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Exploring trophic effects of spotted knapweed (*Centaurea stoebe* L.) on arthropod diversity using DNA metabarcoding

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ABSTRACT

Biological invasions are of particular concern in grasslands, as these systems are highly susceptible to changes in ecosystem energy flows following invasions by exotic plants. Spotted knapweed (*Centaurea stoebe* L.), a Eurasian, perennial forb, is considered one of the most ecologically harmful invasive plants in North American grasslands and may lead to changes in trophic dynamics, particularly within arthropod communities, which depend on plants for food and habitat. Using DNA metabarcoding to assess community dynamics of arthropods collected from pitfall traps and sweep nets, we explored the effects of *C. stoebe* density on the alpha, beta and functional diversity of arthropods in a semi-arid grassland in British Columbia, Canada. We used trait-based approaches to investigate the functional responses of terrestrial arthropod communities to better understand the effects of *C. stoebe* on trophic dynamics. Our study found seasonal differences in the beta-diversity of arthropods, but no differences in arthropod alpha-diversity in knapweed populations. However, our study found a significant reduction in detritivore relative abundance coupled with increases in predator relative abundance, indicating that knapweed density altered the detrital food web. Conversely, herbivores were unaffected by knapweed density, suggesting evidence for greater stability of the grazing food web. Further, predator:prey ratios were highest under high knapweed density. These ratios suggest that top-down effects are likely stronger than the bottom-up effects of *C. stoebe* invasion. DNA metabarcoding provides the tools to develop detailed surveys of species diversity across a range of environments and trophic levels, which could be a useful guide for planning restoration.

1. Introduction

Biological invasions are becoming increasingly common globally, and the introduction and spread of invasive plants alter native plant communities, nutrient cycling, the physical environment and can result in novel trophic interactions (Lau, 2013; Litt et al., 2014; Pearson, 2009; Pimentel et al., 2005). For instance, the dominance of invasive plants can lead to reductions in native plant diversity, which can be particularly unfavorable for arthropods as many species require specific plants for food, reproduction, and habitat. These changes to plant and arthropod communities may result in bottom-up changes in trophic dynamics as arthropods are a large component of the diet of many reptiles, amphibians, small mammals, and birds (Litt et al., 2014; Pearson, 2009). Conversely, invasive plant species may benefit higher trophic levels if they are commonly consumed by herbivores. This creates more available prey for higher-order predators, resulting in top-down changes in trophic cascades (Lau, 2013). Arthropods influence ecosystems by providing important trophic linkages and other services, such as pollination and decomposition, and therefore the effects of plants invasions on arthropods may result in strong ecological consequences (Simao et al., 2010).

Natural grasslands, one of the most endangered ecosystems in North America, are highly susceptible to changes in ecosystem energy flows upon the introduction of invasive plants (Fraser and Carlyle, 2011; Samson and Knopf, 1994). These grasslands provide invaluable ecosystem services to people and the environment, but the use of grasslands by humans is leading to the anthropogenic spread of invasive plants, which can cause declines in native plant and animal biodiversity (e.g. Hansen et al., 2009; Litt and Steidl, 2010). Spotted knapweed (*Centaurea stoebe* L.), a Eurasian, perennial forb, is considered one of the most ecologically harmful invasive plants in western North American grassland communities (Duncan, 2001; Hansen et al., 2009). Once established, *C. stoebe* forms dense, near-monoculture stands and can have direct and indirect effects on ecosystem processes and functions including alterations in soil chemistry (Fraser and Carlyle, 2011; Lejeune and Seastedt, 2001), changes in fire and hydrological regimes (Goodwin and Sheley, 2001; Kulmatiski et al., 2006), increased soil

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erosion, sedimentation and water run-off (Lacey et al., 1989), and reduced native plant diversity (Hansen et al., 2009; Ortega and Pearson, 2005; Tyser and Key, 1988) Establishment of *C. stoebe* is persistent, as the species can tolerate low nutrient soils (Suding et al., 2004) and drought (Mráz et al., 2014) and produces a seed bank that can remain viable for at least 8 years (Davis et al., 1993).

While few studies have explored the trophic effects of *C. stoebe* on arthropod communities, the dominance of *C. stoebe* likely impacts native plant-dependent arthropod species (Hansen et al., 2009; Pearson, 2009), an important consideration on ecosystem function and the potential restoration of an invaded ecosystem. Field studies exploring the effects of *C. stoebe* on arthropod communities have focused on particular functional groups of arthropods, where *C. stoebe* resulted in the homogenization of the carabid community (Hansen et al., 2009) and increased the abundance of native spiders that use vegetation as web substrates (Pearson, 2009). Other studies exploring invasive plants found that some invasive species can alter the functional structure of arthropod communities by producing dietary and habitat shifts (Gomes et al., 2018; Grass et al., 2014; Wong et al., 2019). However, there are a limited number of studies that have used trait-based approaches to investigate the functional responses of terrestrial arthropod communities to biological invasions (Wong et al., 2019).

This study explores the effects of differing densities of spotted knapweed on the alpha-, beta- and functional diversity of arthropods in a semi-arid grassland in British Columbia, Canada. Since traditional methods of biodiversity assessment for arthropods is expensive, time-consuming and relies heavily on taxonomic expertise (Beng et al., 2016; Ji et al., 2013; Yu et al., 2012), we explore the use of DNA metabarcoding in the assessment of arthropod community dynamics to expedite arthropod identification and improve sampling efficiencies (Beng et al., 2016; Ji et al., 2013; Yu et al., 2012). This approach has been successfully used to assign taxonomies to specimens of animals (e.g. Tahir et al., 2016), plants (e.g. Kress et al., 2005), fungi (e.g. Schoch et al., 2012) and other microbes (e.g. Patel et al., 2008). The barcode approach provides large amounts of species-level inventory data and allows for the tracking and measurement of biodiversity over space and time (Yu et al., 2012). We explore the effects of *C. stoebe* invasion on arthropod diversity, as well as changes in arthropod functional groups based on feeding behavior, herbivores, detritivores, omnivores, and predators, to explore the effects of *C. stoebe* invasion on trophic dynamics.

2. Materials and methods

2.1. Study area and site selection

Experimental sites were established in the Lac du Bois Grasslands Protected Area, a 15,000-ha area located Northwest of Kamloops, British Columbia, Canada (50°39′59″ N, 120°19′09″ W). The park and surrounding region are characterized as semiarid, with annual precipitation of 277.6 mm, including 635 mm of snowfall. The average annual daily temperature for the region is 9.3 °C. Lac du Bois is a multi-use area managed for recreation, wildlife, and livestock grazing at low to moderate stocking rates (Bassett and Fraser, 2014; Schmidt et al., 2012). The continuous use of the grasslands by recreational users and ranchers leaves the area susceptible to the introduction of invasive plants, and one species of concern in this grassland is spotted knapweed (*Centaurea stoebe*; Fraser and Carlyle, 2011). In May 2017, twenty 40-m diameter sampling sites were established in the Lac du Bois grassland with varying stem densities of spotted knapweed: 'None' (0–1 stems m^{-2}), 'Low' (2–44 stems m^{-2}), 'Medium' (45–69 stems m^{-2}) and 'High' (>70 stems m^{-2}). To ensure sites shared similar ecosystem properties, all sites were located within a 2 km^2 area (Bode and Maciejewski, 2014). We sampled four 0.5 m × 0.1 m quadrats per site for a total of 80 quadrats across our study plots. In June 2017, vegetation was clipped within each quadrat, sorted, and dried, and sites were reclassified according to the distribution of the data, which resulted in the following categories that use the proportion of spotted knapweed biomass to total aboveground biomass: 'None' (0%), 'Low'(< 30%), and 'High' (>30%).

2.2. Arthropod sampling

Four pitfall traps were installed in a square arrangement, each 2 m apart in the center of each sampling site. Pitfall traps were used to collect ground-dwelling arthropods and consisted of a collection cup (11.5 cm diameter, 7.5 cm depth) dug into the earth flush with ground level filled with 87% denatured ethanol solution to preserve the specimens for DNA analysis. Plywood cover boards (30 cm × 35 cm) were placed approximately 5–10 cm above each pitfall trap to reduce ethanol evaporation. The pitfall traps were opened for five days each month, in the last week of May, June, July, and August 2017. As a result of the phenology of spotted knapweed, we classified the samples as early summer (May and June) and late summer (July and August), because spotted knapweed begins to flower in late June, we expected that the arthropod communities would change as flowering begins.

At the end of each 5-day interval, a 30 cm-diameter canvas wireframe sweep net was used to capture insects along a 20 m transect with 35 sweeps across each site. Sweep netting collected foliar arthropods on top of all plants in each patch to give a better representation of the arthropod community interacting with spotted knapweed plants. All sweep net surveys were completed on days with wind velocities <10 km h^{-1} to increase the probability that arthropods remained on the foliage. Sweep net surveys were conducted by the same researcher to ensure consistency among sites and sampling dates. Three of the four containers from each site and the sweep net samples were pooled together, and the composite sample was stored in a −20 °C freezer with 87% denatured ethanol before DNA extraction. We used four such composite samples from each site for downstream analyses.

2.3. DNA extraction and sequencing

Four sample sites, one sample from each spotted knapweed density, were sent to the Canadian Centre for DNA Barcoding (http://ccdb.ca) to be identified, sequenced, and cataloged into the database (http://www.barcodeoflife.org under project code "LFBC"). The samples sent to the Canadian Centre for DNA Barcoding for sequencing were used to populate the BOLD database. That sample was not used for any statistical analyses in the current study. We used the four composite samples from each site for metabarcoding and statistical analysis. The remaining composite arthropod specimens were extracted from specimen bottles using sterile forceps and left to air dry before DNA extraction. To keep the extracted DNA quantity similar across individual arthropods, the heads from individuals with body length equal to or greater than 5 mm, and the entire bodies of everything smaller, were used (modified from Beng et al., 2016). Tissue samples from each site were homogenized in liquid nitrogen using a pre-cooled and sterilized mortar and pestle; genomic DNA was extracted from ground samples using an E.Z.N.A. Insect DNA Kit (Omega Bio-Tek, Norcross, Georgia, USA) according to the manufacturer's protocol. DNA concentrations were measured using a Qubit 2.0 Fluorometer and a Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

A 402 bp region of the COI mitochondrial gene was amplified via PCR in a Simpli Amp Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific, Waltham, Massachusetts, USA) using degenerate primers (Table 1). Amplifications were carried out in 25 μL with 10 ng genomic DNA, 12.5 μL 2× GoTaq DNA polymerase (Promega Corporation, Madison, Wisconsin, USA), 1 μL each of 10 μM forward and reverse primers and nuclease-free water. PCR reaction conditions were 94 °C for 1 min; 7 cycles of 94 °C for 30 s; 43 °C for 30 s; 72 °C for 40 s; then 30 cycles of 94 °C for 30 s; 55 °C for 30 s; 72 °C for 40 s and finally 72 °C for 5 min (modified from Chuo Beng et al., 2016). Reaction mixtures were then cleaned of DNA <100 bp using an E.Z.N.A. Cycle Pure Kit (Omega

Table 1
PCR primers used in this study.

Primer name	Primer sequence (5′–3′)	Primer source
MHemF (forward)	GCATTYCCACGAATAAATAAYATAAG	Park et al., 2011
dgHCO-2198 (reverse)	TAAACTTCAGGGTGACCAAARAAYCA	Meyer, 2003

Bio-Tek, Norcross, Georgia, USA) according to the manufacturer's instructions; amplicon size was estimated on a 1.5% agarose gel and amplicons were quantified using a Qubit 2.0 Fluorometer.

Using the amplicons from the first round of PCR as a template, the second round of PCR with barcoded primers was completed using the same conditions as before. Second-round PCR primers included barcode and sequencing adaptor sequences; for example, forward primers included the A adaptor sequence (underlined) and a unique IonXpress barcode with a three-base adaptor (bold); reverse primers included the P1 adaptor sequence (underlined and bold): CCATCTCATCCCTGCGTGTCTCCGACTCAG**CTAAGGTAACGAT**GCATTYCCACGAATAAATAAYATAAG, **CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGAT**TAAACTTCAGGGTGACCAAARAAYCA.

Purified adapter and barcode-ligated samples were pooled to equimolar amounts, and quantitative real-time PCR was carried out on an Eco Real-Time PCR System (Illumina Inc., San Diego, California, USA) with an Ion Library TaqMan Quantitation Kit to determine the library concentration for sequencing. Sequencing libraries were templated to Ion Sphere particles, purified and loaded onto Ion 530 chips using an Ion Torrent Ion Chef Instrument. Sequencing was carried out on an Ion S5 XL sequencer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

2.4. Data processing

Sequencing data was processed in Torrent Suite 5.10.0 with PreBaseCaller and BaseCaller Args set to *-disable-all-filters*. The resulting multiplexed BAM file was exported and passed to AMPtk v. 1.0.3 for demultiplexing with the *amptk ion* script using default parameters: minimum read length 100 bases, trim all reads to 300 bases, no barcode mismatches, 2 base primer mismatch allowed (Palmer et al., 2018). Demultiplexed data files were concatenated and then clustered with *amptk cluster* with an Operational Taxonomic Unit (OTU) clustering ratio of 97% and filtered with *amptk filter*.

A database of over 8 million specimens with publicly available taxonomy barcodes was downloaded on September 13th, 2018 from the Barcode of Life Data System (http://v4.boldsystems.org). The database was reformatted using the bold2utax.py script in AMPtk, globally aligned with *amptk database*, subsampled to 90,000 records with bold2amptk.py and converted into a database for local use in *amptk database* according to Palmer (2017). Once the database was prepared, taxonomy was assigned to OTUs using the *amptk taxonomy* script.

OTUs with fewer than 2,000 reads were removed, the data were rarified to the lowest number of reads; the resulting data matrix for analysis included 3,440,055 reads in 265 OTUs. Out of these, only 200 OTUs were classified to order, 174 OTUs were assigned to genera, and 143 OTUs were assigned to species; the remainder were not even classified to the kingdom level. OTUs that remained unclassified were dropped from the final OTU table. Samples with less than 200 reads were also removed from the OTU table. OTUs assigned to species level were retained for statistical analysis, and species were classified into functional groups based on feeding behavior: herbivore, omnivore, predator, and detritivore. Relative abundances of the functional groups were calculated by dividing the number of sequencing reads present for the specific taxa by the total number of reads of all taxa in the sample and used for statistical models.

2.5. Statistical analysis

We used principal coordinate analysis (PCoA) to visualize the community composition of the arthropod community. To run the principal coordinate analysis, we used Bray-Curtis dissimilarity (Bray and Curtis, 1957) followed by permutational multivariate analysis of variance (PERMANOVA) to test the difference in community structure across knapweed density and seasons. For PERMANOVA analysis, we used the Bray-Curtis dissimilarity matrix as a dependent variable with knapweed density and seasons as the independent variables. We also measured the Shannon-Wiener diversity index and examined the differences in alpha diversity across knapweed density and seasons by employing one-way ANOVA models. Shannon-Wiener diversity index was the dependent variable, and knapweed density and seasons were independent variables in the ANOVA models. Further, we inspected the impact of knapweed density and seasons on relative abundances of functional groups within the arthropod community by implementing non-parametric linear models, because normality assumptions were not met.

We conducted all our analysis in R (R Core Team, 2019) using RStudio (RStudio Team, 2016) as an integrated development environment. To calculate the Bray-Curtis dissimilarity, we used the 'vegdist' function from the vegan package (Oksanen, 2018). We fed the Bray-Curtis dissimilarity to the 'adonis' function from the vegan package (Oksanen, 2018) to execute the PERMANOVA analysis. For all ANOVA models, we used the 'aov' function from the base R (version 3.6.1). To run the principal coordinate analysis, we used the 'pcoa' function from the 'ape' package (Paradis and Schliep, 2018). We used the 'tidyverse' package (Wickham, 2017) for data wrangling and visualization.

We also measured the ratio of predator-herbivore, predator-detritivore, and predator- overall prey (herbivore + detritivore), to study prey-predator dynamics. To test the effect of knapweed density and seasons on the prey-predator relationship within the arthropod community, we used a non-parametric model.

3. Results and discussion

PCoA followed by PERMANOVA analysis indicated significant seasonal differences in arthropod communities (pseudo $F = 4.14$, $p < 0.001$; Fig. 1A) between early and late summer seasons, but no differences in arthropod community composition between knapweed populations of different densities (pseudo $F = 0.76$, $p = 0.89$; Fig. 1B). We found no differences in arthropod diversity across seasons ($F = 3.16$; $p = 0.08$, Fig. 2A) or between knapweed densities ($F = 0.72$; $p = 0.08$, Fig. 2B). These results indicate that the community composition of macroinvertebrates in the study site is affected by seasonality, as supported by studies from other ecosystems that show strong seasonal influences on arthropods (Beng et al., 2018; Liu et al., 2013; Sanford and Huntly, 2010). Seasonal changes in climatic factors, such as rainfall and temperature, can drive species compositional shifts due to arthropod phenology (Liu et al., 2013; Sackmann and Flores, 2009). Resource availability may also be correlated with seasonality; for example, Doblas-Miranda et al. (2009) showed that high predator abundance occurred in the fall when prey species were equally abundant.

Two syntheses of the impact of plant invaders on arthropod richness, document overall decreases in arthropod diversity in response to invasive plants (Litt et al., 2014; van Hengstum et al., 2014). Surprisingly, arthropod alpha diversity and community composition in our study were unaffected by knapweed density. Our results are consistent with Farrell et al. (2015), who found no differences in arthropod diversity between native and non-native communities in grasslands in California. Similarly, in an urban woodland habitat, an invasive tree did not affect the beta diversity of many arthropod species (Buchholz et al., 2015). Other studies report that invasive plants can have positive effects on arthropods, either due to the provision of structural support, shelter, or carbon to the arthropod community (Dudek et al., 2016; Tang et al.,

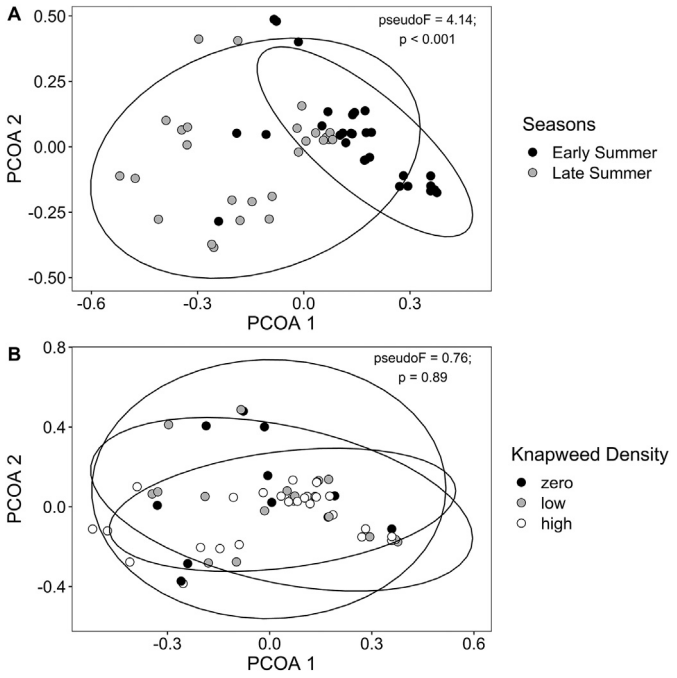

Fig. 1. PCoA of arthropod community composition reveals significant differences in communities between early and late summer in a grassland plant community (A). However, there was no difference in arthropod community composition in response to knapweed density (B).

2012). Thus, the effects of invasive species on arthropods are likely context-dependent. This is unlikely to be a sampling artifact, as other analyses (see below) revealed the response of different facets of the arthropod community to spotted knapweed density and indicate that our sampling scheme can detect changes in arthropod community if present. In this semi-arid protected grassland, the maximum knapweed densities in our study may be below the threshold needed to exert negative impacts on the arthropod community. From a conservation standpoint, our results are tentatively positive and indicate that the arthropod community structure and diversity are not degraded or changed by spotted knapweed invasion.

When we split the arthropods into functional groups, we found that functional groups of arthropods responded in different ways to knapweed density. The relative abundances of omnivores and herbivores were not different between knapweed densities (omnivores: $\chi^2 = 4.78$, $p = 0.091$, herbivores: $\chi^2 = 0.67$, $p = 0.72$). On the other hand, both detritivores and predators responded to knapweed density (detritivores: $\chi^2 = 4.78$, $p = 0.091$, predators: $\chi^2 = 11.29$, $p = 0.0035$). The relative abundance of detritivores was higher when spotted knapweed was absent (Fig. 3), while there was no difference in detritivore relative abundance between low and high densities of spotted knapweed (Fig. 3). Predator relative abundance was higher under high densities of spotted knapweed, but there was no difference in the relative abundance of predators between zero and low densities of spotted knapweed. There were no significant differences between early and late summer in arthropod functional group relative abundance (Fig. 4).

Studies have shown that invasive plants can have both positive and negative effects on arthropod abundance (Gallé et al., 2015; Simao et al., 2010), and invasive plants can have different effects on grazing and detrital food webs in the same ecosystem (McCary et al., 2016). In a meta-analysis by McCary et al. (2016), both herbivores and detritivores were not susceptible to an increased density of plant invaders in grasslands, suggesting stability of grazing and detrital food webs.

Our study found a significant reduction in detritivore relative abundance coupled with increases in predator relative abundance, indicating that spotted knapweed density altered the detrital food web. Conversely, herbivores were unaffected by knapweed density, suggesting evidence for greater stability of the grazing food web, compared to the detrital food web, at the observed densities of the invasive spotted knapweed. Functional redundancy in diverse ecosystems, such as grasslands, may arise from multiple plants performing the same role for herbivores, such that even if the invader reduces the density of some native plants, herbivores can shift to other native plants or use spotted knapweed as a food source. Grasslands in British Columbia exhibit both species and topological diversity (Schmidt et al., 2012; van Ryswyk et al., 1966) and can, therefore, be subject to such functional redundancies. It is also possible that the lack of change in herbivore relative abundance in response to spotted knapweed density may reflect a balance between reduction of native herbivores and an increase in biological control herbivores introduced to control spotted knapweed (Spafford, 2013).

Some of the most successful invasive plants have displayed higher decomposition rates compared to native plants (Allison and Vitousek,

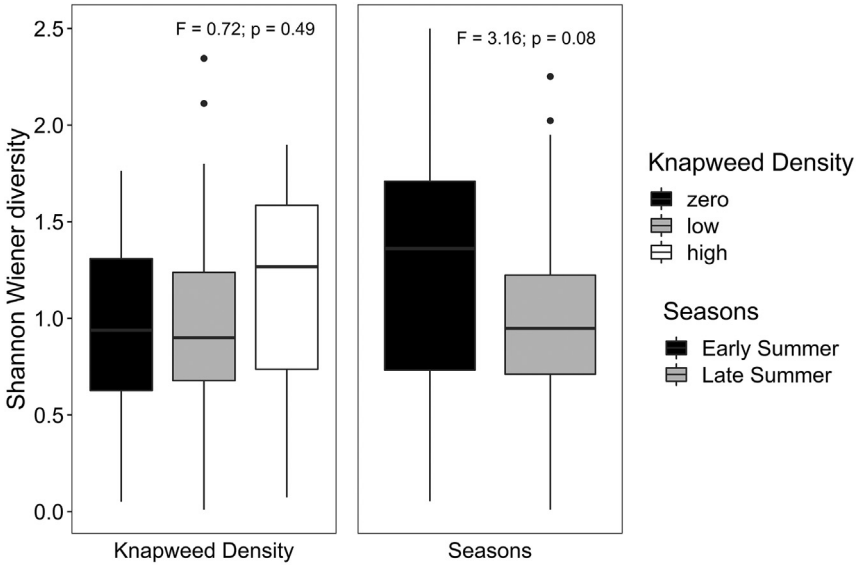

Fig. 2. Shannon Wiener diversity of arthropod communities in a grassland according to knapweed density (A) and seasons (B). The whiskers represent the spread of the data while the box indicates the interquartile range (first quartile, median and third quartile). Dots represent outliers.

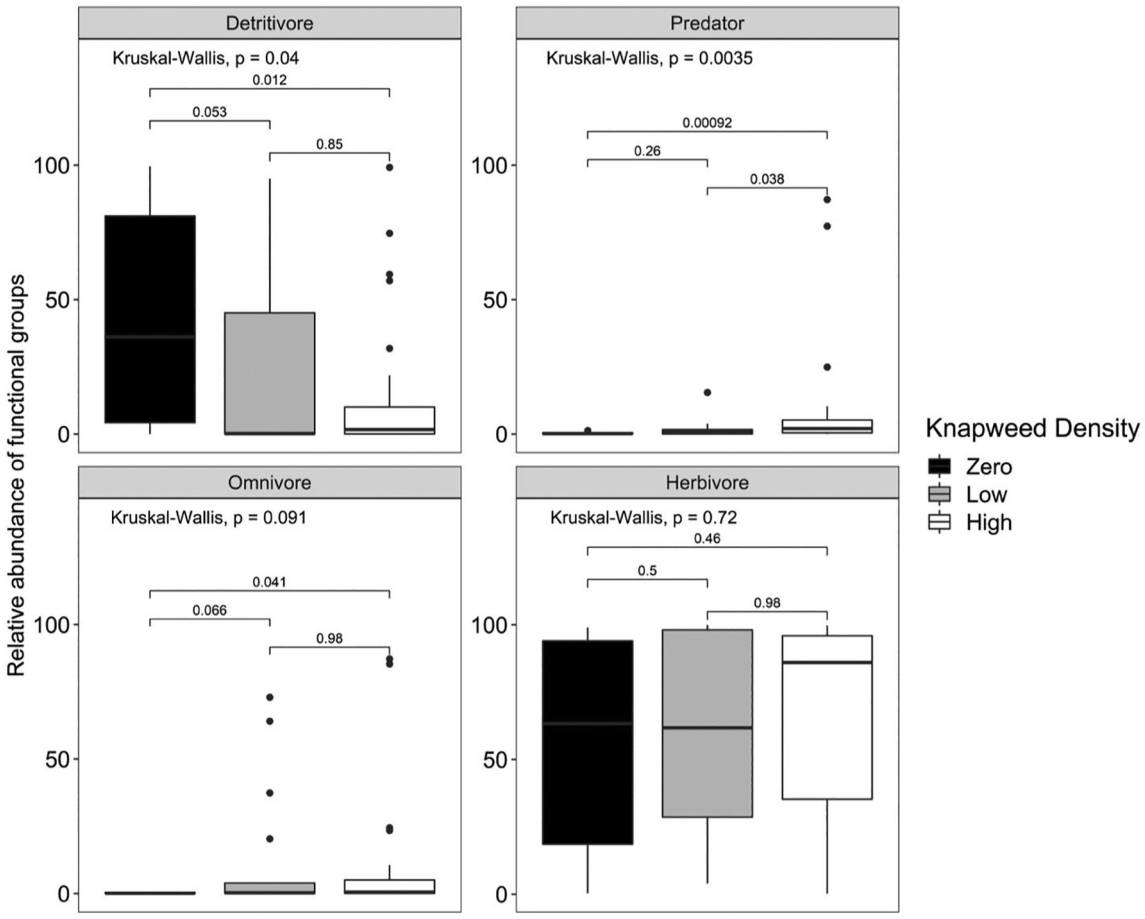

Fig. 3. Response of the four arthropod functional group relative abundances to differences in knapweed density within the grassland community. The whiskers represent the spread of the entire data and the box indicates the interquartile ranges (first quartile, median, and third quartile). Dots represent outliers.

2004; Arthur et al., 2012). Fraser and Carlyle (2011) found a three-fold reduction in litter biomass in spotted knapweed patches compared to native grasslands, indicating higher decomposition rates of knapweed leaves. This may explain the lower relative abundance of detritivores under higher knapweed density. Predator increases under high spotted knapweed densities may be due to structural changes in invasive vegetation (Litt et al., 2014). The reduced litter availability, coupled with possibly increased predation, can lead to reductions in detritivores due to both top-down and bottom-up controls.

All functional group ratios were significantly affected by knapweed density (Fig. 5). The ratio of predators to overall available prey was highest under high knapweed density. Post-hoc tests revealed significant differences in predator:prey ratio between only high and low knapweed densities. The predator:detritivore ratio was significantly different between high and zero knapweed densities and low and zero knapweed densities. Predator:herbivore ratio was significantly different between only high and zero knapweed densities. The functional group ratio was not affected by season (Fig. 6). These ratios suggest that top-down effects are likely stronger than bottom-up effects in these grasslands and that the pressure predators exact upon prey is strongest at the highest densities of knapweed. Predators may be resilient to spotted knapweed density if they have less specialized diets and continue to find food resources regardless of the relative abundance of prey species (Litt and Steidl, 2010; Spafford, 2013).

4. Implications for restoration

Spotted knapweed continues to be a problematic invader in many parts of North America (Akin-Fajiye and Gurevitch, 2018; Harris and Cranston, 1979; Sheley et al., 1998). Spotted knapweed in particular (and invasive plants in general) can change various community and ecosystem properties, such as species composition, ecosystem function, and modify trophic relationships between species (Smith-Ramesh et al., 2017; Van Veen, 2015). In this study, we applied DNA metabarcoding to assess the differences in arthropod communities at different levels of spotted knapweed invasion. Our study indicates that structural changes in the ecosystem could lead to increases in the predator community, likely strengthening top-down effects with increasing invader density. Decreases in the available litter may also reduce material available for decomposition thereby decreasing detritivores in the ecosystem. Our study did not explicitly seek to identify common or rare species; therefore, further studies are required to understand how rare arthropods may vary with seasonality or spotted knapweed density.

Invaders into an ecosystem usually interfere with existing interactions within and across different trophic levels. Arthropod dependence on plants for food, shelter, or structure can change as the plant community changes, therefore measuring the effects of the invader on only a few easily identifiable groups can give an incomplete or biased picture. Hence, the potential success of any restoration program will likely depend on whether the complex nature of organismal interactions is taken account, rather than on restoring single native species (McCary et al., 2016; Smith-Ramesh et al., 2017). For example, if we had observed only one functional group in this study, our conclusion of the impact of the invader would have been completely different. DNA metabarcoding provides the tools to develop detailed surveys of species diversity across a range of environments and trophic levels, and the results thereof should be more useful for guiding restoration measures.

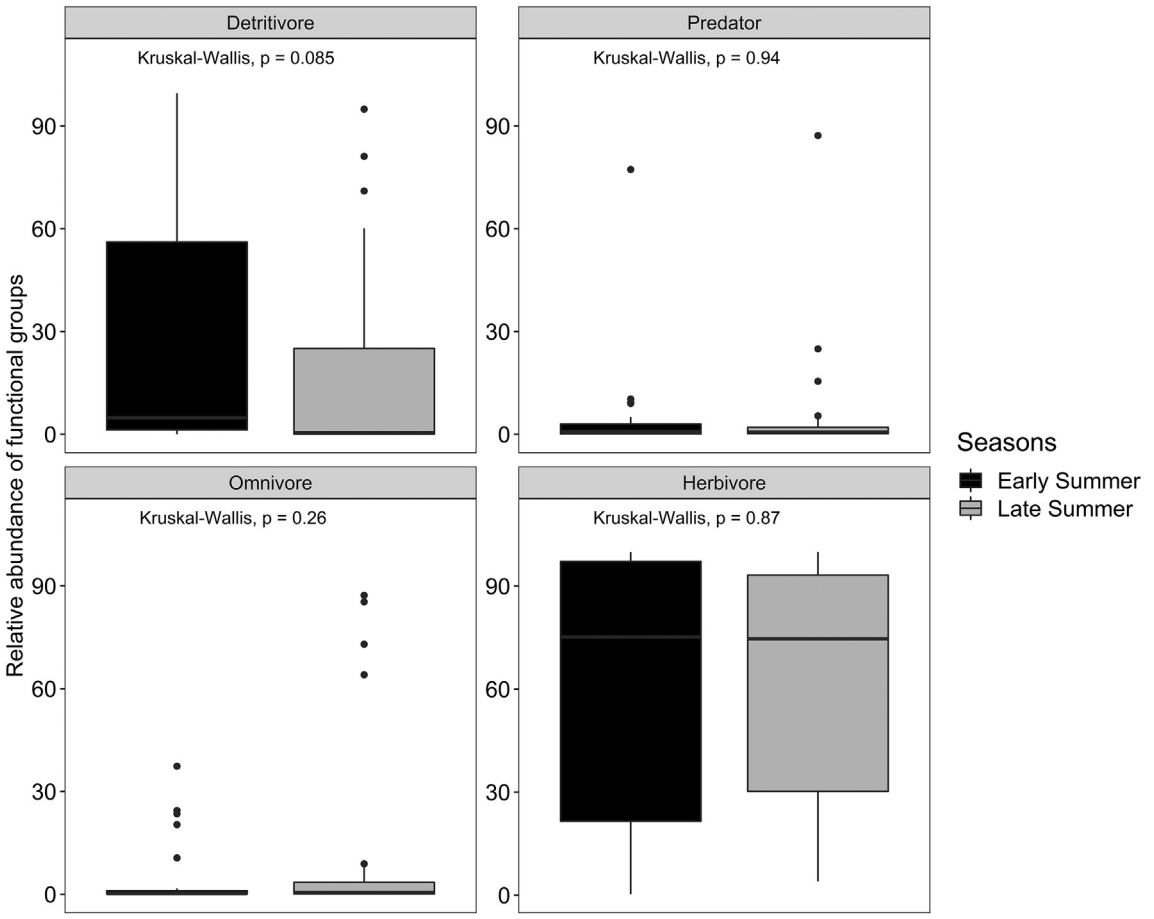

Fig. 4. Seasonal differences in the four arthropod functional group relative abundances within a grassland community. The whiskers represent the spread of the data while the box indicates the interquartile ranges (first quartile, median and third quartile). Dots represent outliers.

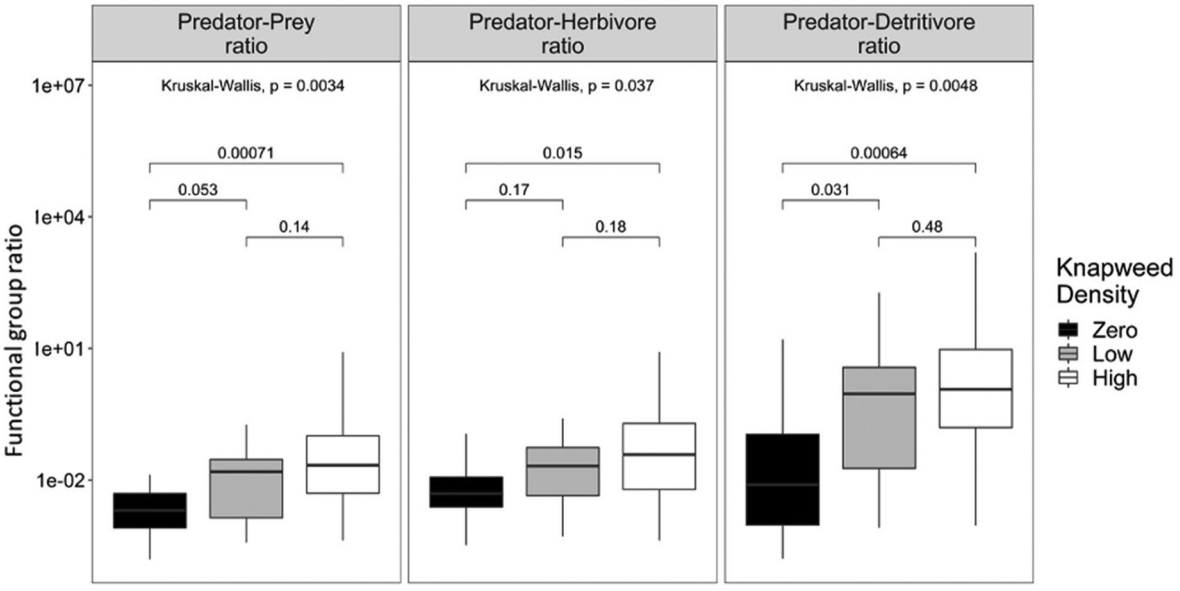

Fig. 5. Response of different functional group ratios to spotted knapweed densities within grassland communities. The whiskers represent the spread of the data while the box indicates the interquartile ranges (first quartile, median and third quartile). Dots represent outliers.

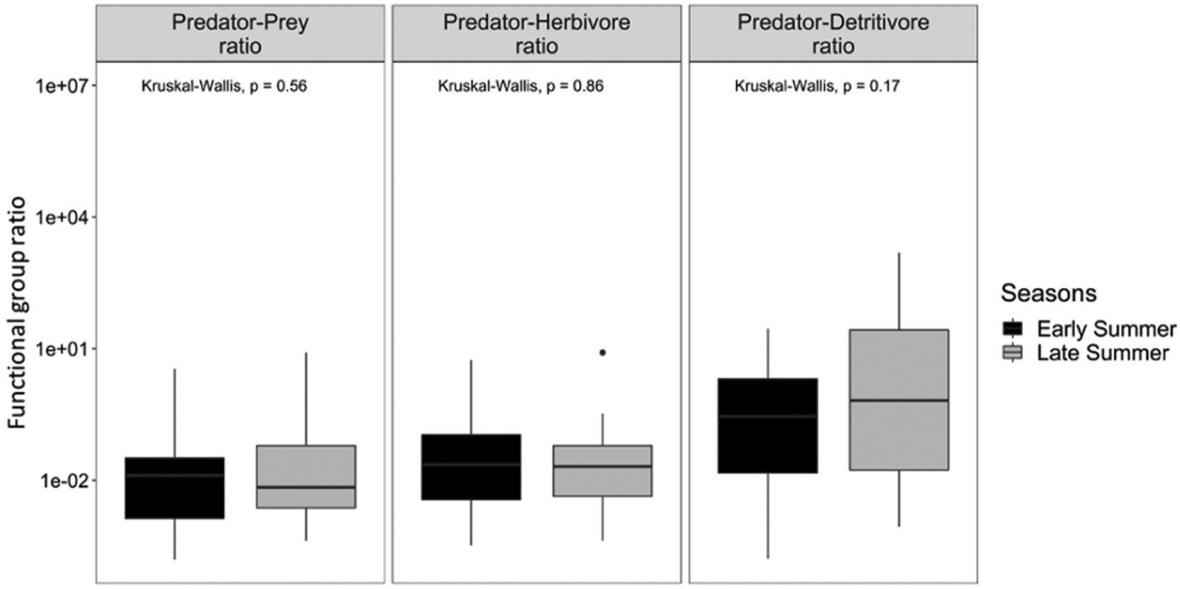

Fig. 6. Seasonal differences between different functional group ratios within the grassland community. The whiskers represent the spread of the data while the box indicates the interquartile ranges (first quartile, median and third quartile). Dots represent outliers.

Declaration of competing interest

We confirm that this manuscript, "Exploring trophic effects of spotted knapweed (*Centaurea stoebe* L.) on arthropod diversity using DNA metabarcoding", is original and has not been published nor submitted for publication elsewhere. We are unaware of any conflicts of interest for this manuscript.

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