unsurprisingly, we saw increases in plant biomass with fertilization. We did not, however, see fertilizer-induced changes in plant richness during our experiment's single growing season. Therefore, while both nutrients and herbivores are capable of affecting productivity in above-ground systems, top-down pressures of herbivores may be more important for maintaining plant diversity over a short period of time (i.e. a single growing season). Previous studies have demonstrated that fertilization may take multiple years to induce shifts in plant richness while biomass effects can be relatively rapid (Dickson & Gross, 2013; Li et al., 2017).

Fertilization also had a significant effect on below-ground community composition and function. We found that fertilizer shifted the relative abundance of multiple bacterial orders (Table 2; Figure S5), but did not affect fungal community composition. Previous studies have highlighted the direct and indirect influence fertilization can have on soil communities. Increased bioavailable C availability in soils, as well as potentially unmeasured changes in soil nutrient levels, likely directly contributed to the observed changes in microbial communities. Contrary to our predictions, fertilization did not decrease soil pH. It is also possible that unmeasured indirect effects could contribute to changes in microbial communities. For example, changes in root exudate composition may shift microbial community composition (Zhalnina et al., 2018), while fertilization may also shift the soil invertebrate community, further driving compositional changes (Fountain et al., 2008). As argued above, the short duration of this field experiment may have limited fungal responses. We hypothesize that, in a longer experiment, ongoing fertilization may shift the biogeochemical template enough that we would see a fungal response. Additionally, Cassman et al. (2016) highlighted that in long-term experiments, fungal and plant communities are often linked in their response to fertilization, suggesting that in subsequent years, we would see an enhanced response of both plant and fungal communities in our study site. Combined, the observed above- and below-ground responses to fertilizer additions suggest that bottom-up controls are important factors structuring both the composition and function of prairie ecosystems.

5 | CONCLUSIONS

We found that herbivore taxonomic composition and functional diversity (i.e. diet) can have strong but different effects on above- and below-ground communities. Herbivore diet drove compositional shifts and functional changes in soil communities, while herbivore taxonomic composition only changed below-ground functions. In accordance with previous work, these results demonstrate that documenting herbivore functional diversity may be a more important metric than taxonomic composition for understanding multitrophic relationships. Future studies would benefit from documenting herbivore diversity via multiple metrics (i.e. functional and taxonomic diversity) to capture their full effect. We also found that herbivore presence may increase C storage below-ground, highlighting a previously underappreciated pathway of soil C sequestration. Additionally, we found that herbivore effects were often mediated by fertilizer additions, emphasizing that top-down and bottom-up effects may interact to shape grassland communities. Combined, this study demonstrates the complex interactions that connect above- and below-ground ecosystems, and highlights the need for a synthesis on the effects of top-down versus bottom-up effects in prairie ecosystems.

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AUTHORS' CONTRIBUTIONS

C.M.P., M.S.S., S.C.P. and A.N.L. conceived of the study and project design; C.M.P. and A.N.L. organized all field work and took plant samples; C.M.P., A.N.L., D.H.B., J.J. and S.C.P. supported plant identification, biomass measurements and plant nutrient analyses; D.H.B. and J.J. collected and identified all grasshoppers used in this study, as well as provided statistical analyses; M.S.S. and J.M.L. collected the soil samples and ran all soil functional assays; J.M.L. performed the microbial sequencing lab work and bioinformatics. All authors contributed significantly to drafting the manuscript.

DATA AVAILABILITY STATEMENT

All microbial sequences, metadata, plant community data and R scripts are available on FigShare.org (https://doi.org/10.6084/m9.figshare.11734374.v1).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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