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Research article

Plant, insect, and soil microbial communities vary across brome invasion gradients in northern mixed-grass prairies

Morgan D. T. Frost[1], Kimberly J. Komatsu[1], Lauren M. Porensky[2], Kurt O. Reinhart[3], Kevin R. Wilcox[1], Zachary L. T. Bunch[1], Ashley D. Jolin[1], Katielyn A. Johnston[1], Gracen E. Trimas[1] and Sally E. Koerner[1]

[1]Department of Biology, University of North Carolina at Greensboro, Greensboro, NC, USA
[2]Rangeland Resources and Systems Research Unit, USDA-ARS, Fort Collins, CO, USA
[3]Livestock and Range Research Laboratory, USDA-ARS, Miles City, MT, USA

Correspondence: Morgan D. T. Frost (mdtrimas@uncg.edu)

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Species interactions shape native plant communities, influencing both composition and ecosystem processes, with invasion by non-native species threatening these dynamic relationships, native species, and function. The consequences of invasive plants in particular may stretch across taxa to impact plant, insect, and soil microbial communities directly and indirectly, with consequences for ecological functioning. In northern mixed-grass prairies in the United States, invasion by two annual brome grasses, *Bromus arvensis* and *B. tectorum*, negatively impacts rangeland plants; however, the simultaneous effects on insects and soil microbes (bacteria and archaea), and the implications for ecological function, have received less attention. Here, using observational field studies conducted at two mixed-grass prairie sites in Montana and Wyoming, we assessed the relationships between plants, insects, and soil microbes across gradients of invasion by *B. arvensis* and *B. tectorum*. Overall, we found differences in plant and insect communities and functional groups with increasing invasion abundance for both brome species. However, associations between invasion and the soil microbial community were species specific, as we only saw these relationships under *B. tectorum* invasion, implying *B. tectorum* may have more substantial consequences for rangeland management. While invasion by annual bromes may cause changes in certain plant and insect functional groups, such as C_4 perennial grasses and certain insect herbivores, soil microbial functional groups may be less impacted, especially under *B. arvensis* invasion. This work sheds light on the need to explore changes in natural communities across taxa and to all invasive species, as ecosystem effects are likely to be contingent upon both.

Keywords: arthropods, *Bromus arvensis*, *Bromus tectorum*, herbivory, soil microbes

Introduction

Species interactions of natural ecosystems are tightly linked and are ecologically/evolutionarily important (Biere and Bennett 2013). Evidence suggests that changes in these interactions can have cascading consequences for overall community structure

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and function (Biere and Bennett 2013). Invasive species are present in nearly every ecosystem type on the planet (IPBES 2023) and they directly and indirectly threaten native communities and the dynamic processes that shape them, including plant–insect–microbe interactions (Schirmel et al. 2016).

Invasive species affect plant community composition and taxonomic richness across ecosystem types (Denley et al. 2019). Plant invasion can lead to loss of certain taxa within plants (Maskell et al. 2006) and insects (Litt et al. 2014). Invasive plants outcompete native plants for limiting resources and drive down plant diversity, often by affecting typical nutrient cycling and soil water dynamics (Walker and Smith 1997, Hejda et al. 2009). This can impact insect composition (Litt et al. 2014), often by decreasing suitable oviposition habitat and forage nutrient content (Bezemer et al. 2014). However, some insect herbivores are relatively insensitive to plant invasion; differential responses depend in part on whether the herbivores are generalists or specialists (Bezemer et al. 2014). Further, invasive plants can affect the structure and composition of soil microbial communities, likely through changes in soil properties (Reinhart and Callaway 2006). For instance, soil microbial diversity and activity can increase under plant invasion due to changes in soil nutrients, pH and root exudates (Xu et al. 2022). Feedbacks between soil communities and invasive plants can be positive or negative, where soil microbes can resist or facilitate invasion and invasives can affect the composition of soil biota (Reinhart and Callaway 2006). In all, invasion can alter complex connections across food webs, and these shifts may enhance or hinder ecosystem stability (Landi et al. 2018).

Both taxonomic and functional identifications are important for understanding the ecosystem consequences of changes in species' abundances (Slade et al. 2007). Community composition changes can alter functional group composition if the species' functions differ, with many shifts in community composition still leading to equivalent function (Biggs et al. 2020). In diverse plant communities, loss of one plant species can be compensated by other species (Joner et al. 2011). Conversely, community composition changes can alter ecological function, even if richness is constant (Spaak et al. 2017). Functional groups used for plants can center around life form, morphology, leaf or root structure, and physiology (Korner 1993), giving insight into productivity, light availability, soil properties, and water/resource usage (McLaren and Turkington 2010). Invasive plants can lead to decreases in insect biomass/diversity and altered function without altering overall insect abundance (e.g. when large insects replace small insects) (Heleno et al. 2009). Insect communities are often grouped into feeding guilds (Novotny et al. 2010), which provide information about trophic structure, such as predator/prey relationships, as well as how insects relate to other communities (e.g. different classifications of herbivores consume plant material differently) (Cagnolo et al. 2002). Functional groups for soil microbes typically center around nutrient cycling, pathogenic behavior, and decomposition, providing insight into resource availability and soil health (Brussaard 1997). Soil microbes are not always functionally redundant, and function can partly depend on specific composition (Lucas et al. 2020), with large differences in function related to disturbance (Berga et al. 2012). However, invasive plants have changed microbial taxa without altering much ecological function (Gibbons et al. 2017). Understanding functional composition can elucidate functional redundancy (Simberloff and Dayan 1991) and ecosystem-scale effects of species shifts, particularly in this time of unprecedented global change as species composition shifts occur (Czortek et al. 2018).

Across the North American Great Plains, including northern mixed-grass prairies, the invasive winter annuals *Bromus arvensis* and *B. tectorum* cause damage to native rangelands by outcompeting native grasses that provide forage later in the growing season (Hulbert 1955). However, it remains unclear how these two annual brome species impact communities simultaneously across plants, insects, and soil microbes. Therefore, using observational field studies in northern mixed-grass prairies of Wyoming and Montana, we explored associations between annual brome abundance and plants, insects, and soil microbes, including their diversity, community composition, and functional composition. We hypothesized 1) richness across plants, insects, and soil microbes would decrease with increasing invasion abundance and 2) both community and functional composition of each group would shift more with increasing invasion. Additionally, we predicted no differences in responses between plants, insects, and microbes (i.e. plants are not more sensitive to invasion than soil microbes) and that all three groups would relate similarly to both brome species.

Material and methods

Site description

We conducted this observational field study at two sites within the Northern Great Plains steppe ecoregion, an area dominated by temperate and semiarid mixed-grass prairie (Martin et al. 1999). The first site, sampled in 2019, is located on private land in a Wyoming shrubland-grassland ecotone in Converse County, WY (43°17'N, 105°02'W). The site exists within the southern end of the Northern Great Plains (an area known locally as the Thunder Basin ecoregion) (Porensky et al. 2018). The second site, sampled from 2020–2022, is located at the Fort Keogh Livestock and Range Research Laboratory in Custer County, MT, central in the Northern Great Plains steppe (46°20'N, 105°59'W).

Both semi-arid sites are moderately grazed in the summer. At the Wyoming site, mean annual precipitation (MAP) is 250–300 mm, with 40–50% falling in April-June, and mean monthly temperature ranges from −5°C in December to 22°C in July (Porensky et al. 2018). In Converse County, WY, annual precipitation in 2019 was 391 mm (NOAA National Centers for Environmental Information 2022). For the Montana site, MAP is 342 mm, with ~50% falling in May-June, and mean monthly temperature ranges from −10°C in January to 24°C in July (Peterson and Reinhart 2012). In Custer County, MT, annual precipitation in 2020 was 251 mm, in 2021 was 257 mm, and in 2022 was 366 mm (NOAA National Centers for Environmental Information 2022). Common plant species include Wyoming big sagebrush *Artemisia tridentata* ssp. *wyomingensis*, blue grama *Bouteloua gracilis*, western wheatgrass *Pascopyrum smithii*, cheatgrass *Bromus tectorum*, field brome *B. arvensis* and six-week fescue *Vulpia octoflora* (Russell et al. 2017, Porensky et al. 2018).

Study design

During the summer of 2019 in Wyoming, we established five blocks each for *B. arvensis* and *B. tectorum* along 10 natural invasion gradients (Supporting information). Each block consisted of five 1-m^2 plots grouped together in space with different invasion levels (1 – very low, 2 - low, 3 - moderate, 4 - high, and 5 – very high cover) of either *B. arvensis* or *B. tectorum* (50 total plots). During the summer of 2020 in Montana, we established blocks along eight natural invasion gradients of *B. arvensis* only. Each block consisted of six 1-m^2 plots across three levels of invasion abundance (1 – very low, 3 – moderate, 5 – very high cover) by *B. arvensis*. In 2020, we collected data from a total of 48 plots, but in 2021 and 2022, we only present data from half (24) of these plots as the other plots were allocated for an additional treatment not presented here. At both sites, invasion levels represent aerial cover categories of the invasive compared to the rest of the plant community.

Field data collection

Field data were collected identically at both sites across all years. During peak growing season each year (June/July), at each plot, we recorded plant species composition by visually estimating foliar cover across the entire 1-m^2 area (i.e. calibrated to estimates generated by a 100 pin-point intercept frame) for each species to the nearest percent. We collected insects for 30 s across each 1-m^2 plot using a modified vacuum leaf blower according to nutrient network protocol (Stewart and Wright 1995) between 11:00–14:00 h at each sampling point. For this, we used a 25-cc Craftsman gas vacuum leaf blower, which we converted to use for arthropod sampling by adding a 3″ × 4″ sewer pipe adapter to the end of the vacuum arm. We used organza bags fitted with wire rings to hold the bags onto the plumbing piece. While not without limitations (e.g. insects moving in response to environmental conditions), this methodology has been shown to be as effective or more effective at arthropod sampling at small spatial scales in grasslands as other methods, such as sweep-netting (Buffington and Redak 1998). All insect samples were frozen after collection until processing. We took three 10-cm deep soil cores from the outer edge of each plot to assess soil microbial (bacterial and archaeal) taxonomic diversity (in 2019 at Wyoming and 2020 at Montana only) and homogenized all three cores in the field before freezing. We collected soil cores between 10:00–15:00 h at each sampling point and sterilized the soil probe and researchers' gloves between plots with 70% isopropyl alcohol to avoid cross contamination.

In 2021 (late June) and 2022 (early June) at Montana only, we collected insect herbivory data according to herbivory variability network protocols (Pearse et al. 2021). To calibrate our visual estimates of herbivory damage, we used the LeafByte application (Getman-Pickering et al. 2020) to ensure our estimates were accurate, and the same researcher collected all herbivory measurements across both sampling periods. In each of the 24 plots, we collected estimates of insect herbivory damage on 12 individuals. Six samples corresponded to invasive bromes (either *B. arvensis* or *B. tectorum*) and six samples corresponded to abundant native species in the plot (native species were selected based on species composition data for each plot, so exact species selected differed between plots). We estimated both total plant herbivory damage and individual leaf damage on all individual leaves, randomly selecting 10 leaves per individual if the plant had greater than 10 leaves (all sampled individuals had at least two leaves).

Laboratory sample processing

We used morphological traits to identify insects to family. After identification, we dried insect samples at 60°C for seven days before collecting total dried insect biomass for each plot. We sent soil microbial samples (collected in 2019 (WY) and 2020 (MT)) to Oregon State University's Center for genome research and biocomputing for extraction, amplification, cleanup and sequencing to generate 250 bp paired-end reads. We then merged the forward and reverse reads using FLASH (Magoč and Salzberg 2011) and the merged reads were quality-controlled using the DADA2 pipeline (Callahan et al. 2016) in Qiime2 (Hall and Beiko 2018). This removed chimeras, and the data were filtered to further remove mitochondria, chloroplast and unassigned sequences. We then removed any amplicon sequence variants with fewer than 10 reads and clustered sequences into operational taxonomic units (OTUs) with a 97% similarity threshold using the Silva database (Pruesse et al. 2007) for reference. We continued to follow the Qiime2 pipeline at a sampling depth of 30 000, which only excluded one plot with less than 8000 reads.

Data analysis

We evaluated changes in plant, insect and soil microbial community and functional composition in Wyoming in 2019 and Montana in 2020 in several ways. First, we assessed community level richness across plant species, insect families, and soil microbial OTUs. To calculate richness, we used the codyn package (Hallett et al. 2016) in R ver. 3.6.2 (www.r-project.org) for plants and insects, and Qiime2 for soil microbial OTUs (level-7 in Qiime2). Second, we used Bray–Curtis dissimilarity matrices (calculated for plants and insects with the 'vegan' package (Dixon 2003) and for microbes with Qiime2) and non-metric multidimensional scaling (NMDS) with two axes to visualize multidimensional community and functional composition of plants, insects and microbes. For plants, we focused on changes in non-brome composition by excluding *B. arvensis* from *B. arvensis* gradients and *B. tectorum* from *B. tectorum* gradients. We chose plots with very little cover of *B. arvensis* in *B. tectorum* plots, and *B. tectorum* cover in *B. arvensis* plots. For functional level responses across plants, insects and microbes, we assigned each plant species to a functional group (C_3 annual grass, C_3 perennial grass, C_4 perennial grass, cactus, forb, sub-shrub/shrub), each insect family to a feeding guild (leaf chewing herbivore, parasitoid, pollen/nectar eating herbivore, predator, sap sucking herbivore, other herbivore) (La Pierre and Smith 2016), and each soil microbial OTU to an ecological function (Faprotax prokaryotic environmental function database; OTUs not found in the database were left as unassigned and OTUs could be assigned to multiple functional groups (Louca et al. 2016)). Third, we determined how each plant, insect and microbial functional group associated with invasion.

For all analyses, we modeled each site, year and invasive species separately. To assess invasion gradient establishment, we evaluated the relationship between absolute percent brome cover and relative percent brome cover using type III mixed-model ANOVAs with a random block effect ('lme4' and 'lmerTest' package (Bates et al. 2015, Kuznetsova et al. 2017), using Satterthwaite's method (Satterthwaite 1941)). We used the same model set-up to compare invasion cover across sites within each invasion level, using Tukey's pairwise comparisons (Tukey 1977) with Benjamini–Hochberg's correction for multiple comparisons (Benjamini and Hochberg 1995). We then used the same model set-up to assess how community richness and each functional group related to invasion abundance. To assess compositional differences among invasion levels for both community and functional level analyses, we used permutational multivariate ANOVA (PERMANOVA) with a random effect of block included. For significant effects, we calculated pairwise comparisons ('RVAideMemoire' package, Hervé 2022).

We then determined how insect herbivory and total biomass changed with invasion in Montana in 2021 and 2022. For this, we tested the effect of invasion level, species status (native or invasive), and their interaction on total plant herbivory using type III ANOVA with a random effect of block included. We also tested this for leaf level herbivory damage, but the results were very similar, so we present only on total plant herbivory. Last, we used the same model set-up to assess the relationship between invasion abundance and total insect biomass for all four study years.

For all regression analyses, we visually assessed plots of the residuals and autocorrelation function/partial autocorrelation function to look for evidence of nonlinearity and autocorrelation, respectively, but we did not find evidence of violations of either assumption in any of our results. To test the assumption of homoscedasticity, we used Levene's test for equality of variances (Levene 1960). We assessed normality of the residuals of all response variables using Shapiro–Wilk, Anderson–Darling, Cramer–von Mises and Kolmogorov–Smirnov tests as part of the 'Olsrr' package (Hebbali 2020), transforming data when necessary to achieve approximate normality and homoscedasticity. Throughout this study, we use $\alpha = 0.05$, but we report results with $0.05 < p < 0.1$ as marginally significant.

Results

Invasion gradients

Selected plots yielded invasion gradients in Montana and Wyoming for *B. arvensis* and *B. tectorum* (Supporting information). Absolute percent cover of bromes significantly increased with relative percent cover across all three gradients (Montana *B. arvensis*: $F_{1,43} = 940.4$, $p < 0.0001$; Wyoming *B. arvensis*: $F_{1,23} = 1034$, $p < 0.0001$; Wyoming *B. tectorum*: $F_{1,23} = 161.2$, $p < 0.0001$) (Supporting information), verifying invasion gradient establishment. Further, at invasion levels 1, 2 and 4, we saw no difference in cover between *B. arvensis* and *B. tectorum*. We found site level differences in *B. arvensis* cover at invasion level 3; we only saw differences in cover between *B. arvensis* and *B. tectorum* at invasion level 5 in Wyoming (Supporting information).

Plant responses

Plant species richness decreased with increasing invasion across all gradients (Fig. 1a, Supporting information). Plant community (Fig. 2a, Supporting information) and functional (Fig. 3a, Supporting information) composition differed significantly across invasion levels. In Montana, *B. arvensis* invasion was negatively correlated with C_3 perennial grass, C_4 perennial grass, forb, and sub-shrub/shrub cover (Supporting information). In Wyoming, *B. arvensis* and *B. tectorum* cover were strongly negatively correlated with C_4 perennial grass cover and weakly negatively correlated with C_3 annual grass cover; *B. tectorum* cover was also negatively correlated with C_3 perennial grass cover (Supporting information).

Insect responses

Insect family richness was not associated with invasion in *B. arvensis* gradients and only marginally associated with invasion in *B. tectorum* gradients, where insect richness slightly increased with increasing invasion (Fig. 1b, Supporting information). However, insect community composition differed significantly with invasion in all three gradients (Fig. 2b, Supporting information). Further, insect functional composition was correlated with invasion in *B. arvensis* gradients in Montana and *B. tectorum* gradients in Wyoming (Fig. 3b, Supporting information). At the functional group scale, leaf-chewing herbivores and sap-sucking herbivores were negatively correlated with *B. arvensis* invasion in Montana (Supporting information), while parasitoid and sap-sucking

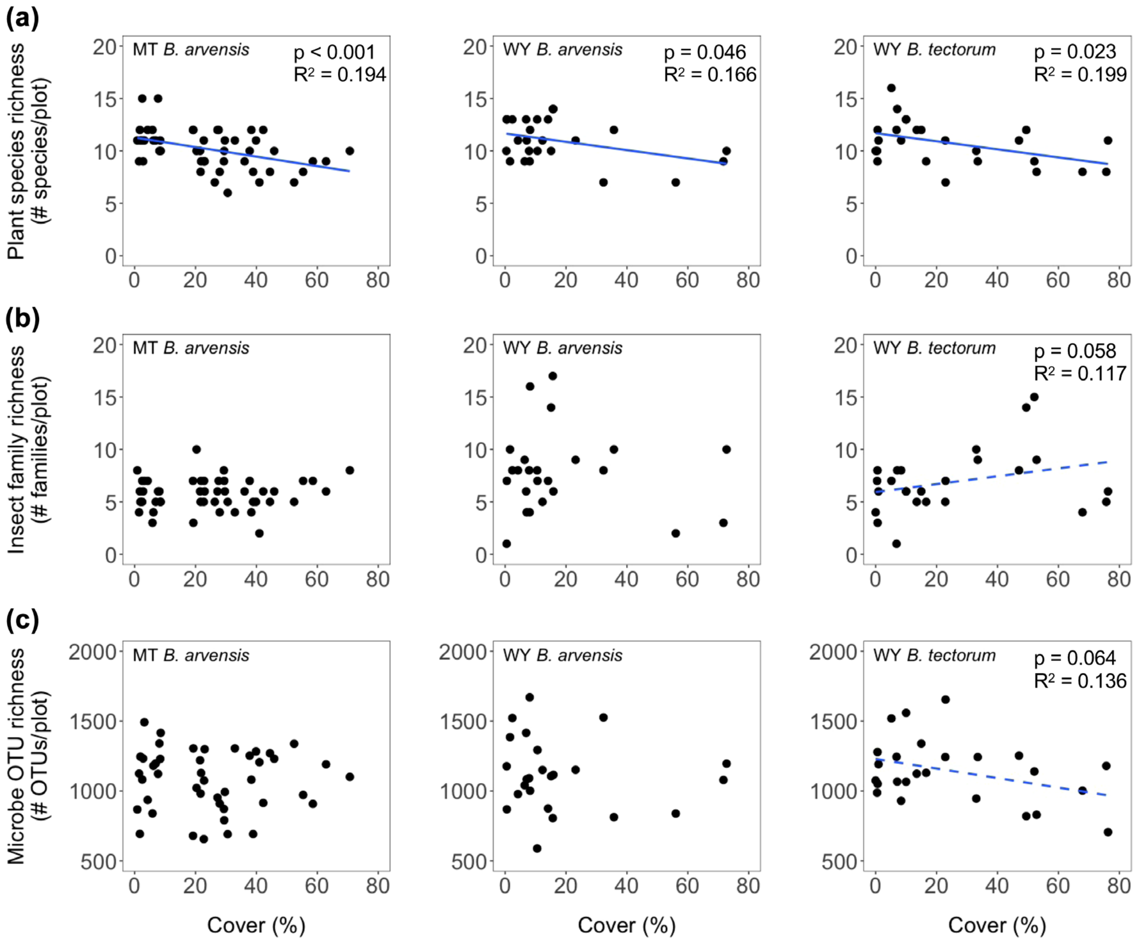

Figure 1. Changes in (a) plant species, (b) insect family, and (c) soil microbial OTU richness with relative invasion cover of *B. arvensis* and *B. tectorum* in Montana (MT) and Wyoming (WY). p-values and marginal R^2 values for significant (solid lines) or marginally significant (dashed line) effects of invasion on richness are shown according to results from mixed-model ANOVAs (Supporting information). One outlier was removed from Montana microbial data to meet model assumptions.

herbivore abundance were positively correlated to *B. arvensis* invasion in Wyoming (Supporting information).

Total insect biomass was not related to invasion across any gradient type or year (Fig. 4a, Supporting information). However, total plant damage due to insect herbivory differed marginally or significantly between native and invasives within every invasion level. Invasion level rarely affected average herbivory, except in 2021 when plant herbivory at the 0% invasion level was marginally lower than plant herbivory at the 100% invasion level (Fig. 4b, Supporting information). Overall, in 2021, average total plant herbivory on invasive annual bromes was 0.84 ± 0.14%, compared to 4.06 ± 0.78% on native plants, and in 2022, herbivory on bromes was 1.02 ± 0.29%, compared to 4.36 ± 0.65% on natives.

Soil microbial responses

Soil microbial OTU richness was not associated with invasion in *B. arvensis* gradients and only marginally associated with invasion in *B. tectorum* gradients, where microbe richness slightly decreased with increasing invasion (Fig. 1c, Supporting information). Likewise, microbial community (Fig. 2c, Supporting information) and functional (Fig. 3c, Supporting information) composition differed with invasion in *B. tectorum* gradients only. At the functional group scale, two soil microbial functional groups (oxygenic photoautotrophy and photosynthetic cyanobacteria) were marginally negatively correlated with *B. arvensis* invasion in Montana, while nine (two positive, seven negative) and 22 (all negative) microbial functional groups were marginally or significantly correlated with *B. arvensis* and *B. tectorum* invasion in Wyoming, respectively (Supporting information). For both bromes in Wyoming, microbial functional groups engaged in some form of phototrophy (phototrophs, photoheterotrophs, etc.) displayed the strongest negative correlations with invasion (Supporting information). For *B. tectorum*, microbial functional groups associated with nitrogen and methanol cycling were also strongly negatively associated with invasion (Supporting information).

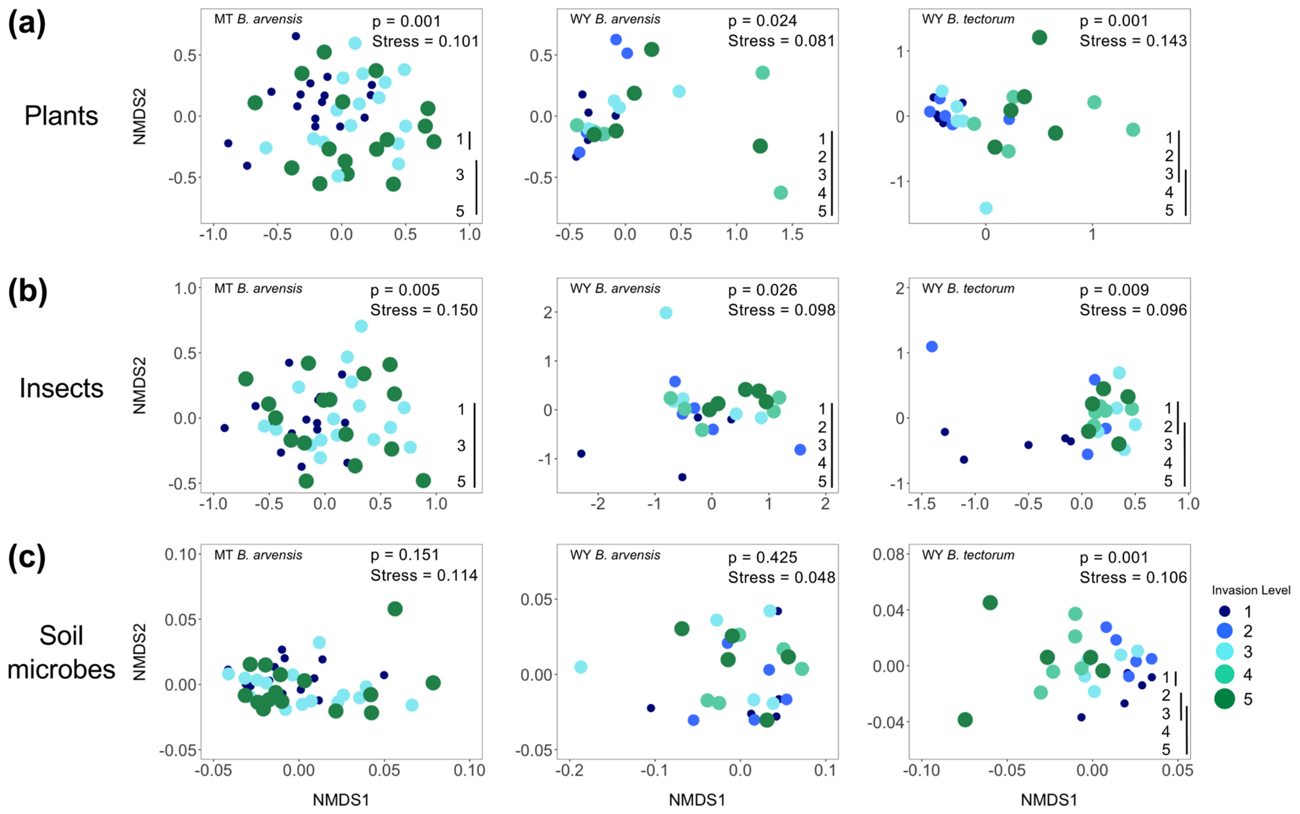

Figure 2. Non-metric multidimensional scaling (NMDS) of (a) plant, (b) insect and (c) soil microbial community compositions in relation to invasion by *B. arvensis* and *B. tectorum* in Montana (MT) and Wyoming (WY). p-values for significant changes in composition are shown according to the PERMANOVA results (Supporting information). Invasion levels connected with vertical black lines are not significantly different based on pairwise comparisons (pairwise PERMANOVA).

Discussion

Our study uniquely assessed the simultaneous responses of plants, insects and soil microbes to gradients of invasion at multiple study sites with two different invasive brome species. Overall, invasive annual brome abundance was more strongly related to plant community composition than insect and soil microbial communities. *Bromus tectorum* also had stronger associations with insects and soil microbes, suggesting communities show differential sensitivity to invasion based on taxa and that even apparently similar invasive species (congeneric C_3 winter annual grasses) may impact communities in unique ways.

Plant species richness significantly decreased with brome invasion, and community and functional composition shifted with invasion, aligning with previous work (Gasch et al. 2013), as annual bromes often have a competitive advantage over native species. *B. arvensis* and *B. tectorum* germinate in the fall or spring depending on climatic conditions, allowing them to utilize available nutrients and moisture earlier than perennial species (Germino et al. 2016). We found declines in C_4 perennial grasses with invasion across all gradients. C_4 perennials, growing later in the season, are sensitive to light availability (Still et al. 2003). As annual bromes use available moisture and decrease light earlier in the season, they outcompete native C_4 species, reducing C_4 abundance (Chambers et al. 2007). In Montana, most plant functional groups were negatively associated with increasing invasion, suggesting *B. arvensis* may create wide-ranging plant compositional shifts in this system.

While insect richness did not vary consistently with invasion, insect community and functional composition significantly differed with invasion across all gradients except one (functional composition in Wyoming *B. arvensis* gradients). As annual bromes shift plant composition, insect communities are also likely to change, given the feedback between available plant species and arthropod presence (Biere and Bennett 2013). For both insects and plants, functional composition results largely paralleled community composition results, suggesting a lack of functional redundancy with potential consequences for ecosystem services such as palatable forage availability, pollination, and pest control (Memmott and Waser 2002, Stout and Morales 2009, Kaiser-Bunbury et al. 2011). When insect functional groups were considered independently, we saw declines in leaf-chewing and sap-sucking herbivores in Montana and increases in parasitoids and sap-sucking herbivores in Wyoming *B. arvensis* gradients. The declines in certain herbivores may be a reflection of less heterogenous habitat (Germino et al. 2016) with increased invasion cover and/or a preference for

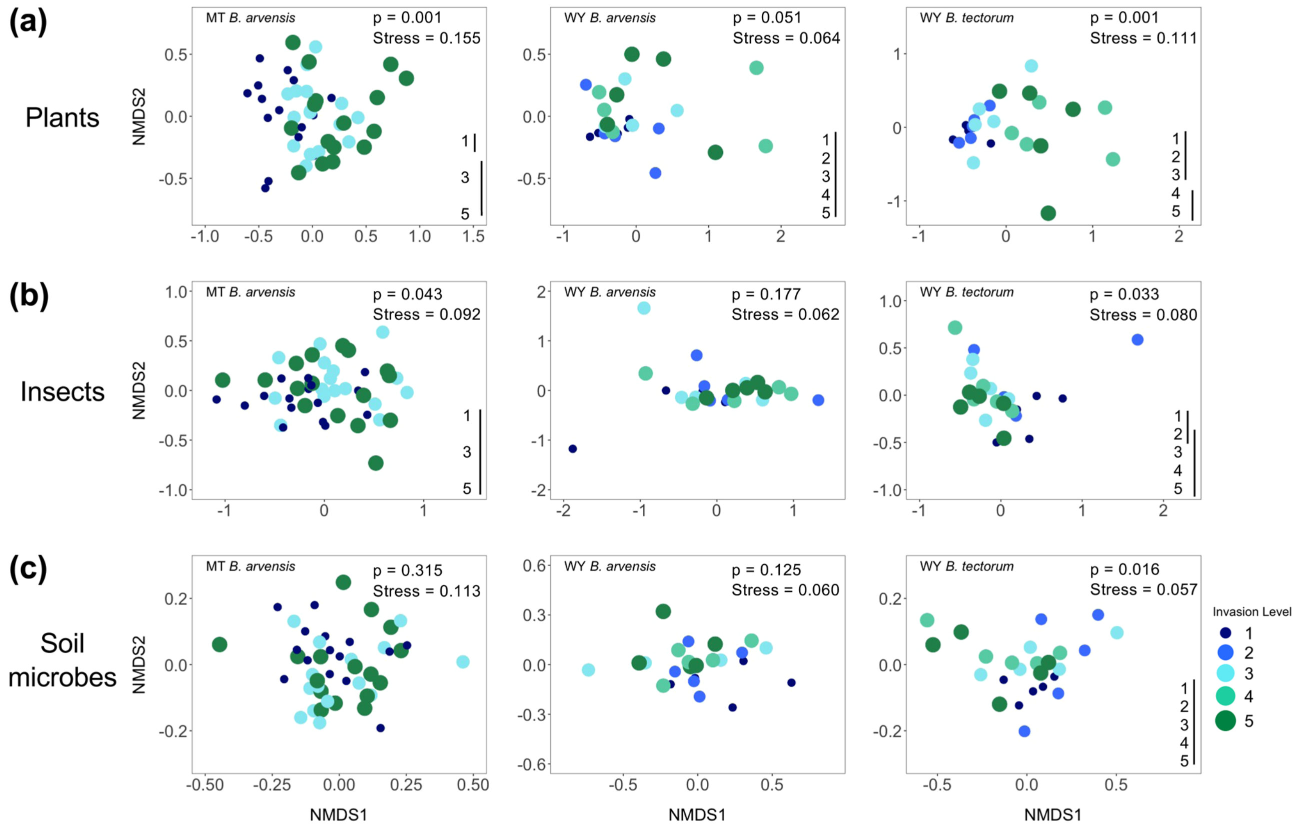

Figure 3. Non-metric multidimensional scaling (NMDS) of (a) plant, (b) insect and (c) soil microbial functional group compositions in relation to invasion by *B. arvensis* and *B. tectorum* in Montana (MT) and Wyoming (WY). p-values for significant changes in functional groups are shown according to the PERMANOVA results (Supporting information). Invasion levels connected with vertical black lines are not significantly different based on pairwise comparisons (pairwise PERMANOVA).

native forage species (Cumberland et al. 2016). However, the increase in parasitoids supports a preference for annual brome species as an oviposition site (Perez-Mendoza et al. 2006). The differences across sites for sap-sucking herbivores likely reflects the differences in C_3 perennial grass availability. Sap-sucking herbivores decrease with less abundant C_3 grasses, a nutritionally rich and preferred food source (Tscharntke and Greiler 1995), at higher invasion levels in Montana. The opposite may be true in Wyoming due to lack of invasion influence on C_3 grasses. Positive relationships between annual brome species and insect abundance in Wyoming also match prior work suggesting that bromes are associated with higher insect abundance in this short-structured prairie (Duchardt et al. 2021).

Insect herbivory was less common on annual bromes than native species. Interestingly, across all years and study sites, invasion level was never related to total insect biomass (Fig. 4, Supporting information). Based on these observations, we measured herbivory in two years in Montana. We found that insect herbivores, under any invasion level, preferred native forage species to invasive bromes. While there is some evidence that certain insect herbivores prefer native species over bromes later in the growing season, insect herbivores will consume bromes earlier in the season (Cumberland et al. 2016). However, we found that both later in the season (2021) and early in the growing season (2022), insect herbivores avoided consuming invasive bromes. This supports our previous finding of a decline in certain herbivore functional group abundances in Montana as well. Combined with a lack of response of total insect biomass to invasion, this could indicate substantial added pressure on native forage species, as insects compete with livestock for forage (Branson and Haferkamp 2014).

We predicted that compositional and functional changes with invasion would occur across plants, insects and soil microbes. However, soil microbial communities were markedly less sensitive to brome invasion than other taxa considered as we saw marginal changes in *B. tectorum* gradients only. At the functional group scale, we found strong declines in functional groups associated with phototrophy, as *Bromus* spp. can increase leaf litter (and thatch) and decrease light availability (Still et al. 2003, Bennett et al. 2014). Given that *B. tectorum* also utilizes large amounts of soil nitrogen (Blank and Morgan 2012), observed negative associations between *B. tectorum* and various nitrogen processing microbes are also logical. While *B. tectorum* has previously been linked to decreased soil microbial abundance (Gasch et al. 2013) and altered community composition (Nasto et al. 2022),

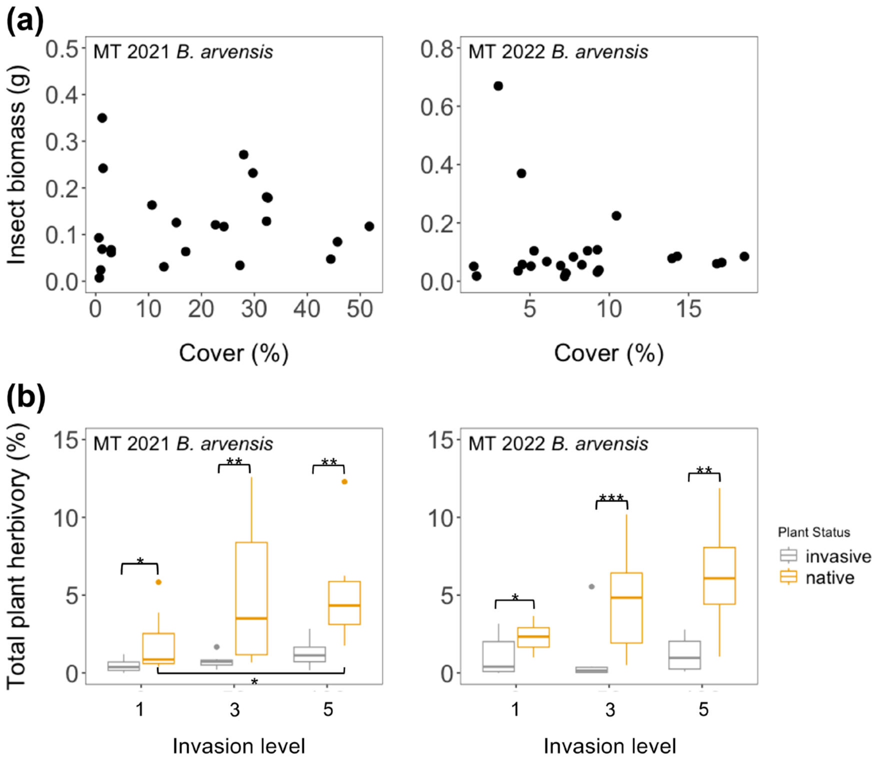

Figure 4. Changes in (a) total insect biomass and (b) total plant herbivory with relative invasion cover of *B. arvensis* in Montana (MT) in 2021 and 2022. Significant differences in plant herbivory from mixed-model ANOVAs (Supporting information) are indicated by asterisks, where * means $p < 0.1$, ** means $p < 0.05$ and *** means $p < 0.001$.

potentially due to soil moisture differences (Gasch et al. 2013), our results newly suggest more limited associations between microbial functional groups and *B. arvensis*, implying that the microbial communities may be resilient to even large shifts in *B. arvensis* abundance, protecting services associated with microbial communities like nutrient cycling (Batten et al. 2006). Alternatively, our sequencing methodology and functional groupings may only partly account for functional differences relative to other methods (e.g. soil enzyme activity or transcriptomics), potentially overinflating functional redundancy in our study (Louca et al. 2016).

We found plants, insects and soil microbes did not relate to *B. arvensis* and *B. tectorum* equally. Given the functional similarity of these two species (both are C_3 winter annual grasses), we expected fairly similar associations between the invasives and plants, insects, and microbes. Our results suggest that *B. tectorum* may have more substantial and far-reaching consequences for community structure than *B. arvensis*. While studies comparing *B. arvensis* and *B. tectorum* are lacking, evidence suggests niche differentiation between the species, particularly in terms of microsite preference (Porensky and Blumenthal 2016). Underlying differences in microsite, including light availability (bare ground cover), soil texture, moisture, and nutrient availability, may help explain the soil microbial differences seen between the species (Porensky et al. 2018). Encouragingly, consequences of invasion for soil functioning may not be too damaging under invasion by *B. arvensis*. In general, this supports the idea that invasion studies and management must consider invasive species identity, as well as functional group (Roscher et al. 2009).

As an observational study, directionality of effects (i.e., causation) between invasive annual bromes and native plants, insects and microbial communities cannot be determined. Some of the responses seen could be indicative of other, non-measured variables or could be themselves influencing brome abundance. For instance, annual bromes respond to changes in microsite variability, including differences in soil texture and moisture (Porensky et al. 2018), and native plant communities in mixed-grass prairies can resist brome invasion (Ashton et al. 2016). While additional experimental work is necessary to isolate any causality in our perceived relationships, previous work demonstrating the negative effects of annual bromes on native grasslands (Melgoza et al. 1990, Knapp 1996, Ashton et al. 2016) does align with our conclusions. Despite the limitations presented by correlation studies, this work is still valuable in informing potentially widespread consequences associated with invasive annual bromes.

Overall, this work sheds light on the understudied consequences of annual brome invasion in northern mixed-grass prairies for plants, insects and soil microbes. Species interactions shape ecosystem functions, and these dynamics are influenced by biodiversity (Thébault and Loreau 2005) and invasion. Annual brome invasion related to plant and insect community structure and function, highlighting the potential for cascading consequences of invasion by *B. arvensis* and *B. tectorum* for rangeland ecosystems. To the contrary, soil microbial communities appeared to be more resistant to change in relation to invasive abundance, suggesting resilience to invasive bromes. Responses to invasion vary across taxa, so further studies are needed to examine cross-community change. Understanding how invasion alters community composition and functional group diversity across rangeland communities is vital not only for long-term rangeland sustainability (DiTomaso 2000), but also for advancing invasion science and management (Ricciardi et al. 2017).

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Author contributions

Morgan D. T. Frost: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Project administration (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Kimberly J. Komatsu:** Conceptualization (supporting); Formal analysis (supporting); Funding acquisition (lead); Methodology (supporting); Project administration (supporting); Resources (equal); Validation (equal); Visualization (equal); Writing – review and editing (equal). **Lauren M. Porensky:** Conceptualization (supporting); Formal analysis (supporting); Funding acquisition (lead); Project administration (supporting); Methodology (supporting); Resources (equal); Supervision (equal); Validation (equal); Visualization (equal); Writing – review and editing (equal). **Kurt O. Reinhart:** Conceptualization (supporting); Formal analysis (supporting); Funding acquisition (lead); Methodology (supporting); Resources (equal); Supervision (equal); Validation (equal); Visualization (equal); Writing – review and editing (equal). **Kevin R. Wilcox:** Conceptualization (supporting); Formal analysis (supporting); Funding acquisition (lead); Methodology (supporting); Validation (equal); Visualization (equal); Writing – review and editing (equal). **Zachary L. T. Bunch:** Data curation (equal); Investigation (equal); Methodology (equal); Writing – review and editing (supporting). **Ashley D. Jolin:** Data curation (equal); Investigation (equal); Methodology (equal); Writing – review and editing (supporting). **Katielyn A. Johnston:** Data curation (equal); Investigation (equal); Methodology (equal); Writing – review and editing (supporting). **Gracen E. Trimas:** Data curation (equal); Investigation (equal); Methodology (equal); Writing – review and editing (supporting). **Sally E. Koerner:** Conceptualization (lead); Data curation (supporting); Formal analysis (equal); Funding acquisition (lead); Investigation (equal); Methodology (equal); Project administration (equal); Resources (lead); Software (lead); Supervision (lead); Validation (lead); Visualization (equal); Writing – original draft (equal); Writing – review and editing (equal);.

Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.fj6q5742p (Frost et al. 2024).

Supporting information

The Supporting information associated with this article is available with the online version.

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