EFFECTS OF THE INVASIVE PLANT SPOTTED KNAPWEED (*Centaurea stoebe* L.) ON GRASSLAND ARTHROPOD COMMUNITITIES: USE OF GENOMIC BARCODING TOOLS FOR ECOSYSTEMS RECLAMATION MANAGEMENT

By

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B.Sc. Carleton University, 2016

A thesis submitted in partial fulfillment of the requirements for the degree of:

MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCE

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ABSTRACT

British Columbia's (BC) grasslands are home to 30 percent of the province's species at risk and are one of Canada's most endangered ecosystems. In BC's Southern interior, human activities such as mining, recreation, and in certain instances, heavy livestock grazing, are altering grassland ecosystems; the increased soil disturbance may leave them susceptible to the colonization of invasive species. Invasive species can cause changes to native plant communities and nutrient cycling, and by doing so, may alter the amount and quality of habitat available for animals such as arthropods. Arthropods are diverse and contribute to energy flow and nutrient cycling, and are therefore an important group to study as a way of determining the effects of changes to ecosystem function. Spotted knapweed (*Centaurea stoebe* L.), a perennial forb native to Eastern Europe, is considered one of the most ecologically harmful invasive species in Western North America. The objectives of this study were (1) to determine if spotted knapweed is altering arthropod community structure and density in grassland habitats; and (2) to DNA metabarcode all arthropod specimens collected using methodology that could be implemented to expedite site restoration efforts. To address these objectives, pitfall traps were installed at sites that were colonized, in differing densities, by spotted knapweed, and DNA metabarcoding was conducted on specimens collected.

The results suggest that spotted knapweed density indirectly correlates with arthropod functional groups through changes in plant community composition. These indirect effects show different correlations between the functional groups; suggesting that both top down and bottom up control is at play upon the introduction of spotted knapweed. Decreases in herbivore and detritivore biomass was associated with increasing spotted knapweed density. Omnivore, predator, and parasite biomass had more intricate interactions. DNA metabarcoding results indicated a more complex interaction between Orthoptera and spotted knapweed density than suggested by a simple positive correlation. All other arthropod orders sampled were not obviously influenced by spotted knapweed.

This study describes a relatively rapid and inexpensive technique for monitoring arthropod biodiversity with a DNA metabarcoding methodology applicable to both invasive species conservation efforts and for guiding remediation work in disturbed grassland sites.

Keywords: Invasive plant, arthropod, functional groups, metabarcoding, grassland, reclamation, remediation

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ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. Lauchlan Fraser, for his exceptional guidance and support throughout the course of my project; Dr. Jon Van Hamme for his expertise throughout the sequencing process and his constant helpful advice and support with data processing; Dr. Rob Higgins for his fieldwork methodology advice; and Dr. Louis Gosselin for written reviews and suggestions.

I would also like to specially thank Dr. Afsana Islam for her continuous direction and guidance throughout the rigorous PCR optimization aspect of the project; Dr. Eric Bottos for his unwavering help and advice throughout the bioinformatics workflow and data analysis; Dr. Shane Rollans for his persistence in helping me to use R throughout my statistical analyses; and Breanne McAmmond for her willingness to pass on her genomics lab knowledge.

Everyone at the Fraser Lab and the TRUgen Lab have contributed to a friendly and educational working and learning environment. I am thankful for the encouragement, assistance with field work, and discussion from my lab peers and research assistants: Matthew Coghill, Colton Stephens, Chantalle Gervan, Janelle Paulson, and Garret O'Connell. I would also like to specially thank my parents, Janet and John Foster, for their loving support, and my partner, Jesse Godlington, who has constantly reminded me to explore beautiful British Columbia as a distraction from my thesis work.

This research was supported by the Natural Sciences and Engineering Research Council of Canada, the Canada Industrial Chairs Program, Genome BC, and Thompson Rivers University.

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CHAPTER 1: GENERAL INTRODUCTION

The introduction and spread of invasive plants is a global ecological concern (Vitousek et al. 1996). Alterations to native plant communities and nutrient cycling, which can occur with the invasion of non-native plants (Vitousek et al. 1996), can alter the amount and quality of habitat available for animals at multiple trophic levels, including arthropods (Litt et al. 2014). When non-native plants are introduced into an area, they are often able to colonize at very high rates compared to native plants. It is suggested that because many invasive plant species lack predators in their new environments they are able to grow unrestrained as described by the enemy-free hypothesis (Andonian et al. 2011; Siemann et al. 2006). This typically leads to increased dominance of invasive plants and decreased productivity and diversity of native plants (Bartomeus et al. 2008).

Grasslands

Natural grasslands make up less than 1% of British Columbia's land mass, are one of the most endangered ecosystems in Canada, and are highly susceptible to changes in ecosystem energy flows upon introduction of invasive plants (Fraser and Carlyle 2011). Grasslands provide invaluable services to people and the environment including carbon sequestration (Wilson 2009; Costanza et al. 1998), water filtration (Wilson 2009), wildlife management, forage for grazing livestock (Havstad 2008), and recreational areas. However, the use of grasslands by humans is leading to the anthropogenic spread of invasive plants, causing a decline in plant and animal biodiversity (e.g. Litt and Steidl 2010; Hansen et al. 2009).

Arthropods

Arthropod community biomass and composition is largely dependent on plant community density and composition (Haddad et al. 2001). Therefore, changes to plant community, biomass, or composition, caused by the introduction of invasive plants, could largely affect arthropod communities because many arthropod species need specific plants for their diet or as sites for reproduction and protection (Tallamy et al. 2010). Native arthropods may not recognize or be able to use non-native plants as a resource (Tallamy 2004), which could lead to changes in diversity and biomass of insect communities (Litt et al. 2014).

Arthropods, which make up the majority of animal species in terrestrial habitats (Havastad 2008), are an important group to study as a way of determining the effects of

environmental changes to ecosystem functioning. Insect guilds can be affected differently by changes in plant community structures in response to the introduction of invasive plant species (Litt et al. 2014). For example, an herbivorous insect that feeds only on a particular native plant that is being outcompeted by an invasive plant would be affected by the invasive plant, whereas a predatory insect feeding on the herbivore may also be indirectly affected through a decrease in its food source (Litt et al. 2014). Studying the effects of plant community changes on specific insect guilds may give us insight into trophic level interactions throughout arthropod food chains.

Spotted knapweed

Spotted knapweed (*Centaurea stoebe* L. subsp. *micranthos*; (Ochsmann 2001)) is an invasive perennial forb introduced from Europe to Northwestern North America in the 1890s (Fraser and Carlyle 2011). It is a large taprooted plant that grows to the height of 0.2-1.8 m, with solitary pink, purple, or occasionally cream-coloured flowering heads at the ends of branches (Province of British Columbia 2002). The principal stem leaves are divided pinnately and have smooth margins that narrow toward the top of the shoot. The deeply lobed rosette leaves are up to 15 cm long (Figure 1.1).

Figure 1.1 *Spotted knapweed (Centaurea stoebe L.)*

Image source: https://search.creativecommons.org/

Spotted knapweed outcompetes native plants in disturbed North American grasslands, which lowers available wildlife and livestock forage, and alters availability of soil mineral nutrients (Fraser and Carlyle 2011). Hill et al. (2006) found that spotted knapweed can have a greater ability to uptake soil water than native grasses such as Bluebunch wheatgrass

(*Pseudoregneria spicata*) and Western wheatgrass (*Pascopyrum smithii*). Spotted knapweed is thought to secrete allelochemicals through its roots into surrounding soils that can shift microbial community structure and function, increase soil phosphorus and potassium availability (Thorpe et al. 2006), and reduce soil nitrogen availability for surrounding plants to uptake (Suding et al. 2004). This can hinder the growth of native plants by changing essential nutrient availability, resulting in functional ecosystem changes that may impact arthropods by reducing plant biomass and increasing bare ground compared with grasslands without spotted knapweed.

DNA metabarcoding

DNA metabarcoding is a method that can be used to assess biodiversity of microbial, plant, animal, and insect communities. The approach combines two technologies: high-throughput DNA sequencing and phylogenetics (Ji et al. 2013). DNA metabarcoding methods allow researchers to differentiate specimens of animals, plants, and fungi using short sequences of DNA, also known as 'DNA barcodes'. To differentiate animals, a 658 base pair sequence in the mitochondrial cytochrome c oxidase subunit I gene (COI) is used as a phylogenetic marker (Ji et al. 2013). Following amplification and sequencing of target genes from specimen genomic DNA or environmental metagenomic DNA, computation methods can be used to group sequences into Operational Taxonomic Units (OTUs), which, under ideal conditions, represent individual species. Online public databases, such as the Barcode of Life Database (BOLD), which is a growing global public reference library of species identifiers (http://www.barcodeoflife.org), can be used to assign taxonomies to the calculated OTUs using sequence alignment methodologies.

Databases such as BOLD are established using DNA barcoding methods. DNA barcoding for plants, animals, and arthropods involves morphologically identifying specimens, sequencing individual barcode genes, and assigning those data unique Barcode Index Numbers (BINs). DNA barcoding of individual specimens uses Sanger sequencing (Sanger et al. 1977) to obtain high quality amplicon sequences (Gibson et al. 2014). All known specimens are assigned a BIN, which is used as a proxy for a formal Linnaean species name. Official BINs in databases are associated with curated specimens that have been morphologically identified by subject matter experts.

Sanger sequencing technology, used in DNA barcoding, is limited to sequencing a single gene fragment per specimen per sequencer lane (Ji et al. 2013). While some modern sequencers using Sanger chemistry can handle 16 plates each containing 384 wells, this is considered a low throughput sequencing approach as each specimen must be processed individually. High-throughput sequencing technologies used for DNA metabarcoding can simultaneously sequence millions of individual DNA molecules from a mixture of specimens (Kircher and Kelso 2010) (Figure 1.2). DNA metabarcoding, as described extensively by Ji et al. (2013), uses degenerate Polymerase Chain Reaction (PCR) primers that target a diversity of arthropod species, to mass-amplify a barcode gene (such as the COI gene) from large mixed samples of organisms simultaneously. PCR products, or amplicons, can then be sequenced en masse using a high-throughput sequencer. Each individual specimen in the sample results in the production of numerous amplicons that are, following sequencing, clustered into OTUs (Ji et al. 2013). From there, researchers can refer to online databases to assign specimen taxonomic identifiers.

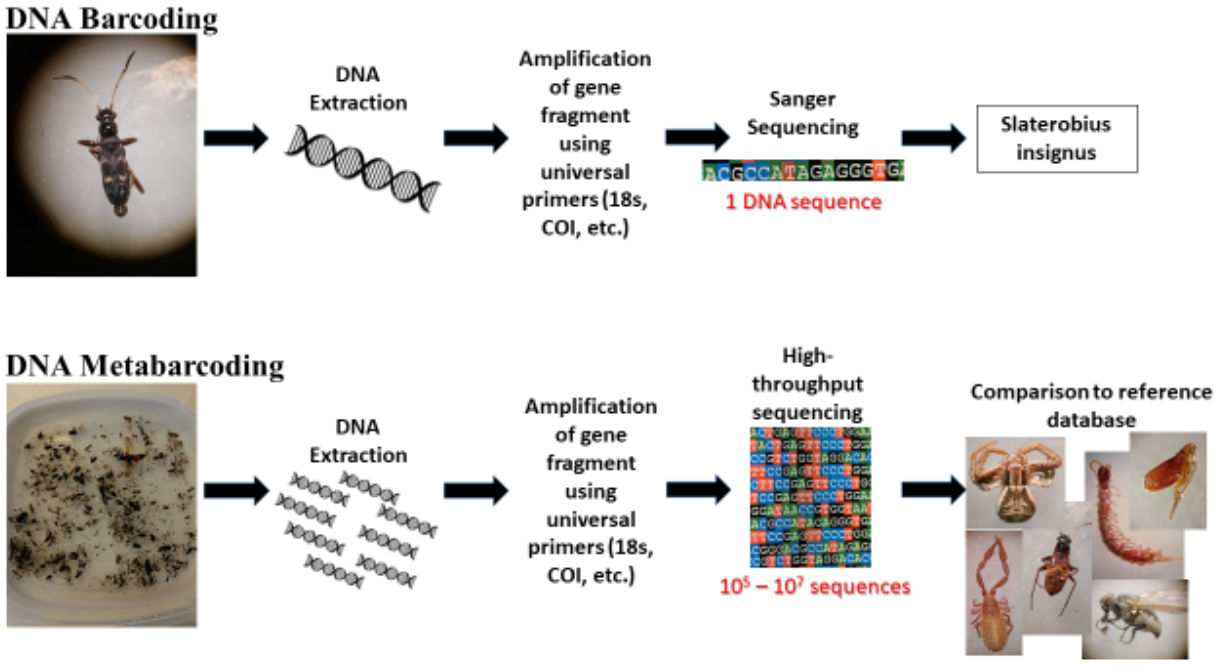

Figure 1.2. *Visual representation of the methods of DNA barcoding and DNA metabarcoding.*

Image source: modified from https://search.creativecommons.org/

Identifying individual species morphologically can be time consuming and expensive; usually an experienced taxonomist is needed. If a specimen is damaged or is in an immature stage of development, even specialists may be unable to accurately identify certain specimens

(Gibson et al. 2015). Once methods are developed, DNA metabarcoding can be less time consuming when dealing with large numbers of samples, and most researchers are able to take tissue samples from specimens for DNA extraction and amplicon sequencing. DNA metabarcoding is a way to advance large-scale arthropod sampling for purposes such as conducting environmental assessments or for monitoring site remediation. If we are able to sample and characterize diverse arthropod communities, we will have a better understanding of how ecosystems should function following anthropogenic and natural disturbances.

Objectives

The objective of Chapter 2 was to determine if the density of spotted knapweed patches influence the community composition of arthropods in a semi-arid grassland ecosystem. I morphologically identified arthropods and grouped into functional guilds (using Marshall 2006). I compared differences in biomass, species richness, and functional diversity of arthropod guilds caught in pitfall traps with differing spotted knapweed density to determine if epigeal arthropods are affected by the density of spotted knapweed patches. I hypothesized that herbivore and omnivore guild species richness and biomass would decrease in the presence of spotted knapweed, leading indirectly to decreases in predator and parasite guild biomass due to changes in prey items. I also hypothesize that detritivore biomass would increase due to increasing dead plant litter that has been associated with the colonization of spotted knapweed in past studies (Alerding and Hunter 2013).

In Chapter 3, I implemented DNA metabarcoding methods (Hebert et al. 2003) to determine if knapweed biological control agents, like *Larinus* (Coleoptera: Curculionidae), or how taxa such as Orthoptera or Coleoptera were affected by the density of spotted knapweed. This project will be used to help investigate DNA metabarcoding as a fast and cost-effective method to expedite large-scale arthropod sampling following site remediation efforts**.**

Chapter 4 presents general research conclusions, future research, and management implications that could be applied based on the findings from this study.

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CHAPTER 2: ARE ARTHROPOD COMMUNITIES IN GRASSLAND ECOSYSTEMS AFFECTED BY THE ABUNDANCE OF AN INVASIVE PLANT?

INTRODUCTION

There are more than 13,000 alien plant species in the world but only about a tenth are invasive, imposing negative effects on an area's economy, public health, and environment (van Kleunen et al. 2015). Plant species invasions are a global conservation concern, leading to changes in native plant community composition and soil chemistry (Vitousek et al. 1996; Ehrenfeld 2003).

Arthropods represent the group with the largest animal biomass and make up the majority of animal species in terrestrial habitats (Havastad 2008), including grasslands. They contribute to ecosystem function in their roles as pollinators, foragers, soil engineers, and food for other organisms (Higgins and Lindgren 2006; Bourn and Thomas 2002; Tscharntke and Greiler 1995). The diversity of functional roles of arthropod communities in grasslands makes them useful groups when trying to understand changes to ecosystem structure with the introduction of invasive plants.

At least 90% of arthropod herbivores feed on plants in a single family or a few genera (Bernays and Graham 1988). With the decrease in native plant diversity, due to colonization by invasive plants, subsequent decreases in the diversity of native herbivore and omnivore arthropods have been observed (Litt et al. 2014; Vila et al. 2011). However, Tallamy et al. (2010) found that invasive plants, such as Norway maple and crepe myrtle, can potentially support generalist North American herbivore arthropod species in certain circumstances, such as in urban environments. Litt and Steidl (2010) observed decreases in overall abundance and richness of arthropods with increasing nonnative grasses in a grassland ecosystem. Although the response of overall herbivore and omnivore diversity to non-native plant invasion is hard to predict, generalist species are less diverse but far more abundant than specialist species (Tallamy 2004).

Indirectly, predator and parasite arthropod guilds can be adversely affected by changes in prey items or vegetation structure due to the colonization of invasive plants (Gratton and Denno

2005). This may lead to subsequent changes in ecosystem services due to the decline of these arthropod groups. However, detritivore arthropod biomass might increase due to an alien plant invasion because increases in decaying ground litter associated with highly productive invasive plants with palatable high-nutrient leaf tissue could provide extra food (Levin et al. 2006). It is nevertheless expected that any changes to native arthropod diversity, such as decreases in the diversity of all arthropod guilds except detritivores, would lead to changes in guild dynamics. This could result in a chain of effects throughout the ecosystem (Grant et al. 2017; Pearson 2009). For example, decreases in predators could act as a top-down control for the population of their preys' population being unrestrained by predation. Alternatively, herbivores could become less dense due to bottom-up control of limited native plant biomass for consumption with the introduction of plant competition.

Grasslands are one of the most endangered ecosystems in Canada and are highly susceptible to changes in ecosystem energy flows due to the introduction of invasive plants (Aguair 2005). Grasslands provide invaluable services to people and the environment including carbon sequestration (Wilson 2009; Costanza et al. 1998), water filtration (Wilson 2009), wildlife management, forage for grazing livestock (Aguair 2005), and recreational areas. However, the use of grasslands by humans, in the form of both permanent changes to the ecosystem and changes to natural disturbance regimes, is leading to the anthropogenic spread of invasive plants such as spotted knapweed, altering plant and animal biodiversity (Mack et al. 2000).

Centaurea stoebe L. subsp. *micranthos* (spotted knapweed) is a deeply tap-rooted perennial forb native to Eastern Europe that was first introduced into North America in the 1890s (Fraser and Carlyle 2011). It is considered one of the most ecologically harmful invasive plant species in Western North America (Hansen and Ortega 2009). Spotted knapweed is an extremely competitive plant in part due to high seed production rates and the ability of seeds to remain viable for eight years or more (Davis et al. 1993). Spotted knapweed is also able to alter soil properties by elevating phosphorous within its rhizosphere and by decreasing bioavailable soil carbon and nitrogen pools (Fraser and Carlyle 2011). Spotted knapweed is suspected of releasing allelopathic chemicals through its roots, allowing it to spread rapidly by making the soil inhospitable to native plant species (Callaway and Ridenour 2004). Spotted knapweed primarily

colonizes grassland communities by forming dense, almost-monoculture stands once established (Hansen and Ortega 2009). This study explores the effects of differing densities of spotted knapweed on the functional groups of grassland arthropods in the semi-arid grasslands of Southern Central British Columbia. Research contributing to our understanding of invasive plants is important in informing conservation management strategies essential for combatting the spread of invasive plants to reduce biodiversity loss.

We predict that spotted knapweed density will have differing effects on the biomass of arthropod functional groups. We hypothesize that herbivore and omnivore functional group biomass will decrease in the presence of spotted knapweed. This will lead indirectly to decreases in predator and parasite biomass due to changes in abundance or diversity of prey items. Detritivore biomass is hypothesized to increase with increasing spotted knapweed density due to the increase in food availability and plant litter, with the colonization of spotted knapweed (Alerding and Hunter 2013).

METHODS

Study area

In May 2017, arthropod sampling sites were established in the upper grasslands of Lac du Bois (LDB) (Figure 2.1), a 15,000-ha grassland area located Northwest of Kamloops, British Columbia (BC) (50º39’59’’ N, 120º19’09’’ W). LDB is a protected shortgrass and shrub-steppe ecosystem that occurs in the rain shadow of the BC Coast Mountains. The park and surrounding region is characterized as semiarid, with annual precipitation of 277.6 mm, (including 63.5 cm of snowfall). Average annual daily temperature for the region is 9.3ºC (Environment Canada 2010). Dominant grasses in the region include bluebunch wheatgrass (*Pseudoroegneria spicata*) and rough fescue (*Festuca altaica*). Common shrubs include big sagebrush (*Artemisia tridentata*), rabbit brush (*Ericameria nauseosa*), prickly rose (*Rosa acicularis*) and grey horsebrush (*Tetradymia canescens*) (Lee et al. 2014). LDB is a multi-use area managed for recreation, wildlife, and livestock grazing at low to moderate stocking rates (Bassett and Fraser 2015; Schmidt et al. 2012; Evans 2011). The continuous use of the grasslands by recreational users and ranchers leaves the area susceptible to the introduction of invasive plants through high propagule pressure such as hitchhiking seeds attached to clothing, boots, vehicle tires, and other means. This makes it an important study area due to the numerous invasive plants currently in the park

(eg. Fraser and Carlyle 2011; BC Forest Services 1999) and the potential for further human seed dispersal of invasive species.

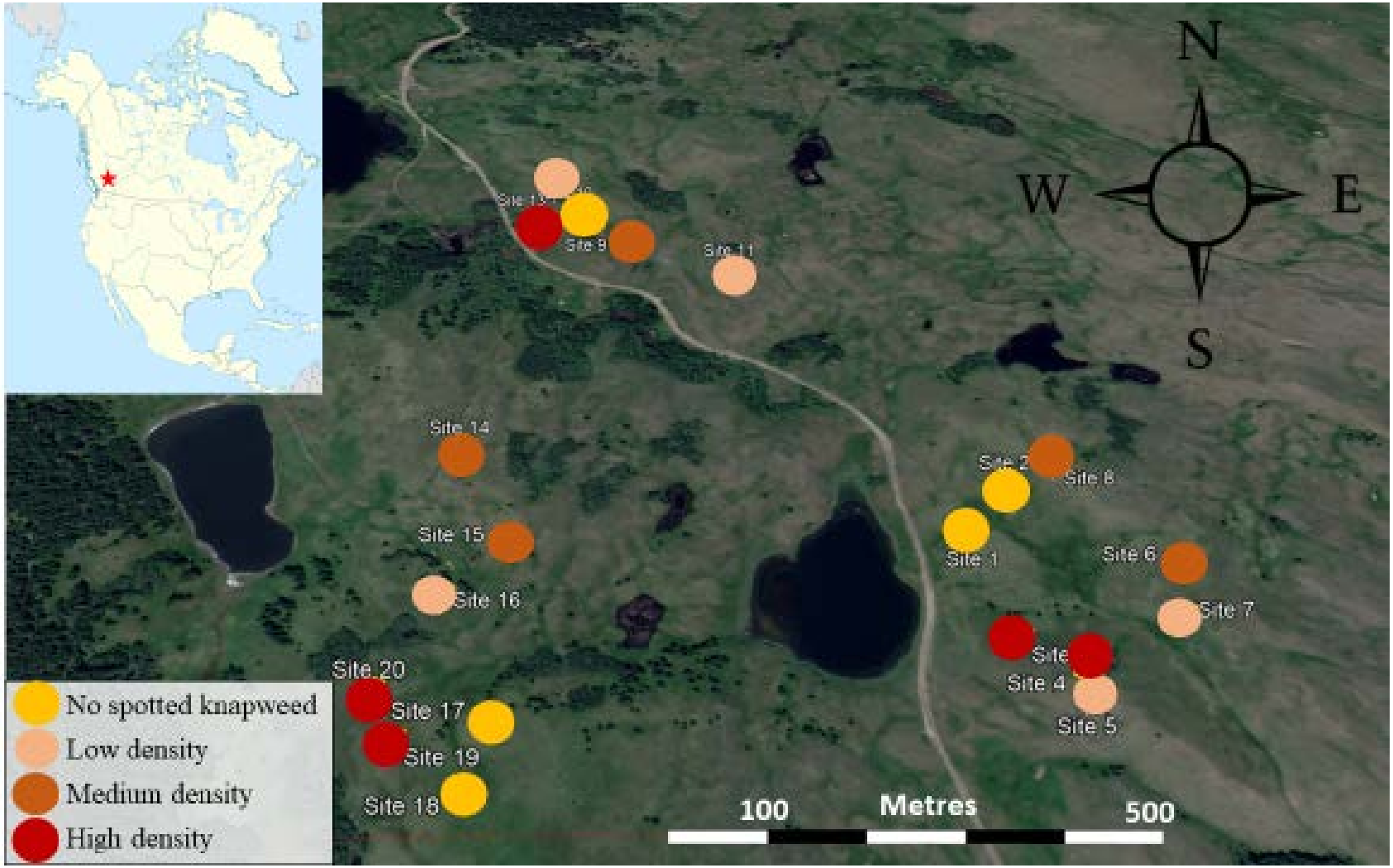

Figure 2.1. *Location of sampling sites in the upper grasslands of Lac du Bois Grasslands protected area, Northwest of Kamloops, British Columbia, Canada.*

Image source: Google Earth

Figure 2.2. *Diagram of a sweep netting transect and placement of the pitfall traps at one sampling site infested with purple-flowered spotted knapweed.*

Image source: modified from https://search.creativecommons.org/

Site selection

Twenty 40 m diameter sampling sites were located in the LDB grasslands with varying 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}) (Fig. 2.1). The sites were all located within <2 km^2 (Fig 2.1) to ensure that they shared similar ecosystem properties to allow observed differences to be more meaningful (Bode and Maciejewski 2014).

Sampling protocol

In the centre of each sampling site, 4 pitfall traps were installed in a square arrangement, each 2 m apart (Figure 2.2). Pitfall traps are small epigeal arthropod collection traps that consist of a collection cup (11.5 cm diameter, 7.5 cm depth) dug into the earth flush with ground level (Bassett and Fraser 2015) (Figure 2.3). The collection cups were 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. Spotted knapweed seedlings emerge in early May (Schirman 1981). The pitfall traps were

opened for a period of five days each month, in the last week of May, June, July, and August 2017.

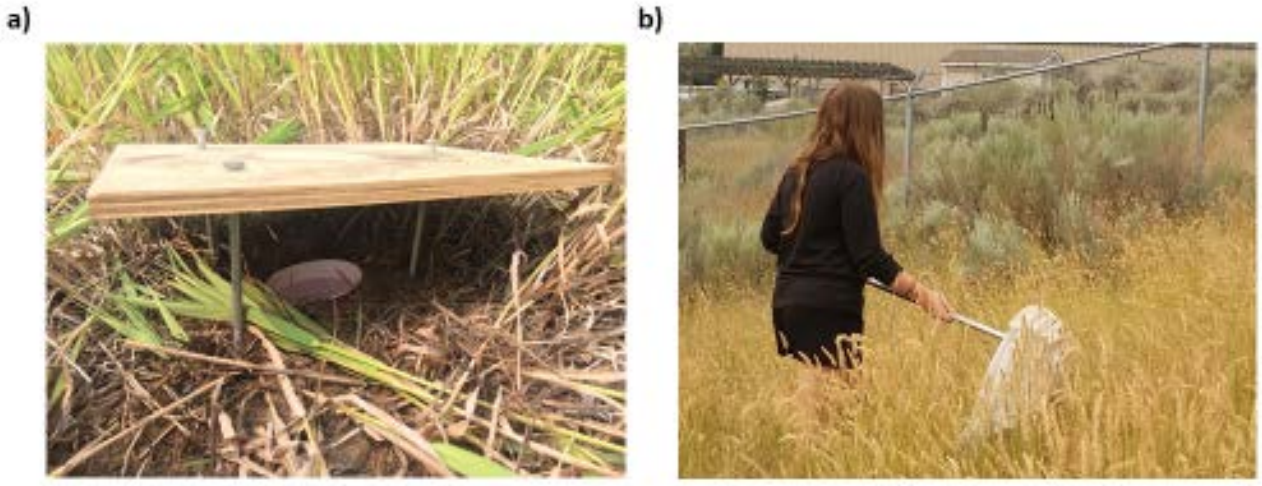

Figure 2.3. *Photos of a pitfall trap (a) and a sweep net survey (b).*

Image source: J. Foster

In Addition, at the end of each 5-day period, a 30 cm-diameter canvas wire-frame 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 (Doxon et al. 2011). All sweep net surveys were completed on days with wind velocities <10 km/h to increase the probability that arthropods remained on the foliage. Sweep net surveys were conducted by the same researcher to ensure consistency between sites and sampling dates. The sweep net samples were not used in Chapter 2, but retained for DNA metabarcoding in Chapter 3

Spotted knapweed has been observed to increase soil temperature and surface water runoff (Fraser and Carlyle 2011; Lacey et al. 1989). As arthropod species can be highly affected by changes in temperature (Bokhorst et al. 2008), it is important to consider this additional covariable in the experimental set up. Soil temperature data loggers (DS1921G-F#5 Maxim Integrated, San Jose, CA, USA) were installed at 5 cm depth in the centre of each site, and these were set to record soil temperature at 2-hour intervals.

Vegetation was sampled June 20-28th, 2017 at each of the twenty sites. 1 m × 1 m quadrats were placed two metres North of each pitfall trap, totalling eighty quadrats. Within each

quadrat, counts were made of the number of spotted knapweed stems and the percent cover of all plants, bare ground, and litter were recorded. All plant species in each quadrat was identified and the percent cover of each species within the quadrat was recorded. Additionally, live standing biomass was quantified by clipping all plats within a 0.5 m × 0.1 m section of each quadrat at ground level. The plant biomass samples were separated as spotted knapweed as one component and all other live plants as the other component. The plant biomass samples were stored in brown paper bags and dried in a Yamato oven (Model No. DKN8132) at 65ºC for 48 hours (as per Bassett and Fraser 2015) and weighed with an analytical balance to the nearest 0.00001 g (Fisher Scientific accuseries 225D). The biomass data were then converted into g/m^2.

The Shannon-Wiener Index of plant community diversity was calculated with the species cover data for each plot using the equation:

$$Shannon - Wiener\ Diversity\ Index\ (H) = -\sum_{i}^{s} = {}_1p_i lnp_i$$

The Simpson diversity index was calculated using the same data with the equation:

$$Simpson's\ Recipricol\ Diversity\ Index\ (D) = \frac{1}{\sum_{i}^{s} = {}_1p_i^2}$$

Where *p* is the proportion (n/N) of plant biomass of a particular species (n) divided by the total biomass (N) and *s* is the number of species (see Colwell, 1988).

Arthropod specimen sorting

Specimens were stored in a -20ºC freezer in 150-mL containers unique to each pitfall trap filled with 87% denatured ethanol. All arthropods captured in one of the four containers from each sampling site at each sampling date were taxonomically identified and sorted using forceps and sorting dishes into functional groups. The specimens were sorted based on adult life stage diet (using Marshall 2006). Each individual was assigned to one of the following functional groups: herbivore, omnivore, detritivore, predator, or parasite. After being sorted into functional groups, specimens were dried in an oven at 65ºC for 48 hours and weighed with an analytical balance to measure biomass (as per Harrower 2016). Species richness was calculated, and functional Shannon-Wiener diversity and Simpson diversity were calculated using the number of individuals of each functional group.

The remaining three of the four containers from each site and the sweep net samples were kept in a freezer with ethanol for DNA metabarcoding (Chapter 3).

Data analysis

All data were analyzed statistically using RStudio integrated under R 3.4.4 "Someone to Lean On" (The R Foundation for Statistical Computing). The data were checked for normality using boxplots and residual plots. Homogeneity of variance was assessed using the Fligner-Killeen test, and when necessary, the data were transformed using a natural logarithm transformation or a log (x+1) transformation for biomass and species richness data that contained zeros. All data analyses were tested for significance at the 5% probability level and noted within the 10% probability level to recognize possible trends in the data.

A one-way analysis of variance (ANOVA) and post hoc Tukey test were done to test the effects of the density of spotted knapweed (no, low, medium, and high density) on the biomass, species richness, and functional diversity of each arthropod guild captured. The arthropod guilds included herbivore, omnivore, predator, detritivore, and parasite.

Finally, four principal components analyses (PCA) were conducted, one for each of the four summer months, to examine the most influential functional group associated with arthropod community composition. Stepwise multiple regressions in both directions using Akaike information criterion (AIC) values were run using the principal components and the significant site variables to determine the best fitting model that each principal component represented. These regressions helped to explore interacting effects of site variables, spotted knapweed density, and functional groups.

RESULTS

Plant community characteristics

Overall plant biomass, excluding spotted knapweed, was significantly lower (P=0.046) in plots with high spotted knapweed density (126.1±20.9 g/m^2) compared to no spotted knapweed (404.6±85.0 g/m^2) (Table 2.1). High spotted knapweed density sites also resulted in the lowest plant ground litter cover (P=0.001, 19.6±2.1 %) and the highest amount of bare ground (P=0.099, 23.0±1.8 %). Sites without spotted knapweed also had the lowest daily ground temperature throughout the summer (P=0.003, 2.9 ^oC colder).

Sites with high spotted knapweed density had the highest invasive plant cover (P<0.001, 41.4±3.0 %) and lowest native plant cover (P<0.001, 48.0±6.2 %). Sites with no spotted knapweed had the highest native plant cover (94±6.0 %) and lowest invasive plant cover (1.5±0.5 %). A complete list of native and invasive plants identified is in Appendix A. Plant community diversity, measured using two diversity indices, was higher in sites with low densities of spotted knapweed compared to sites with no spotted knapweed present (P=0.061 and 0.089), while plant diversity at sites with medium and high densities of spotted knapweed did not differ between each other or between low and no densities of spotted knapweed.

Table 2.1. *Results of one-way analyses of variance examining the relationship of spotted knapweed density on site variables, ±SE, n=20, df=3.*

	Knapweed Density				**F**	**P**
Site Variables	**None**	**Low**	**Medium**	**High**		
Plant Biomass (g/m^2)	$404.6±85.0^{a}$	$212.4±67.8^{bc}$	$168.6±25.3^{bc}$	$126.1±20.9^{c}$	3.37	**0.046***
Ground Litter Cover (%)	$64.7±9.8^{ab}$	$35.4±8.1^{bc}$	$46.1±3.0^{abc}$	$19.6±2.1^{d}$	8.20	**0.001***
Bare Ground Cover (%)	$6.4±3.2^{b}$	$15.9±7.6^{ab}$	$16.8±4.6^{ab}$	$23.0±1.8^{a}$	2.05	**0.099**
Daily Ground Temperature(^{o}C)	$18.8±0.6^{b}$	$23.6±1.2^{a}$	$21.7±0.8^{ab}$	$22.2±0.8^{a}$	5.15	**0.003***
Native Plant Cover (%)	$94±6.0^{a}$	$53.1±8.7^{b}$	$68.9±6.8^{b}$	$48.0±6.2^{b}$	11.9	**<0.001***
Invasive Plant Cover (%)	$1.5±0.5^{c}$	$20.3±6.4^{b}$	$24.2±1.6^{b}$	$41.4±3.0^{a}$	20.1	**<0.001***
Shannon-Wiener Diversity	$3.9±0.5^{b}$	$5.2±0.2^{a}$	$4.8±0.2^{ab}$	$4.4±0.2^{ab}$	3.01	**0.061**
Simpson Diversity	$5.6±1.5^{b}$	$9.7±1.3^{a}$	$8.0±1.0^{ab}$	$6.0±0.5^{ab}$	2.62	**0.089**

Bold values indicate statistical significance at P<0.1, * indicates significance at P<0.05

Arthropod functional biomass and diversity

Arthropod specimens from a total of 80 pitfall traps were counted, sorted, and weighed. Appendix B shows sampling frequency of arthropod families that were morphologically identified.

The arthropod samples were collected monthly, thus a repeated measures design. However, there were several arthropod community variables that were not correlated with the sampling date (Table 2.2): Simpson diversity, herbivore biomass, detritivore biomass, and parasite biomass. Theses variables were therefore grouped for analysis.

Table 2.2*. Multiple analyses of variance results of the relationship of spotted knapweed density and date sampled on arthropod community functional groups, n=80, df=3.*

	Knapweed Density		**Date**	
Response Variable	**F**	**P**	**F**	**P**
Overall Species Richness (n/trap)	1.776	0.164	13.445	**<0.001***
Overall Biomass (mg/trap)	1.788	0.162	3.646	**0.033***
Shannon-Wiener Diversity	1.402	0.249	3.625	**0.034***
Simpson Diversity	1.643	0.192	0.950	0.394
Herbivore Biomass (mg/trap)	2.849	**0.047***	0.266	0.767
Omnivore Biomass (mg/trap)	2.529	**0.068**	5.952	**0.001***
Predator Biomass (mg/trap)	1.006	0.389	7.982	**0.001***
Detritivore Biomass (mg/trap)	1.536	0.660	1.281	0.287
Parasite Biomass (mg/trap)	1.154	0.337	1.072	0.350
Daily Ground Temperature (^{o}C)	7.450	**<0.001**	15.854	**<0.001***

Bold values indicate statistical significance at P<0.1, * indicates significance at P<0.05

Herbivore biomass was greater at no spotted knapweed (18.6±8.9 g) compared to sites where spotted knapweed was present (Table 2.3). Detritivore biomass was largest in the absence of spotted knapweed (3±2.5 g) but only at 10% probability.

Table 2.3*. Analysis of variance results of the relationship of spotted knapweed density on arthropod community biomass and functional group biomass for the entire summer, ±SE, n=80, df=3.*

	Knapweed Density				**F**	**P**
Response Variable	**None**	**Low**	**Medium**	**High**		
Overall Summer						
Overall Biomass	192.7±60.3	181.3±66.6	161.3±56.1	202.7±74.5	1.788	0.162
Shannon-Wiener Diversity	1.2±0.2	1.3±0.2	1.7±0.1	1.6±0.1	1.402	0.249
Simpson Diversity	1.7±0.2	1.8±0.1	2.2±0.1	2.1±0.1	1.780	0.158
Herbivore Biomass	18.6±8.9^{a}	4.0±1.6^{b}	3.8±0.9^{b}	5.2±2.5^{b}	2.857	**0.043***
$^{\phi}$Omnivore Biomass	27.3±8.0	23.6±13.5	9.4±3.4	19.0±4.3	1.943	0.131
Predator Biomass	85.4±29.7	128.8±56.4	123.9±52.5	104.7±26.4	1.006	0.389
Detritivore Biomass	8.8±4.8^{a}	3.9±2.4^{ab}	0.1±0.09^{b}	0.4±0.2^{b}	2.739	**0.066**
Parasite Biomass	3.3±2.5	0.6±0.3	0.1±0.07	0.4±0.2	1.832	0.148

Biomass is measure in mg per pitfall trap.
Bold values indicate statistical significance at P<0.1, * indicates significance at P<0.05.

ϕNote that biomass is not an accurate measurement for omnivores caught in pitfall traps, as most omnivores caught (>90%) were ants (Formicidae), which are eusocial animals (Andersson, 1984) that follow scent trails into pitfall traps; not giving accurate representations of the population. Instead, see Appendix C with species richness data.

Functional groups were correlated differently by the density of spotted knapweed at different sampling periods throughout the summer (Table 2.4). May and July sampling yielded no significant differences of arthropod community composition at different spotted knapweed densities. However, May sampling yielded much higher overall insect biomass than the other months.

In June, herbivore biomass was 10-25× higher in the absence of spotted knapweed than at sites with spotted knapweed. Omnivore biomass was lowest where no spotted knapweed were present and detritivore biomass decreased with increasing spotted knapweed density. August sampling had the highest predator biomass at low spotted knapweed densities and the lowest predator biomass at no spotted knapweed density.

Table 2.4. *Analysis of variance results of the relationship of spotted knapweed density on arthropod community functional group biomass sampled each month, ±SE, n=20, df=3.*

	Knapweed Density				**F**	**P**
Response Variable	**None**	**Low**	**Medium**	**High**		
May						
Overall Biomass	106.1	525.9	346.1	463.7	1.850	0.179
Shannon-Wiener Diversity	1.2	1.8	1.7	1.6	0.481	0.700
Simpson Diversity	1.7	2.3	2.2	2.2	0.680	0.577
Herbivore Biomass	7.0	6.2	4.2	3.2	0.214	0.885
ϕOmnivore Biomass	11.5	23.8	6.8	19.7	1.393	0.281
Predator Biomass	86.7	441.6	335.0	440.0	1.532	0.245
Detritivore Biomass	0.3	1.9	0.04	0.6	0.394	0.759
Parasite Biomass	1.3	1.2	0.01	0.03	0.831	0.496
June						
Overall Biomass	324.6	76.6	157.4	240.3	1.546	0.241
Shannon-Wiener Diversity	1.8	1.3	2.1	1.8	0.829	0.497
Simpson Diversity	2.1	1.8	2.4	2.1	0.484	0.698
Herbivore Biomass	38.6^{a}	1.5^{b}	3.8^{b}	2.6^{b}	3.513	**0.039***
ϕOmnivore Biomass	8.3^{a}	48.4^{b}	12.8^{ab}	31.4^{ab}	2.721	**0.079**
Predator Biomass	163.2	54.6	140.1	204.7	1.294	0.311
Detritivore Biomass	61.1^{a}	12.1^{b}	0.4^{b}	0.01^{b}	4.008	**0.026***
Parasite Biomass	10.2	0.0	0.3	1.4	1.070	0.390
Daily Ground Temperature	16.5	20.5	18.9	18.9	2.277	0.119
July						
Overall Biomass	81.2	101.3	136.0	82.2	0.714	0.558

Shannon-Wiener Diversity	1.1	1.1	1.6	1.9	1.036	0.403
Simpson Diversity	1.8	1.6	2.1	2.3	0.572	0.642
Herbivore Biomass	4.1	3.6	2.9	0.8	0.823	0.500
ϕOmnivore Biomass	33.8	9.6	16.9	21.8	1.207	0.339
Predator Biomass	30.7	4.7	20.0	32.9	2.220	0.125
Detritivore Biomass	2.0	0.1	3.7	0.1	0.400	0.755
Parasite Biomass	0.8	1.1	0.2	0.2	0.397	0.757
Daily Ground Temperature	20.2^{a}	25.9^{b}	23.9^{ab}	24.4^{ab}	2.644	**0.085**
August						
Overall Biomass	6.3^{b}	21.6^{a}	5.4^{b}	24.7^{a}	2.732	**0.055**
Shannon-Wiener Diversity	0.7	1.1	1.4	1.0	0.448	0.772
Simpson Diversity	1.4	1.5	2.1	1.9	1.018	0.441
Herbivore Biomass	8.9	4.1	3.9	5.2	0.760	0.533
ϕOmnivore Biomass	16.6	2.3	0.9	2.9	1.634	0.221
Predator Biomass	0.9^{b}	31.5^{a}	0.6^{b}	7.1^{ab}	2.235	**0.087**
Detritivore Biomass	3.9	1.0	0	0.6	0.424	0.739
Parasite Biomass	0.8	0	0	0	1.000	0.418
Daily Ground Temperature	19.6^{a}	24.3^{b}	22.3^{ab}	23.3^{ab}	2.703	**0.082**

Biomass is measure in mg per pitfall trap.
Bold values indicate statistical significance at P<0.1, * indicates significance at P<0.05.
ϕSee appendix C for omnivore and other species richness data.

Arthropod community trophic interactions

A PCA using the overall summer biomass of all five functional groups' showed components 1 and 2 accounted for about 60% of the variation in functional group biomass among differing spotted knapweed density sites (Figure 2.4; Table 2.5). Components 2 and 3 accounted for about 42% of the variation. Component 1, controlled by spotted knapweed biomass (Table 2.6), negatively predicts herbivore and parasite biomass (r = -0.666 & -0.683, Table 2.6). Component 2, controlled by litter cover and ground temperature (Table 2.7), negatively predicts omnivore and predator biomass (r = -0.676 & -0.685, Table 2.5). Finally, component 3, controlled by plant biomass and litter cover (Table 2.8), negatively predicts detritivore biomass (r = -0.929, Table 2.5). The vectors reveal a negative relationship between herbivores/parasites versus predators/omnivores at all sites (Figure 2.4). See Appendix D-F for monthly scale PCA results.

Table 2.5*. Factor loadings of the overall summer principal components analysis, n=80.*

	Component 1	**Component 2**	**Component 3**
Herbivore	-0.666	0.108	0.233
Omnivore	-0.163	-0.676	-
Predator	-0.127	-0.675	-0.255
Detritivore	-0.217	0.254	-0.929
Parasite	-0.683	0.101	0.124
Standard Deviation	1.357	1.078	0.976
Variance (%)	36.8	23.3	19.0
Cumulative Variance (%)	36.8	60.1	79.1

Table 2.6*. Three multiple regression analyses for significant site variables predicting principal component 1, 2, and 3, n=80, df=56.*

	Component 1 *F-stat=2.39, P=0.078, R^2=0.130*				***Component 2*** *F-stat=7.56, P=0.001, R^2=0.222*				***Component 3*** *F-stat=2.59, P=0.062, R^2=0.132*			
Variable	**Estimate**	**SE**	**T**	**P**	**Estimate**	**SE**	**T**	**P**	**Estimate**	**SE**	**T**	**P**
Intercept	-1.872	1.172	-1.597	0.116	-3.668	1.005	-3.648	**0.001***	-2.550	2.136	-1.194	0.238
Spotted Knapweed Biomass (g/m^2)	0.201	1.109	2.400	**0.019***								
Plant Biomass (g/m^2)									0.890	0.386	2.300	**0.025***
Litter Cover (%)					1.868	0.573	3.257	**0.002***	-1.800	0.988	-1.823	**0.074**
Bare Ground Cover (%)	-1.729	1.373	-1.259	0.213								
Daily Ground Temperature (^oC)	0.076	0.030	1.259	0.213	0.118	0.034	3.411	**0.001***	-0.039	0.043	-0.912	0.366

Bold values indicate statistical significance at P<0.1, * indicates significance at P<0.05.

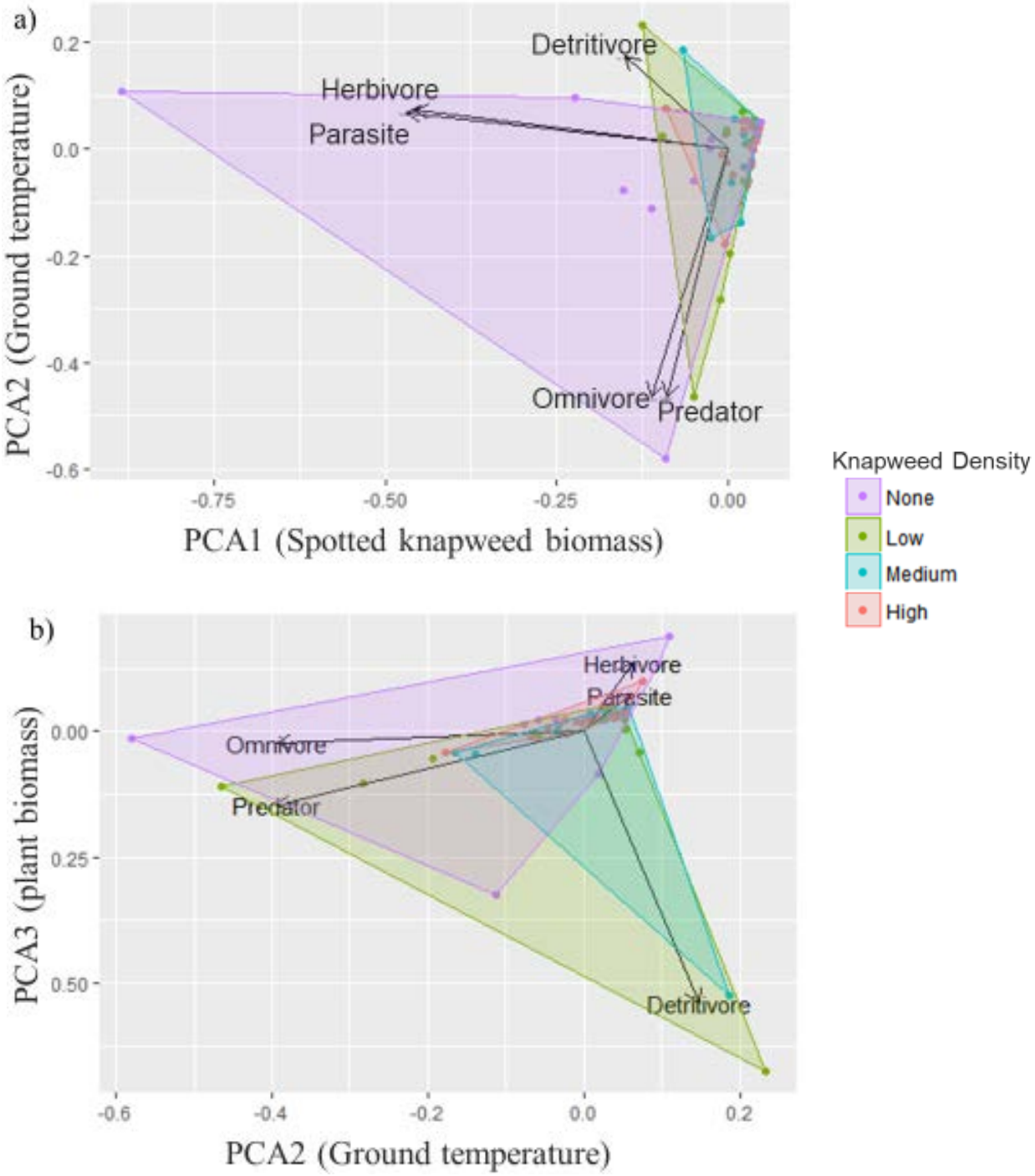

Figure 2.4*. Overall principal components analyses for the entire summer examining the influence of each functional group on overall arthropod community composition graphed using (a) components 1 and 2, and (b) components 2 and 3, n=80.*

DISCUSSION

This study explored the effects of differing densities of the highly invasive plant spotted knapweed on grassland arthropod communities in the semi-arid grasslands of Southern Central British Columbia. As predicted, spotted knapweed presence had differing correlations on the biomass of different arthropod functional groups. These correlations may have been the absence of foraging or reproduction opportunities (Bernays and Graham 1988), or through changes in native plant community through competition (Callaway and Ridenour 2004; Hansen and Ortega 2009), and changes in abiotic ecosystem factors such as amount of bare ground or litter cover and soil temperatures (Fraser and Carlyle 2011).

Plant community characteristics

Contrary to previous findings (Fraser and Carlyle 2011), spotted knapweed density did not correlate with plant community diversity, but higher spotted knapweed densities negatively correlated with overall biomass of the plant community. Spotted knapweed is thought to secrete allelochemicals through its roots into surrounding soils that can shift microbial community structure and function, increase soil phosphorus and potassium availability (Thorpe et al. 2006), and reduce soil nitrogen availability (Suding et al. 2004; Fraser and Carlyle 2011). This change in soil chemistry within dense spotted knapweed stands makes the environment less hospitable for all plants, reducing plant biomass. This was further confirmed by the observation of bare ground cover being highest and plant litter cover being lowest at high spotted knapweed densities. As found by Fraser and Carlyle (2011), the increase in bare ground likely lead to increased soil temperatures in the highest density spotted knapweed stands when compared to sites with no knapweed. It is important to note that this is inference based on past studies exploring spotted knapweed altering soil characteristics (Suding et al. 2004; Thorpe et al. 2006; Fraser and Carlyle 2011). Whether site characteristics determine spotted knapweed distribution or spotted knapweed influences site characteristics cannot be determined through our data at these sites. Regardless, spotted knapweed presence can result in functional changes to the habitat for arthropods.

Overall arthropod functional biomass and diversity

Arthropod functional diversity was not correlated with spotted knapweed density. Past studies have shown both increases (e.g. Kappes et al. 2007; Alerding and Hunter 2013) and decreases (e.g. Burghardt et al. 2010; Bultman and DeWitt 2008; Ernst and Cappuccino 2005) in arthropod community diversity with the introduction of invasive plants. It is possible that any negative effects of spotted knapweed on specific arthropod functional groups were counteracted by positive effects to other functional groups. This can be seen in Table 2.3, where no obvious trend exists in overall arthropod biomass with differing spotted knapweed densities.

As predicted, the biomass of specific arthropod functional groups was uniquely negatively and positively correlated with differing densities of spotted knapweed in the grassland ecosystems. This suggests that indirect changes to arthropod habitat through the introduction of spotted knapweed was the driving force in the changes observed to functional group biomass. Any changes to arthropod functional groups can lead to changes in community dynamics that could have cascading effects throughout the ecosystem.

There were also differences to arthropod community measures – except herbivore, detritivore, and parasite biomass – on a temporal scale throughout the summer.

Herbivores

Herbivores are generally unable to use a plant as a food source when they do not share an evolutionary history with that plant (Tallamy 2004). Bernays and Graham (1988) found that 90% of all arthropod herbivores feed on plants in only a single family or a few genera. In a review paper by Litt et al. (2014), 42 out of 87 studies found that herbivorous arthropod abundance, species richness, or biomass decreased due to the presence of invasive plant species, with the rest of the studies showing no differences or increases. Our study showed the same negative association between herbivore biomass and spotted knapweed biomass.

Herbivores are one of only two functional groups observed throughout the course of the summer to have higher biomass with no knapweed than with any knapweed. Many species in the orders Thysanoptera (thrips), Hemiptera (true bugs), and Coleoptera (beetles) were found in our samples and can be adversely affected by novel plants because they are considered host specific during some or all life stages (Triplehorn and Johnson, 2005). Therefore, herbivores could have lower biomass in spotted knapweed sites due to bottom up control of limited native plant

biomass for consumption. These indirect effects could have influenced another frequently sampled herbivorous order, Orthoptera (grasshoppers), which has been observed to decrease in areas dominated by invasive plants (Litt and Steidl 2010; Yoshioka and Kadoya 2010).

Decreases in herbivorous arthropods can adversely affect higher trophic levels, especially grassland birds, which feed on large herbivores such as Lepidoptera (butterflies and moths) and Orthoptera (Wiens and Rotenberry 1979). Decreased herbivore biomass could also have been influenced by predaceous arthropod functional groups through top-down control. When predator biomass was high, herbivore biomass was low. The ratio of predator:herbivore biomass bell curved with spotted knapweed density (none=4.59; low=32.2; medium=32.6; high=20.1, suggesting that the habitat created by spotted knapweed could have facilitated better hunting conditions for predators or adverse refuge for herbivores at intermediate densities.

Omnivores

The omnivore biomass did not show obvious trends based on spotted knapweed density, and also differed greatly between months. Omnivores are a difficult group to predict and analyze because they play many ecosystem roles and have varying diets and environmental needs (Triplehorn and Johnson 2005; Trigos-peral et al. 2018). This could make the group more adaptable to the introduction of invasive species and subsequent changing of the habitat (Wolkovich et al. 2009).

Over 90% of the omnivore samples collected were from the family Formicidae (ants), which are eusocial animals. Eusocial animals follow scent trails (Andersson 1984), which could give inaccurate results of functional group biomass with numerous individuals following one another into the trap. It is still important to understand the effects of changing ecosystems on Formicidae because they are such a diverse functional group that are easy to identify and play important environmental roles as seed dispersers, and prey items (Schmidt et al. 2012).

Predators

Past studies have shown predators being adversely correlated with invasive plants indirectly through changes in prey items (Bultman and DeWitt 2008; Gratton and Denno 2005). However, the predator biomass in this study followed a non-significant unimodal distribution of more biomass at intermediate spotted knapweed densities and lowest biomass at pristine sites and at highly dense sites. Site characteristics including higher ground temperatures, less litter

cover, and more bare ground at intermediate spotted knapweed densities could all contribute to improved mobility and preferred hunting habitat for predaceous Lycosidae (wolf spiders) and Carabidae (ground beetles) that were frequently found in traps. Carabidae have been observed to hunt more actively and effectively in warmer temperatures (Frank and Bramböck 2016) and several Araneae (spiders) and other predators have had increased hunting mobility and web-creating availability in the presence of invasive plants (Pearson et al. 2009). These site characteristics persist at high spotted knapweed densities; however, the lack of other functional group biomass at these sites drastically reduces prey availability and could counteract the abiotic hunting advantages for predators.

Most studies exploring changes in predaceous arthropod biomass associated with invasive plants are observation-based studies, not controlled experiments that lead to cause and effect relationships (Litt et al. 2014). Observation-based interpretations, such as this study, are complicated and usually require more information about ecosystem food webs, prey capture techniques, and other habitat needs for predators.

Parasites

Parasite biomass did not differ with spotted knapweed density. These non-significant results might be due to the large standard error associated with the samples. When looking at general trends in the data, one can see that there were more parasites in pristine sites than any other. Parasite host animals such as birds (Hickman et al. 2006), small mammals (Bateman and Ostoja 2012), and larger arthropods (Bultman and DeWitt 2008) have been shown to prefer pristine grassland areas compared with areas invaded by novel plants. However, the data collected from this study does not portray this.

Detritivores

Detritivore biomass was near zero in medium and high spotted knapweed densities and was lower than pristine site biomass ($P<0.1$). This finding was surprising as other studies reviewed by Litt et al. (2014) found that detritivores are most likely to benefit from a plant invasion, as was observed in 58 out of 87 studies reviewed, and no studies documenting decreases. Detritivores are likely to benefit from the introduction of invasive plants because invasive plants are generally more productive, which increases ground litter and decaying vegetation (Siemann et al. 2006; Bartomeus et al. 2008). This should provide more food and

preferred habitat conditions for detritivores (Longcore 2003) such as Collembola (springtails) and Microcoryphia (jumping bristletails), which were frequently sampled. An explanation for our unexpected results is the peculiar site characteristics associated with spotted knapweed invaded sites in this study. High density spotted knapweed sites had significantly less litter cover and higher bare ground cover, as was also observed in this region by Fraser and Carlyle (2011). This is the opposite of what is expected at high density invasive plant patches (Alerding and Hunter 2013). The high density spotted knapweed sites in our study may have been affected by high winds in the upper grasslands of LDB, where the dominant grass species is rough fescue (*Festuca scabrella*). Rough fescue is a densely tufted perennial grass, which grows in large clumps and has persistent old sheaths and leaf bases that form large dead vegetation litter mats (Parish et al. 1996). These dense mats are ideal microclimates for detritivorous arthropods (Alerding and Hunter 2013).

Spotted knapweed outcompeting rough fescue in this specific habitat may lead to decreased litter cover and adverse habitat conditions for detritivorous arthropods in this specific study site. Duplicating this observational study at other semi-arid grassland locations in Western North America will give us a better understanding of the effects of spotted knapweed on detritivores.

Arthropod community trophic interactions

PCA suggests that there are numerous site characteristics and interacting trophic relationships that contribute to differing biomass of arthropod functional groups in this grassland ecosystem. All three components used in the PCA are associated with different site characteristics that have differing influences on functional groups. The result that more spotted knapweed density correlated with less herbivore and parasite biomass was likely due to the interacting effects of spotted knapweed outcompeting native plants, providing less food sources, and acting as a bottom-up control for herbivores (Triplehorn and Johnson 2005; Litt and Steidl 2010), as well as less host organisms for parasites using invaded sites (Bultman and DeWitt 2008; Bateman and Ostoja 2012). Herbivores and parasites were almost exclusively grouped into the pristine no-knapweed sites. More litter cover could lead to more difficult hunting for predators (Frank and Bramböck 2016), explaining the negative relationship with predator biomass.

The negative relationship between herbivores/parasites and predators/omnivores at all sites suggests top-down control, with more predators leading to fewer herbivores, at sites with more spotted knapweed and less litter cover (Hairston et al. 1960). The introduction of spotted knapweed seems to facilitate ideal hunting habitat with less litter cover for predators to control the density of herbivores (Frank and Bramböck 2016). Additionally, higher parasite abundance at sites without spotted knapweed can act as a top down control for host predator and omnivore species (Bultman and DeWitt 2008; Gratton and Denno 2005).

Conclusion

The results from this study suggest that the density of spotted knapweed patches in semi-arid grasslands have varying effects on arthropod functional groups. High density of spotted knapweed was correlated with decreases in plant biomass, possibly working as a bottom-up control of less forage decreasing herbivorous arthropod biomass. This had no significant correlation on omnivore or parasite biomass. The presumed allelopathic chemicals released into the soil from spotted knapweed may have suppressed germination of native plants, which may have resulted in more bare ground, higher ground temperatures, and less litter cover in sites with spotted knapweed, thus providing a better hunting habitat for predator biomass at intermediate spotted knapweed densities. A top-down control on herbivores was presumably facilitated, judging by an increasing predator:herbivore ratio observed with increasing spotted knapweed density. Detritivore biomass was highest at pristine, no-knapweed grassland sites and significantly lower at spotted knapweed invaded sites, which may have been due to the lack of food availability with limited ground litter cover. Any changes to arthropod functional groups due to the introduction of invasive species would lead to changes in overall community dynamics felt throughout the ecosystem.

Although this study resulted in several interesting findings, functional guilds are not a comprehensive method of grouping arthropods. The complexity of differing ecosystem services that different members of a specific functional guild performs (Higgins and Lindgren 2006; Bourn and Thomas 2002; Tscharntke and Greiler 1995) makes it hard to definitively make conclusions about ecological implications when simplifying arthropods into groups. Identifying specimens using genomic techniques that can accurately identify genera, such as DNA metabarcoding (Ji et al. 2013), could help gain a better understanding of specific arthropod community changes.

Future studies could include manipulation trials to assess arthropod community rebounds with the removal of spotted knapweed in an area to better understand the legacy effects of spotted knapweed after site restoration. It would also be interesting to explore the mechanisms behind the biomass of specific arthropod functional groups (i.e. palatability trials, reproduction locations, hunting site characteristics, etc.).

Human activities such as mining, recreation, and farming are altering British Columbia grassland ecosystems; leaving them increasingly susceptible to anthropogenic-caused changes, such as the colonization of invasive plants. These grasslands provide invaluable services to people and the environment. The results from this study contribute to our growing understanding of invasive plants in British Columbia grasslands. This information will inform conservation management strategies important in combatting the spread of invasive plants and the subsequent shifts in ecological productivity and biodiversity

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CHAPTER 3: DNA BARCODING AS AN EXPEDITED METHOD OF ARTHROPOD IDENTIFICATION

INTRODUCTION

Invasive plant species have broad ecological and economic impacts and are one of the main global threats to biodiversity (Vitousek et al. 1996). When invasive plants are introduced into an area they are often able to establish at high rates and densities compared to native plants in direct competition. This is because many invasive plant species lack predators in their new environments and grow unrestrained by predation (Siemann et al. 2006). This typically leads to increased dominance of invasive plants and decreased biomass and diversity of native plants (Benesperi et al. 2012; Vilà et al. 2011), all of which can disrupt energy dynamics at higher trophic levels throughout the ecosystem (Schirmel et al. 2016; Tallamy et al. 2010).

Arthropods represent the group with the largest animal biomass and make up the majority of animal species in terrestrial habitats (Havstad 2008). They contribute to ecosystem function in their various and diverse roles as pollinators, foragers, soil engineers, and food for other organisms (e.g. Higgins and Lindgren 2006; Bourn and Thomas 2002; Tscharntke and Greiler 1995). Therefore, a deeper knowledge of the impact of invasive plants on arthropods is important in understanding changes to ecosystem energy flows higher in the food chain.

Spotted knapweed is a deeply tap-rooted perennial forb native to Eastern Europe that was first introduced into North America in the 1890s (Fraser and Carlyle 2011). It is considered one of the most ecologically harmful invasive plant species in Western North America (Hansen and Ortega 2009). It is an extremely competitive plant through copious production of seeds that can remain viable for over eight years (Davis et al. 1993). Spotted knapweed can alter soil properties by increasing phosphorous within its rhizosphere, thus benefitting spotted knapweed persistence and growth, and decreasing soil carbon and nitrogen pools (Fraser and Carlyle 2011). Spotted knapweed is suspected to exude an allelochemical through its roots, making the soil inhospitable to native plant species (Callaway and Ridenour 2004). Once established, spotted knapweed primarily colonizes grassland communities by forming dense, near-monoculture stands (Hansen and Ortega 2009). Spotted knapweed has adverse economic impacts as it causes greater surface water runoff, increases sediment loading (Lacey et al. 1989), and because the plant is generally unpalatable for wild and domestic grazing ungulates.

The province of British Columbia released its first biological control agents against spotted knapweed in 1970. Insect agents were chosen based on their ability to self-perpetuate, self-distribute, and create a long-term balance between insect feeding on knapweed (Gayton and Miller 2012; Powell et al. 1994). Numerous agents were released in the Lac du Bois Grasslands Protected Area, including *Larinus obtusus* (knapweed flower weevil), which is now an established, self-perpetuating population (Gayton and Miller 2012).

Although there are studies on the effects of plant invaders on arthropods (e.g. Alerding and Hunter 2013; Litt and Steidl 2010; Pearson et al. 2009), our knowledge is incomplete, given the megadiversity of arthropods and their ecological traits (Kadlec et al. 2018). New strategies of expedited arthropod identification could improve our sampling efficiencies and ability to understand arthropod community dynamics.

Traditional methods of biodiversity assessment for cryptic and diverse organisms such as arthropods is expensive and time consuming (Ji et al. 2013; Yu et al. 2010). Morphological identification can usually only be conducted by experienced taxonomists. If a specimen is damaged or if the specimen is a juvenile, specialists may be unable to accurately identify to the species level (Gibson et al. 2015). DNA metabarcoding is a semi-automated, higher throughput method that only requires specimen tissue samples to obtain genetic barcodes for identification (e.g. Marizzi et al. 2018). DNA metabarcoding combines two technologies: DNA taxonomy, and high-throughput sequencing (HTS) (Ji et al. 2013) as a tool to rapidly assess biodiversity in bulk, mixed samples (Chuo Beng et al. 2016; Valentini et al. 2016). HTS technology allows for the simultaneous analysis of large numbers of specimens in multiplex polymerase chain reactions (PCR) rather than a single specimen per PCR, as is done with Sanger sequencing (Sanger et al. 1977).

DNA metabarcoding has been successfully used to assign taxonomies to specimens of animals (e.g. Naseem and Tahir 2016), plants (e.g. Kress et al. 2005), fungi (e.g. Schoch et al. 2012), and other microbes (e.g. Patel et al. 2008). When properly deployed, this approach can reliably and cost-effectively provide relative abundance of species assemblages along environmental gradients (Chuo Beng et al. 2016), which is important in the application of conservation management and environmental assessments.

According to Herbert et al. (2003ab), the 658-base pair (bp) mitochondrial cytochrome c oxidase subunit 1 (COI) gene can serve as 'the core of a global bio-identification system for animals'. The COI gene is preferentially used to differentiate animals as it is present in all eukaryotes, and it is easy to amplify and sequence due to its short length and the robust capabilities of universal PCR primers (Naseem and Tahir 2016; Folmer et al. 1994). COI also appears to possess sufficient variation in nucleotide sequences to discriminate closely allied species (Herbert et al. 2003ab).

The main objective of this study was to assess the effects of spotted knapweed patch density on epigeal arthropod community assemblages. In particular: (i) Does the ordination of overall arthropod communities vary with spotted knapweed density at a temporal scale? (ii) Are there particular arthropod orders that are differentially impacted by spotted knapweed density? (iii) Is arthropod diversity affected by spotted knapweed density? (iv) And is DNA metabarcoding an appropriate method for expedited arthropod sampling compared to morphological identification?

METHODS

Arthropod samples were collected in the Lac du Bois Grasslands Protected Area in the summer of 2017 (refer to Chapter 2). Samples not identified in Chapter 2 were kept at -20℃ in 87% denatured ethanol prior to DNA extraction. 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 catalogued into the database (http://www.barcodeoflife.org under project code "LFBC") This was to ensure that all specimens sampled in this region (additionally, Garris et al. 2016, project code "NGNNA") were available for building custom DNA barcoding databases for the local region.

DNA extraction

Arthropod specimens were extracted from specimen bottles using sterile forceps and left to air dry prior to DNA extraction. In order 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 Chuo 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).

PCR conditions

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 3.1). Amplifications were carried out in 25 μL with 10 ng genomic DNA, 12.5 μL 2X GoTaq DNA polymerase (Promega Corporation, Madison, Wisconsin, USA), 1 μL each of 10 μM forward and reverse primers, and nuclease-free water. PCR cycling 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 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.

Table 3.1. *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

Using the amplicons from the first round of PCR as a template, a 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 three base adaptor (bold); reverse primers included the P1 adaptor sequence (underlined and bold): CCATCTCATCCCTGCGTGTCTCCGACTCAG**CTAAGGTAACGAT**GCATTYCCACGAA TAAATAAYATAAG,
CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGATTAAACTTCAGGGT GACCAAARAAYCA.

Sequencing

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).

Data processing

Sequencing data was processed in Torrent Suite 5.10.0 with Pre-BaseCaller and BaseCaller Args set to *-disable-all-filters*. The resulting multiplexed BAM file was exported and passed to AMPtk v. 1.0.3 (Palmer et al., 2018) 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). Demultiplexed data files were concatenated and then clustered with *amptk cluster* with an 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* as per the instructions on https://amptk.readthedocs.io/en/latest/taxonomy.html. Once the database was prepared, taxonomy was assigned to OTUs using the *amptk taxonomy* script.

OTUs with fewer than 2000 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, with 173 OTUs assigned to genera and 136 OTUs assigned to species (Appendix G).

Statistical analysis

All data were analyzed statistically in RStudio integrated under R 3.4.4 “Someone to Lean On” (The R Foundation for Statistical Computing). When necessary, data were natural logarithm or arcsine transformed. All data analyses were tested for a significance at the 5% probability level and noted within the 10% probability level to recognize possible trends in the data.

A non-parametric multidimensional scaling analysis (nMDS) was conducted to visualize the arthropod community data. Clear compositional changes were observed over time, so each month of arthropod sampling was analyzed separately to address the differences in changing arthropod communities throughout the summer. Arthropod OTUs were grouped into orders and into functional groups as per the previous chapter to better visualize community structures. Shannon-Wiener alpha diversity was calculated for the overall arthropod communities and orders at each month using the vegan package in R.

Arthropod order and functional group species richness were input into regression analysis models with spotted knapweed density as the predictor variable to determine which arthropod groups were most affected. A regression was conducted with *Larinus obtusus* reads as the predictor variable to explore correlations with a knapweed biological control agent and knapweed presence. Additionally, one-way analyses of variance (ANOVAs) and post hoc Tukey tests were done to test the effects of the density of spotted knapweed broken into categorical data (no, low, medium, and high density) on the density and species richness of each arthropod functional guild. The arthropod guilds included herbivore, omnivore, predator, detritivore, and parasite.

RESULTS

The arthropod communities showed a clear structure, grouping by month in the ordination plot (Figure 3.1). Further data analyses were grouped by month due to this temporal separation. See appendix H for nMDS ordination plots for each month of sampling.

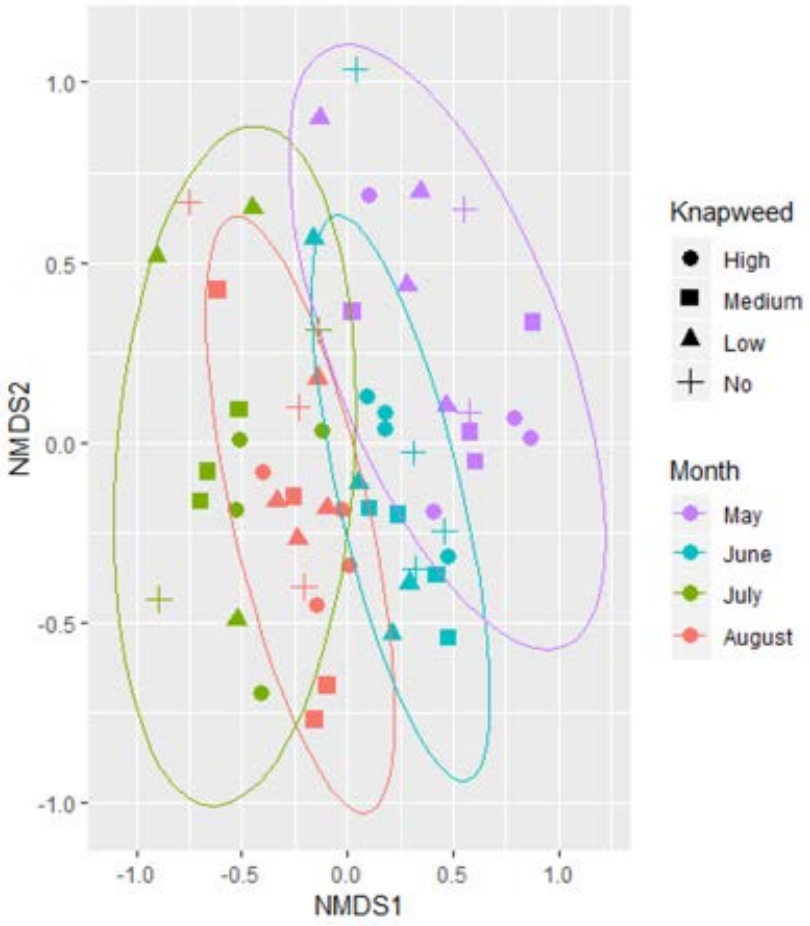

Figure 3.1*. Ordination (nMDS) plot illustrating the similarities and differences in insect OTU composition across four spotted knapweed density categories (high, medium, low, and no) and across each month of sampling (May, June, July, and August), n=56.*

Biological control agent

Larinus obtusus density was positively correlated with spotted knapweed density in the month of July (Table 3.2; Figure 3.2). Other months did not show significant trends.

Table 3.2. *Linear regression results of Larinus obtusus (knapweed flower weevil) density and spotted knapweed density, n=56.*

L. obtusus	F	P	df
May	n/a	n/a	n/a
June	0.074	0.790	15
July	5.033	**0.049***	12
August	0.091	0.768	14

Bold values indicate statistical significance at P<0.1, * indicates significance at P<0.05

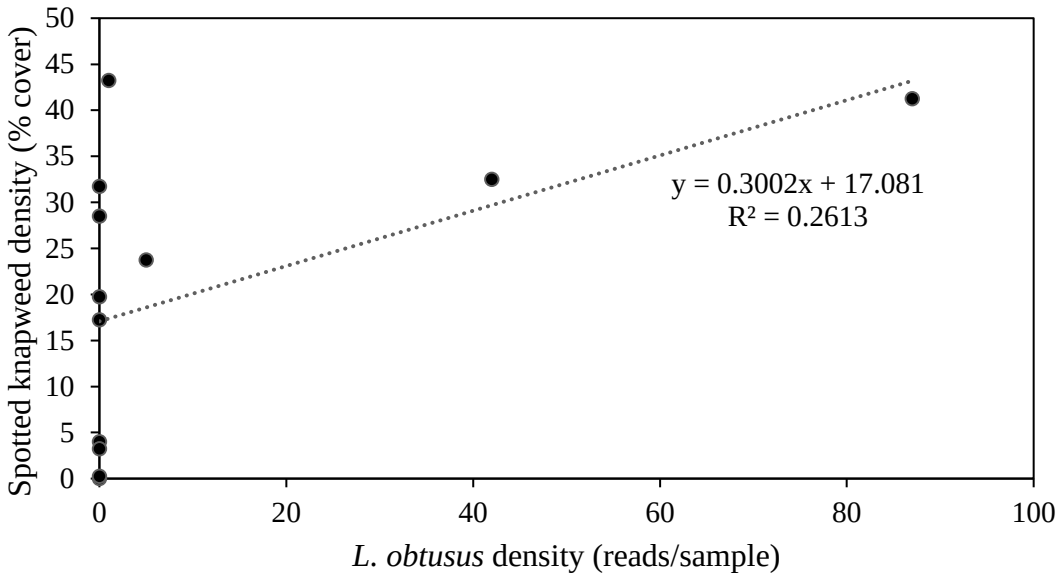

Figure 3.2*. Linear regression of Larinus obtusus density vs spotted knapweed percent cover sampled in the month of July, n=56.*

Spotted knapweed effects on arthropod orders

There were few significant correlations of spotted knapweed density on species richness by arthropod order (Table 3.3). Total species richness, and the species richness of Diptera and Coleoptera were negatively correlated depending on month (Figure 3.3). Orthoptera and Araneae were positively correlated, but also depending on month (Figure 3.3).

Table 3.3*. Regression analyses results of the effect of spotted knapweed density on arthropod order species richness, n=56, df=49.*

Knapweed Density	**F**	**P**	**df**
Whole Summer			
Total Richness	<0.001	0.993	55
Orthoptera	4.367	**0.042***	55
Hemiptera	0.095	0.759	55
Araneae	2.739	**0.100**	55
Diptera	0.996	0.322	55
Coleoptera	0.618	0.435	55
Hymenoptera	0.537	0.467	55
Archaeognatha	0.984	0.326	55
May			
Total Richness	3.206	**0.098**	14
Orthoptera	<0.0001	0.999	14
Hemiptera	0.513	0.488	14
Araneae	1.167	0.301	14
Diptera	5.141	**0.043***	14
Coleoptera	5.945	**0.031***	14
Hymenoptera	0.044	0.837	14
Archaeognatha	2.246	0.160	14
June			
Total Richness	0.085	0.774	15
Orthoptera	10.41	**0.006***	15
Hemiptera	0.171	0.685	15
Araneae	0.554	0.469	15
Diptera	1.776	0.204	15
Coleoptera	0.183	0.676	15
Hymenoptera	1.377	0.260	15
Archaeognatha	0.065	0.803	15
July			
Total Richness	0.763	0.403	12
Orthoptera	0.477	0.505	12
Hemiptera	0.742	0.409	12

Diptera	0.488	0.500	12
Coleoptera	0.072	0.794	12
Hymenoptera	0.725	0.414	12
Archaeognatha	0.062	0.512	12
August			
Total Richness	0.181	0.677	14
Orthoptera	0.258	0.620	14
Hemiptera	0.637	0.439	14
Araneae	2.670	0.126	14
Diptera	0.128	0.726	14
Coleoptera	0.035	0.854	14
Hymenoptera	0.079	0.783	14
Archaeognatha	0.153	0.702	14

Bold values indicate statistical significance at P<0.1, * indicates significance at P<0.05

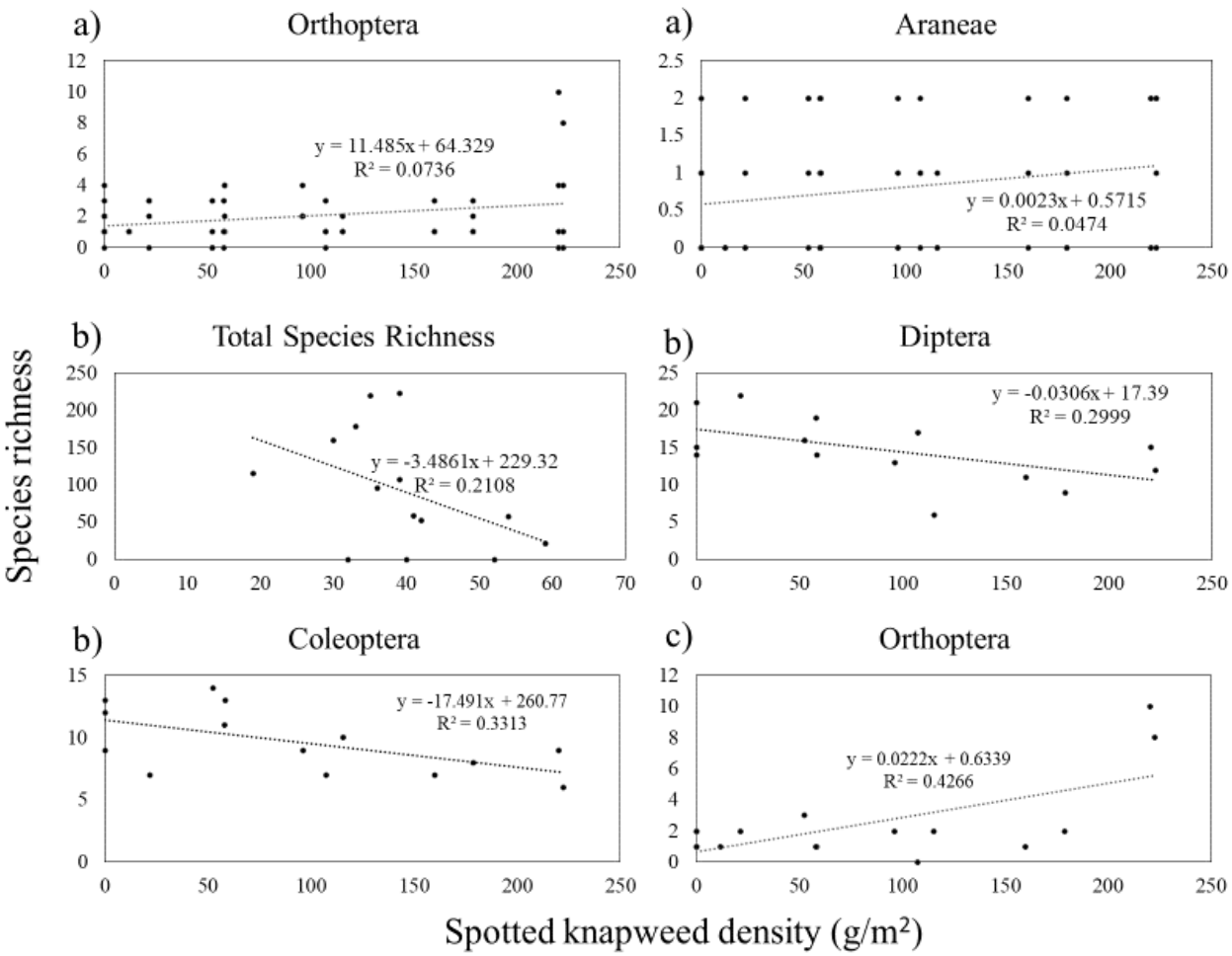

Figure 3.3*. Effect of spotted knapweed density on arthropod order species richness during a) the overall summer, b) May, c) June, n=56, df=49.*

Orthoptera diversity was consistently positively correlated to increasing spotted knapweed density (Table 3.4). This was most noticeable in the overall summer, June, and August sampling. Diptera diversity was negatively correlated to spotted knapweed in July, and Coleoptera diversity was positively correlated in August (Figure 3.4).

Table 3.4*. Regression analyses results of the effect of spotted knapweed density on arthropod order Shannon-Wiener diversity, n=56, df=49.*

Knapweed Density	**F**	**P**	**df**
Whole Summer			
All arthropod diversity	0.006	0.936	55
Coleoptera diversity	0.202	0.655	55
Diptera diversity	1.122	0.294	55

Hemiptera diversity	0.209	0.649	55
Hymenoptera diversity	1.94	0.169	55
Orthoptera diversity	6.566	**0.013***	55
May			
All arthropod diversity	0.216	0.650	14
Araneae diversity	1.273	0.281	14
Coleoptera diversity	0.031	0.863	14
Diptera diversity	0.220	0.648	14
Hemiptera diversity	0.696	0.420	14
Hymenoptera diversity	0.002	0.962	14
Orthoptera diversity	0.278	0.607	14
June			
All arthropod diversity	0.856	0.370	15
Araneae diversity	0.140	0.713	15
Archaeognatha diversity	3.546	**0.081**	15
Coleoptera diversity	0.23	0.639	15
Diptera diversity	0.079	0.783	15
Hemiptera diversity	0.005	0.943	15
Hymenoptera diversity	0.236	0.635	15
Orthoptera diversity	6.707	**0.021***	15
July			
All arthropod diversity	1.544	0.242	12
Archaeognatha diversity	1.933	0.195	12
Coleoptera diversity	0.060	0.811	12
Diptera diversity	4.387	**0.063**	12
Hemiptera diversity	0.421	0.531	12
Hymenoptera diversity	1.171	0.304	12
Orthoptera diversity	0.049	0.830	12
August			
All arthropod diversity	0.360	0.560	14
Araneae diversity	0.364	0.557	14
Archaeognatha diversity	1.37	0.263	14
Coleoptera diversity	3.636	**0.079**	14
Diptera diversity	0.0001	0.990	14

Hemiptera diversity	1.458	0.249	14
Hymenoptera diversity	0.571	0.463	14
Orthoptera diversity	5.673	**0.033***	14

Bold values indicate statistical significance at P<0.1, * indicates significance at P<0.05

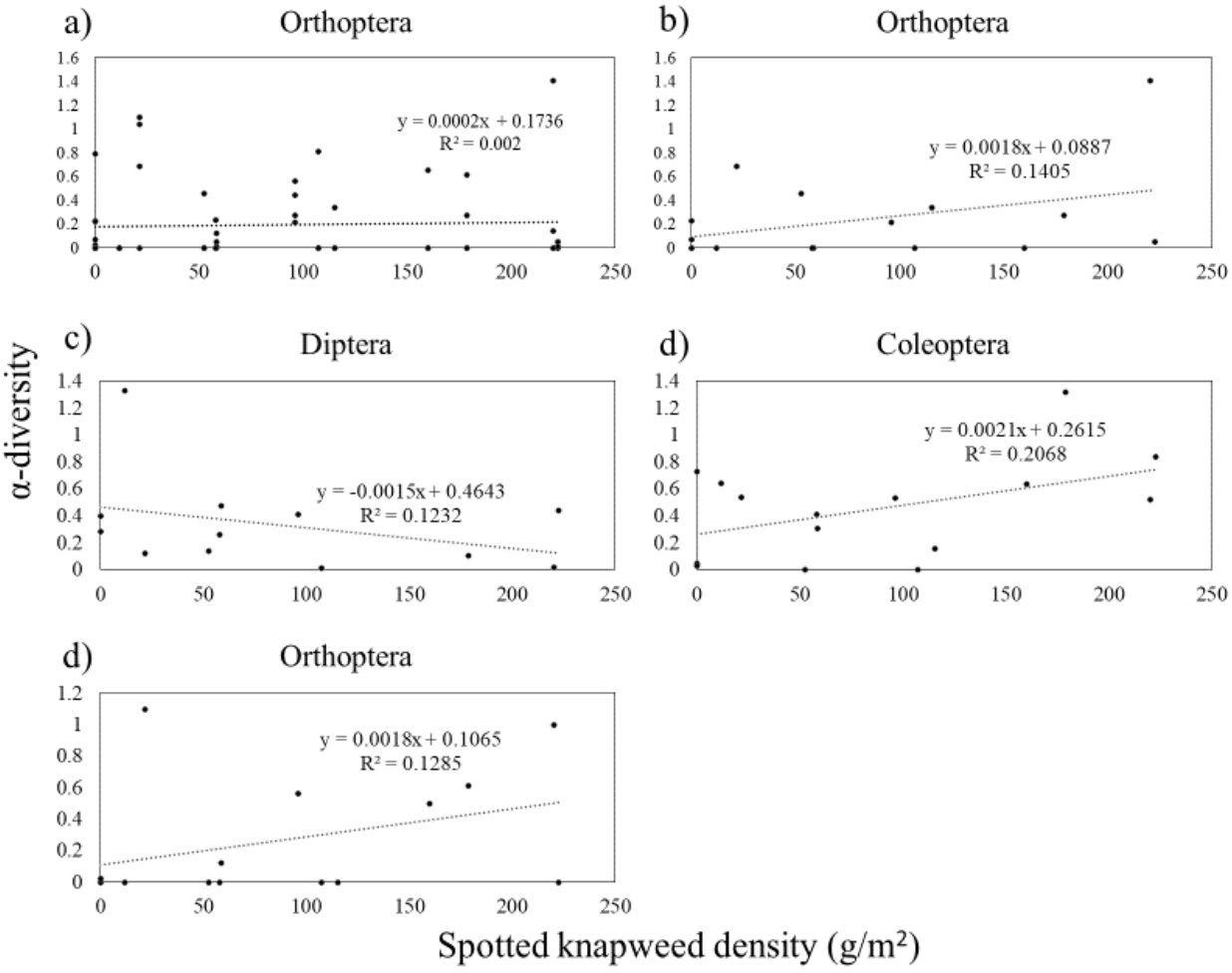

Figure 3.4*. Effect of spotted knapweed density on arthropod order Shannon-Wiener diversity during a) the overall summer, b) June, c) July, d) August, n=56, df=49.*

Spotted knapweed and arthropod functional groups

Herbivore density was consistently positively correlated to the density of spotted knapweed throughout most of the summer (Table 3.5, Figure 3.5 & 3.6).

Table 3.5*. Regression analyses results on the effect of spotted knapweed density (number of reads) on arthropod functional group density, n=56, df=51.*

Knapweed Density	F	P	df
Whole Summer			
Herbivore	3.842	**0.055**	55
Omnivore	1.501	0.226	55
Predator	0.296	0.588	55
Detritivore	3.419	**0.069**	55
Parasite	0.043	0.837	55
May			
Herbivore	1.166	0.302	14
Omnivore	3	0.109	14
Predator	0.034	0.855	14
Detritivore	0.111	0.243	14
Parasite	0.001	0.977	14
June			
Herbivore	0.262	0.616	15
Omnivore	0.003	0.986	15
Predator	0.169	0.687	15
Detritivore	0.564	0.465	15
Parasite	0.237	0.634	15
July			
Herbivore	5.482	**0.041***	12
Omnivore	1.737	0.217	12
Predator	1.883	0.200	12
Detritivore	2.068	0.181	12
Parasite	2.031	0.184	12
August			
Herbivore	3.847	**0.072**	14
Omnivore	<0.0001	0.998	14
Predator	0.184	0.675	14
Detritivore	0.284	0.603	14
Parasite	0.113	0.742	14

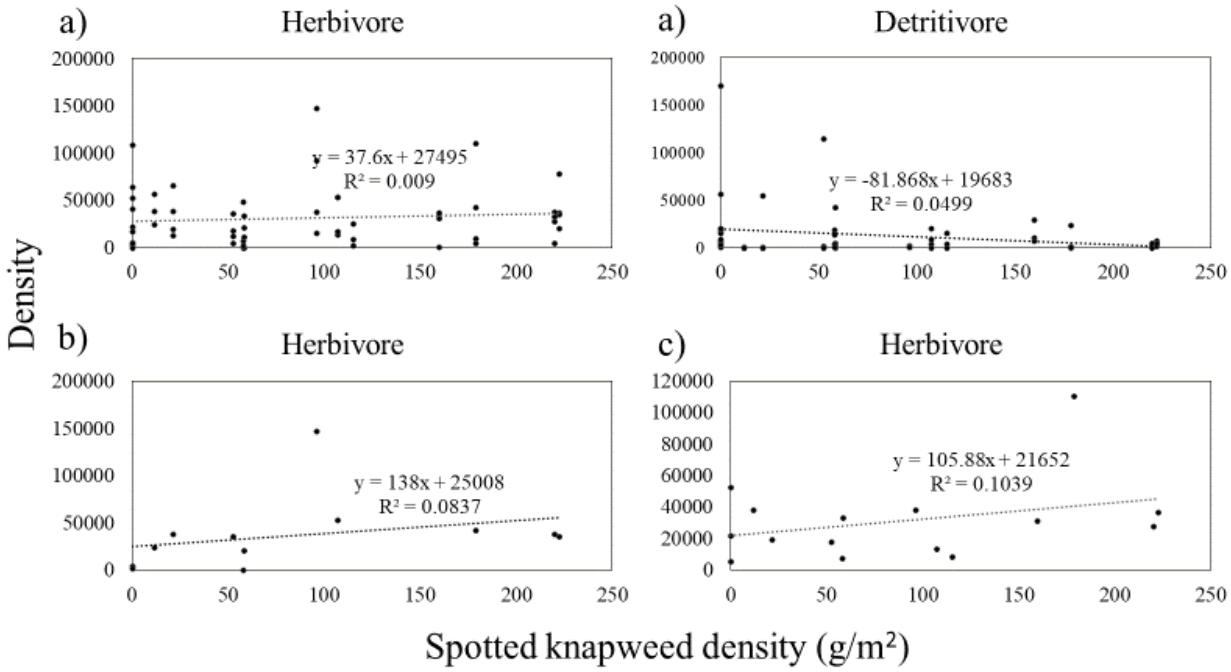

Figure 3.5*. Effects of spotted knapweed density on functional group density (total number of reads) during a) the overall summer, b) July, c) August, n=56, df=51.*

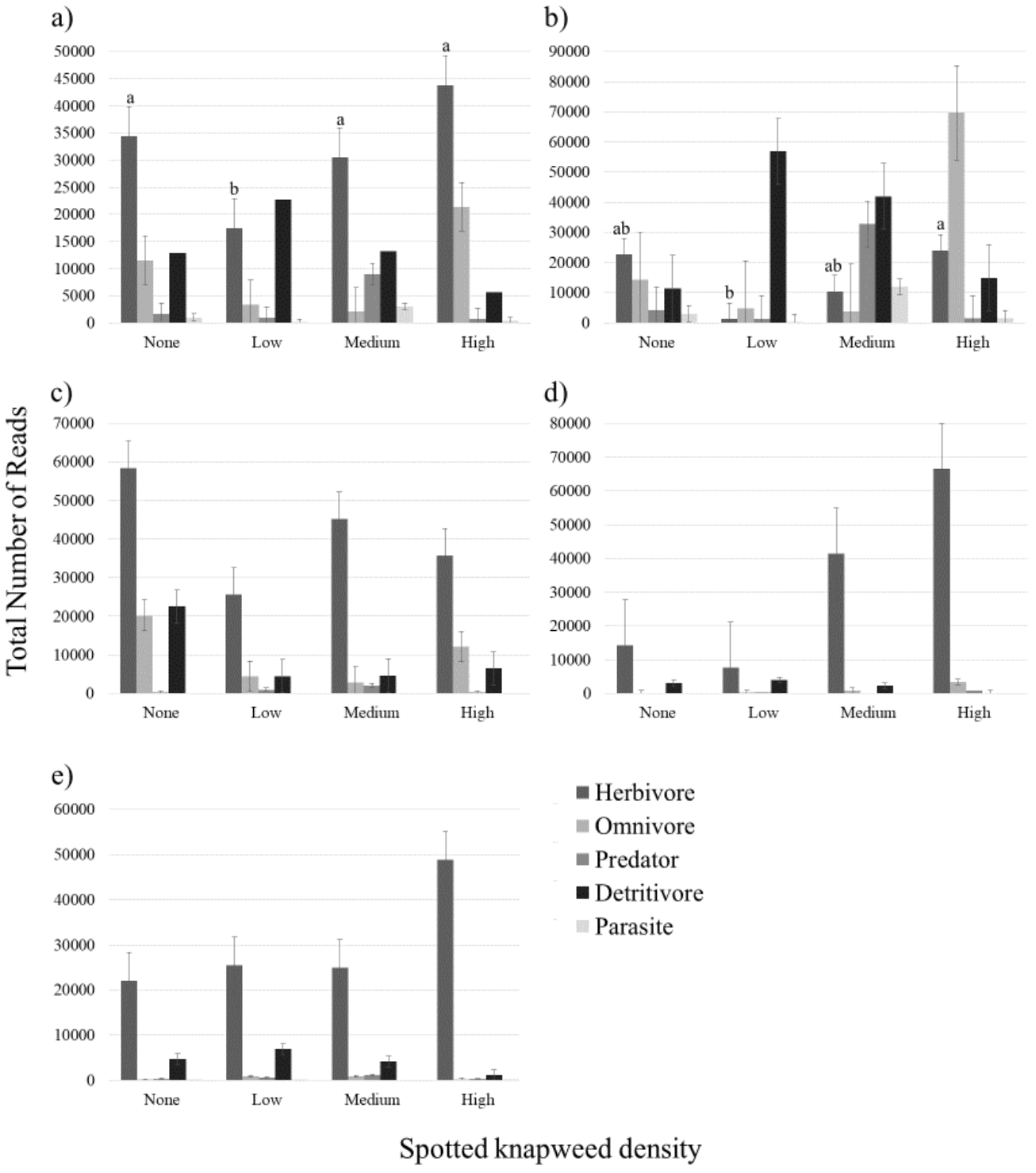

Figure 3.6*. Arthropod functional group density (total number of reads) at no, low, medium, and high spotted knapweed density patches (±SE), during a) overall summer, b) May, c) June, d) July, e) August, n=20 per functional group.*

There was no significant relationship between spotted knapweed density and arthropod functional group richness.

Table 3.6. *Regression analyses of spotted knapweed density predicting arthropod functional group species richness, n=56, df=51.*

	Knapweed Density	**F**	**P**	**df**
Whole	Herbivore	0.075	0.786	55
Summer	Omnivore	0.073	0.788	55
	Predator	0.004	0.949	55
	Detritivore	0.106	0.745	55
	Parasite	0.272	0.638	55
May	Herbivore	0.582	0.460	14
	Omnivore	2.288	0.156	14
	Predator	0.558	0.469	14
	Detritivore	2.638	0.130	14
	Parasite	1.802	0.204	14
June	Herbivore	0.374	0.551	15
	Omnivore	0.046	0.833	15
	Predator	0.317	0.582	15
	Detritivore	0.132	0.722	15
	Parasite	0.405	0.535	15
July	Herbivore	0.787	0.396	12
	Omnivore	1.924	0.196	12
	Predator	3.654	**0.085**	12
	Detritivore	0.036	0.852	12
	Parasite	1.229	0.293	12
August	Herbivore	0.149	0.706	14
	Omnivore	<0.001	0.975	14
	Predator	0.002	0.964	14
	Detritivore	0.085	0.776	14
	Parasite	0.646	0.436	14

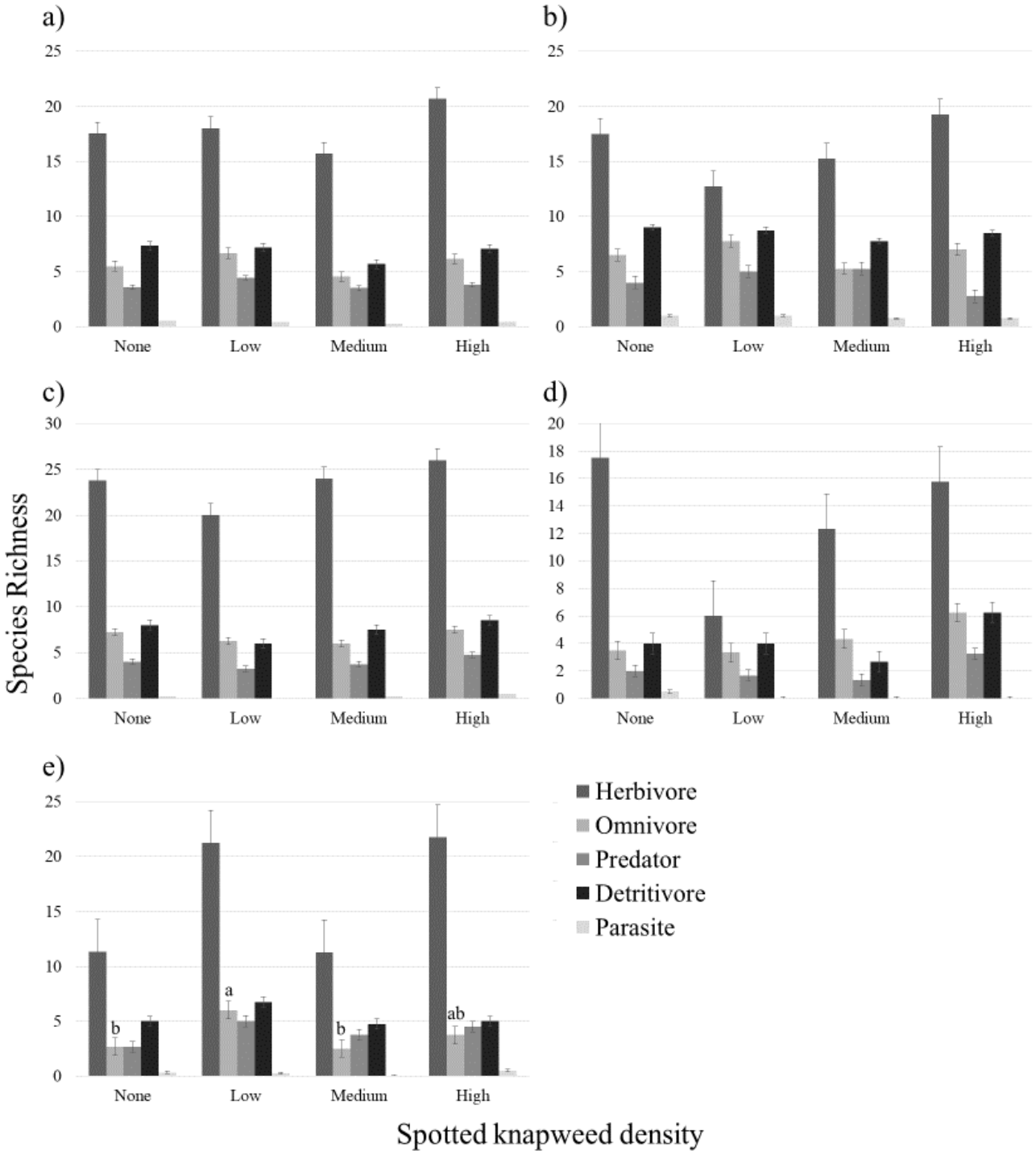

Figure 3.7*. Arthropod functional group species richness at no, low, medium, and high spotted knapweed density patches (±SE), during a) overall summer, b) May, c) June, d) July, e) August, n=20 per functional group.*

DISCUSSION

This study explored the effects of differing densities of the highly invasive plant spotted knapweed on grassland epigeal arthropod communities in the semi-arid grasslands of Southern Central British Columbia. As predicted, spotted knapweed presence had differing correlations with the density of different arthropod orders and functional groups. These correlations may have been through the absence of foraging or reproduction opportunities (Bernays and Graham 1988), or through changes in native plant community through competition (Hansen and Ortega 2009; Callaway and Ridenour 2004), and changes in abiotic ecosystem factors such as amount of bare ground (Fraser and Carlyle 2011). As predicted, spotted knapweed density was positively correlated with the density of a knapweed biological control agent, *Larinus obtusus*.

Biological control agent

Larinus obtusus is a species of true weevil known as the knapweed flower weevil. It was released as a biological control agent in the Lac du Bois Grasslands in 1992 (Gayton and Miller 2012). *L. obtusus* is a host specific species that only feeds on the flowering heads of *Centaurea* species. The population has proliferated since its introduction and is now a common arthropod found in our sampling. Increased activity of *L. obtusus* in spotted knapweed areas may have resulted from the need for these beetles to search for suitable host plants to feed on. Frid et al. (2009) estimated the return on biocontrol investment for *C. maculosa* in British Columbia at $17 for each dollar spent (Gayton and Miller 2012). Our findings show that *L. obtusus* is still at healthy populations within spotted knapweed patches in the Lac du Bois Grasslands where the weevils were first released almost thirty years ago.

Spotted knapweed effects on arthropod orders

In a mosaic of natural grasslands as seen in Lac du Bois Provincial Park, spotted knapweed is not significantly correlated with most arthropod communities. However, Orthoptera (grasshoppers, locusts, and crickets) alpha diversity showed a positive relationship with spotted knapweed density for the overall summer data and specifically in June and August. An increase in Orthoptera diversity with high invasive species density was not predicted in this study but could be explained by understanding that grasshoppers prefer open-spaced environments. Increasing spotted knapweed density was correlated with more bare ground

(Chapter 2). A similar study observed higher Orthoptera richness in more open land use habitats when compared with natural ones (Chuo Beng et al. 2016).

Spotted knapweed and arthropod functional groups

Correlations between spotted knapweed density on arthropod functional group density and species richness were mostly non-existent but were also positive or negative for some functional groups. This is consistent with previous reports that functional groups differ in their responses to invasive species (Chuo Beng et al. 2016; Litt et al. 2013). The only significant functional group trends were an increase in herbivore density with increasing spotted knapweed density in July and August. These unexpected results are highly peculiar when compared to numerous other studies looking at herbivorous arthropods decreasing in density, species richness, or diversity in the presence of invasive plants (eg. Tallamy et al. 2010; Litt and Steidl 2010; Yoshioka and Kadoya 2010). These results also contradict those found in Chapter 2. A possible explanation for these discrepancies in data could have been the additional sweep net samples that were included in this Chapter. Leafhoppers (Cicadellidae) are a family of foliar true bugs that suck plant sap from grass, shrubs, or trees (Turner et al. 2010; Alyokhin et al. 2004). Many leafhoppers were found in our samples (Appendix G) for Chapter 3 that were not included in Chapter 2 due to their foliar habitat preference. Leafhoppers are highly adaptable generalist species that have been used as biological control agents for other invasive plants (Turner et al. 2010), thus making them a great candidate to be present at high abundance and diversity in spotted knapweed patches.

There was a perceived pattern of detritivore density decreasing with increasing spotted knapweed density when grouped into categorical data (Figure 3.6). Although not statistically significant, this correlation can be seen throughout all months of sampling and in Chapter 2. Higher density spotted knapweed sites were observed to have significantly less detritus material (Chapter 2). Low density spotted knapweed and pristine sites were dominated by rough fescue (*Festuca scabrella*), which grows in large clumps and has persistent old sheaths and leaf bases that provide ample detritus for detritivores to thrive on (Parish et al. 1996). It is expected that detritivores would not choose sites with low or no detritus available.

It is worth noting that this study had small sample sizes of only 20 samples per month. It is difficult to obtain statistically significant results with such small sample size; however, trends in the data indicate a need for further sampling over a larger time scale to confirm.

DNA metabarcoding

Arthropods are the most plentiful and varied animals on Earth, but comprehensive information on large-scale patterns of richness, endemism, and biogeography are lacking (Chuo Beng et al. 2016). There is a high cost in time, money, and labour associated with sorting and identifying samples from large-scale inventories. However, there is a growing representation of arthropods in biodiversity databases such as the Barcode of Life Database (BOLD). Continuous contribution to these databases will allow cheap and efficient monitoring methods, such as presented in this study, to be the preferred method for arthropod biodiversity assessments.

A major limitation of the approach in this study is the accuracy of the primers at identifying Araneae (spiders). There were numerous spider species/genera manually observed in Chapter 2, that were not picked up by the primers in sequencing. We are currently unsure as to the reason for some of the Araneae manually identified not appearing in our sequenced results, as this primer combination has been effectively used in past studies including spiders (Chuo Beng et al. 2016). However, it should be noted that an additional forward and reverse primer should be used in combination with the ones in this study to accurately identify arthropod orders from interior BC.

Comparisons with morphological arthropod identification

When directly compared with morphological identification from Chapter 2, DNA metabarcoding is the more efficient and accurate method when it comes to identifying large samples and using species richness as a data parameter. This method will only become more robust and accurate as further samples are added to public reference databases, such as BOLD.

However, number of reads, defined as each time an individual insect's DNA was counted, from the sequencing output was not as reliable of a parameter for calculating relative density of arthropod communities when compared with drying and weighing arthropod biomass as done in chapter 2. This can be seen by the results of herbivores having higher densities in higher density spotted knapweed patches. Chapter 2 resulted in opposite findings for herbivores. This might have been due to many of the herbivores being larger-bodied individuals (such as

grasshoppers and moths) compared to other functional groups. Although steps were taken to try to avoid this (see Chuo Beng et al. 2016) more DNA from these individuals might have resulted in additional reads being amplified and a perceived higher density. Ji et al. simply stated that because the field of metabarcoding is advancing so rapidly, data sets are continuously subject to error and loss of information (2013). Many studies in the field to date have been to validate metabarcoding against standard morphological sampling and to develop more efficient pipelines (Ji et al. 2013).

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CHAPTER 4: RESEARCH CONCLUSIONS

RESEARCH SYNTHESIS

The conclusions from Chapter 2 suggest that the density of spotted knapweed patches in semi-arid grasslands create numerous changes to site characteristics and interacting trophic relationships that contribute to differing biomass of arthropod functional groups. High densities of spotted knapweed were negatively correlated with plant biomass, leading to less forage and subsequent decreases in herbivorous arthropod biomass. The presumed allelopathic chemicals released into the soil from spotted knapweed possibly suppressed germination of native plants and resulted in more bare ground, higher ground temperatures, and less litter cover in sites with spotted knapweed. This provided a better hunting habitat for predator biomass at intermediate spotted knapweed densities, which was negated at high densities due to the lack of prey availability. Detritivore biomass was highest at pristine grassland sites and significantly lower at spotted knapweed invaded sites due to the lack of food availability with limited litter cover.

Chapter 3 provides deeper insight into spotted knapweed effects on arthropod orders. Orthoptera alpha diversity was positively correlated with spotted knapweed density. However, other arthropod orders were not correlated with spotted knapweed density. *Larinus obtusus,* a biological control agent released in Lac du Bois in 1992 to control spotted knapweed, was observed at higher relative densities at higher spotted knapweed density sites (Gayton and Miller 2012).

This research will expand our knowledge of DNA metabarcoding methodology and of the growing database of Western North American arthropods, which will be directly applicable to future reclamation methods and environmental assessments on human-disturbed ecosystems.

LIMITATIONS AND FUTURE RESEARCH

One of the major limitations of this study is the lack of manipulation trials. It was not feasible on the current timescale to add an additional experimental trial to this project. However, this project could be greatly expanded by exploring species-specific interactions with spotted knapweed versus other native plants. Future researchers could conduct trials where chosen arthropod species are exposed to different plant food sources and observed throughout the period of their lifespan to determine the direct effects of invasive plants such as spotted knapweed on

specific arthropods. Additionally, continued sampling over the course of several years would give us better insight into spotted knapweed changing site characteristics and corresponding arthropod community changes through a timescale.

Although DNA metabarcoding gave us greater depth of arthropod orders and specific species, several *Araneae* species manually identified did not appear in the sequencing results. This is likely a limitation of the primers used in our methodology not being universal enough to separate *Araneae* from other organisms. It is suggested that an additional forward and reverse primer be used in combination with the ones in this study to accurately identify arthropod orders from interior British Columbia.

MANAGEMENT IMPLICATIONS

Human activities such as mining, recreation, and farming are altering British Columbia grassland ecosystems; leaving them increasingly susceptible to anthropogenic-caused changes, such as the colonization of invasive plants. These grasslands provide invaluable services to people and the environment. The results from this study contribute to our growing understanding of invasive plants in British Columbia grasslands.

Invasive species

The spread of invasive species is strongly shaped by trends in human trade and transport and can be dated as far back as the first human trade routes (Preston et al. 2004). Grasslands are one of the most endangered ecosystems in Canada and are highly susceptible to changes in ecosystem energy flows due to the introduction of invasive plants (Aguair 2005). Grasslands provide invaluable services to people and the environment including carbon sequestration (Wilson 2009; Costanza et al. 1998), water filtration (Wilson 2009) wildlife management, forage for grazing livestock (Aguair 2005), and recreational areas. However, the constant use of grasslands by humans is leading to the anthropogenic spread of invasive plants – such as spotted knapweed – throughout grasslands, altering plant and animal biodiversity (Mack et al. 2000).

With the help of studies, such as this one, that give us better insight into specific correlations associated with invasions, such as patch density influencing arthropod communities, we can inform the public and policy makers to better manage invasion potential. Knowing that patch density of spotted knapweed negatively correlates with herbivores and detritivores lets us

target specific invaded areas where, for example, an herbivorous arthropod species of interest inhabits. More in depth identification of herbivorous arthropods such as Orthoptera species will be possible with the expansion of barcoding databases. The spread of invasive species is one of the main global threats to biodiversity (Vitousek et al. 1996) and one of the hardest threats to manage. Lac du Bois grasslands protected area has been managed for the invasion of spotted knapweed since 1978 through chemical spot treatments and the release of biocontrol agents (Ministry of Environment, Lands and Parks 2000). However, there has still been continual spread of the plant through the grassland in part due to lack of education by recreational park users. This research may help gain public awareness of spotted knapweed in the Lac du Bois region. Any knowledge gained about specific species interactions of invasions is beneficial in educating management at a local level.

Mining site reclamation

Mining is a significant industry that contributes to Canada's economy by employing more than 596,000 workers and contributing $57.6 billion to Canada's gross domestic product in 2016 (Mining Association of Canada 2017). Although it is economically beneficial and an essential industry to support society, mining adversely affects the surrounding terrestrial environment, including grassland ecosystems. Some environmental impacts of mining include erosion, the formation of sinkholes, landscape alterations (Mol and Ouboter 2004), changes to vegetation communities, and the contamination of soil, groundwater, and surface water (Salomons 1995). It is mandatory in Canada that mining operations track the changes to biodiversity and ecosystem functioning impacted by their mining activities (New Gold Inc. 2015). They must also demonstrate the effectiveness of programs implemented for site reclamation.

Historically, land reclamation efforts had the primary goal of reestablishing vegetation, but this narrow view can result in failed efforts (Simmers 2010; Fagan 2008). A recent trend considers mine reclamation in terms of the whole ecosystem, including biodiversity targets and considerations for all ecosystem functions and services (Garris 2016; Fraser 2015). However, the industry lacks reliable and sensitive tools to assess environmental health, from the microbial level to the full ecosystem level. Ecosystem-based approaches informed by molecular tools, such as DNA metabarcoding, have the power, sensitivity and the accessibility for application in developing healthy soils, building food web structures, increasing species biodiversity, and addressing specific species of interest (Fraser 2015).

There is currently a lack of standardized methods and procedures in reclamation practice within British Columbia. Through the help of this study, standard operating procedures (SOPs) and guidelines can be drafted to help mine personnel apply and use the biological information produced by this project and through BOLD. This study implemented DNA metabarcoding as an expedited method for arthropod community identification that can be directly implemented for pre- and post- mining site environmental assessments and reclamation strategies.

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Appendix A. *Plant species identified from all sites in Lac du Bois.*

Common Name	Latin Name	Native or Non-native
Alfalfa	*Medicago sativa*	Non-Native
American vetch	*Vicia americana*	Native
Arrowleaf balsamroot	*Balsamorhiza sagittata*	Native
Baltic rush	*Juncus balticus*	Native
Black medick	*Medicago lupulina*	Non-Native
Blue wildrye	*Elymus glaucus*	Native
Bluebunch wheatgrass	*Pseudoroegneria spicata*	Native
Brown eyed susan	*Rudbeckia triloba*	Native
Canada thistle	*Cirsium arvense*	Non-Native
Chicory	*Cichorium intybus*	Non-Native
Chocolate lily	*Fritillaria affinis*	Native
Common dandelion	*Taraxacum officinale*	Non-Native
Common harebell	*Campanula rotundifolia*	Native
Corn brome	*Bromus squarrosus*	Non-Native
Crested wheatgrass	*Agropyron cristatum*	Non-Native
Diffuse knapweed	*Centaurea diffusa*	Non-Native
Fairy candelabra	*Androsace occidentalis*	Non-Native
Fern-leaved desert parsley	*Lomatium dissectum*	Native
Field locoweed	*Oxytropis campestris*	Native
Field pussytoes	*Antennaria neglecta*	Native
Field sedge	*Carex praegracilis*	Native
Graceful cinquefoil	*Potentilla gracilis*	Native
Hillside milk vetch	*Astragalus collinus*	Native
Holboells rockcress	*Arabis holboellii*	Native
Junegrass	*Koeleria macrantha*	Native
Kentucky bluegrass	*Poa pratensis*	Native
Lemonweed	*Oxytropis campestris*	Native
Long leaved daisy	*Erigeron corymbosus*	Native
Meadow death camas	*Toxicoscordion venenosum*	Native
Narrow leaved collomia	*Collomia linearis*	Native
Narrow leaved hawkweed	*Hieracium umbellatum*	Native
Needle and thread grass	*Hesperostipa comata*	Native
Nodding onion	*Allium cernuum*	Native
Nootka rose	*Rosa nutkana*	Native
Orange arnica	*Arnica fulgens*	Native
Oval leaved blueberry	*Vaccinium ovalifolium*	Native
Pale comandra	*Comandra umbellata*	Native
Parsnip flowered buckwheat	*Eriogonum heracleoides*	Native
Pumpbelly brome	*Bromus inermis pumpellianus*	Native
Purple peavine	*Lathyrus nevadensis*	Native
Rosy pussytoes	*Antennaria rosea*	Native
Rough fescue	*Festuca scabrella*	Native
Round-leaved alumroot	*Heuchera cylindrica*	Native

Sagebrush mariposa lily	*Calochortus macrocarpus*	Native
Sandberg's bluegrass	*Sandbergs bluegrass*	Native
Slender hawksbeard	*Crepis atribarba*	Native
Small flowered woodland star	*Lithophragma parviflorum*	Native
Spotted knapweed	*Centaurea stoebe*	Non-Native
Spreading needlegrass	*Hesperostipa comata*	Native
Stiff needlegrass	*Achnatherum occidentale*	Native
Thompsons paintbrush	*Castilleja thompsonii*	Native
Timber milk vetch	*Astragalus miser*	Native
Tower mustard	*Turritis glabra*	Native
Unknown flower spp 1	*N/A*	N/A
Unknown flower spp 2	*N/A*	N/A
Unknown flower spp 3	*N/A*	N/A
Western spring beauty	*Claytonia lanceolata*	Native
Western stickseed	*Lappula occidentalis*	Native
Yarrow	*Achillea millefolium*	Native
Yellow rattle	*Rhinanthus minor*	Native
Yellow salsify	*Tragopogon dubius*	Non-Native

Appendix B. *Arthropod families sampled from each functional group ranked as most to least frequently sampled.*

Sampling Frequency	Herbivore	Omnivore	Predator	Detritivore	Parasite
Most Frequent ↓ **Least Frequent**	• Drosophilidae (small fruit flies) • Rhopalidae (scentless plant bugs) • Curculionidae (weevils) • Thysanoptera (thrips) • Carabidae (ground beetles) → genus Amara • Acrididae (grasshopper family) • Cicadellidae (leafhoppers) • Tephritidae (fruit flies) • Pentatomidae (stink bugs) • Erebidae (moth family) • Tipulidae (crane flies)	• Formicidae (ants) • Gryllidae (crickets) • Forficulidae (earwigs) • Muscidae (house flies) • Syrphidae (hover flies) • Crabronidae (wasp family)	• Carabidae (ground beetles) • Lycosidae (wolf spiders) • Thomisidae (crab spiders) • Araneae* → numerous other spiders unidentified • Staphylinidae (rove beetles) • Lithobiomorpha (stone centipedes) • Elateridae (click beetles) • Dolichopodidae (long-legged flies) • Chrysopidae (green lacewings)	• Collembolla* (springtails) • Microcoryphia* (jumping bristletails) • Diptera* (numerous flies unidentified) • Diplura* (two-pronged bristletails) • Oniscidae (sow bugs) • Armadillidiidae (pill bugs) • Silphidae (carrion beetles)	• Demodecidae (parasitic mites) • Trombiculidae (chigger mites) • Ixodidae (hard ticks) • Culicidae (mosquito) • Pulicidae (fleas) • Tachinidae (parasitoid flies)

*Did not distinguish to family level, only order

Appendix C. *Analysis of variance results of the effects of spotted knapweed density on arthropod functional group species richness, n=20.*

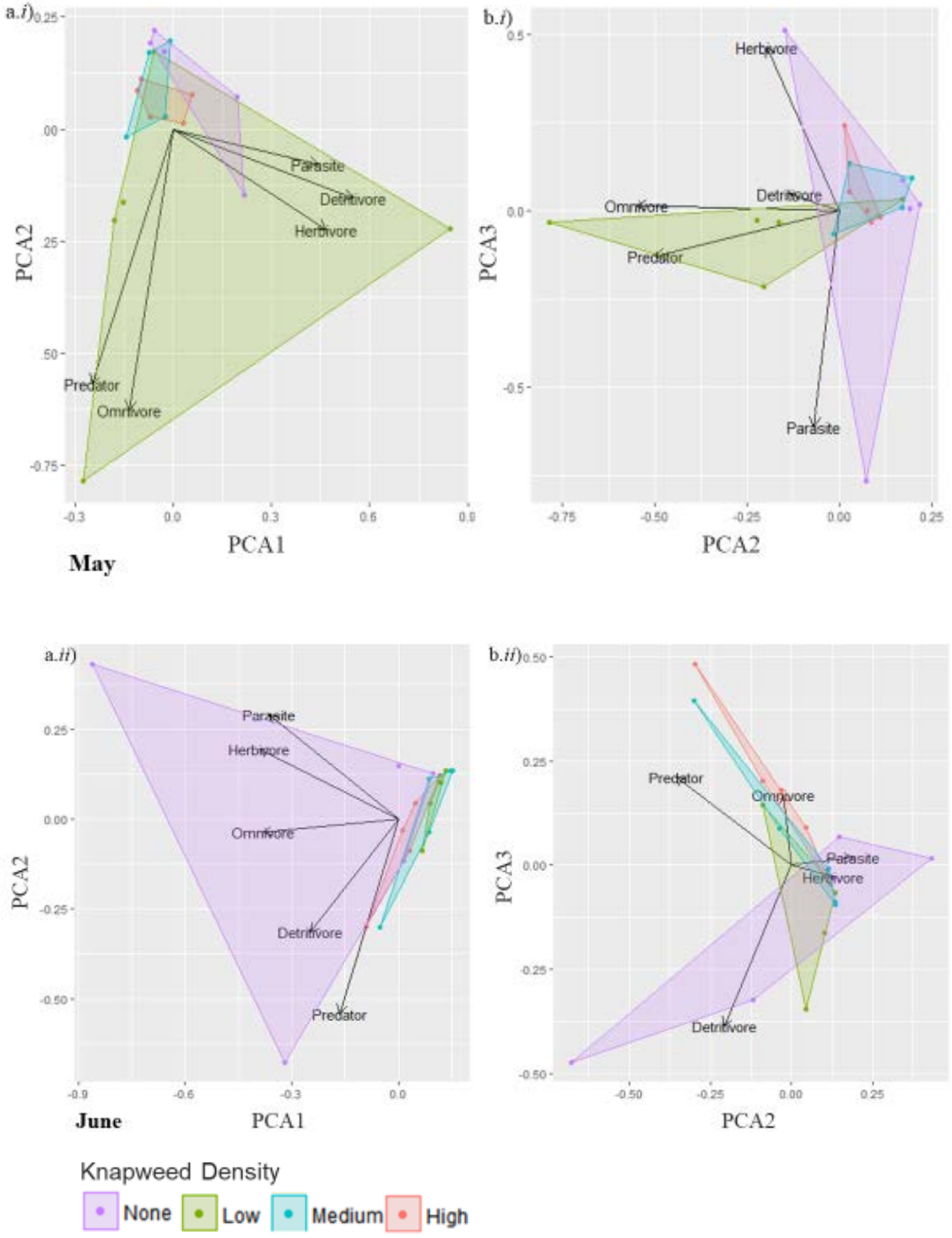
	Mean				df	F	P
Knapweed Density	**None**	**Low**	**Medium**	**High**			
Summer 2016							
Overall Species Richness	5.8	5.0	5.7	6.7	3	0.587	0.626
Herbivore Richness	1.1	1.0	1.1	1.2	3	0.035	0.991
Omnivore Richness	1.8	2.1	2.2	2.6	3	0.638	0.593
Predator Richness	1.9	1.2	1.6	2.0	3	1.109	0.351
Detritivore Richness	0.5	0.4	0.5	0.4	3	0.197	0.898
Parasite Richness	0.5	0.3	0.3	0.5	3	0.557	0.645
May							
Overall Species Richness	4.6	7.8	5.4	5.6	3	1.133	0.365
Herbivore Richness	0.4	1.6	0.8	1.0	3	1.786	1.190
Omnivore Richness	1.8^{b}	4.0^{a}	1.8^{b}	1.8^{b}	3	2.521	**0.095**
Predator Richness	2.2	1.6	2.4	2.0	3	0.265	0.849
Detritivore Richness	0.2	0.2	0.2	0.4	3	0.143	0.933
Parasite Richness	0.2	0.4	0.2	0.4	3	0.266	0.848
June							
Overall Species Richness	9.4	6.4	8.6	10.8	3	0.820	0.502
Herbivore Richness	2.4	1.0	1.2	1.4	3	1.611	0.226
Omnivore Richness	2.2	2.2	3.0	3.6	3	0.866	0.479
Predator Richness	2.2	2.4	2.0	3.8	3	0.939	0.445
Detritivore Richness	1.4	0.8	1.6	0.6	3	1.554	0.239
Parasite Richness	1.0	0.2	0.8	1.0	3	0.735	0.546
July							
Overall Species Richness	5.4	4.0	6.4	7.2	3	1.738	0.199
Herbivore Richness	1.0	0.6	1.4	0.8	3	0.833	0.495
Omnivore Richness	1.8	1.8	3.2	3.4	3	1.529	0.246
Predator Richness	2.0^{a}	0.4^{b}	1.6^{a}	2.0^{a}	3	7.564	**0.002***
Detritivore Richness	0.2	0.4	0.2	0.2	3	0.222	0.879
Parasite Richness	0.6	0.6	0.2	0.6	3	0.381	0.768
August							
Overall Species Richness	4.0	2.0	2.4	3.2	3	0.354	0.787
Herbivore Richness	0.6	1.0	1.0	1.4	3	0.688	0.572
Omnivore Richness	1.6^{a}	0.4^{b}	0.8^{ab}	1.4^{ab}	3	3.061	**0.058**
Predator Richness	1.4	0.4	0.6	0.4	3	0.405	0.752
Detritivore Richness	0.2	0.2	0	0.2	3	0.333	0.801
Parasite Richness	0.2	0	0	0	3	1.000	0.418

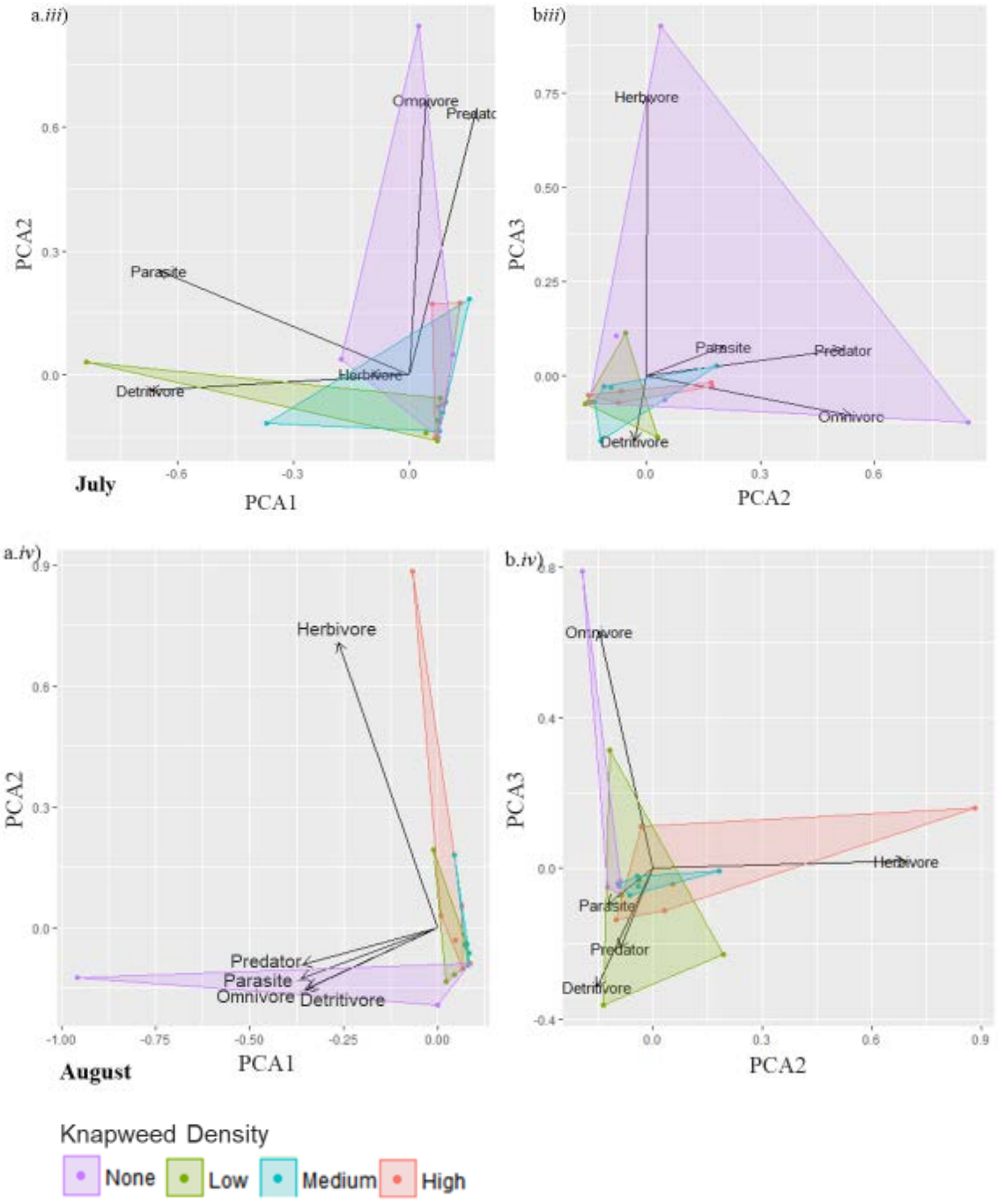
Bold values indicate statistical significance at P<0.1, * indicates significance at P<0.05.

Appendix D. *Factor loading of the monthly summer principal components analysis.*

	Component 1	Component 2	Component 3
May			
Herbivore	0.523	-0.251	0.594
Omnivore	-0.150	-0.703	-
Predator	-0.276	-0.637	-0.163
Detritivore	0.616	-0.172	-
Parasite	0.497	-	-0.785
Standard Deviation	1.442	1.235	0.842
Variance (%)	41.6	30.5	14.2
Cumulative Variance (%)	41.6	72.1	86.3
June			
Herbivore	-0.543	0.271	-
Omnivore	-0.529	-	0.357
Predator	-0.231	-0.754	0.449
Detritivore	-0.343	-0.438	-0.816
Parasite	-0.504	0.404	-
Standard Deviation	1.743	1.105	0.782
Variance (%)	60.8	24.4	12.2
Cumulative Variance (%)	60.8	85.2	97.4
July			
Herbivore	-0.105	-	0.956
Omnivore	-	0.697	-0.137
Predator	0.180	0.666	-
Detritivore	-0.702	-	-0.224
Parasite	-0.680	0.262	-
Standard Deviation	1.342	1.259	1.029
Variance (%)	36.0	31.7	21.2
Cumulative Variance (%)	36.0	67.7	88.9
August			
Herbivore	-0.348	0.935	-
Omnivore	-0.455	-0.198	0.849
Predator	-0.473	-0.121	-0.287
Detritivore	-0.466	-0.207	-0.424
Parasite	-0.481	-0.169	-0.129
Standard Deviation	2.046	0.751	0.390
Variance (%)	83.7	11.3	3.0
Cumulative Variance (%)	83.7	95.0	98.0

Appendix E. *Principal components analyses for each month of sampling; May (i), June (ii), July (iii), and August (iv); examining the influence of each functional group on overall arthropod community composition graphed using (a) components 1 and 2, and (b) components 2 and 3***.**




a.iii)
Omnivore
Parasite
Herbivore
Detritivore
PCA2
PCA1
July
b.iii)
Herbivore
Parasite
Predator
Omnivore
Detritivore
PCA3
PCA2
a.iv)
Herbivore
Predator
Parasite
Omnivore
Detritivore
PCA2
PCA1
August
b.iv)
Omnivore
Herbivore
Parasite
Predator
Detritivore
PCA3
PCA2
Knapweed Density
None
Low
Medium
High

Appendix F. *Multiple regression analyses for significant site variables predicting principal component 1, 2, and 3 for each month of sampling, n=20, df=56.*

						Variables					
						Intercept	Spotted knapweed biomass (g/m^2)	Plant biomass (g/m^2)	Litter Cover (%)	Bare Ground Cover (%)	Daily Ground Temperature (^oC)
		Model Values									
		F-stat	**P**	**R^2**	**df**						
May	Component 1	1.670	0.212	0.323	18	SE 4.650	SE 0.171	SE 0.871	SE 2.79 0	SE 2.914	-
						T -0.357	T 1.742	T 0.206	T 0.445	T -1.085	
						P 0.726	P 0.103	P 0.840	P 0.663	P 0.296	
	Component 2	1.445	0.271	0.292	18	SE 4.072	SE 4.072	SE 0.763	SE 2.443	SE 2.552	-
						T -1.957	T -1.957	T 1.296	T 0.681	T 0.861	
						P 0.071	**P 0.071**	P 0.216	P 0.507	P 0.404	
	Component 3	0.297	0.875	0.078	18	SE 3.170	SE 0.122	SE 0.594	SE 1.902	SE 1.986	-
						T 0.170	T 0.327	T 0.053	T -0.552	T -0.118	
						P 0.867	P 0.749	P 0.958	P 0.590	P 0.908	
June	Component 1	1.391	0.286	0.332	19	SE 7.441	SE 0.222	SE 1.091	SE 3.301	SE 3.854	SE 0.198
						T -1.619	T 1.988	T 0.705	T 0.136	T -0.686	T 1.961
						P 0.128	**P 0.067**	P 0.493	P 0.893	P 0.504	**P 0.017***
	Component 2	0.289	0.911	0.094	19	SE 5.494	SE 0.164	SE 0.806	SE 2.442	SE 2.846	SE 0.146
						T 0.097	T -0.344	T -0.260	T 0.606	T 0.022	T -0.113
						P 0.924	P 0.736	P 0.799	P 0.554	P 0.983	P 0.912
	Component 3	1.625	0.217	0.367	19	SE 3.249	SE 0.097	SE 0.476	SE 1.444	SE 1.683	SE 0.086
						T 1.242	T 0.457	T -2.098	T 0.886	T 0.214	T 0.021
						P 0.235	P 0.655	P 0.054	P 0.391	P 0.833	P 0.983

July	Component 1	0.455	0.802	0.140	19	SE 6.503	SE 0.193	SE 0.955	SE 2.889	SE 3.260	SE 0.122
						T 0.033	T -0.490	T -0.484	T 0.948	T 1.314	T -0.336
						P 0.974	P 0.632	P0.636	P 0.359	P 0.210	P 0.742
	Component 2	0.521	0.757	0.157	19	SE 6.041	SE 0.179	SE 0.887	SE 2.684	SE 3.029	SE 0.114
						T -0.136	T -1.055	T 0.404	T 0.166	T 0.784	T -0.605
						P 0.894	P 0.309	P0.692	P 0.870	P 0.446	P 0.555
	Component 3	1.615	0.220	0.366	19	SE 4.281	SE 0.127	SE 0.629	SE 1.902	SE 2.146	SE 0.081
						T 1.791	T -1.781	T -1.443	T 0.715	T 0.835	T -1.983
						P 0.095	**P 0.097**	P 0.171	P 0.487	P 0.418	**P 0.067**
Aug	Component 1	0.715	0.622	0.203	19	SE 9.543	SE 0.283	SE 1.402	SE 4.239	SE 4.784	SE 0.180
						T 0.166	T 0.514	T -1.147	T 1.145	T -0.583	T 0.944
						P 0.871	P 0.616	P0.271	P 0.272	P0.569	P 0.361
	Component 2	1.56	0.235	0.358	19	SE 3.146	SE 0.093	SE 0.462	SE 1.398	SE 1.577	SE 0.059
						T 0.493	T 1.286	T -1.478	T 0.595	T -0.652	T 1.02
						P 0.629	P 0.219	P 0.162	P 0.561	P 0.525	P 0.324
	Component 3	0.313	0.897	0.101	19	SE 0.093	SE 0.005	SE 0.028	SE 0.085	SE 0.097	SE 0.036
						T -0.210	T -1.032	T 0.534	T -0.397	T 0.119	T -0.002
						P 0.837	P 0.319	P 0.601	P 0.697	P 0.907	P 0.999

Appendix G. *Associated taxonomic identification for each OTU in sequenced results.*

OTU ID	Taxonomy	Phylum	Class	Order	Family	Genus	Species
001	GS|100.0|BOLD:AAZ1768	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Litomiris	*s:Litomiris curtus*
002	GS|99.7|BOLD:AAV0219	p:Arthropoda	c:Insecta	o:Hemiptera	f:Rhyparochromidae	g:Slaterobius	*s:Slaterobius insignis*
003	GS|100.0|BOLD:ACY9729	p:Arthropoda	c:Insecta	o:Coleoptera	f:Tenebrionidae	g:Eleodes	*s:Eleodes vandykei*
004	GS|100.0|BOLD:AAB3500	p:Arthropoda	c:Insecta	o:Diptera	f:Tephritidae	g:Urophora	*s:Urophora cardui*
005	GS|100.0|BOLD:AAB5081	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Leptopterna	*s:Leptopterna dolabrata*
006	GS|100.0|BOLD:AAA8278	p:Arthropoda	c:Insecta	o:Hemiptera	f:Scutelleridae	g:Homaemus	*s:Homaemus aeneifrons*
007	GS|100.0|BOLD:AAG5507	p:Arthropoda	c:Insecta	o:Diptera	f:Chironomidae	g:Chironomus	*s:Chironomus atrella*
008	GS|100.0|BOLD:AAB8638	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Litomiris	*s:Litomiris debilis*
009	GS|99.7|BOLD:AAF7101	p:Arthropoda	c:Insecta	o:Diptera	f:Fanniidae	g:Fannia	*s:Fannia canicularis*
010	GS|99.7|BOLD:AAA8764	p:Arthropoda	c:Insecta	o:Orthoptera	f:Acrididae	g:Camnula	*s:Camnula pellucida*
011	GS|91.3|BOLD:ABX4018	p:Arthropoda	c:Insecta	o:Coleoptera	f:Dermestidae	g:Dermestes	*s:Dermestes caninus*

012	GS|100.0|BOLD:ACE5391	p:Arthropoda	c:Insecta	o:Diptera	f:Tephritidae	g:Chaetorellia	
013	GS|99.7|BOLD:AAH4153	p:Arthropoda	c:Insecta	o:Diptera	f:Chloropidae		
014	GS|99.3|BOLD:AAH0413	p:Arthropoda	c:Insecta	o:Coleoptera			
015	GS|100.0|BOLD:AAD0642	p:Arthropoda	c:Insecta	o:Diptera	f:Muscidae	g:Helina	*s:Helina reversio*
016	GS|100.0|BOLD:AAD7982	p:Arthropoda	c:Insecta	o:Diptera	f:Culicidae	g:Aedes	*s:Aedes campestris*
017	GS|100.0|BOLD:AAE0406	p:Arthropoda	c:Insecta	o:Hymenoptera	f:Formicidae	g:Formica	*s:Formica lasioides*
018	GS|100.0|BOLD:AAB1098	p:Arthropoda	c:Insecta	o:Diptera	f:Culicidae	g:Aedes	*s:Aedes excrucians*
019	GS|99.6|BOLD:AAG8797	p:Arthropoda	c:Insecta	o:Hemiptera	f:Cicadellidae	g:Diplocolenus	*s:Diplocolenus configuratus*
020	GS|100.0|BOLD:ACJ0304	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Phytocoris	
021	GS|99.7|BOLD:AAA5195	p:Arthropoda	c:Insecta	o:Lepidoptera	f:Noctuidae	g:Lacinipolia	*s:Lacinipolia pensilis*
022	GS|99.7|BOLD:AAQ0072	p:Arthropoda	c:Insecta	o:Coleoptera	f:Elateridae	g:Selatosomus	*s:Selatosomus semimetallicus*
023	GS|100.0|BOLD:AAG4333	p:Arthropoda	c:Insecta	o:Coleoptera	f:Staphylinidae	g:Xantholinus	*s:Xantholinus linearis*
024	GS|99.7|BOLD:AAF7969	p:Arthropoda	c:Insecta	o:Hemiptera	f:Pentatomidae	g:Thyanta	*s:Thyanta pallidovirens*
025	GS|97.0|BOLD:ACJ8319	p:Arthropoda	c:Insecta	o:Diptera	f:Chloropidae	*g:Meromyza*	

026	GS|99.3|BOLD:ACF4015	p:Arthropoda	c:Insecta	o:Diptera	f:Tephritidae	g:Campiglossa	*s:Campiglossa genalis*
027	GS|100.0|BOLD:AAG3286	p:Arthropoda	c:Insecta	o:Diptera	f:Phoridae	*g:Megaselia*	
028	GS|99.7|BOLD:ACE6932	p:Arthropoda	c:Insecta	o:Orthoptera	f:Acrididae	g:Arphia	*s:Arphia pseudonietana*
029	GS|100.0|BOLD:AAB8817	p:Arthropoda	c:Insecta	o:Diptera	f:Muscidae	g:Muscina	*s:Muscina levida*
030	GS|100.0|BOLD:AAZ0082	p:Arthropoda	c:Insecta	o:Coleoptera	f:Tenebrionidae	g:Helops	
031	GS|99.7|BOLD:AAE3073	p:Arthropoda	c:Insecta	o:Coleoptera	f:Silphidae	g:Thanatophilus	*s:Thanatophilus lapponicus*
032	GS|100.0|BOLD:AAG8799	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Chlamydatus	*s:Chlamydatus keltoni*
033	GS|100.0|BOLD:ACF1257	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Adelphocoris	*s:Adelphocoris lineolatus*
034	GS|100.0|BOLD:AAG8821	p:Arthropoda	c:Insecta	o:Hemiptera	f:Cicadellidae	g:Doratura	*s:Doratura stylata*
035	GS|99.7|BOLD:AAC8204	p:Arthropoda	c:Insecta	o:Lepidoptera	f:Noctuidae	g:Euxoa	*s:Euxoa bochus*
036	GS|98.3|BOLD:AAI4455	p:Arthropoda	c:Arachnida	o:Araneae	f:Araneidae	g:Metepeira	*s:Metepeira palustris*
037	GS|99.7|BOLD:AAG2875	p:Arthropoda	c:Insecta	o:Hemiptera	f:Cicadellidae	g:Ceratagallia	*s:Ceratagallia cinerea*
039	GS|99.3|BOLD:AAM7341	p:Arthropoda	c:Insecta	o:Diptera	f:Heleomyzidae	g:Oecothea	*s:Oecothea fenestralis*
040	GS|99.0|BOLD:ACA6220	p:Arthropoda	c:Insecta	o:Orthoptera	f:Tettigoniidae	g:Steiroxys	*s:Steiroxys cf trilineata*

041	GS|98.7|BOLD:AAB4891	p:Arthropoda	c:Insecta	o:Coleoptera	f:Silphidae	g:Nicrophorus	*s:Nicrophorus investigator*
042	GS|100.0|BOLD:AAB7600	p:Arthropoda	c:Insecta	o:Coleoptera	f:Curculionidae	g:Larinus	*s:Larinus obtusus*
043	GS|100.0|BOLD:ACB0946	p:Arthropoda	c:Insecta	o:Diptera	f:Chironomidae	g:Smittia	
044	GS|99.7|BOLD:AAV0264	p:Arthropoda	c:Insecta	o:Hemiptera	f:Cicadellidae	g:Latalus	*s:Latalus mundus*
045	GS|100.0|BOLD:AAG5198	p:Arthropoda	c:Insecta	o:Coleoptera	f:Curculionidae	g:Otiorhynchus	*s:Otiorhynchus ovatus*
046	GS|100.0|BOLD:AAG0243	p:Arthropoda	c:Insecta	o:Coleoptera	f:Silphidae	g:Nicrophorus	*s:Nicrophorus guttula*
047	GS|98.9|BOLD:AAA8278	p:Arthropoda	c:Insecta	o:Hemiptera	f:Scutelleridae	g:Homaemus	*s:Homaemus aeneifrons*
048	GS|99.7|BOLD:AAF9891	p:Arthropoda	c:Insecta	o:Orthoptera	f:Gryllidae	g:Oecanthus	*s:Oecanthus nigricornis*
050	GS|99.3|BOLD:ACN9284	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae		
051	GS|98.7|BOLD:AAY8854	p:Arthropoda	c:Insecta	o:Coleoptera			
052	GS|99.3|BOLD:ABZ1405	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Lygus	*s:Lygus borealis*
053	GS|100.0|BOLD:AAP7560	p:Arthropoda	c:Insecta	o:Diptera	f:Chloropidae	*g:Meromyza*	
054	GS|98.6|BOLD:ACM7148	p:Arthropoda	c:Insecta	o:Coleoptera			
055	GS|99.3|BOLD:AAD0591	p:Arthropoda	c:Insecta	o:Hemiptera	f:Rhopalidae	g:Harmostes	*s:Harmostes reflexulus*

056	GS|99.7|BOLD:AAL2821	p:Arthropoda	c:Insecta	o:Diptera	f:Muscidae	g:Phaonia	*s:Phaonia apicalis*
057	GS|81.5|BOLD:AAG8886	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	*g:Phytocoris*	
058	GS|100.0|BOLD:AAY2830	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Polymerus	*s:Polymerus balli*
059	GS|99.7|BOLD:AAB8851	p:Arthropoda	c:Insecta	o:Diptera	f:Drosophilidae	g:Drosophila	*s:Drosophila affinis*
060	GS|100.0|BOLD:AAI9341	p:Arthropoda	c:Insecta	o:Hemiptera	f:Thyreocoridae	g:Corimelaena	*s:Corimelaena incognita*
061	GS|100.0|BOLD:AAA7374	p:Arthropoda	c:Insecta	o:Diptera	f:Syrphidae	g:Sphaerophoria	*s:Sphaerophoria philanthus*
062	GS|100.0|BOLD:AAC2855	p:Arthropoda	c:Insecta	o:Diptera	f:Sepsidae	g:Sepsis	*s:Sepsis neocynipsea*
063	GS|100.0|BOLD:AAA5308	p:Arthropoda	c:Insecta	o:Diptera	f:Chironomidae	*g:Cricotopus*	
064	GS|100.0|BOLD:ACB6454	p:Arthropoda	c:Insecta	o:Diptera	f:Mycetophilidae	g:Cordyla	
065	GS|79.3|BOLD:ACB6454	p:Arthropoda	c:Insecta	o:Diptera	f:Mycetophilidae	g:Cordyla	
067	GS|99.3|BOLD:AAB1850	p:Arthropoda	c:Insecta	o:Hemiptera	f:Aphrophoridae	g:Philaenus	*s:Philaenus spumarius*
068	GS|100.0|BOLD:AAZ1925	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Coquillettia	*s:Coquillettia insignis*
069	GS|100.0|BOLD:AAI5560	p:Arthropoda	c:Insecta	o:Hemiptera	f:Scutelleridae	g:Eurygaster	*s:Eurygaster amerinda*
070	GS|100.0|BOLD:ACL3236	p:Arthropoda	c:Insecta	o:Diptera	f:Fanniidae	g:Fannia	*s:Fannia coracina*

071	GS|97.7|BOLD:ACB9088	p:Arthropoda	c:Insecta	o:Diptera	f:Chloropidae	g:Aphanotrigonum	*s:Aphanotrigonum trilineatum*
072	GS|100.0|BOLD:ACA6322	p:Arthropoda	c:Insecta	o:Coleoptera			
073	GS|100.0|BOLD:AAC7993	p:Arthropoda	c:Insecta	o:Hemiptera	f:Alydidae	g:Alydus	*s:Alydus conspersus*
074	GS|100.0|BOLD:AAC8322	p:Arthropoda	c:Insecta	o:Coleoptera	f:Carabidae	g:Harpalus	*s:Harpalus opacipennis*
076	GS|100.0|BOLD:AAG9373	p:Arthropoda	c:Insecta	o:Hemiptera	f:Cicadellidae	g:Balclutha	*s:Balclutha rhenana*
077	GS|77.3|BOLD:AAN9052	p:Arthropoda	c:Insecta	o:Diptera			
078	GS|89.0|BOLD:AAE3943	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Plagiognathus	*s:Plagiognathus chrysanthemi*
079	GS|99.7|BOLD:AAC5564	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Adelphocoris	*s:Adelphocoris rapidus*
080	GS|99.7|BOLD:AAA2218	p:Arthropoda	c:Insecta	o:Odonata	f:Coenagrionidae	g:Enallagma	*s:Enallagma cyathigerum*
081	GS|72.1|BOLD:AAX3548	p:Arthropoda	c:Insecta	o:Odonata			
082	GS|99.7|BOLD:ACG1868	p:Arthropoda	c:Insecta	o:Diptera	f:Ephydridae	g:Scatella	*s:Scatella stagnalis*
083	GS|99.3|BOLD:AAF2735	p:Arthropoda	c:Insecta	o:Coleoptera	f:Silphidae	g:Nicrophorus	*s:Scatella stagnalis*
084	GS|99.7|BOLD:AAE0456	p:Arthropoda	c:Insecta	o:Dermaptera	f:Forficulidae	g:Forficula	*s:Scatella stagnalis*
085	GS|99.0|BOLD:AAY7701	p:Arthropoda	c:Insecta	o:Coleoptera			

086	GS|100.0|BOLD:AAN8307	p:Arthropoda	c:Insecta	o:Hemiptera	f:Cicadellidae	g:Balclutha	*s:Balclutha manitou*
087	GS|100.0|BOLD:AAW1617	p:Arthropoda	c:Insecta	o:Coleoptera	f:Staphylinidae	g:Aleochara	*s:Aleochara rubricalis*
088	GS|96.7|BOLD:AAZ1768	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Litomiris	*s:Litomiris curtus*
089	GS|96.7|BOLD:ACA3662	p:Arthropoda	c:Insecta	o:Coleoptera			
090	GS|100.0|BOLD:ABX3926	p:Arthropoda	c:Insecta	o:Hemiptera	f:Cicadellidae	g:Auridius	*s:Auridius auratus*
091	GS|99.0|BOLD:AAD0482	p:Arthropoda	c:Insecta	o:Diptera	f:Chironomidae	*g:Psectrocladius*	
092	GSL|89.2|BOLD:ACE7981	p:Arthropoda	c:Insecta	o:Orthoptera	f:Acrididae	*g:Aeropedellus*	
093	GS|99.3|BOLD:AAA2674	p:Arthropoda	c:Insecta	o:Diptera	f:Drosophilidae	g:Drosophila	*s:Drosophila subquinaria*
095	GS|100.0|BOLD:AAQ0054	p:Arthropoda	c:Insecta	o:Coleoptera	f:Carabidae	g:Harpalus	*s:Harpalus laticeps*
096	GS|100.0|BOLD:ACP4088	p:Arthropoda	c:Insecta	o:Diptera	f:Chloropidae	*g:Incertella*	
097	GS|99.7|BOLD:AAF6788	p:Arthropoda	c:Insecta	o:Hymenoptera	f:Formicidae	g:Formica	*s:Formica neogagates*
098	GS|95.2|BOLD:AAZ1768	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Litomiris	*s:Litomiris curtus*
100	GS|100.0|BOLD:AAG2123	p:Arthropoda	c:Insecta	o:Diptera	f:Tachinidae	g:Dinera	*s:Dinera grisescens*
101	GS|100.0|BOLD:ACG7900	p:Arthropoda	c:Insecta	o:Diptera	f:Chloropidae	*g:Meromyza*	

102	GS|100.0|BOLD:ACI7163	p:Arthropoda	c:Arachnida	o:Araneae	f:Araneidae	g:Metepeira	*s:Metepeira palustris*
103	GS|99.7|BOLD:AAN5526	p:Arthropoda	c:Insecta	o:Diptera	f:Dolichopodidae		
105	GS|99.7|BOLD:AAE1941	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Stenodema	*s:Stenodema vicina*
106	GS|100.0|BOLD:ACB0907	p:Arthropoda	c:Insecta	o:Diptera	f:Chironomidae		
107	GS|97.3|BOLD:AAZ0977	p:Arthropoda	c:Insecta	o:Coleoptera			
108	GS|97.7|BOLD:AAD5789	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Leptopterna	*s:Leptopterna amoena*
109	GS|97.4|BOLD:AAQ0072	p:Arthropoda	c:Insecta	o:Coleoptera	f:Elateridae	g:Selatosomus	*s:Selatosomus semimetallicus*
111	GS|93.3|BOLD:AAG6647	p:Arthropoda	c:Insecta	o:Diptera	f:Bibionidae	g:Bibio	
113	GS|99.0|BOLD:AAG0666	p:Arthropoda	c:Insecta	o:Orthoptera	f:Gryllidae	g:Gryllus	*s:Gryllus veletis*
116	GS|100.0|BOLD:ACY9994	p:Arthropoda	c:Insecta	o:Coleoptera	f:Tenebrionidae	g:Eleodes	*s:Eleodes pimelioides*
118	GS|99.7|BOLD:AAG8948	p:Arthropoda	c:Insecta	o:Hemiptera	f:Reduviidae	g:Sinea	*s:Sinea diadema*
120	GS|95.0|BOLD:ACR7501	p:Arthropoda	c:Insecta	o:Coleoptera	f:Carabidae	g:Calosoma	*s:Calosoma wilkesii*
122	GS|76.4|BOLD:ACT1583	p:Arthropoda	c:Insecta	o:Diptera	f:Chironomidae		
124	GS|100.0|BOLD:AAV0257	p:Arthropoda	c:Insecta	o:Hemiptera	f:Cicadellidae	g:Sorhoanus	*s:Sorhoanus uhleri*

125	GS|99.3|BOLD:ABY5363	p:Arthropoda	c:Insecta	o:Diptera	f:Sarcophagidae	*g:Metopia*	
127	GS|100.0|BOLD:ACR8648	p:Arthropoda	c:Insecta	o:Diptera	f:Lauxaniidae	g:Minettia	*s:Minettia lupulina*
128	GS|99.7|BOLD:AAD5789	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Leptopterna	*s:Leptopterna amoena*
129	GS|92.0|BOLD:ABA2351	p:Arthropoda	c:Insecta	o:Diptera	f:Fanniidae	g:Fannia	*s:Fannia mutica*
130	GS|100.0|BOLD:AAD6543	p:Arthropoda	c:Insecta	o:Hymenoptera	f:Formicidae	g:Aphaenogaster	*s:Aphaenogaster occidentalis*
133	GS|99.7|BOLD:AAN6531	p:Arthropoda	c:Collembola	o:Entomobryomorpha	f:Tomoceridae		
134	GS|90.3|BOLD:AAC5779	p:Arthropoda	c:Insecta	o:Orthoptera	f:Acrididae	g:Gomphocerippus	*s:Gomphocerippus rufus*
135	GS|100.0|BOLD:AAL7755	p:Arthropoda	c:Insecta	o:Diptera	f:Sphaeroceridae	g:Spelobia	*s:Spelobia tufta*
136	GS|100.0|BOLD:AAA4555	p:Arthropoda	c:Insecta	o:Orthoptera	f:Acrididae	g:Melanoplus	*s:Melanoplus borealis*
140	GS|99.7|BOLD:ACJ8319	p:Arthropoda	c:Insecta	o:Diptera	f:Chloropidae	*g:Meromyza*	
141	GS|100.0|BOLD:ACD1208	p:Arthropoda	c:Insecta	o:Diptera	f:Phoridae	*g:Megaselia*	
142	GS|97.7|BOLD:AAV1530	p:Arthropoda	c:Insecta	o:Archaeognatha	f:Machilidae		
143	GS|94.3|BOLD:ACI3534	p:Arthropoda	c:Insecta	o:Coleoptera	f:Chrysomelidae	*g:Acanthoscelides*	
144	GS|96.4|BOLD:AAB8638	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Litomiris	*s:Litomiris debilis*

146	GS|98.3|BOLD:ACZ0225	p:Arthropoda	c:Insecta	o:Coleoptera	f:Tenebrionidae	g:Eleodes	*s:Eleodes rotundipennis*
147	GS|95.7|BOLD:ACA6220	p:Arthropoda	c:Insecta	o:Orthoptera	f:Tettigoniidae	g:Steiroxys	*s:Steiroxys cf trilineata*
149	GS|97.0|BOLD:AAN6561	p:Arthropoda	c:Collembola	o:Entomobryomorpha	f:Entomobryidae		
152	GS|93.3|BOLD:ABX3926	p:Arthropoda	c:Insecta	o:Hemiptera	f:Cicadellidae	g:Auridius	*s:Auridius auratus*
153	GS|100.0|BOLD:AAG2721	p:Arthropoda	c:Insecta	o:Hemiptera	f:Pentatomidae	*g:Thyanta*	
154	GS|100.0|BOLD:ACE5283	p:Arthropoda	c:Insecta	o:Hemiptera	f:Coreidae	g:Coriomeris	*s:Coriomeris humilis*
156	GS|100.0|BOLD:ACI3068	p:Arthropoda	c:Insecta	o:Hemiptera	f:Cicadellidae	g:Ceratagallia	*s:Ceratagallia siccifolia*
158	GS|99.0|BOLD:ACI7834	p:Arthropoda	c:Insecta	o:Diptera	f:Chironomidae	*g:Cricotopus*	
159	GSL|91.5|BOLD:AAG2875	p:Arthropoda	c:Insecta	o:Hemiptera	f:Cicadellidae	*g:Ceratagallia*	
161	GS|100.0|BOLD:AAZ1109	p:Arthropoda	c:Insecta	o:Coleoptera	f:Tenebrionidae	g:Coniontis	*s:Coniontis ovalis*
163	GS|99.7|BOLD:AAG1503	p:Arthropoda	c:Insecta	o:Diptera	f:Chloropidae	g:Tricimba	*s:Tricimba melancholica*
166	GS|90.7|BOLD:AAZ1768	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Litomiris	*s:Litomiris curtus*
168	GS|99.7|BOLD:ACI4503	p:Arthropoda	c:Insecta	o:Diptera	f:Lonchaeidae		
170	GS|98.8|BOLD:AAA6381	p:Arthropoda	c:Arachnida	o:Araneae	f:Tetragnathidae	g:Tetragnatha	*s:Tetragnatha laboriosa*

171	GS|99.3|BOLD:AAG2721	p:Arthropoda	c:Insecta	o:Hemiptera	f:Pentatomidae	*g:Thyanta*	
172	GS|100.0|BOLD:AAG7284	p:Arthropoda	c:Insecta	o:Diptera	f:Sphaeroceridae	g:Pullimosina	*s:Pullimosina pullula*
173	GS|88.5|BOLD:ACM7148	p:Arthropoda	c:Insecta	o:Coleoptera			
174	GS|87.0|BOLD:ACU2890	p:Arthropoda	c:Insecta	o:Hymenoptera			
176	GS|100.0|BOLD:AAH0256	p:Arthropoda	c:Insecta	o:Coleoptera	f:Latridiidae	g:Corticarina	*s:Corticarina cavicollis*
177	GS|95.2|BOLD:AAF4462	p:Arthropoda	c:Insecta	o:Hemiptera	f:Rhyparochromidae	g:Megalonotus	*s:Megalonotus chiragra*
180	GS|75.0|BOLD:AAN9052	p:Arthropoda	c:Insecta	o:Diptera			
187	GS|99.3|BOLD:AAG6903	p:Arthropoda	c:Insecta	o:Diptera	f:Heleomyzidae	g:Trixoscelis	*s:Trixoscelis fumipennis*
189	GS|100.0|BOLD:AAH4141	p:Arthropoda	c:Insecta	o:Diptera	f:Chloropidae	*g:Neodiplotoxa*	
191	GS|99.3|BOLD:AAC0814	p:Arthropoda	c:Insecta	o:Hemiptera	f:Rhopalidae	g:Stictopleurus	*s:Stictopleurus punctiventris*
193	GS|82.9|BOLD:ADC9253	p:Arthropoda	c:Insecta	o:Hemiptera	f:Cicadellidae	*g:Ceratagallia*	
197	GS|99.6|BOLD:ADL3427	p:Arthropoda	c:Insecta	o:Hemiptera	f:Delphacidae	g:Javesella	*s:Javesella atrata*
198	GS|96.7|BOLD:ACY9729	p:Arthropoda	c:Insecta	o:Coleoptera	f:Tenebrionidae	g:Eleodes	*s:Eleodes vandykei*
203	GS|100.0|BOLD:AAG8800	p:Arthropoda	c:Insecta	o:Hemiptera	f:Rhyparochromidae	g:Trapezonotus	*s:Trapezonotus arenarius*

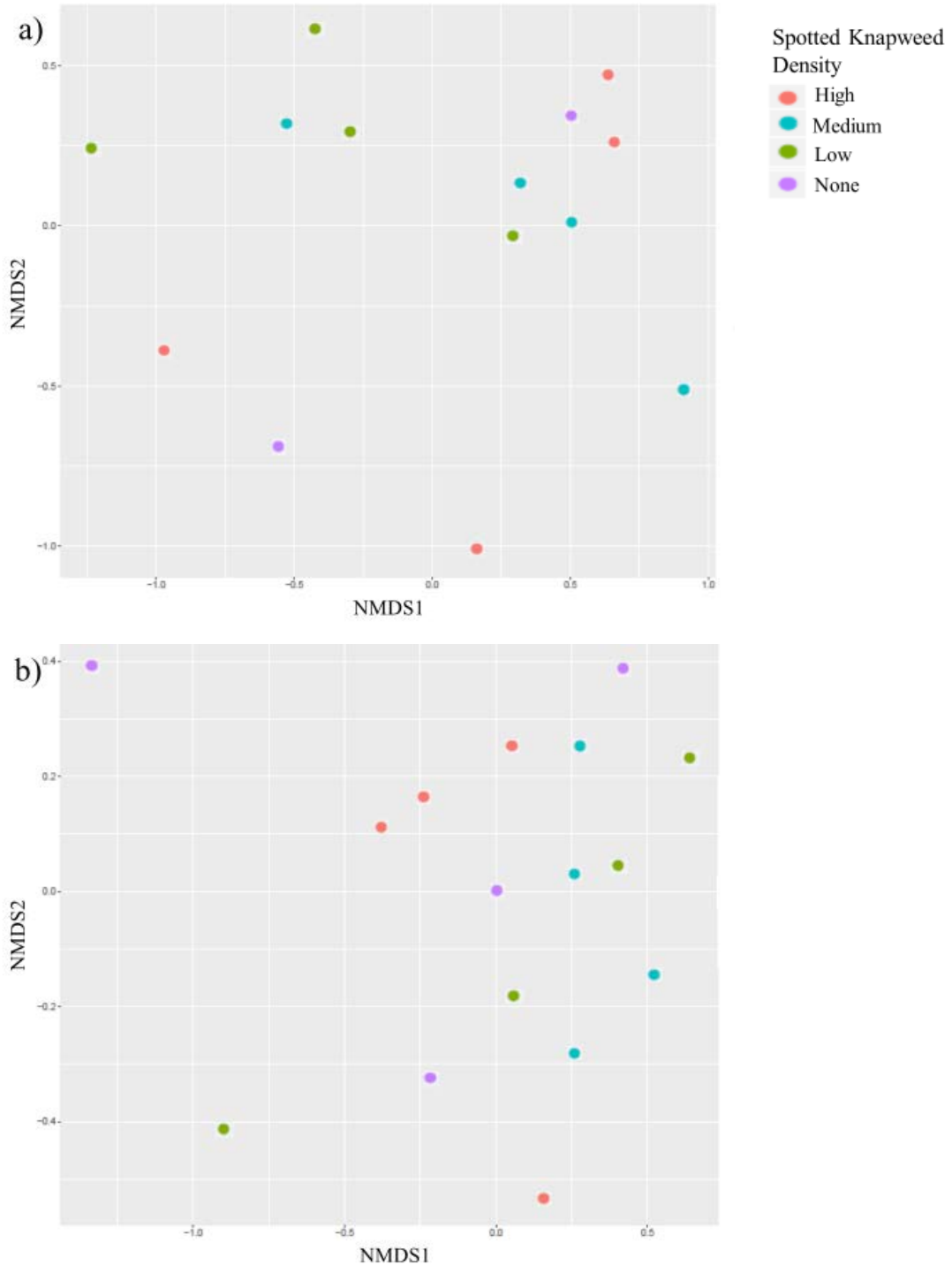
204	GS|96.0|BOLD:AAG8943	p:Arthropoda	c:Insecta	o:Hemiptera	f:Pentatomidae	g:Chlorochroa	*s:Chlorochroa uhleri*
205	GS|100.0|BOLD:AAG7275	p:Arthropoda	c:Insecta	o:Diptera	f:Sphaeroceridae	*g:Pullimosina*	
206	GS|96.3|BOLD:ACL5084	p:Arthropoda	c:Insecta	o:Orthoptera	f:Tettigoniidae	*g:Steiroxys*	
218	GSL|84.4|BOLD:AAB4425	p:Arthropoda	c:Insecta	o:Orthoptera	f:Acrididae	*g:Aeropedellus*	
221	GS|96.0|BOLD:AAD7721	p:Arthropoda	c:Insecta	o:Orthoptera	f:Acrididae	g:Arphia	*s:Arphia conspersa*
222	GS|97.9|BOLD:AAQ0072	p:Arthropoda	c:Insecta	o:Coleoptera	f:Elateridae	g:Selatosomus	*s:Selatosomus semimetallicus*
223	GS|96.3|BOLD:ACR7501	p:Arthropoda	c:Insecta	o:Coleoptera	f:Carabidae	g:Calosoma	*s:Calosoma wilkesii*
232	GS|81.6|BOLD:AAG6186	p:Arthropoda	c:Insecta	o:Archaeognatha			
237	GS|70.6|BOLD:ADK0436	p:Arthropoda	c:Insecta	o:Lepidoptera	f:Geometridae	g:Elophos	*s:Elophos caelibaria*
239	GS|96.7|BOLD:AAZ1768	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Litomiris	*s:Litomiris curtus*
241	GS|100.0|BOLD:AAB8143	p:Arthropoda	c:Insecta	o:Hemiptera	f:Nabidae	g:Nabis	*s:Nabis americoferus*
252	GS|97.0|BOLD:AAG5198	p:Arthropoda	c:Insecta	o:Coleoptera	f:Curculionidae	g:Otiorhynchus	*s:Otiorhynchus ovatus*
253	GS|99.7|BOLD:AAV4063	p:Arthropoda	c:Insecta	o:Diptera	f:Ephydridae	g:Philygria	*s:Philygria punctatonervosa*
254	GS|95.3|BOLD:ACL5084	p:Arthropoda	c:Insecta	o:Orthoptera	f:Tettigoniidae	*g:Steiroxys*	

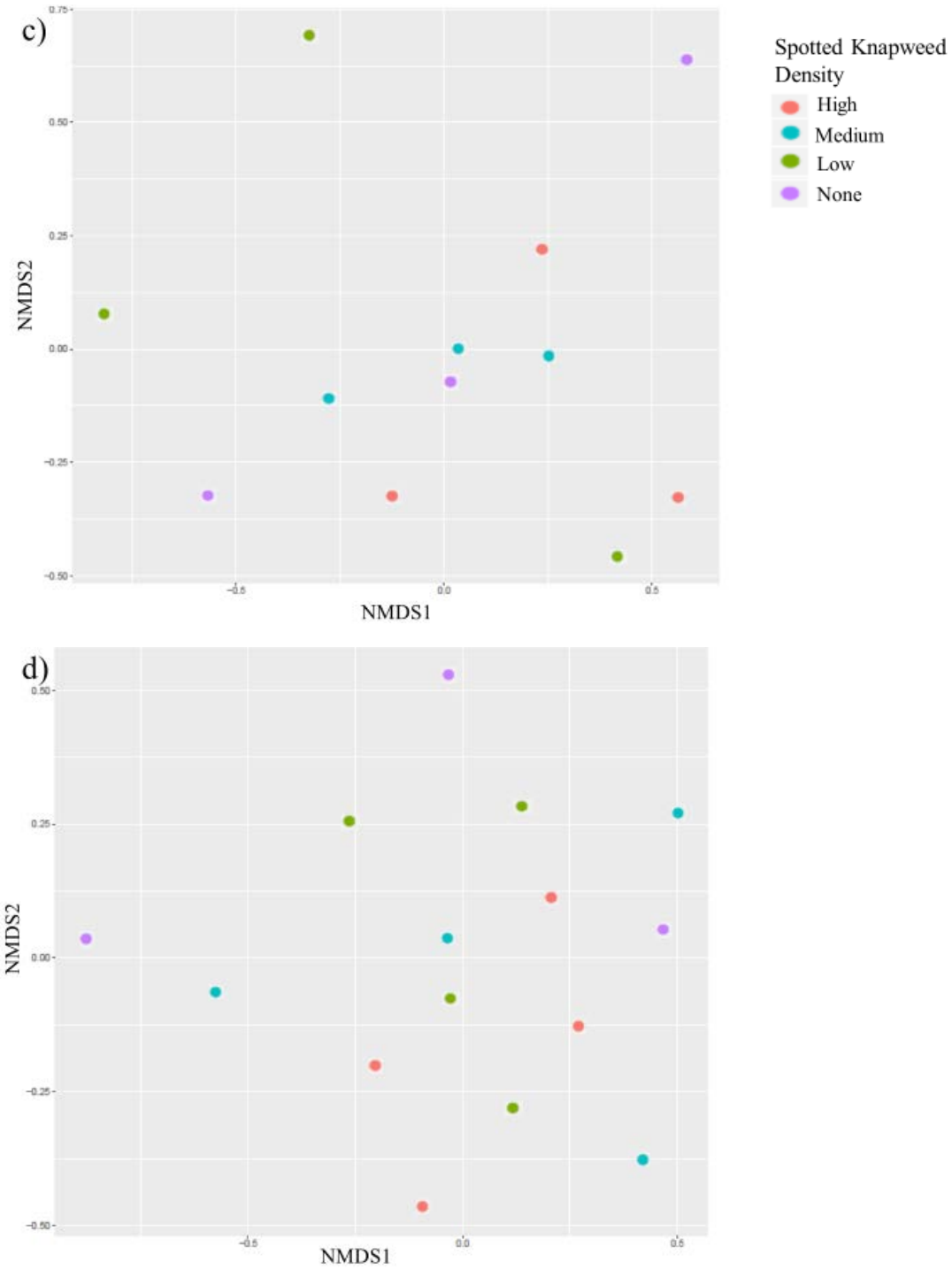
259	GS|100.0|BOLD:AAJ2923	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Labopidea	*s:Labopidea lenensis*
261	GS|100.0|BOLD:AAH4227	p:Arthropoda	c:Insecta	o:Diptera	f:Heleomyzidae		
265	GS|88.1|BOLD:AAC5779	p:Arthropoda	c:Insecta	o:Orthoptera	f:Acrididae	g:Gomphocerippus	*s:Gomphocerippus rufus*
267	GS|97.3|BOLD:ABX3925	p:Arthropoda	c:Insecta	o:Hemiptera	f:Cicadellidae	g:Auridius	*s:Auridius aurigineus*
268	GS|83.1|BOLD:AAC5779	p:Arthropoda	c:Insecta	o:Orthoptera	f:Acrididae	g:Gomphocerippus	*s:Gomphocerippus rufus*
271	GS|90.7|BOLD:ACL5088	p:Arthropoda	c:Insecta	o:Orthoptera	f:Tettigoniidae	*g:Steiroxys*	
279	GS|96.3|BOLD:AAG0666	p:Arthropoda	c:Insecta	o:Orthoptera	f:Gryllidae	g:Gryllus	*s:Gryllus veletis*
288	GS|100.0|BOLD:AAA5299	p:Arthropoda	c:Insecta	o:Diptera	f:Chironomidae	g:Cricotopus	*s:Cricotopus sylvestris*
291	GS|91.7|BOLD:AAA8764	p:Arthropoda	c:Insecta	o:Orthoptera	f:Acrididae	g:Camnula	*s:Camnula pellucida*
293	GS|98.7|BOLD:AAG0666	p:Arthropoda	c:Insecta	o:Orthoptera	f:Gryllidae	g:Gryllus	*s:Gryllus veletis*
297	GS|99.3|BOLD:AAA5308	p:Arthropoda	c:Insecta	o:Diptera	f:Chironomidae	*g:Cricotopus*	
300	GS|99.7|BOLD:AAG6822	p:Arthropoda	c:Insecta	o:Diptera	f:Muscidae	*g:Schoenomyza*	
301	GS|97.7|BOLD:AAF4462	p:Arthropoda	c:Insecta	o:Hemiptera	f:Rhyparochromidae	g:Megalonotus	*s:Megalonotus chiragra*
304	GS|81.3|BOLD:ACC0275	p:Arthropoda	c:Insecta	o:Diptera	f:Mycetophilidae	g:Cordyla	

309	GS|99.7|BOLD:AAC4369	p:Arthropoda	c:Insecta	o:Hemiptera	f:Pentatomidae	g:Aelia	*s:Aelia americana*
315	GS|95.0|BOLD:AAA8764	p:Arthropoda	c:Insecta	o:Orthoptera	f:Acrididae	g:Camnula	*s:Camnula pellucida*
320	GS|90.7|BOLD:AAC5779	p:Arthropoda	c:Insecta	o:Orthoptera	f:Acrididae	g:Gomphocerippus	*s:Gomphocerippus rufus*
326	GS|93.3|BOLD:AAA8278	p:Arthropoda	c:Insecta	o:Hemiptera	f:Scutelleridae	g:Homaemus	*s:Homaemus aeneifrons*
341	GS|100.0|BOLD:ACC2700	p:Arthropoda	c:Insecta	o:Diptera	f:Phoridae	*g:Megaselia*	
344	GS|99.4|BOLD:AAC6116	p:Arthropoda	c:Insecta	o:Hemiptera	f:Scutelleridae	g:Homaemus	*s:Homaemus bijugis*
352	GS|93.3|BOLD:ACL5084	p:Arthropoda	c:Insecta	o:Orthoptera	f:Tettigoniidae	*g:Steiroxys*	
354	GS|95.3|BOLD:AAC6116	p:Arthropoda	c:Insecta	o:Hemiptera	f:Scutelleridae	g:Homaemus	*s:Homaemus bijugis*
355	GS|99.7|BOLD:ABX3968	p:Arthropoda	c:Insecta	o:Hemiptera	f:Cicadellidae	g:Orocastus	*s:Orocastus tener*
379	GS|98.0|BOLD:ABZ1405	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Lygus	*s:Lygus borealis*
381	GS|84.0|BOLD:ABY2035	p:Arthropoda	c:Arachnida	o:Mesostigmata	f:Parasitidae		
382	GS|99.7|BOLD:ACK6348	p:Arthropoda	c:Insecta	o:Hemiptera	f:Pentatomidae	g:Holcostethus	*s:Holcostethus limbolarius*
384	GS|90.0|BOLD:AAE3943	p:Arthropoda	c:Insecta	o:Hemiptera	f:Miridae	g:Plagiognathus	*s:Plagiognathus chrysanthemi*
388	GS|92.0|BOLD:AAC5779	p:Arthropoda	c:Insecta	o:Orthoptera	f:Acrididae	g:Gomphocerippus	*s:Gomphocerippus rufus*

391	GS|99.3|BOLD:AAC6116	p:Arthropoda	c:Insecta	o:Hemiptera	f:Scutelleridae	g:Homaemus	*s:Homaemus bijugis*
396	GS|91.0|BOLD:ACA6391	p:Arthropoda	c:Insecta	o:Orthoptera	f:Tettigoniidae	g:Anabrus	*s:Anabrus simplex*
399	GS|80.7|BOLD:AAG6186	p:Arthropoda	c:Insecta	o:Archaeognatha			
410	GS|96.7|BOLD:ACL5084	p:Arthropoda	c:Insecta	o:Orthoptera	f:Tettigoniidae	*g:Steiroxys*	
411	GS|85.8|BOLD:ABX2762	p:Arthropoda	c:Insecta	o:Orthoptera	f:Acrididae	g:Stenobothrus	*s:Stenobothrus stigmaticus*
416	GS|99.3|BOLD:ACE5823	p:Arthropoda	c:Insecta	o:Orthoptera	f:Acrididae	g:Chorthippus	*s:Chorthippus curtipennis*

Appendix H. *Ordination (nMDS) plot illustrating the similarities and differences in OTU composition across four spotted knapweed density categories (high, medium, low, and none) and across each month of sampling a) May, b) June, c) July, and d) August, n=56.*




c)
NMDS2
NMDS1
d)
NMDS2
NMDS1
Spotted Knapweed Density
High
Medium
Low
None