GUT BACTERIAL COMMUNITIES IN CARABID BEETLES: HOST TAXONOMY, EXTRINSIC FACTORS, AND FEEDING HABITS

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Abstract

Bacteria in animal guts (= the gut bacterial community, GBC) can be crucial to host survival and physiology. These gut bacteria may aid in digestion, synthesis of nutrients, or protection from pathogens. To fully understand the biology of the host, it is therefore necessary to study their gut bacteria. Ground beetles, or carabids (Coleoptera: Carabidae), are a diverse family of insects with ~40,000 species, 2,700 of which are in North America. Ground beetles commonly use their voracious appetites to feed on many agricultural pests and are found in many different habitats. The diversity of carabids plus the ease with which many of them can be collected allows for an examination of the effects of host taxonomy, extrinsic factors, and feeding habits on the GBC. Few studies have examined how these factors affect GBCs. Here, I characterized the GBC of nine carabid subfamilies, 23 genera, and 47 species from various natural settings. First, I used genetic relatedness of the host at three taxonomic levels to assess how host taxonomy relates to GBC diversity. I then examined how GBC diversity varies across habitat, geographic region, season, or years, as well as feeding habits of cropland carabids in Alberta. The results showed that GBCs can differ depending on all those factors, especially host taxonomy and feeding habit. The diversity among some congeneric carabids was especially distinct. As is consistent with previous studies, the two predominate bacterial phyla were Pseudomonadota and Bacillota. The three most common bacterial genera across carabids studied here were *Enterococcus*, *Gilliamella*, and an unidentified member of Yersiniaceae. This study also found evidence of core gut bacteria and endosymbiotic bacteria for some carabid groups examined.

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List of Abbreviations

AB	Alberta
ANCOM-BC	Analysis of Composition with Bias Correction
ASV	Amplicon Sequence Variant
COI	Cytochrome Oxidase Subunit I
DA	Differential Abundance
DNA	Deoxyribonucleic Acid
GBC	Gut Bacterial Community
GMB	Gut Microbiome
GQ	Genome Quebec
K-W	Kruskal-Wallis
LAB	Lactic Acid Bacteria
MT	Montana
mtDNA	Mitochondrial DNA
n	Sample Size
NGS	Next Generation Sequencing
p	P-value
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PE	Paired End
PERMANOVA	Permutational Multivariate Analysis of Variance
q	Q-value
RA	Relative Abundance
rDNA	Ribosomal DNA
rRNA	Ribosomal RNA
SSU	Small Subunit
V1-V9	Gene Region of 16S rDNA

Chapter 1: General Introduction

1.1 Introduction

Multicellular organisms (e.g., plants, animals, mushrooms) form associations with communities of microscopic bacteria, archaea, fungi, protists, or viruses. These various microbes are collectively termed a microbiome. Some of the most profound relationships known in the animal kingdom are between insects and the associated bacterial communities in their microbiomes. For instance, *Wolbachia* infects the cells of approximately 66% of all insect species and can determine the host's success at producing offspring (Hilgenboecker et al., 2008). An insect's bacterial community can be crucial to the health and reproduction of the host, such that to fully understand the biology of the host, we need to consider its bacterial communities. This idea is captured by the holobiont concept, which states that a host and the associated microbial community living in or on it can be viewed as a single evolutionary unit (Pita et al., 2018). The holobiont concept relies heavily upon microbial lineages staying the same as they are transferred from parent to offspring. An extension of the holobiont concept is the hologenome concept, which states that a host's genome functions are intertwined with the genome functions of its microbial community (Rosenberg & Zilber-Rosenberg, 2018). An example of the hologenome concept is when honey bees *Apis* (Hymenoptera: Apidae) cannot acquire crucial amino acids from their nutritionally limited food sources and therefore rely on the genes of their gut bacteria to synthesize them (Zhang et al., 2022).

1.2 Location and Transmission

Members of a host’s bacterial community may be present within its cells and body tissues (endosymbionts), within the contents of the gut lumen (exosymbionts), or on its outer surface (ectosymbionts). Although the gut lumen is within the body of the host its contents are not considered part of body. Cellular endosymbionts are the most intimately associated components of a host’s microbiome. They are usually acquired maternally through transovarial transmission when the intracellular bacteria infect eggs forming within the host (Durden et al., 2019; Porter & Sullivan, 2023). Exosymbionts and ectosymbionts are acquired either through exposure and recruitment from the environment or through application of bacteria directly to the host’s eggs upon deposition (Geerinck et al., 2022; Grond et al., 2017; Janke et al., 2022; Liu et al., 2019; Ohbayashi et al., 2015).

The composition of the bacterial community can be similar from one generation to the next within a host species due to horizontal recruitment from a similar environment or vertical inheritance from a parent (Robinson et al., 2019; Roughgarden, 2020). Recruitment from the environment involves providing the proper environment/ecosystem for bacteria that are likely at low relative abundances (RA) when not associated with a host-provided ecosystem. Once they reach the host-provided ecosystem, the bacteria proliferate rapidly (Jin et al., 2022; Unzueta-Martínez et al., 2022). Horizontal recruitment only keeps host bacterial communities similar across generations if the needed microbes are present in the host’s environment. Vertical inheritance from a parent allows bacteria to evolve with their host and can keep microbiomes very similar across generations.

1.3 Role of GBC

Of particular relevance within the context of this thesis are bacterial communities within the gut lumen of the host, hereafter referred to as the gut bacterial community (GBC). Bacteria are the major constituent of nearly all gut microbiomes (GMB) (Dias et al., 2020). Some of these bacteria aid in digestion, immune functions, behavior, thermal tolerance, and more (Douglas, 2022). The benefit of the GBC to the host becomes very clear when that animal feeds on nutrient-poor foods such as animal blood or plant sap (Douglas, 2022). For example, to survive on ingested blood, females of the mosquito *Aedes aegypti* (Diptera: Culicidae) require gut bacteria that synthesize B vitamins (Duron & Gottlieb, 2020). Many herbivores and detritivores require digestive help from their GBC. For instance, the termite *Psammotermes hypostoma* (Blattodea: Termitidae) uses wood-degrading enzymes produced by gut bacteria to break down complex polysaccharides into digestible molecules (Maurice & Erdei, 2018). Components of the GBC may also produce antimicrobial agents or other compounds to help protect the host from pathogens and pesticides (Davoodabadi et al., 2015; de Almeida et al., 2017). The gut bacterium *Gilliamella*, which is common in honey bees (*Apis mellifera*), shows distinct resistance to the bacterial pathogen *Melissococcus plutonius* (Lang et al., 2023). Pathogenic microbes can also have a hard time colonizing a gut if it is already colonized by bacteria that compete effectively for resources (O'Loughlin et al., 2015). In those cases, the application of antimicrobial agents gives pathogens an opportunity to colonize as it removes existing microbes. In recent years, researchers have found extensive networks of nerves and neurons that transmit signals between the gut and the brain (Hoffman & Lumpkin, 2018) and the presence of certain bacteria and their associated metabolites can influence the gut-brain signaling (O'Riordan et al., 2022).

1.4 Advances in Research

Prior to advances in genetic sequencing that started about 20 years ago, the methods for examining bacterial species and their relative abundances (RA) in a gut lumen sample were limited to viewing samples under a microscope, growing lab cultures, and testing for the presence of specific proteins or metabolites (Escobar-Zepeda et al., 2015). Viewing samples with a microscope can be useful for protozoa (avg. size 5.0 µm to 1.0 mm), but much less useful for bacteria (avg. size 0.2 to 2.0 µm). Growing lab cultures from a gut lumen sample is ineffective if the specific conditions required for the growth of each bacterial type are not present. Technological advances in the early 2000s allowed us to use next generation sequencing (NGS) (Fig. 1.1), which is a culture-independent method that provides a more complete view of bacterial taxonomy and their relative abundances in a sample through parallel sequencing. At first, NGS was too expensive for most large-scale studies, but starting in about 2012, NGS became much less expensive (Escobar-Zepeda et al., 2015) and is now more accessible. One type of NGS uses a platform called Illumina MiSeq.

NGS on the Illumina MiSeq platform consists of four main steps (Fig. 1.1). Step 1 is sample preparation in which template DNA is extracted from the sample. Step 2 involves the amplification of the DNA to generate thousands to millions of short (150-350 base pairs) DNA sequences called reads. Specific primers can be used to target either specific genes or parts of the genome for amplification instead of the whole genome. In step 3, sequences are recorded by measuring fluorescence of each nucleotide as it is attached to the growing sequence. In step 4, a bioinformatic process is used to quality filter and then align the reads to publicly available databases of genetic sequences to discover the identity and relative abundance of organisms. For example, to identify bacterial species in a sample, the hypervariable 16S rRNA gene regions flanked by conserved regions can be sequenced (Bukin et al., 2019; Zhang et al., 2018). The V4 region is currently used by researchers studying prokaryotes, but there are eight other partial gene regions (V1-V9) that also have been used, depending on the prokaryotic species (Bukin et al., 2019; Zhang et al., 2018).

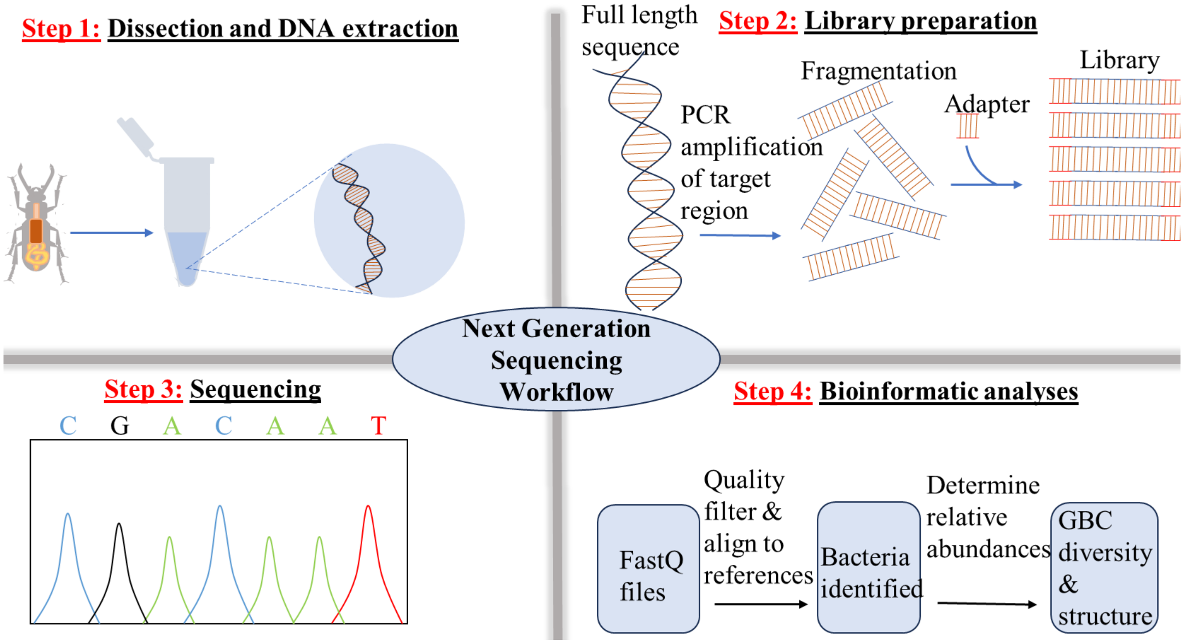


Figure 1.1. Steps of NGS on Insect Gut Samples: Next generation sequencing (NGS) on an Illumina MiSeq platform to analyze the gut bacterial community in insects consists of four main steps. Step 1) insect mid and hind guts are dissected out and DNA is extracted from them. Step 2) after polymerase chain reaction (PCR) amplification of gene region of interest, DNA is fragmented. Adapters are added to the fragments to allow them to attach to Illumina sequencing lanes and a library of reads (fragments with adapters) is formed. Step 3) Fluorescence of each nucleotide is measured as a way of recording sequence information. Step 4) sequences and their quality levels are stored in FastQ files which are then quality filtered, assigned a bacterial taxon, and statistically analyzed to understand GBC diversity and structure.

1.5 Insects as GBC Study Subjects

We now have the ability to study GBCs of many animals with large numbers of samples. Insects are an excellent choice for a large GBC study because: 1) they occupy almost all terrestrial and freshwater habitats, 2) they are very speciose, with over 1 million described species, 3) they are involved in many important ecological functions, and 4) we can use them as model study systems for animals that are harder to collect or rear in a lab (Jenkins, 2020; Jones et al., 2013; Rainio & Niemela, 2003). In addition to some GBC functions that are found throughout the animal kingdom, insects also have their own unique relationships with bacteria (Gupta & Nair, 2020). For instance, bacteria in the hemolymph of some insects confer protection against parasitism (Blow & Douglas, 2019). Some insects have specialized cells called bacteriocytes for housing and controlling their symbiotic bacteria (Blow & Douglas, 2019). Cellular endosymbionts are more common in insects than any other group of organisms and researchers are trying to understand how infection by endosymbionts such as *Wolbachia* may confound insect microbiome analyses or affect the insect GBC itself (Wilches et al., 2021).

Although recent technological advances have allowed for an increase in bacterial taxa identification accuracy and scope, research on insect GBCs is not new. Most insect groups studied involve human health and agriculture. In agriculture, honey bees (Hymenoptera: Apidae: *Apis* spp.) have received the most attention as pollinators of crops, but other insects have received attention because they are agricultural pests (de Almeida et al., 2017; Zhang et al., 2022). On the subject of human health, primarily mosquitos but also other insects have been studied to help develop control measures related to human diseases (Douglas, 2022). To study human GBCs, the GBCs of vinegar flies *Drosophila melanogaster* (Diptera: Drosophilidae) have been used as model systems (Ji *et al.*, 2022).

The most common bacterial phyla found in insect GBCs are: Pseudomonadota, Bacillota, Actinomycetota, Bacteroidota, and Mycoplasmatota (Douglas, 2022; Oren & Garrity, 2021). Of those phyla, Pseudomonadota (γ-Proteobacteria and α-Proteobacteria) and Bacillota (Lactobacillaceae and *Clostridium*) is usually the most abundant (Douglas, 2022). Several studies have investigated how extrinsic characteristics of the host's environment or the intrinsic characteristics of host itself (e.g., host physiology) may affect the composition of GBCs (Rocha et al., 2022). Characteristics of the host insect's environment that may influence GBCs include habitat, season, geographical region, or year-to-year changes. In addition, other characteristics of the host may influence GBCs including evolutionary relationships (e.g., genetic distance or taxonomic groups) and feeding habits (e.g., carnivore or herbivore).

1.6 Extrinsic Factors Affecting GBC

Horizontal transmission of GBC components requires recruitment from the surrounding environment. Different environments have different types of microbes (Bahram et al., 2018; Chowdhury et al., 2019). The GBC is often dynamic in that some insects are able to lose an important bacterial species from their GBC and recruit a different microbial species from the environment to fill the same role (Koga et al., 2022). Thus, members of the same host species may have a different GBC depending on their environment.

Several studies on diverse groups of insects have shown that habitat affects GBC diversity, but not as much as feeding habit or host species (Do et al., 2022; Karimian et al., 2022; Kudo et al., 2019; Neupane & Nayduch, 2022; Rocha et al., 2022; Tiede et al., 2017). Environmental habitat was found to be a significant factor in GBC composition in a study that included 218 species from across 21 insect orders (Yun et al., 2014). The environmental habitats were divided into sky (aerial), ground (terrestrial), underground (subterranean), and aquatic. The GBC differences found when comparing environmental habitats were most apparent when looking at ratios of aerobic, anaerobic, and facultatively anaerobic bacteria. Aerial insects had the highest relative abundances of aerobes while subterranean and aquatic insects had the highest relative abundances of anaerobes (Yun et al., 2014). Because many insect species are only found in one or a few habitats, it can be difficult to investigate natural populations of the same species found in different habitats. We can however much more easily see if all insects from a specific location (e.g., a coniferous forest) have similar bacterial constituents while controlling for other factors such as season, feeding habits, or geographic region.

Temporal differences such as seasonal change or year-to-year changes may affect GBC composition due to differences in temperature, food item availability, and precipitation. Growth and replication rates of bacteria are usually closely related to temperature. Insects are exothermic and their GBCs are likely affected by temperature changes in the environment (Li et al., 2023). For example, *Escherichia coli* only grows and replicates at temperatures between 23° and 40°C (Kumar & Libchaber, 2013). A study of the GBC of the pea aphid, *Acrythosiphon pisum* (Hemipteran: Aphididae), showed that temperature was the best indicator for the presence of certain bacterial symbionts (Smith et al., 2021). Seasonality is also important. Crickets, *Gryllus veletis* (Orthoptera: Gryllidae), have seasonal shifts in their GBC composition that corresponded with overwintering and immune system changes (Ferguson et al., 2018). A study of the GBC of the house fly, *Musca domestica* (Diptera: Muscidae), found significant differences between the time of year that the samples were collected and the abundance of *Bacillus, Lactobacillus*, unclassified *Lactococcus*, *Peptostreptococcus*, and Actinomycetota (Neupane & Nayduch, 2022). Precipitation is also important as soil microbiomes are impacted heavily by drought conditions (Wang & Allison, 2021), which may directly or indirectly affect animal-associated microbiomes (Williams et al., 2022). An insect abundance study conducted over an eighteen-year period showed that population sizes of insects can fluctuate by eight-fold from year to year (Owen & Owen, 1990). With the quantities of some insects fluctuating so much from year to year, it is likely to cause variation in both competition and predator/prey dynamics that in turn affect the abundance and type of food that is available for an insect. The type and abundance of food items a host eats may control what species of bacteria a host is exposed to.

Even if the habitats where two insects are collected are essentially the same (e.g., riverbank), the habitats may have differences that correspond with the geographical region where they are found. A study of the leafhopper *Psammotettix alienus* (Hemiptera: Cicadidae) in wheat fields of China showed differences in GBC composition depending on which region (highlands or lowlands) of the country they occurred (Wang et al., 2019). A study of freshwater insect GBCs across North America found that GBCs had more similarities associated with geographic region than host taxonomy (Ayayee et al., 2022).

1.7 Intrinsic Factors

Intrinsic characteristics of the insect host can influence GBC composition and include evolutionary relationships and feeding habits. Given that members of the GBC can be vertically transmitted from generation to generation via egg cytoplasm, anal smearing, or other methods, arguably the GBCs may be more similar among insect species that share more recent evolutionary histories. Also, an insect's digestive system can have physiological differences (e.g., pH) that aid in the digestion of different food items and provide optimal conditions to harbor specific beneficial microbes. If other factors are controlled for, then there is a likelihood that GBC diversity patterns will correlate with feeding habits (e.g., herbivores or carnivores). Studies of how GBC diversity relates to feeding habit have concluded that it is a strong determinant of GBC composition (Kolasa et al., 2019; Kudo et al., 2019; Yun et al., 2014).

Another factor affecting GBC is the life cycle of the host. An insect may have different GBC needs depending on its life stage. For example, Shukla et al. (2016) showed that cellulose degrading bacteria are only required in the larval stage of some dung beetles (*Euoniticellus intermedius* and *E. triangulatus*) and not in the adult stage. The bacteria do however have to persist in the females of the species so they can be passed down to offspring through anal smearing on the egg cases upon deposition. As a result, some important bacteria may only be present within adult females but not adult males leading to sexual dimorphism of GBCs in some species (Shukla et al., 2016).

1.8 Carabids for GBC Study

For my MSc thesis, I focus on the GBCs of ground beetles or carabids (Coleoptera: Carabidae). There are about 40,000 carabid species worldwide (Lovei et al., 1996) with about 2500 of those species in North America (Larochelle & Larivière, 2003). They are almost always beneficial to ecosystems and are natural enemies of pest species (Larochelle, 1990) and some have even been imported into North America as biological control agents (Weseloh, 1985). Most carabids are predatory or granivorous, but a few are detritivores, parasitoids, or scavengers (Larochelle, 1990). The beneficial effects of carabids are most evident in agroecosystems where they eat close to their own body weight in food every day during active seasons (Hill et al., 2017; Satpathi, 2021). Many carabid species do not have wings (Venn, 2016) and species that are capable of flight may only fly a couple of times during their lives. This limited mobility can restrict them to a relatively small habitat and make them relatively easy to catch by hand or with pitfall traps. Multiple aspects of carabid biology and ecology have been well studied, but little is known about the diversity of their GBCs.

Of the relatively few studies that have examined the GBC diversity in carabid beetles, results vary with the species studied, methods, and sample sizes (Table 1.1). In their comparison across different families of beetles, Kolasa et al. (2019) characterized the microbiome of carabids based on only four species from the genus *Bembidion*. They concluded that the GBCs of carabids have high diversity warranting further study. In a study of 27 carabid species from 12 genera, Kudo et al. (2019) found that feeding habit was a stronger determinant of GBC composition than habitat, however, the GBCs of carabids did differ significantly between forest and grassland habitats (Kudo et al., 2019).

One limitation with Kudo et al. (2019) is that 17 of the 27 species examined only contained three or less specimens and may not have captured the GBC diversity within each species. Silver et al. (2021) examined the effect of diet manipulation for three species of carabids (two carnivores, one granivore) in different genera. Their results showed that GBCs were resistant to changes in diet, with the bacterial genus *Enterococcus* significantly more abundant in the two carnivorous species than in the granivorous species. Gut bacterial communities were more similar within beetle species than within diet manipulation types (Silver et al., 2021).

Table 1.1. Summary of main findings of previous research on carabid gut bacterial communities (GBC). Sample sizes are shown after each species.

Carabidae species	Main findings	Reference
***Duvalius muieri* (n = 1) and *D. lesu* (n = 9)**	Cave-dwelling carabids have unique GBC diversity not reported in other studies.	Moldovan, et al. (2023)
***Chlaenius pallipes* (n = 32)**	High GBC diversity between individuals of the same carabid species. Habitat had significant effects on GBC.	Do, et al. (2022)
***Pterostichus melas italicus* (n = 30)**	Herbicide (pendimethalin) exposure altered GBC.	Giglio, et al. (2021)
***Harpalus pensylvanicus* (n = 4) and *Anisodactylus sanctaecrucis* (n = 6)**	GBCs had low diversity. Each carabid species had a unique GBC composition.	Lundgren, et al. (2007)
***Brachinus elongatulus* (n = 37)**	*Dysgonomonas* may have a role in defensive chemical production.	McManus, et al. (2018)
27 species (n = 116)	GBC diversity varied significantly among feeding habits and habitats. Some patterns in the GBC coincided with fungi in the gut.	Kudo, et al. (2019)
***Poecilus chalcites* (n = 58)**	Majority of bacterial taxa also detected in other insect GBC studies. Lab reared beetles had fewer bacterial taxa.	Lehman, et al. (2009)
***Harpalus pensylvanicus* (n = 75)**	Beetles fed antibiotics consumed fewer seeds, beetles with *Enterococcus faecalis* in their GBC consumed more seeds.	Lundgren and Lehman (2010)
***Pseudoophonus rufipes* (n = 29)**	GBC diversity varied between conventional and organic cropping systems.	Magagnoli, et al. (2022)
***Anisodactylus similis, Pterostichus serripes,* and *Brachinus elongatulus* (n = 36)**	GBCs were more similar within beetle species than within diet manipulation treatments.	Silver, et al. (2021)
***Bembidion decorum, B. modestum, B. punctulatum,* and *B. varicolor* (n = 39)**	The carabids used had different major microbiome components than other beetle families.	Kolasa, et al. (2019)
***Calosoma peregrinator* (n = 3)**	Host taxonomy is a strong determinant of GBC composition, but feeding habit can be stronger depending on which feeding habit.	Colman, et al. (2012)
***Harpalus pensylvanicus* (n = 1)**	Metagenomic bacterial data are consistent with studies that used metagenetic bacterial data.	Kieran (2020)

1.9 Research Objectives

My research expanded on the existing knowledge of carabid GMBs, specifically the bacterial community, and examined some of the factors that may affect GBC diversity. To address some limitations of previous studies and apply novel approaches, I used larger sample sizes (on average) and a greater diversity of taxonomic groups within the family Carabidae. I used an updated bacterial sequence database (2019) to allow for more taxonomic resolution of bacteria. I also collected beetles from a larger variety of habitats and some that had not been studied well from a GBC perspective (e.g., more habitats (including cropland), years, and regions). In Chapter 2, I used 47 carabid species from 23 genera in nine subfamilies to test the hypothesis that GBC composition would be most similar among closely related beetles (e.g., species in the same genus). In Chapter 3, I examined how spatial and temporal differences in carabid sample collection related to GBC diversity. Spatial comparisons looked at the host's habitat and geographical location. Temporal differences examined year-to-year and seasonal changes in GBCs. I also looked for patterns related to feeding habits of the hosts. In both chapters 2 and 3 I attempted to identify core members of the carabid GBC. Chapter 4 summarized key findings and placed them into a broader context and discussed future research directions.

1.10 References

Ayayee PA, Wesner JS & Ouellette SP.(2022) Geography, taxonomy, and ecological guild: Factors impacting freshwater macroinvertebrate gut microbiomes. *Ecology and Evolution*, **12**(12), e9663.

Bahram M, Hildebrand F, Forslund SK, Anderson JJ, Soudzilovskaia NA, Bodegom PM, *et al*. (2018) Structure and function of the global topsoil microbiome. *Nature* (London), **560**(7717), 233-237.

Blow F & Douglas AE (2019) The hemolymph microbiome of insects. *Journal of Insect Physiology*, **115**, 33-39.

Bukin YS, Galachyants YP, Morozov IV, Bukin SV, Zakharenko AS & Zemskaya, TI (2019) The effect of 16S rRNA region choice on bacterial community metabarcoding results. *Scientific Data*, **6**(1), 190007.

Chowdhury TR, Lee J-Y, Bottos EM, Brislawn CJ, White III RA, Bramer, LM, *et al*. (2019) Metaphenomic responses of a native prairie soil microbiome to moisture perturbations. *mSystems*, **4**(4), e00061-19.

Colman DR, Toolson EC, & Takacs-Vesbach CD. (2012) Do diet and taxonomy influence insect gut bacterial communities? *Molecular Ecology*, **21**(20), 5124-5137.

Davoodabadi A, Dallal MMS, Foroushani AR, Douraghi M, Yazdi MKS & Harati, FA (2015) Antibacterial activity of *Lactobacillus spp.* isolated from the feces of healthy infants against enteropathogenic bacteria. *Anaerobe*, **34**, 53-58.

De Almeida LG, Beraldo De Moraes LA, Trigo JR, Omoto C & Consoli FL (2017) The gut microbiota of insecticide-resistant insects houses insecticide-degrading bacteria: A potential source for biotechnological exploitation. *PloS One,* **12,** e0174754.

Dias AM, Cordeiro G, Estevinho MM, Veiga R, Figueira L, Reina-Couto M*, et al.* (2020) Gut bacterial microbiome composition and statin intake—A systematic review. *Pharmacology Research & Perspectives,* **8,** e00601.

Do Y, Park J-K, Park W-B & Kim M-S (2022) The gut bacterial community of *Chlaenius pallipes* (Coleoptera: Carabidae) associates with their habitat and morphology. *Insects*, **13,** 1099.

Douglas AE (2022) Insects and Their Beneficial Microbes. Princeton, NJ, USA. Princeton University Press.

Durden L, Wang D, Panaccione D & Clay K (2019) Decreased root-knot nematode gall formation in roots of the morning glory *Ipomoea tricolor* symbiotic with ergot alkaloid-producing fungal *Periglandula* sp. *Journal of Chemical Ecology*, **45,** 879-887.

Duron O & Gottlieb Y (2020) Convergence of nutritional symbioses in obligate blood feeders. *Trends in Parasitology*, **36**(10), 816-825.

Escobar-Zepeda A, Vera-Ponce De León A & Sanchez-Flores A (2015) The road to metagenomics: from microbiology to DNA sequencing technologies and bioinformatics. *Frontiers in Genetics,* **6,** 348.

Ferguson LV, Dhakal P, Lebenzon JE, Heinrichs DE, Bucking C & Sinclair BJ (2018) Seasonal shifts in the insect gut microbiome are concurrent with changes in cold tolerance and immunity. *Functional Ecology,* **32,** 2357-2368.

Geerinck MWJ, Van Hee S, Gloder G, Crauwels S, Colazza S, Jacquemyn H, *et al.* (2022) Diversity and composition of the microbiome associated with eggs of the southern green stinkbug, *Nezara viridula* (Hemiptera: Pentatomidae). *Microbiology Open,* **11,** e1337.

Giglio A, Vommaro ML, Gionechetti F & Pallavicini A (2021) Gut microbial community response to herbicide exposure in a ground beetle. *Journal of Applied Entomology,* **145,** 986-1000.

Grond K, Lanctot RB, Jumpponen A & Sandercock BK (2017) Recruitment and establishment of the gut microbiome in arctic shorebirds. *FEMS Microbiology Ecology,* **93,** fix142.

Gupta A & Nair S (2020) Dynamics of insect-microbiome interaction influence host and microbial symbiont. *Frontiers in Microbiology*, **11**, 1357.

Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A & Werren JH (2008) How many species are infected with *Wolbachia*? - A statistical analysis of current data. *FEMS Microbiology Letters*, **281**(2), 215-220.

Hill MP, Macfadyen S & Nash MA (2017) Broad spectrum pesticide application alters natural enemy communities and may facilitate secondary pest outbreaks. *PeerJ,* **5,** e4179.

Hoffman BU & Lumpkin EA (2018) A gut feeling. Gut-brain signaling involves neurotransmission from gut sensory epithelial cells. *Science*, **361**(6408), 1203-1204.

Janke RS, Moog S, Weiss B, Kaltenpoth M & Florez LV (2022) Morphological adaptation for ectosymbiont maintenance and transmission during metamorphosis in *Lagria* beetles. *Frontiers in Physiology,* **13,** 979200.

Jenkins S (2020) *Insects by the Numbers: A Book of Infographics,* New York, Boston, Houghton Mifflin Harcourt.

Ji D, Sun H, Yang W, Gao M & Xu H (2022) Transfer of human microbiome to *Drosophila* gut model. *Microorganisms (Basel),* **10**(3), 553

Jin B-J, Liu X-P, Le Roux X, Bi Q-F, Li K-J, Wu C-Y, *et al*. (2022) Biochar addition regulates soil and earthworm gut microbiome and multifunctionality. *Soil Biology & Biochemistry,* **173**, 108810.

Jones RT, Sanchez LG & Fierer N (2013) A cross-taxon analysis of insect-associated bacterial diversity. *PloS One,* **8,** e61218.

Karimian F, Koosha M, Choubdar N & Oshaghi MA (2022) Comparative analysis of the gut microbiota of sand fly vectors of zoonotic visceral leishmaniasis (ZVL) in Iran; host-environment interplay shapes diversity. *PLOS Neglected Tropical Diseases,* **16,** e0010609.

Kieran TJ (2020) Mitochondrial, metagenomic, and phylogenetic analysis of the ground beetle *Harpalus pensylvanicus* (Coleoptera: Carabidae). *Gene,* **740**, 144540.

Koga R, Moriyama M, Onodera-Tanifuji N, Ishii Y, Takai H, Mizutani M, *et al*. (2022) Single mutation makes *Escherichia coli* an insect mutualist. *Nature Microbiology,* **7**(8), 1141.

Kolasa M, Scibior R, Mazur MA, Kubisz D, Dudek K & Kajtoch Ł (2019) How hosts taxonomy, trophy, and endosymbionts shape microbiome diversity in beetles. *Microbial Ecology,* **78,** 995-1013.

Kudo R, Masuya H, Endoh R, Kikuchi T & Ikeda H (2019) Gut bacterial and fungal communities in ground-dwelling beetles are associated with host food habit and habitat. *The ISME Journal,* **13,** 676-685.

Kumar P & Libchaber A (2013) Pressure and temperature dependence of growth and morphology of *Escherichia coli*: experiments and stochastic model. *Biophysical Journal,* **105**(3), 783-793.

Lang H, Liu Y, Duan H, Zhang W, Hu X, & Zheng H (2023) Identification of peptides from honeybee gut symbionts as potential antimicrobial agents against *Melissococcus plutonius*. *Nature Communications*. **14**(1), 7650-7661

Larochelle A (1990) Food of Carabid Beetles (The):(Coleoptera: Carabidae, Including Cicindelinae). Quebec, QC, Canada. Association des Entomologistes du Québec.

Larochelle A & Larivière M-C (2003) A Natural History of the Ground-Beetles (Coleoptera: Carabidae) of America North of Mexico. Sofia, Bulgaria. Pensoft.

Lehman RM, Lundgren JG & Petzke LM (2009) Bacterial communities associated with the digestive tract of the predatory ground beetle, *Poecilus chalcites*, and their modification by laboratory rearing and antibiotic treatment. *Microbial Ecology*, **57**(2), 349-358.

Li J, Bates KA, Hoang KL, Hector TE, Knowles SCL & King KC (2023) Experimental temperatures shape host microbiome diversity and composition. *Global Change Biology*, **29**(1), 41-56.

Liu F, Hewezi T, Lebeis SL, Pantalone V, Grewal PS & Staton ME (2019) Soil indigenous microbiome and plant genotypes cooperatively modify soybean rhizosphere microbiome assembly. *BMC Microbiology,* **19,** 201.

Lovei GL & Sunderland KD (1996) Ecology and behavior of ground beetles (Coleoptera: Carabidae). *Annual Review of Entomology,* **41,** 231-256.

Lundgren JG & Lehman RM (2010) Bacterial gut symbionts contribute to seed digestion in an omnivorous beetle. *PloS One*, **5**(5), e10831.

Lundgren JG, Lehman RM & Chee-Sanford J (2007) Bacterial communities within digestive tracts of ground beetles (Coleoptera: Carabidae). *Annals of the Entomological Society of America,* **100,** 275-282.

Magagnoli S, Alberoni D, Baffoni L, Martini A, Marini F, Di Gioia D, Mazzon M, *et al*. (2022) The ground beetle *Pseudoophonus rufipes* gut microbiome is influenced by the farm management system. *Scientific Reports*, **12**(1), 22638.

Maurice N & Erdei L (2018) Termites and Sustainable Management: Volume 1 – Biology, Social Behavior and Economic Importance (pp. 69-99). *Springer Nature*.

McManus R, Ravenscraft A & Moore W (2018) Bacterial associates of a gregarious riparian beetle with explosive defensive chemistry. *Frontiers in Microbiology*, **9**, 2361.

Moldovan OT, Carrell AA, Bulzu P-A, Levei E, Bucur R, Sitar C, *et al.* (2023) The gut microbiome mediates adaptation to scarce food in Coleoptera. *Environmental Microbiome,* **18,** 80.

Neupane S & Nayduch D (2022) Effects of habitat and sampling time on bacterial community composition and diversity in the gut of the female house fly, *Musca domestica* Linnaeus (Diptera: Muscidae). *Medical and Veterinary Entomology*, **36**(4), 435-443.

O'Loughlin JL, Samuelson DR, Braundmeier-Fleming AG, White BA, Haldorson GJ, Stone JB, *et al*. (2015) The intestinal microbiota influences *Campylobacter jejuni* colonization and extraintestinal dissemination in mice. *Applied and Environmental Microbiology*, **81**(14), 4642-4650.

O'Riordan KJ, Collins MK, Moloney GM, Knox EG, Aburto MR, Fülling C, *et al*. (2022) Short chain fatty acids: Microbial metabolites for gut-brain axis signalling. *Molecular and Cellular Endocrinology,* **546**, 111572.

Ohbayashi T, Takeshita K, Kitagawa W, Nikoh N, Koga R, Meng X, *et al.* (2015) Insect's intestinal organ for symbiont sorting. *Proceedings of the National Academy of Sciences*, **112,** E5179-E5188.

Oren A & Garrity GM (2021) Valid publication of the names of forty-two phyla of prokaryotes. *International Journal of Systematic and Evolutionary Microbiology*, **71**(10), 005056.

Owen DF & Owen J (1990) Assessing insect species-richness at a single site. *Environmental Conservation*, **17**(4), 362-364.

Pita L, Rix L, Slaby BM, Franke A & Hentschel U (2018) The sponge holobiont in a changing ocean: From microbes to ecosystems. *Microbiome,* **6,** 46.

Porter J & Sullivan W (2023) The cellular lives of *Wolbachia*. *Nature Reviews Microbiology*, **21**(11),750-766.

Rainio J & Niemela J (2003) Ground beetles (Coleoptera: Carabidae) as bioindicators. *Biodiversity and Conservation,* **12**(3), 487-506.

Robinson CD, Bohannan BJ & Britton RA (2019) Scales of persistence: transmission and the microbiome. *Current Opinion in Microbiology,* **50,** 42-49.

Rocha FP, Ronque MU, Lyra ML, Bacci Jr M & Oliveira PS (2022) Habitat and host species drive the structure of bacterial communities of two neotropical trap-jaw *Odontomachus* ants. *Microbial Ecology,* **86,** 699–712.

Rosenberg E & Zilber-Rosenberg I (2018) The hologenome concept of evolution after 10 years. *Microbiome*, **6**(1), 78.

Roughgarden J (2020) Holobiont evolution: mathematical model with vertical vs. horizontal microbiome transmission. *Philosophy, Theory, and Practice in Biology*, **12**(20220112).

Satpathi CR (2021) Ground Beetles and Their Role in Management of Crop Pests. Boca Raton, FL, USA. CRC Press.

Schmid RB, Lehman RM, Brözel VS & Lundgren JG (2014) An indigenous gut bacterium, *Enterococcus faecalis* (Lactobacillales: Enterococcaceae), increases seed consumption by *Harpalus pensylvanicus* (Coleoptera: Carabidae). *Florida Entomologist,* **97,** 575-584.

Shukla SP, Sanders JG, Byrne MJ & Pierce NE (2016) Gut microbiota of dung beetles correspond to dietary specializations of adults and larvae. *Molecular Ecology*, **25**(24), 6092-6106.

Silver A, Perez S, Gee M, Xu B, Garg S, Will K, *et al.* (2021) Persistence of the ground beetle (Coleoptera: Carabidae) microbiome to diet manipulation. *PloS One,* **16,** e0241529.

Smith AH, O'Connor MP, Deal B, Kotzer C, Lee A, Wagner B*, et al.* (2021) Does getting defensive get you anywhere?—Seasonal balancing selection, temperature, and parasitoids shape real-world, protective endosymbiont dynamics in the pea aphid. *Molecular Ecology,* **30,** 2449-2472.

Tiede J, Scherber C, Mutschler J, McMahon KD & Gratton C (2017) Gut microbiomes of mobile predators vary with landscape context and species identity. *Ecology and Evolution*, **7**(20), 8545-8557.

Unzueta-Martínez A, Scanes E, Parker LM, Ross PM, O'Connor W & Bowen JL (2022) Microbiomes of the sydney rock oyster are acquired through both vertical and horizontal transmission. *Animal Microbiome*, **4**(1), 32.

Venn S (2016) To fly or not to fly: Factors influencing the flight capacity of carabid beetles (Coleoptera: Carabidae). *European Journal of Entomology*, **113**(1), 587-600.

Wang B & Allison SD (2021) Drought legacies mediated by trait trade-offs in soil microbiomes. *Ecosphere*, **12**(6), e03562

Wang H, Wu N, Liu Y, Kundu JK, Liu W & Wang X (2019) Higher bacterial diversity of gut microbiota in different natural populations of leafhopper vector does not influence WDV transmission. *Frontiers in Microbiology*, **10**, 1144.

Weseloh RM, (1985) Predation by *Calosoma sycophanta* L (Coleoptera, Carabidae)-evidence for a large impact on gypsy-moth, *Lymantria dispar* L (Lepidoptera, Lymantriidae), pupae. *Canadian Entomologist*, **117**(9), 1117-1126.

Wilches DM, Coghlin PC & Floate KD (2021) Next generation sequencing, insect microbiomes, and the confounding effect of *Wolbachia*: a case study using spotted-wing Drosophila (*Drosophila suzukii*) (Diptera: Drosophilidae). *Canadian Journal of Zoology,* **99,** 588-595.

Williams CE, Kueneman JG, Nicholson DJ, Rosso AA, Folfas E, Casement B*, et al.* (2022) Sustained drought, but not short-term warming, alters the gut microbiomes of wild anolis lizards. *Applied and Environmental Microbiology*, **88**(19), e0053022.

Yun J-H, Roh SW, Whon TW, Jung M-J, Kim M-S, Park D-S, *et al*. (2014) Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. *Applied and Environmental Microbiology*, **80**(17), 5254-5264.

Zhang J, Ding X, Guan R, Zhu C, Xu C, Zhu B, et al. (2018) Evaluation of different 16S rRNA gene V regions for exploring bacterial diversity in a eutrophic freshwater lake. *Science of the Total Environment*, **618**, 1254-1267.

Zhang Z, Mu X, Shi Y & Zheng H (2022) Distinct roles of honeybee gut bacteria on host metabolism and neurological processes. *Microbiology Spectrum*, **10,** e0243821.

Chapter 2: How Carabid Taxonomy Relates to Diversity of Gut Bacterial Communities

2.1 Abstract

Bacteria in animal guts (= the gut bacterial community (GBC)) can be crucial to host survival. These gut bacteria may aid in digestion, synthesis of vitamins and nutrients, or protect from pathogens. To fully understand the biology of the host, it is therefore necessary to study their gut bacteria. Ground beetles, or carabids (Coleoptera: Carabidae), are a family of insects with diverse taxonomy across ~40,000 species, 2500 of which are in North America. Ground beetles are beneficial to ecosystems and are generally considered beneficial in cropland. The diversity of carabids plus the ease at which many of them can be collected allows for an examination of the effects of host taxonomy on characteristics of the gut bacterial community. Previous studies have not focused on how GBCs differ between different taxonomic groups of carabids. I used next generation sequencing to characterize the GBCs in nine carabid subfamilies, 23 genera, and 47 species from 210 specimens. Thirty-eight of the 47 carabid species are represented by five specimens each. My study examined if patterns of GBCs correspond to host taxonomy. The results show that GBCs can differ between carabid subfamilies, genera in a subfamily, and especially species within a genus. As is consistent with previous studies of carabid GBCs, the two predominantly relative abundant (RA) bacterial phyla across carabids were Pseudomonadota and Bacillota at 43.9% and 41.0%, respectively. The three bacterial genera with the highest RA across carabids studied here were *Enterococcus* (19.1%), *Gilliamella* (8.1%), and an unidentified member of the family Yersiniaceae (7.8%). Evidence of core gut bacteria and endosymbiotic bacteria for some carabid taxonomic groups was also found.

2.2 Introduction

Multicellular organisms (e.g., plants, animals, mushrooms) always form associations with microscopic communities of bacteria, archaea, fungi, viruses, or protists. These various microbes are collectively termed a microbiome. A microbiome can profoundly affect the health and reproduction of a host to the point that its survival depends on which kind of microbes are present. In many situations a host's microbiome, or components of it, may evolve side-by-side with the host. This is known as the holobiont concept, which states that a host and its microbiome can be viewed as a single evolutionary unit (Pita *et al.*, 2018). Similarly, the hologenome concept states that a host's genome functions are intertwined with the genome functions of its microbiome (Rosenberg & Zilber-Rosenberg, 2018). An example of the hologenome concept is found in honey bees *Apis* (Hymenoptera: Apidae) who do not have their own nuclear genes to synthesize crucial amino acids and therefore rely on the genes of their gut bacteria to do it (Zhang *et al.*, 2022).

A microbiome can be located in, on, or around a host and fall into three main groups: endosymbionts, ectosymbionts, and exosymbionts, respectively. For example, the contents of an animal's gut lumen are considered outside of its body such that the microbiome contained within are exosymbionts. Cellular endosymbionts are usually acquired when the intracellular microbes infect gametes as they form within a parent (Durden *et al.*, 2019, Porter & Sullivan, 2023). Acquisition of other microbiome components is done either through recruitment from the environment or vertical transmission through the application of microbiome material directly to the host's eggs, seeds, or offspring (Geerinck *et al.*, 2022, Grond *et al.*, 2017, Janke *et al.*, 2022, Liu *et* *al.*, 2019, Ohbayashi *et al.*, 2015). Vertical transmission of microbes directly from parents, rather than horizontal transmission, better facilitates maintaining a stable microbiome throughout a species or population (Michaud *et al.*, 2020).

Prior to advances in genetic sequencing that started about 20 years ago, the methods for examining a microbiome provided a limited view of the organisms contained within it (Escobar-Zepeda *et al.*, 2015). The emergence of next-generation sequencing (NGS) allows for massive parallel sequencing of DNA within a microbiome sample to ascertain microbial identities and their relative abundances (RA). Starting in ~2012, NGS became much more affordable (Escobar-Zepeda *et al.*, 2015) and was therefore accessible to a much wider range of research.

Bacteria are the major constituent of nearly all gut microbiomes and gut bacterial communities (GBC) are the main focus of this thesis (Dias *et al.*, 2020). Some of the benefits these bacteria may provide include aiding in digestion, immune functions, nutrient synthesis, and thermal tolerance (Maurice & Erdei, 2018, Blow & Douglas, 2019, Duron & Gottlieb, 2020, Smith *et al.*, 2021). Some bacteria in GBCs are transient and have varying timespans before they leave the digestive system. Other bacteria are permanent residents of the gut and remain within the digestive system unless particular events, such as the application of antibacterials, occur. The term “core-microbiome” has more than one definition, but for the purposes of this study, a core-microbiome refers to microbes that are found in nearly every sample (Risely & Tate, 2020) in a carabid group.

Insects are an excellent choice for a GBC study with hundreds of samples and host diversity because: 1) they occupy almost all terrestrial and freshwater habitats, 2) they are very speciose, with over 1 million described species, and 3) they have their own unique relationships with microbes in addition to some that are similar throughout the animal kingdom (Gupta & Nair, 2020, Jenkins, 2020, Jones *et al.*, 2013, Rainio & Niemela, 2003). Until recently, most insect groups studied focused on human health or agriculture. For example, mosquitos have been studied to help develop control measures related to human diseases (Douglas, 2022). In agriculture, honey bees have received the most attention as pollinators of crops, while other studied insects are agricultural pests or biological control agents (Douglas, 2022).

Taxonomy of the insect host may influence GBC composition. For example, leafhoppers in the subfamily Ledrinae (Hemiptera: Cicadellidae) have symbionts that provide specific benefits but depending on which leafhopper species is examined that symbiont can be a fungus or bacteria (Nishino *et al.*, 2016). In addition, some members of the GBC can be transmitted from generation of the host to next via egg cytoplasm, anal smearing, or other methods. Therefore, it stands to reason that GBCs may be more similar in insect species that are more closely related taxonomically or phylogenetically.

For my MSc thesis, I focus on ground beetles or carabids (Coleoptera: Carabidae) to examine how host phylogeny relates to GBC diversity. There are about 40,000 carabid species worldwide (Lovei *et al.*, 1996) with about 2500 of those species in North America (Larochelle & Larivière, 2003). They are almost always beneficial to ecosystems and are natural enemies of pest species (Larochelle, 1990). Most carabids are predatory carnivores or granivores, but a few are parasitoids, detritivores, or scavengers (Larochelle, 1990). The beneficial effects of carabids are most evident in cropland agroecosystems where they eat close to their own body weight in food every day during active seasons to reduce pest populations without the harmful non-target effects associated with pesticide use (Hill *et al.*, 2017, Satpathi, 2021). Many ground beetles are not avid fliers or cannot fly at all (Venn, 2016). They may only fly a couple times during their lives. This limited mobility makes many of them relatively easy to catch by hand or with pitfall traps. Multiple aspects of carabid biology and ecology have been well studied, but more could be known about how the diversity of their GBCs may be the result of differences in host taxonomy.

The goal of my study was to expand knowledge of the GBC in carabids while examining how taxonomy of the host relates to GBC diversity. To address limitations of previous studies, sterile techniques and dissections were used to isolate the gut and its associated microbiome as opposed to the full body microbiome which has been used in some carabid studies (Kolasa *et al.*, 2019). I used larger sample sizes (on average) and a greater diversity of carabid taxonomic groups (more carabid subfamilies, genera, and species than previous studies) (Kudo *et al.*, 2019). I hypothesize that GBC composition will be most similar among closely related taxonomic groups (e.g., species in the same genus). I also looked for evidence of core-microbiome members at different carabid taxonomic levels.

2.3 Methods

2.3.1 Beetle Collection

My goal for beetle collections was to examine the GBC in as many carabid species as possible while also having adequate sample sizes. Beetles were collected between June 2021 and July 2023 from late spring to early fall. Sampled habitats included cropland, woodland, prairie, riparian zones, and urban settings in western Montana and southern

Alberta. A list of samples and their collection details are in Appendix 2. Most beetles were captured by pitfall trapping, but some were collected by hand or butterfly net. Beetles were captured alive and then most were immediately placed in 70%-100% ethanol to preserve the DNA and bacterial diversity at time of death. Some samples were captured alive then immediately frozen for several weeks at -20°C before they were sorted and placed in ethanol.

2.3.2 Beetle Identification

Beetles were first keyed to the species level using morphological traits (Lindroth, 1961) after which any questionable identifications were verified through DNA barcoding. For barcoding, DNA extracted from gut tissues (see section 2.3.3) was used to amplify a portion of the mitochondrial DNA (mtDNA) cytochrome oxidase I (COI) gene with the universal primers HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') and LCO1490 (5'- GGT CAA CAA ATC ATA AAG ATA TTG G – 3') (Folmer *et al.*, 1994) and general methods described in Hebert *et al.* (2003). The amplified DNA was sent to Génome Québec Innovation Centre at McGill University (Montréal, Canada) (GQ) for sequencing. The returned COI sequences were edited in MEGA5 to remove primers, chromatograms were checked and edited (Tamura *et al.*, 2011) and then sequences compared with those in GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/) and BOLD (https://www.boldsystems.org/) to identify beetles to the species level.

2.3.3 Beetle Dissections and DNA Extractions

For DNA extraction, each beetle sample first had their elytra carefully removed before being surface sterilized using a 0.6% solution of sodium hypochlorite (bleach) and finally being rinsed with sterile water. It was not possible to perform gut dissections on small species < 3.5 mm in length; i.e., *Bembidion quadrimaculatum*, *Trechus oregonensis*, *Syntomus americanus*, *Apristus constrictus*. In these cases, DNA was extracted from whole abdomens after removal of legs and elytra. For large species, *Carabus*, *Pasimachus*, *Chlaenius*, and *Harpalus pensylvanicus*, the gut was dissected out and divided in two with the DNA extracted from each half in a separate kit and then the DNA from each extraction then combined. Beetle midguts and hindguts were dissected out using sterile techniques, small scissors, and tweezers. After cutting and folding back the dorsal integument of the abdomen, the gut was cut just posterior to the thorax and again at the end of the abdomen. Extractions were performed using DNeasy® Blood and Tissue Kits (Qiagen Group, Hilden, Germany) after which the DNA concentration was measured using a nanodrop spectrophotometer. Isolates with insufficient concentrations (< 10 ng/ ul) were replaced using a different beetle specimen if it was available. DNA samples were kept frozen at -20°C until they were sent for sequencing.

Two separate sets of extracted DNA samples were sent to GQ, one in fall 2022 and a second in fall 2023 and some of the same sample groups were sent in each batch to check for batch effects across years. Genome Quebec performed PCR amplifications of the V4 hypervariable region of prokaryote 16S SSU rDNA using the 515FB-806RB primer pair and their amplification protocols (25 ul, Roche FastStart High Fi Taq, 58^{o}C, 26 cycles) before sequencing on a MiSeq Illumina platform, following the manufacturer's instructions (Illumina, 2019), to generate the requested PE 250 base pair reads targeting an average of 100,000 sequences per sample.

Demultiplexed sequence data were received from GQ and then quality filtering, denoising, paired-end merging (250 base pair PE reads merged to form ~292 base pair V4 hypervariable gene region), and feature-table construction were performed using the default settings of the dada2 (Callahan *et al.*, 2016) plug-in in Qiime 2 (version 2023.2) software (Estaki *et al.*, 2020). Trimming of sequences was done to remove ends with a Phred score below 30, which was only a single base on the 3’ end of each of the forward and reverse reads on about 33% of all reads prior to paired-end merging. Before sequences are assigned a bacterial taxonomic identification, they are amplicon sequence variants (ASVs). An ASV is a single, unique DNA sequence from a high-throughput sequencing system which is then assigned taxonomic identity using the SILVA rRNA database (silva-138-99-515-806-nb-classifier) in Qiime 2. Given the large number of ASVs identified, those represented by fewer than 20 sequences (in one sample or combined across all samples) were considered trace levels and excluded to simplify analyses while not losing many samples due to small libraries. This threshold is consistent with other studies because if a sample with a small library (e.g., ~500 sequences) had 20 sequences for a rare ASV, then ~5% of the library would be removed (Kudo *et al.*, 2019; Kolasa *et al.*, 2019). Qiime 2 was also used for taxonomic filtering of all non-bacterial sequences (e.g., mitochondrial DNA) and statistical analyses. Bacterial sequences assigned to *Wolbachia* were also removed because it is an obligate intracellular endosymbiont (Nikoh *et al.*, 2014), *Wolbachia* cannot occupy the gut lumen and, therefore, cannot be part of the GBC. Other potential endosymbionts such as

Spiroplasma were left in the analyses because of reports they can exist outside of cells (Gilad *et al.*, 2003) and were more likely to have originated within the gut lumen. At the end of this process, each beetle sample was associated with a library of bacterial sequences characterizing the host's GBC.

2.3.4 GBC Analyses

To determine whether GBC diversity reflects the host's evolutionary history, analyses were done at three carabid taxonomic levels: subfamilies within family Carabidae, genera within a subfamily, and species within genera. For this thesis chapter, a maximum of five specimens per species were used. Unless otherwise noted, mean values reported here include standard deviation.

To describe local and regional species diversity, three measures of alpha diversity were used; richness is a measure of how many ASVs are found in a sample group, evenness measures how evenly the relative abundances (RA) of ASVs are spread through the sample group, and a Shannon index incorporates both richness and evenness (Kers & Saccenti, 2022). For all three measures, Kruskal-Wallis (K-W) (all groups and pairwise) tests determined significance (Ruxton & Beauchamp, 2008). Pairwise Kruskal-Wallis measures were only mentioned here if the Kruskal-Wallis all-groups measure showed significance. A Benjamini-Hochberg correction is the Qiime 2 default method for correcting pairwise p-values (p) to generate q-values (q) (Ferreira & Zwinderman, 2006).

To visualize the beta diversity of GBCs, principal coordinate analyses (PCoA) were plotted to show Bray-Curtis dissimilarities between carabid samples (Kers &

Saccenti, 2022). Bray-Curtis measures the presence and absence of ASVs while also placing higher weight on ASVs with higher RA. The PCoA places samples with more similar microbiomes closer together in multivariate space. Permutational multivariate analysis of variance (PERMANOVA) was performed on the PCoA to test for significant separation between carabid taxonomic groupings.

Because some statistical measures perform best with standardization and because of variation in the size of sequence libraries across beetle samples, prior to analyses of alpha diversity (richness, evenness, and Shannon) and beta diversity (Bray-Curtis), sequence counts were standardized across libraries using rarefaction (Willis, 2019). A rarefaction level was set for each group to retain the largest number of ASVs while ensuring retention of as many beetle samples as possible. Note, however, that rarified libraries were not used for the measures of RA or differential abundance (DA) shown in tables or taxa-bar-plots, meaning no samples were removed.

Analyses of differential abundances (DA) were used to statistically test differences in the abundance of bacterial taxa between sample groups to help identify which bacterial taxa are driving separation between samples plotted in the PCoA. To examine which bacterial taxa at the phylum and genus level had significant DA amongst carabid taxonomic groups, an analysis of composition with bias correction (ANCOM-BC) was performed on the data (Lin & Peddada, 2020). An ANCOM-BC test takes into account not only how many samples the bacterial taxa appeared in, but also measures the true absolute abundances of sequences assigned to an ASV to eliminate overshadowing by robust predominant bacterial taxa sequences (Cappellato *et al.*, 2022). Observing the true absolute abundances of sequences in a sample library can generate a significant value that is different than what is seen in the RA. Due to possible variance in sequence counts created by inconsistencies in DNA extraction and amplification, the sequence library size is not always an indicator of the original bacterial quantities in a sample. For example, if you generate one sequence library with 1000 sequences and another with 500 sequences it does not mean that one of those sample had twice as much bacteria as the other, but it is evidence that one had better sequencing success than the other. A strict Bonferroni correction was used on p-values to generate q-values used to detect significant differences among groups (Armstrong, 2014). For most of the bacteria noted in this thesis, I have only reported them at the phylum or genus level. For bacterial phyla, a cutoff of 0.5% relative abundance (RA) allowed retention of almost all differentially abundant bacterial taxa present at biologically meaningful levels. Because of their greater diversity within a sample, a cutoff of 1.5% RA was applied for bacterial genera. These cutoff levels are consistent with what other studies have reported (Silver *et al.*, 2021, Geerinck *et al.*, 2022, Rocha *et al.*, 2022).

2.3.5 Beetle Phylogeny

To illustrate the diversity and relatedness of the carabid species used in the current study, a phylogenetic tree was constructed using 658 base pair (bp) COI sequences from 30 of my samples (GenBank ascension numbers: PQ150013, PQ150015-PQ150043) and additional sequences from NCBI for a total of 52 sequences from different species (GenBank numbers in Fig 2.1). A closely related non-carabid species, *Trachypachus gibbsii*, was used as an outgroup. The evolutionary history was inferred by using the maximum likelihood method and General Time Reversible model (Nei & Kumar, 2000) in MEGA11 (Tamura *et al.*, 2021). A consensus tree was constructed from 200 bootstrap replicates (Felsenstein, 1985) to represent the evolutionary history. Bootstrap values less than 70% support were not reported. Initial trees for the heuristic search were obtained automatically by applying the maximum parsimony method. A discrete gamma distribution was used to model evolutionary rate differences among sites (four categories (+G, parameter = 0.4182)). The rate variation model allowed for some sites to be evolutionarily invariable ([+/], 26.82% sites).

2.4 Results

2.4.1 Beetle Collections and Phylogeny

I collected specimens representing 47 species in 23 genera, spanning nine of the 14 subfamilies present in North America (Table 2.1). From 86 of these beetles, COI sequences were obtained to confirm species' identification and for phylogenetic analysis. For the seven subfamilies in the tree represented by at least two species, phylogenetic analysis showed only the subfamilies Omophroninae and Cicindelinae to be monophyletic (Fig. 2.1). All genera represented by at least two species were monophyletic except for *Amara* and *Pterostichus*.

2.4.2 Sequence Libraries

I obtained 16S rDNA sequences to characterize the gut bacterial community of 282 beetles. These 282 sequence libraries included a total of 25,714,548 sequences and had an average Phred score of 37; i.e., they were of high quality (Ewing *et al.*, 1998). After quality filtering, merging reads, and removing non-bacterial and *Wolbachia* sequences, 2,492,935 total sequences remained for 280 beetles. For these beetles, the average sequence library contained 8,903 sequences (range: 20 to 39,741; median: 8,535) representing a total of 7,358 ASVs (average: 339 ASVs per sample). In this chapter, 210 of the 280 beetles were selected to compare between taxonomic groups (subfamily, genus, or species). Carabid species were represented by 3-5 samples and those represented by < 3 samples were grouped with others in the analyses of carabid subfamily or genus taxonomic level.

2.4.3 GBC Comparisons Among Carabidae Subfamilies

To observe patterns of GBC diversity between carabid subfamilies, 210 beetle samples were grouped by subfamilies (Table 2.1). After rarefaction, samples with fewer than 508 sequences were removed (Appendix 1.1, Fig. S1) leaving 189 samples (Table 2.1) for alpha diversity measures of richness, evenness, and Shannon diversity and the beta diversity measure of Bray-Curtis dissimilarity (Fig. 2.3). Unrarefied data (all samples) were used for the RA and DA analyses (Tables 2.2 and 2.3) and the bar plot of bacterial genera (Fig. 2.4).

Alpha diversity among subfamilies

No significant differences in alpha diversity between subfamily groupings were found when using Kruskal-Wallis (all groups) to test evenness (mean = 0.897 ± 0.076, H = 6.268, p = 0.617). However, significant differences were detected among subfamilies for richness (K-W test, $H = 50.590$, $p < 0.001$, Fig. 2.2a) and Shannon diversity (K-W test, $H = 45.154$, $p < 0.001$, Fig. 2.2b). There was significantly low richness in Trechinae compared to all other subfamilies besides Patrobinae (K-W test, $H \geq 5.835$, $q \leq 0.040$, Fig. 2.2a). Patrobinae had significantly low richness compared to all subfamilies besides Trechinae, Elaphrinae, and Scaritinae (K-W test, $H \geq 6.244$, $q \leq 0.0345$, Fig. 2.2a). Elaphrinae had significantly low richness compared to Harpalinae and Omophroninae (K-W test, $H \geq 6.663$, $q \leq 0.030$, Fig. 2.2a). Significant Shannon diversity is found when comparing Trechinae to all subfamilies except Elaphrinae and Patrobinae (K-W test, $H \geq 6.342$, $q \leq 0.042$, Fig. 2.2b), when comparing Patrobinae to Carabinae, Harpalinae, and Omophroninae (K-W test, $H \geq 7.260$, $q \leq 0.032$, Fig. 2.2b), and when comparing Elaphrinae to Harpalinae (K-W test, $H = 7.018$, $q = 0.032$, Fig. 2.2b) .

Beta diversity among subfamilies

Beta diversity was measured with a Bray-Curtis dissimilarity matrix and plotted in a PCoA (Fig. 2.3a). The first three axes represent 13.26% of the total beta diversity between these beetle samples. Results show the separation of samples along three transects (columns). This general “tripod” pattern of separation is an artefact of sparse data, meaning that a large number of ASVs are absent or nearly so in any one sample and there is little overlap among samples to create significance.

Results of the PERMANOVA test showed significant separation (pseudo-F = 1.896, $p = 0.001$) among subfamilies indicating that there were meaningful differences in some carabid subfamily GBCs. For example, Cicindelinae, Omophroninae, and

Scaritinae occupy a distinct area of the PCoA from Elaphrinae, Patrobinae, Trechinae, and Nebriinae, which mostly occupy the same area (Fig. **2.**3b). Harpalinae, one of the most sampled subfamilies in the dataset, is found throughout the PCoA. Interestingly, some of the Harpalinae form a distinct group that spreads along axes 2 (4.38%) and 3 (2.94%) and sits in a central position on axis 1 (5.94%) (Fig. **2.**3a).

Bacterial phyla: For the 210 samples combined across the nine subfamilies examined here, the eight bacterial phyla above the cutoff of 0.5% RA accounted for 98.7% of the RA (Table 2.2). Overall, the two bacterial phyla with predominant RA were Pseudomonadota (43.9%) and Bacillota (41.0%) which are present at over 10% in each subfamily. Only three bacterial phyla, Pseudomonadota, Bacillota, and Bacteroidota, are present across all subfamilies above the RA threshold (Table 2.2).

When the absolute sequence counts were examined using differential abundance (DA), eight of the top ten bacterial phyla had a significant DA among one or more carabid subfamilies ($q < 0.05$) (Table 2.2). All subfamilies have a significantly high or low DA of the two most common bacterial phyla, Pseudomonadota and Bacillota, with the exception of Harpalinae which has a significant midrange DA for Pseudomonadota (Table 2.2).

Bacterial genera: The ASVs found in carabid beetles were also examined at a higher taxonomic resolution; normally at the genus level, but when that level of classification is unknown, Class (c_), Order (o_) or Family (f_) is presented for the unknown genera. For the 210 samples combined across the nine subfamilies examined here, the 14 bacterial genera above 1.5% RA accounted for 66.5% of the RA (Table 2.3). The highest RA was *Enterococcus* (19.1%) followed by three bacterial genera at between 5-10% *Gilliamella* (8.1%), f_Yersiniaceae (7.8%), and *Lactobacillus* (5.7%). Within individual carabid subfamilies, the number of bacterial genera above 1.5% RA ranged from eight in Elaphrinae and Omophroninae to 17 in Scaritinae and total RA above the threshold within each subfamily ranged from 60.1-93.9%. No bacterial genera were found above the 1.5% RA threshold across all beetle subfamilies. Two subfamilies, Patrobinae and Trechinae, did not have the most frequent bacteria in other subfamilies, *Enterococcus*, above 1.5% RA; they were also the only two subfamilies with *Spiroplasma* RA above 12%. *Gilliamella*, the bacteria group with the second highest total RA, is in eight of nine subfamilies at 2.7-44.7%; it is only below 1.5% RA in Scaritinae. Among the bacterial taxa creating the most separation between the left and right transects formed in the PCoA of beetles is a genus of o_Lactobacillales, the left group of beetles having higher levels and the right group having less or none (Table 2.3).

Ten of the top 56 bacterial genera had significant DA ($q < 0.05$) in at least one carabid subfamily (Table 2.3). The two carabid subfamilies with significantly high DA of *Enterococcus* also have significantly high DA of *Gilliamella*, which is a common pattern throughout this study. Omophroninae has significantly high DA in six of its eight bacterial genera above 1.5% RA. All three of Scaritinae’s bacterial genera with significant DA have significantly low DA and this is also the carabid subfamily with the most bacterial genera above 1.5% RA (Table 2.3).

Bacterial genera bar plot

Results for 208 individual carabid samples used in Chapter 2 are displayed in a box plot of the 30 bacterial genera with the highest relative abundance (Fig. 2.4).

2.4.4 Within a Subfamily (Harpalinae)

Ninety-three Harpalinae samples across six genera provided the best opportunity to examine patterns of GBC diversity among carabid genera within a subfamily (Table 2.1). After rarefaction, samples with fewer than 508 sequences were removed leaving 91 samples (Table 2.1) for alpha diversity measures of richness, evenness, and Shannon diversity (Fig. 2.5) and the beta diversity measure of Bray-Curtis dissimilarity (Fig. 2.6). Unrarefied data (all samples) were used for the RA and DA analyses (Tables 2.4 and 2.5).

Alpha diversity within a subfamily (Harpalinae)

No significant differences in alpha diversity between Harpalinae genera were found when using Kruskal-Wallis (all groups) to test evenness (mean = 0.898 ± 0.053, $H = 7.535$, $p = 0.184$). However, significant differences were detected among Harpalinae genera for richness (K-W test, $H = 12.097$, $p = 0.033$, Fig. 2.5a) and Shannon diversity (K-W test, $H = 12.569$, $p = 0.028$, Fig. 2.5b). A pairwise test detected significantly low richness in *Agonum* compared to *Pterostichus* (K-W test, $H = 9.204$, $p = 0.036$); however, Kruskal-Wallis (pairwise) comparisons did not detect significant differences between Harpalinae genera for Shannon diversity ($H < 7.727$, $q > 0.082$, Fig. 2.5b).

<u>*Beta Diversity within a subfamily (Harpalinae)*</u>

The PCoA using a Bray-Curtis dissimilarity matrix explained 15.94% of the variation in Harpalinae GBCs in its three axes (Fig. 2.6). Results of a PERMANOVA test showed significant separation (pseudo-F = 2.204, p = 0.001) among samples by carabid genera. For example, 38 of the 41 samples of *Amara* and *Harpalus* form a cluster distinct from *Agonum* and *Chlaenius* (Fig. 2.6 and see inset).

<u>*Bacterial phyla.*</u> For the 93 samples combined across the six Harpalinae genera examined here, the ten bacterial phyla above the cutoff of 0.5% RA accounted for 99.6% of the RA (Table 2.4). The most abundant were Pseudomonadota (49.7%), Bacillota (29.9%) and Actinomycetota (8.2%), which were present in all genera at high levels (≥ 2.7%) except for Actinomycetota in *Agonum*. Within each of the Harpalinae genera, the amount of bacterial phyla with RA above the 0.5% threshold ranged from 4 to 10. Among the bacterial taxa creating a similar distribution for samples of *Amara* and *Harpalus* in the PCoA (Fig. 2.6) are high levels of Actinomycetota (>10%) (Table 2.4).

Eight of the top 14 bacterial phyla had significant DA ($q < 0.05$) between Harpalinae genera (Table 2.4) with indications of similarities between certain genera (Table 2.4). *Agonum* and *Pterostichus* had significantly high DA of Pseudomonadota, Bacillota, and Bacteroidota. *Amara* and *Harpalus* had significantly high DA for Actinomycetota and Chloroflexota.

Bacterial genera. Combined across all 93 Harpalinae samples, 14 bacterial genera exceeded the cutoff threshold of 1.5% RA and comprised 62.1% of the total RA (Table 2.5). Representation of these bacteria in the Harpalinae genera ranged from eight (*Calathus*) to 19 (*Amara*) with the bacteria *Enterococcus* having the highest total RA at 10.4%. The 19 most RA bacterial genera in *Amara* and the 11 most RA bacterial genera in *Harpalus* only added up to be 54.9% and 62.6% of the total RA in those beetle genera respectively. The other ~40% of the RA in those two carabid genera are trace levels of bacteria below the 1.5% cutoff. The most prevalent bacterial genera across all six Harpalinae genera were f_Enterobacteriaceae (in six of six genera), *Enterococcus* (in five genera), and f_Yersiniaceae (in five genera). *Pterostichus* had relatively high RA of f_Yersiniaceae (27.6%) with the next closest RA of that bacteria being in Calathus (9.9%).

Nineteen of the top 34 bacterial genera by RA had significant DA ($q < 0.05$) between Harpalinae genera (Table 2.5). The two Harpalinae genera that had significantly high DA of *Enterococcus* also had significantly high DA of *Gilliamella* and *Amara* had significantly low DA of *Gilliamella*. The member of f_Enterobacteriaceae above 1.5% RA in all Harpalinae genera only had significantly high DA in *Calathu*s. *Calathus* had eight bacterial genera above 1.5% RA and six of those bacterial genera had significantly high DA. The three bacterial genera that *Pterostichus* had significantly low DA in are three that are above 1.5% RA in *Amara* (Table 2.5).

2.4.5 GBC Diversity Within a Genus of Carabid

From the samples used in this chapter, GBC comparisons among species were made for each of nine carabid genera. In each of these genera, there were at least two species, each represented by a minimum of three individuals. In this chapter, results are reported for three carabid genera; *Agonum*, *Amara,* and *Cicindela* each of which contained a minimum of three species. Results of the remaining six genera (*Bembidion*, *Carabus*, *Harpalus*, *Nebria*, *Omophron*, *Pterostichus*) are provided in Appendix 1.2. This was done solely to limit the size of this chapter to a manageable size while answering the question of how GBC diversity is related to host taxonomy.

2.4.5.1 *Agonum*

The GBC diversity for three species of *Agonum, A. muelleri* (n = 5)*, A. piceolum* (n = 5), and *A. placidum* (n = 5), were examined (Table 2.1). With rarefaction, the sequence libraries for these samples each contained 4923 sequences and all specimens were included in the analyses.

Alpha diversity among species of Agonum

Kruskal-Wallis tests detected no significant difference across species for measures of richness (H = 2.911, p = 0.233), evenness (H = 1.220, p = 0.543) or Shannon diversity (H = 4.220, p = 0.121). For all *Agonum* samples combined, mean ± SD (min-max) values were richness = 33.8 ± 12.0 (21-54), evenness = 0.896 ± 0.061 (0.704-0.939) and Shannon diversity = 4.482 ± 0.566 (3.488-5.296).

Beta diversity among species of Agonum

Beta diversity of GBCs in *Agonum* samples using Bray-Curtis dissimilarities plotted in a PCoA (Fig. 2.7) showed significant separation (PERMANOVA, pseudo-F = 2.886, p = 0.001) by species. The three PCoA axes collectively captured 49.46% of the total GBC diversity. On the first two axes each species generally formed its own cluster, the exception being a single *A. placidum* that clustered with *A. muelleri*. *Agonum piceolum* separated from the other two species on axis 1 (25.27%) while spreading within the species on axis 3 (11.62%) (Fig. 2.7).

Bacterial phyla. The four bacterial phyla with RA above 0.5% had a total RA ≥ 99.3% for all samples combined and for samples within species (Table 2.6). The two bacteria phyla with the highest RA were Bacillota and Pseudomonadota which combined for 83.5% of bacterial RA at the phylum level. One major difference between *A. piceolum* and the two other *Agonum* species is the low RA of Bacteroidota (1.1% vs 19.8% and 11.9%) which may explain most of the separation on the first axis of the PCoA (Fig. **2.**7). Each of the five bacterial phyla with an RA above 0.5% had significant DA (q < 0.05) in at least one *Agonum* species. Each *Agonum* species has a different pattern of significant DA (Table 2.6).

Bacterial genera. The ten bacterial genera with RA above 1.5% had a total RA of 94.6% for all samples combined (Table 2.7). Three to six of these genera comprised 91.5-98.5% of the RA for individual *Agonum* species, but with pronounced differences. For *A.*

piceolum, *Leuconostoc* was common, but *Dysgonomonas* was rare. The opposite pattern was observed for *A. muelleri* and *A. placidum* and likely explains most of the separation on axis 1 of the PCoA (Fig. 2.7). *Spiroplasma* was common in *A. placidum* but rare in *A. muelleri* and *A. piceolum*, which likely explains most of the separation of samples on axis 2 of the PCoA (Fig. 2.7).

Five of the 13 bacterial genera with an RA above 1.5% had significant DA ($q < 0.05$) between at least one *Agonum* species (Table 2.7). Patterns identified by differences in DA across species were consistent with patterns indicated with RA, meaning that the bacterial genera at high RA also had robust sequence counts (Table 2.7).

2.4.5.2 *Amara*

Most samples of the four *Amara* species (Table 2.1) had robust sequence counts, so a rarefaction of 1479 sequences per sample was chosen which resulted in removing two *A. quenseli* samples from the analyses of richness, evenness, Shannon diversity, and the PCoA (Table 2.1).

Alpha diversity among species of Amara

Kruskal-Wallis tests detected no difference across species for measures of richness ($H = 4.635$, $p = 0.201$), evenness ($H = 4.527$, $p = 0.210$) or Shannon diversity ($H = 4.738$, $p = 0.192$). For 18 specimens of *Amara* combined, mean ± SD (min-max) values were: richness = 84.9 ± 60.8 (23-222), evenness = 0.924 ± 0.043 (0.804-0.959), and Shannon diversity = 5.643 ± 1.079 (4.010-7.444).

<u>Beta diversity among species of</u> <u>Amara</u>

The Bray-Curtis PCoA (Fig. 2.8) showed the first three axes combined to explain 20.54% of the beta diversity present in *Amara* samples. Results of the PERMANOVA test did not show significant separation (pseudo-F = 0.985, p = 0.706) among samples by species.

<u>Bacterial phyla.</u> For the 20 samples combined across the four *Amara* species examined here, the nine bacterial phyla with an RA above 0.5% totaled 98.7% (Table 2.8). The three bacterial phyla with predominant RA were Pseudomonadota (31.2%), Bacillota (25.8%), and Actinomycetota (20.8%). Each *Amara* species had 9-10 bacterial phyla above 0.5% RA (Table 2.8). Eight of those bacterial phyla were above 0.5% RA in all *Amara* species.

Eight of the 13 bacterial phyla above 0.5% RA had significant DA ($q < 0.05$) in at least one *Amara* species (Table 2.8). The DA of Actinomycetota was significantly high in *A. carinatai*, but significantly low in the other *Amara* species. The significantly low DA in the three bacterial phyla at the highest RA in *A. quenseli* is likely an artifact of high levels of *Wolbachia* sequences being removed from the sequence libraries of all five samples leaving low sequence counts for the remaining bacterial taxa (Table 2.8).

<u>Bacterial genera</u>. For all *Amara* samples combined, the 20 bacterial genera over 1.5% RA totaled 55.1% meaning that > 45% of the RA remaining was trace amounts of bacteria at < 1.5%. No bacterial genera were predominant and total RA was relatively even across bacterial genera (Table 2.9). Within each *Amara* species total RA ranged

from 52.9-85.5%. The two bacterial genera with RA > 5% for all *Amara* combined were *Lactococcus* (6.7%) and f_Enterobacteriaceae (6.4%) (Table 2.9).

Only 15 of the top 46 bacterial genera had significant DA ($q < 0.05$) between *Amara* species (Table 2.9). *Lactococcus* was above the RA cutoff in all *Amara* species and had a significantly high DA in *A. farcta* and *A. littoralis* (Table 2.9).

2.4.5.3 *Cicindela*

To examine GBC diversity within four species of the *Cicindela* genus, 20 samples (five per species) were compared: *C. lengi*, *C. oregona*, *C. purpurea*, and *C. repanda* (Table 2.1). Sequence libraries were rarefied to 299 sequences per sample while not losing any samples from the analyses and allowing diversity to plateau on a rarefaction chart (Appendix 1.3, Fig. S9).

Alpha diversity among species of Cicindela

Significant differences ($p < 0.05$) in alpha diversity between *Cicindela* species groupings were found in Kruskal-Wallis (all groups) measures of evenness (K-W test, $H = 11.537$, $p = 0.009$, Fig 2.8), but not richness ($H = 5.617$, $p = 0.132$) or Shannon diversity ($H = 4.097$, $p = 0.251$). For all specimens of *Cicindela* combined, mean ± SD (min-max) values were richness = 29.3 ± 19.3 (4-74) and Shannon diversity = 4.138 ± 1.072 (1.975-5.799). A Kruskal-Wallis (pairwise) comparison showed significant differences in evenness with *Cicindela lengi* and *C. purpurea* having more evenness and *C. repanda* and *C. oregona* having less evenness (Fig. 2.9).

Beta diversity among species of Cicindela

The PCoA using a Bray-Curtis dissimilarity matrix explained 29.82% of the variation in *Cicindela* GBCs in its three axes (Fig. 2.10). Results of the PERMANOVA show significant separation (pseudo-F = 1.689, p = 0.001) among samples by species. Axis 1 (13.93%) and axis 2 (10.25%) grouped *C. lengi* and *C. purpurea* into a tight cluster while the other two species show more variation and very little clustering.

Bacterial phyla. For all *Cicindela* samples combined, the seven bacterial phyla above 0.5% RA totaled 98.9% (Table 2.10). The two bacterial phyla with predominant RA were Bacillota (50.0%) and Pseudomonadota (32.2%) followed by only one other bacterial phylum above 3.0% (Actinomycetota = 10.6%). For individual carabid species, *Cicindela purpurea* had almost 10x as much RA of Actinomycetota (33.3%) than the other *Cicindela* species (2.7-3.7%). The three highest RA bacterial phyla in *C. oregona* and *C. repanda*, both of which have fewer bacteria phyla than the other two *Cicindela* species, have almost identical distributions and are within 1% of each other (Table 2.10).

Eight of the twelve bacterial phyla above 0.5% RA had significant ($q < 0.05$) DA in at least one *Cicindela* species (Table 2.10). Each species has, more or less, its own pattern of significant DA in bacterial phyla (Table 2.10).

Bacterial genera. For all *Cicindela* samples combined across the four species examined here, 11 bacterial genera exceeded a threshold of 1.5% RA and contained 71.3% of the GBC (Table 2.11). Within each *Cicindela* species, the number of bacterial genera above

1.5% RA ranged from a low of three in *C. repanda* and four in *C. oregona* to 11 in *C. lengi* and 15 in *C. purpurea*. No bacterial genera above 1.5% RA were present in more than two *Cicindela* species. *Cicindela oregona* and *C. repanda* share their top three RA bacterial genera. *Cicindela lengi* and *C. purpurea* share three of their top four RA bacterial genera. The main differences between the *C. oregona*/*C. repanda* group and the other two species is the former contained high RA (~70%) of the bacterial phylum Bacillota (mostly *Enterococcus* and *Leuconostoc*) and the bacterial genus *Gilliamella* (15.5-22.4%) (Tables 2.10 and 2.11). In *C. lengi and C. purpurea* there are three bacterial genera from Bacillota and Pseudomonadota along with sparce data creating most of the clustering for those *Cicindela* species in the PCoA (Fig. 2.10 and Table 2.11).

Only four of the 27 bacterial genera above 1.5% RA had significant DA ($q < 0.05$) in at least one *Cicindela* species (Table 2.11). *Enterococcus* had significantly high DA in *C. oregona* and *C. repanda*. *Cicindela repanda* also had a significantly high DA of *Leuconostoc* and *Gilliamella*. *Cicindela lengi* and *C. purpurea* had no bacterial genera with significant DA (Table 2.11).

2.5 Discussion

In this chapter, I set out to test the hypothesis that the taxonomic relatedness of host carabid species is reflected in the similarity of their GBC. I did this by comparing GBCs across carabid subfamilies, across genera in the same subfamily, and by comparing species in the same genus. I also set out to identify core members of the carabid GBC.

2.5.1 Inferred Evolutionary Relationships of Beetles

Results of the phylogenetic tree developed to reinforce the taxonomic relatedness of the beetle species I studied (Fig. 2.1) were generally consistent with expectations, albeit with some notable exceptions (López-López & Vogler, 2017, Ober & Maddison, 2008). I expected species in the same subfamily to be placed in the same clade, but this only happened with Cicindelinae and Omophroninae. Elaphrinae and Patrobinae were only represented by a single species each so it is unknown whether species of that subfamily would form a clade. In contrast, all of the subfamilies studied here are shown to be monophyletic in phylogenies from other studies. For example, López-López & Vogler (2017) created a carabid phylogeny using combined analysis of mitogenomes and nuclear 18S rRNA that places *Bembidion* and *Trechus* together in a clade for the subfamily Trechinae. Discrepancies between the phylogeny created here and accepted taxonomic classifications are likely due to the use of only a single mitochondrial gene (COI). Other studies use a combination of nuclear and (or) mitochondrial genes for better accuracy (Kavanaugh *et al.*, 2021). I also note that bootstrap values assigning membership to different clades in my phylogeny were typically below 70, indicating a low level of confidence in clade assignment (Hillis & Bull, 1993). Thus, the phylogenetic tree I developed (Fig. 2.1) is better suited to show the diversity of species studied across subfamilies than critically assessing their evolutionary relationships.

2.5.2 General Patterns of GBC in Carabidae

When examined at the level of bacterial phyla, the GBCs of the carabid species studied here were consistent with previous studies showing high RA of Pseudomonadota, Bacillota, Actinomycetota, Bacteroidota, and Fusobacteriota in carabid beetles (Table 2.12). These results are also consistent with studies of other animal GBCs (e.g., humans) (Dias *et al.*, 2020). An apparent discrepancy is that six previous studies detected high RA of Mycoplasmatota (Table 2.12). However, this is likely due to the presence of *Spiroplasma* (Tables 2.12 and 2.13), which is classified as belonging to the phylum Mycoplasmatota in some databases but in the phylum Bacillota in the SILVA database used in this study (Oren, 2024).

My results are also generally consistent with previous similar studies when bacterial genera are compared (Table 2.13). *Enterococcus* has been commonly reported to be abundant in carabid GBCs as was shown here (Table 2.3). Two of the three previous studies that did not report *Enterococcus* in the six most RA bacterial genera were studies of *Harpalus pensylvanicus*; the third study looked at two species of cave dwelling carabids (Lundgren *et al.*, 2007, Lundgren & Lehman, 2010, Moldovan *et al.*, 2023). This current study did not find *Enterococcus* above the 1.5% RA threshold in *H. pensylvanicus* (Table S6). This is a surprising result considering that two previous studies reported a link between *H. pensylvanicus* and *Enterococcus* (Lundgren & Lehman, 2010, Schmid *et al.*, 2014). *Enterococcus* for all samples combined in this study (19.1%) is at double the RA of the next closest bacterial genus, *Gilliamella* (8.1%). *Spiroplasma* is reported in six of ten previous studies that examined bacterial genera and, in this thesis chapter, was in the top ten most RA bacterial genera when all the samples are combined. It is a common microbiome member of many arthropod species (Williamson *et al.*, 1998) and is usually found in the guts and hemolymph where they can influence host reproduction (Anbutsu & Fukatsu, 2003). Only one of ten previous carabid GBC studies showed a non-*Serratia* member of Yersiniaceae in the top six RA bacterial genera, but this study showed a non-*Serratia* member of that bacterial family as the 3rd highest RA bacterial genus (Table 2.13). Differences between this study and previous studies may also reflect advances or differences in NGS, bioinformatics, and bacterial databases.

2.5.3 Comparisons of GBC Among Carabid Subfamilies

To my knowledge, this study is the first to compare microbiomes among carabid subfamilies. Most previous studies focused on closely related beetle species or multiple species within Harpalinae. For example, Kudo *et al.* (2019) examined 27 carabid species (12 genera), all samples were from the Harpalinae subfamily except a single sample from Cicindelinae and six samples across two Carabinae species.

There was some evidence that the patterns of GBC diversity in carabid subfamilies matched the patterns of carabid evolutionary relationships. Genetic phylogenies place Elaphrinae, Patrobinae, and Trechinae in a common clade (Fig. 2.1 (Ribera *et al.*, 2005). In the current study, the GBCs of these subfamilies had similarly low levels of richness and diversity (Fig. 2.2) and grouped together with beta diversity (Fig. 2.3b). Patrobinae and Trechinae also were the only two subfamilies studied here that do not have *Enterococcus* above 1.5%, have *Spiroplasma* above 12%, and had an unidentified genus of the order Lactobacillales above 9% (Table 2.3).

There was also other GBC differentiation between carabid subfamilies. For example, at the bacterial phylum level there seemed to be a somewhat unique distribution of low Pseudomonadota (10.1% RA) and high Bacillota (78.5% RA) in Omophroninae compared to other subfamilies (Table 2.2). Omophroninae also had a higher enrichment of *Enterococcus*, as well as other prominent bacterial genera, than any other subfamily studied here. An antimicrobial agent may be what kept the GBCs uniform throughout Omophroninae. Fungi are among well-known sources of antimicrobials (Chanthasena *et al.*, 2021).

Harpalinae stands out by having the most diverse GBC, which suggests that its GBC could be used as an approximate representation of almost all the GBC diversity in the Carabidae family, with only a few exceptions. However, this result might reflect the greater diversity of Harpalinae samples used in the current study; ten genera versus one to three genera for the other subfamilies examined here (Table 2.1). This finding suggests that detecting associations between host taxa and their GBCs requires a finer level of resolution than obtained with comparisons of GBC among carabid subfamilies.

2.5.4 Comparisons of GBC Among Carabid Genera within Harpalinae

When examining differences within the subfamily Harpalinae for the current study, different carabid genera more clearly exhibit aspects of distinct GBCs. Similarities among samples of *Amara* and *Harpalus* force all but one sample into a tight cluster in the PCoA (Fig. 2.5) and most have high levels of the bacterial phylum Actinomycetota (Table 2.4). Samples of *Agonum* and *Chlaenius* are distinct from those of *Amara* and *Harpalus*, with further separation of *Chlaenius* from some *Agonum* samples (Fig. 2.5). Different carabid genera also show large differences in numbers of bacteria phyla above a relative abundance (RA) threshold of 0.5%; three for *Badister* versus ten for *Harpalus* (Table 2.4). Further differences are apparent when examining the GBC at the level of bacterial genera. For example, the RA of f_Yersiniaceae is about three times higher in *Pterostichus* (27.6%) than in the next closest Harpalinae genera, *Calathus* (9.9%) (Table 2.5).

Only two of the few previous studies to compare GBCs among carabid genera have included congeneric species (Table 2.13). Kolasa *et al.* (2019) report the GBC for four species of the non-Harpalinae genus *Bembidion* but make no comparisons with other carabid genera. Kudo *et al.* (2019) report the GBCs for congeneric species in ten Harpalinae genera including *Pterostichus*, *Chlaenius*, *Harpalus* and *Amara*. However, they compare effects of diet and habitat on GBCs rather than host taxonomic affinity. A major take-away from Kudo *et al.* (2019) is that fungal gut symbionts are more likely to occur in *Pterostichus* and *Chlaenius* species (both carnivores) than in *Harpalus* and *Amara* species (both granivores) which they attribute to different feeding habits. Fungal gut symbionts may have a role in regulating the growth of some bacterial taxa.

Results of these previous studies (Table 2.13) allow for broad comparisons with the GBCs of carabid genera studied here. Although different species were used, this study (Table S2) and Kolasa *et al.* (2019) both identified *Rickettsia* and a member of family Orbaceae among the most RA bacterial genera in *Bembidion*. The previous study did not find high RA of *Serratia*, *Lactococcus*, *Weissella*, or *Acinetobacter* in *Bembidion* species like this study did (Table 2.13 and Appendix 1.2.1 - Table S2). In Kudo *et al.* (2019), ASVs that could not be classified to the level of order were identified as “unclassified”, which comprised the majority of the GBC reported for *Harpalus* species. In the current study of *Harpalus* GBCs, only two ASVs could not be classified to the level of order, one was a bacterium that could not be classified to phylum and the other was c_Alphaproteobacteria (Table 2.5) which dominated (21.9% RA) the GBC for the *Harpalus* species examined. For *Amara* species in Kudo *et al.* (2019), many bacterial taxa could not be classified to the order level but all of the bacterial taxa above the threshold in *Amara* for this study could be classified beyond that taxonomic level. The bacterial taxa in *Amara* that were classified to order in that paper (Streptococcaceae and Enterobacteriaceae) are consistent with what is reported for this thesis. For *Pterostichus* and *Chlaenius* species in the Kudo *et al.* (2019) paper, the family Orbaceae and Enterococcaceae were both found in most samples which is consistent with what is reported for this paper (Orbaceae: *Gilliamella* and Enterococcaceae: *Enterococcus*). Four of the six most RA bacterial genera reported in another previous study of a *Chlaenius* species (Do *et al.*, 2022) were also reported for the *Chlaenius* genus in the current study above the threshold and in similar orders of descending RA with *Enterococcus* and *Gilliamella* being the highest (Table 2.5). These collective findings provide evidence for differences in the GBCs associated with different carabid genera.

2.5.5 Comparisons of GBCs Among Carabid Species Within Genera

Examining the composition of GBC among congeneric species provides the strongest evidence that even closely related host taxa can have distinct GBCs. For each of nine genera, I compared the GBCs of at least two species. Results for species of *Agonum*, *Amara*, and *Cicindela* are emphasized here. Solely to limit the length of this chapter, results of the other six carabid genera (*Bembidion* (Appendix 1.2.1), *Carabus* (Appendix 1.2.2), *Harpalus* (Appendix 1.2.3), *Nebria* (Appendix 1.2.4), *Omophron* (Appendix

1.2.5), *Pterostichus* (Appendix 1.2.6)) are provided in Appendix 1.2 and are only briefly discussed here.

Results for six of the nine genera examined showed significant separation of species by their GBCs (*Agonum*, *Bembidion*, *Cicindela*, *Harpalus, Nebria, Pterostichus*) with the clearest separation being between species within *Cicindela* and *Agonum*. Some of this separation can be attributed to the relative abundance of one or a few specific bacterial genera. In *Agonum*, *Spiroplasma* was predominant in *A. placidum* (34% RA) but rare in *A. muelleri* and *A. piceolum* (< 1.5% RA, Table 2.7). In *Harpalus*, the dominance (87.4% RA) of a member of the Alphaproteobacteria class in *H. pensylvanicus* distinguishes its GBC from all other *Harpalus* species (Appendix 1.2.3, Fig. S5). The four *Cicindela* species examined here form two phylogenetic clades (*C. repanda* + *C. oregona* vs. *C. lengi* + *C. purpurea*) (Fig. 2.1, (Gough *et al.*, 2019)) with corresponding similarities in their GBCs. There may have been a split in GBC structure that coincided with speciation. Previous studies have linked changes in GBC structure to speciation events (An *et al.*, 2023). The distribution of f_Yersiniaceae, *Enterococcus*, and *Gilliamella* all follow a pattern in *P. adstrictus* and *P. oregonus* that resembles how those beetles are in a clade together that is separate from the other *Pterostichus* species in the phylogeny for this study (Fig. 2.1 and Appendix 1.2.6 (Table S12)).

In contrast, results for *Amara*, *Carabus,* and *Omophron* did not identify distinct differences in GBCs among species for any measure of either alpha or beta diversity (Fig. 2.7, Appendix 1.2.2 and 1.2.5). Of possible significance for *Amara*, however, is the occurrence of the bacteria Bdellovibrionota. This bacterium was only detected above the 0.5% RA threshold in the current study for *Amara quenseli* and *A. littoralis* (Table 2.8), which clade together (albeit with a low bootstrap value) in the phylogenetic tree (Fig. 2.1).

Two previous studies (Giglio *et al.*, 2021, Silver *et al.*, 2021) looked at the GBC of two *Pterostichus* species not studied here and found *Spiroplasma* at higher levels than what is reported here for *Pterostichus*. Giglio *et al.* (2021) also found the Yersiniaceae genus *Serratia* as the most RA bacterial genus in *Pterostichus melas italicus* (Table 2.13) although the current study only classified ASVs from *Bembidion carinula, Cicindela oregona,* and the carabid genus *Amara* as *Serratia* bacteria (Appendix 1.2.1 (Table S2) and Tables 2.9 and 2.11).

Comparisons within genera provide the additional insight that measures of alpha diversity are of limited value in distinguishing among GBC for congeneric species. With the exception of two cases, values for richness, Shannon diversity, and evenness did not differ significantly among congeneric species. In the first case, a significant difference in evenness was detected between *Cicindela* species corresponding to the split in diversity between the two sets of sister species (Fig. 2.8). In the second case, a difference in evenness was indicated ($p = 0.052$) between species of *Harpalus* (Appendix 1.2.3 - Fig. S4) driven by the aforementioned dominance of a member of the Alphaproteobacteria class in *H. pensylvanicus* (Appendix 1.2.3 - Table S6).

2.5.6 Bacterial Taxa of Specific Interest

The overall results of the current study identified a subset of bacteria that may be particularly important in structuring their hosts' GBC. Factors that may regulate or influence these GBCs are host taxonomy, vertical transmission of microbes, extrinsic factors (e.g., habitat), feeding habits, gut physiology, Eukaryotic microbes (e.g., fungi), Archaea, or particularly dominant bacterial taxa.

A member of the bacterial class Alphaproteobacteria was the particularly dominant RA bacterium in *Harpalus pensylvanicus* (87.4%) (Appendix 1.2.3 - Table S6) and was not found in any other carabid samples in this study, even at trace levels. Lundgren *et al.* (2010) also found an unidentified Alphaproteobacteria in *H. pensylvanicus* samples from Illinois, USA. The sequences from Lundgren *et al.* (2010) and this study were blasted on the NCBI GenBank and produced the same results, hinting at a bacterial endosymbiont similar to *Candidatus Mesenet longicola* which was isolated from coconut beetles (*Brontispa longissimi*) (Takano *et al.*, 2021). The sequences from *H. pensylvanicus* produced a 97% match to GenBank sequences for the coconut beetle endosymbiont which has been shown to create cytoplasmic incompatibility which leads to selective reproduction of only infected hosts (Takano *et al.*, 2021).

Many of the bacterial genera found here are members of the order Lactobacillales, also known as lactic acid bacteria (LAB), including *Lactobacillus, Lactococcus, Enterococcus, Leuconostoc*, and *Weisella*. At least one member of LAB was found above the cutoff threshold in virtually all carabid species studied here. They are effective at breaking down carbohydrates to produce lactic acid, short-chain fatty acids, amines, bacteriocins, vitamins, and exopolysaccharides (Wang *et al.*, 2021). Some may be transient members of the GBC and others may be resident and adapted to living in the digestive tract of the host (Endo *et al.*, 2010).

There was an unknown member of the Yersiniaceae family common in several carabid groups, especially some *Pterostichus* species. Some Yersiniaceae bacteria are pathogenic to many insects, but they are occasionally present within an insect for beneficial reasons such as to protect from parasites (Vaughan *et al.*, 2022, Boyd *et al.*, 2021, Engel *et al.*, 2012). The RA of Yersiniaceae was high enough and in enough samples of some carabid groups (i.e., average of >20% RA) that if they are members of the *Yersinia* or *Serratia* genera of Yersiniaceae and were not present in a beneficial manner then those may have been sickly carabid individuals (Kingry *et al.*, 2023). The GBC of almost all samples of *Pasimachus elongatus*, *Calathus ingratus,* and three *Pterostichus* species had a member of the Yersiniaceae family at relatively high RA which also appeared in the two other species of *Pterostichus* and in several other carabid taxa, but not in all samples and at lower RA (Fig. 2.4; Table 2.5). Within *Pterostichus*, *P. testaceus* was particularly high (54.5% RA) in that member of Yersiniaceae (Fig. 2.4; Appendix 1.2.6 - Table S12) and varying levels of Yersiniaceae may be what caused the scattering of the *Pterostichus* samples in the Harpalinae genera PCoA (Fig. 2.5).

For all samples combined, *Spiroplasma* had the 7th highest RA at 3.6%. It was found in many samples spread across carabid subfamilies, genera, and species. Previous studies of carabids and other arthropods have found higher RA of that facultatively endosymbiotic bacteria. For example, six of ten carabid GBC studies that reported bacterial genera found *Spiroplasma* in the six most RA bacteria with three of those studies reporting *Spiroplasma* in the top two highest for RA. Because *Spiroplasma* can be at high concentrations in host cells and hemolymph, a study that does not remove the gut for analysis may report higher RA of that bacteria and it is possible that even the cells of the gut lining can make it appear that *Spiroplasma* is at high RA in the gut lumen.

Wolbachia was common in the majority of samples from 12 carabid species (average RA = ~50%) (Table 2.1) but excluded from consideration in this study as they are almost never part of the GBC found in the gut lumen. However, its presence could be a factor affecting the GBC via the epithelial cells lining the gut or affecting host immune responses. More research is needed to see if *Wolbachia* affects the GBC itself or just the procedure of analyzing the GBC (Wilches *et al.*, 2021).

2.5.7 Core Gut Bacteria

A key objective of this research was to look for evidence of possible core gut bacteria at different taxonomic levels of carabids; i.e., at the level of family, subfamily, genera, or species. In this study, a core-microbiome is referred to as microbes that are found in nearly every sample (Risely & Tate, 2020). Carabidae as a family had the phyla Pseudomonadota, Bacillota, and Bacteroidota at levels greater than the 0.5% RA threshold in every subfamily, which could indicate that they are core bacterial phyla. These bacterial phyla are common in GBCs of humans and many other animals, with members of Pseudomonadota and Bacillota usually being predominant (Gilroy *et al.*, 2021, Binda *et al.*, 2018, Kho & Lal, 2018). For the current study, when viewing bacterial genera, there were none above the 1.5% RA threshold in all subfamilies. Most prevalent above this threshold were *Gilliamella* (8 of 9 subfamilies) and *Enterococcus* (7 of 9 subfamilies) (Table 2.3). *Gilliamella* is very common in honey bees (Zhang *et al.*, 2022) and was found in several previous carabid GBC studies (Table 2.13). *Enterococcus* is common throughout many animals (Shah & Varahan, 2024) including most carabid GBC studies (Table 2.13). Similarly, there is of yet no strong evidence of core bacteria specific to a carabid subfamily, although the Harpalinae subfamily is the only one that has had its microbiome studied very well.

There is greater evidence of core bacteria in particular carabid genera or species, especially if they have specialized diets (e.g., scavengers) (Kudo *et al.*, 2019). With the exception of *H. pensylvanicus* samples, which were dominated by a member of the class Alphaproteobacteria, samples of *Amara* and *Harpalus* in the current study were clustered tightly in the PCoA (Fig. 2.5). The underlying mechanism creating GBC similarities in *Harpalus* and *Amara* may be their feeding habit which is mostly granivorous (Larochelle, 1990). All *Amara* and *Harpalus* species had the bacterial phylum Actinomycetota above the cutoff threshold. For possible core gut bacteria in carabid species the LAB genus *Leuconostoc* was in all samples of *Cicindela repanda* and *Agonum piceolum* at moderate RA (average: > 30%) and had significantly high DA while not being present at all in most other carabid samples. Bacteria in the class Alphaproteobacteria associated with the species *H. pensylvanicus* could be a core member of the gut microbiome specific to that species if it resides in the gut lumen, but it is more likely to be an endosymbiont residing in the cells of the host. This same observation applies to the occurrence of *Spiroplasma*, which has been identified as both an endosymbiont and an exosymbiont depending on circumstances (Anbutsu & Fukatsu, 2003).

2.5.8 Conclusions

This study was an ambitious project to characterize the GBC of field collected carabid beetles at many different taxonomic levels while using adequate sample sizes. As such, my findings reflect the diversity of species collected, the number of individuals per species, and their gut contents at the time of collection. Patterns of GBC diversity coincided with carabid taxonomic groups, especially GBC diversity showing separation among species of some carabid genera. The clearest separation of GBC diversity being between the closest taxonomically related carabids hints at the association between speciation events and changes in GBCs.

The analyses in this chapter ignore differences in extrinsic factors and feeding habits of the carabid host. Some of the GBC patterns appearing in this thesis chapter do not seem to align with carabid taxonomy very well. Previous studies have determined that factors such as habitat, season, and feeding habit can influence GBC composition (Do *et al.*, 2022, Karimian *et al.*, 2022, Kudo *et al.*, 2019, Smith *et al.*, 2021). In the analyses of carabid subfamily GBCs in this thesis chapter, eight of the nine subfamilies were represented by hosts that were almost entirely carnivorous or omnivorous while Harpalinae was the only subfamily to include herbivorous/granivorous species and genera (*Amara* and *Harpalus*) (Larochelle, 1990). The next chapter of this thesis addresses extrinsic factors and feeding habits of the carabid host.

2.6 References

An Y, Braga MP, Garcia SL, Grudzinska-Sterno M, Hambäck PA & Sveriges L (2023) Host phylogeny structures the gut bacterial community within *Galerucella* leaf beetles. *Microbial Ecology,* **86,** 2477-2487.

Anbutsu H & Fukatsu T (2003) Population dynamics of male-killing and non-male-killing Spiroplasmas in *Drosophila melanogaster*. *Applied and Environmental Microbiology,* **69,** 1428-1434.

Armstrong RA (2014) When to use the Bonferroni correction. *Ophthalmic & Physiological Optics*, **34,** 502-508.

Binda C, Lopetuso LR, Rizzatti G, Gibiino G, Cennamo V & Gasbarrini A (2018) Actinobacteria: A relevant minority for the maintenance of gut homeostasis. *Digestive and Liver Disease,* **50,** 421-428.

Blow F & Douglas AE (2019) The hemolymph microbiome of insects. *Journal of Insect Physiology,* **115,** 33-39.

Boyd BM, Chevignon G, Patel V, Oliver KM & Strand MR (2021) Evolutionary genomics of APSE: a tailed phage that lysogenically converts the bacterium *Hamiltonella defensa* into a heritable protective symbiont of aphids. *Virology Journal,* **18,** 219.

Callahan BJ, Mcmurdie PJ, Rosen MJ, Han AW, Johnson AJ & Holmes SP (2016) DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods,* **13,** 581-583.

Cappellato M, Baruzzo G & Di Camillo B (2022) Investigating differential abundance methods in microbiome data: A benchmark study. *PLoS Computational Biology,* **18,** e1010467.

Chanthasena P, Penkhrue W, Khowangklang P, Sritangos P & Nantapong N (2021) Antimicrobial potential of fungi isolated from soils of dry dipterocarp forest in northeast Thailand. *Chiang Mai Journal of Science,* **48,** 793-807.

Dias AM, Cordeiro G, Estevinho MM, Veiga R, Figueira L, Reina-Couto M, *et al.* (2020) Gut bacterial microbiome composition and statin intake—A systematic review. *Pharmacology Research & Perspectives,* **8,** e00601.

Do Y, Park J-K, Park W-B & Kim M-S (2022) The gut bacterial community of *Chlaenius pallipes* (Coleoptera: Carabidae) associates with their habitat and morphology. *Insects,* **13,** 1099.

Douglas AE (2022) *Insects and Their Beneficial Microbes,* Princeton, NJ, USA., Princeton University Press.

Durden L, Wang D, Panaccione D & Clay K (2019) Decreased root-knot nematode gall formation in roots of the morning glory *Ipomoea tricolor* symbiotic with ergot alkaloid-producing fungal *Periglandula sp*. *Journal of Chemical Ecology,* **45,** 879-887.

Duron O & Gottlieb Y (2020) Convergence of nutritional symbioses in obligate blood feeders. *Trends in Parasitology,* **36,** 816-825.

Endo A, Futagawa-Endo Y & Dicks LMT (2010) Diversity of *Lactobacillus* and *Bifidobacterium* in feces of herbivores, omnivores and carnivores. *Anaerobe,* **16,** 590-596.

Engel P, Martinson VG & Moran NA (2012) Functional diversity within the simple gut microbiota of the honey bee. *Proceedings of the National Academy of Sciences,* **109,** 11002-11007.

Escobar-Zepeda A, Vera-Ponce De León A & Sanchez-Flores A (2015) The road to metagenomics: from microbiology to DNA sequencing technologies and bioinformatics. *Frontiers in Genetics,* **6,** 348.

Estaki M, Jiang L, Bokulich NA, Mcdonald D, González A, Kosciolek T, *et al.* (2020) QIIME 2 enables comprehensive end-to-end analysis of diverse microbiome data and comparative studies with publicly available data. *Current Protocols in Bioinformatics,* **70,** e100.

Ewing B, Hillier L, Wendl MC & Green P (1998) Base-calling of automated sequencer traces using Phred. I. accuracy assessment. *Genome Research,* **8,** 175-185.

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution,* **39,** 783-791.

Ferreira JA & Zwinderman AH (2006) On the Benjamini-Hochberg method. *The Annals of Statistics,* **34,** 1827-1849.

Folmer O, Black M, Hoeh W, Lutz R & Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology,* **3,** 294.

Geerinck MWJ, Van Hee S, Gloder G, Crauwels S, Colazza S, Jacquemyn H, *et al.* (2022) Diversity and composition of the microbiome associated with eggs of the southern green stinkbug, *Nezara viridula* (Hemiptera: Pentatomidae). *Microbiology Open,* **11,** e1337.

Giglio A, Vommaro ML, Gionechetti F & Pallavicini A (2021) Gut microbial community response to herbicide exposure in a ground beetle. *Journal of Applied Entomology,* **145,** 986-1000.

Gilad R, Porat A & Trachtenberg S (2003) Motility modes of *Spiroplasma melliferum BC3*: a helical, wall-less bacterium driven by a linear motor. *Molecular Microbiology,* **47,** 657-669.

Gilroy R, Ravi A, Getino M, Pursley I, Horton DL, Alikhan N-F*, et al.* (2021) Extensive microbial diversity within the chicken gut microbiome revealed by metagenomics and culture. *PeerJ ,* **9,** e10941.

Gough HM, Duran DP, Kawahara AY & Toussaint EFA (2019) A comprehensive molecular phylogeny of tiger beetles (Coleoptera, Carabidae, Cicindelinae). *Systematic Entomology,* **44,** 305-321.

github Carabid GBC: https://github.com/kingfishertile/Carabid-gut-bacterial-communities.git Archived supplemental data from this thesis.

Grond K, Lanctot RB, Jumpponen A & Sandercock BK (2017) Recruitment and establishment of the gut microbiome in arctic shorebirds. *FEMS Microbiology Ecology,* **93,** fix142.

Gupta A & Nair S (2020) Dynamics of insect-microbiome interaction influence host and microbial symbiont. *Frontiers in Microbiology,* **11,** 1357.

Hebert PDN, Cywinska A, Ball SL & Dewaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society. B, Biological Sciences,* **270,** 313-321.

Hill MP, Macfadyen S & Nash MA (2017) Broad spectrum pesticide application alters natural enemy communities and may facilitate secondary pest outbreaks. *PeerJ,* **5,** e4179.

Hillis DM & Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology,* **42,** 182-192.

Illumina. (2019). MiSeq System Guide. Document # 15027617 v05 Material # 20000262. Available online from https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/miseq/miseq-system-guide-for-local-run-manager-15027617-05.pdf [accessed 9 June 2024]

Janke RS, Moog S, Weiss B, Kaltenpoth M & Florez LV (2022) Morphological adaptation for ectosymbiont maintenance and transmission during metamorphosis in *Lagria* beetles. *Frontiers in Physiology,* **13,** 979200.

Jenkins S (2020) *Insects by the Numbers: A Book of Infographics,* New York, Boston, Houghton Mifflin Harcourt.

Jones RT, Sanchez LG & Fierer N (2013) A cross-taxon analysis of insect-associated bacterial diversity. *PloS One,* **8,** e61218.

Karimian F, Koosha M, Choubdar N & Oshaghi MA (2022) Comparative analysis of the gut microbiota of sand fly vectors of zoonotic visceral leishmaniasis (ZVL) in Iran; host-environment interplay shapes diversity. *PLOS Neglected Tropical Diseases,* **16,** e0010609.

Kavanaugh DH, Maddison DR, Simison WB, Schoville SD, Schmidt J, Faille A*, et al.* (2021) Phylogeny of the supertribe Nebriitae (Coleoptera, Carabidae) based on analyses of DNA sequence data. *Zookeys,* **1044,** 41-152.

Kers JG & Saccenti E (2022) The power of microbiome studies: Some considerations on which alpha and beta metrics to use and how to report results. *Frontiers in Microbiology,* **12,** 796025.

Kho ZY & Lal SK (2018) The human gut microbiome - A potential controller of wellness and disease. *Frontiers in Microbiology,* **9,** 1835.

Kingry LC, Gladney L & Petersen JM (2023) Yersiniaceae*, p.1-4. *Manual of Clinical Microbiology*, 13th Edition. ASM Press, Washington, DC.

Kolasa M, Scibior R, Mazur MA, Kubisz D, Dudek K & Kajtoch Ł (2019) How hosts taxonomy, trophy, and endosymbionts shape microbiome diversity in beetles. *Microbial Ecology,* **78,** 995-1013.

Kudo R, Masuya H, Endoh R, Kikuchi T & Ikeda H (2019) Gut bacterial and fungal communities in ground-dwelling beetles are associated with host food habit and habitat. *The ISME Journal,* **13,** 676-685.

Larochelle A (1990) *Food of Carabid Beetles (The):(Coleoptera: Carabidae, Including Cicindelinae),* Quebec, QC, Canada., Association des Entomologistes du Québec.

Larochelle A & Larivière M-C (2003) *A Natural History of the Ground-Beetles (Coleoptera: Carabidae) of America North of Mexico,* Sofia, Bulgaria, Pensoft.

Lin H & Peddada SD (2020) Analysis of compositions of microbiomes with bias correction. *Nature Communications,* **11,** 3514.

Lindroth CH (1961) The ground beetles (Carabidae, excl. Cicindelinae) of Canada and Alaska, Part 2-6. *Opuscula Entomologica***,** pp 1192.

Liu F, Hewezi T, Lebeis SL, Pantalone V, Grewal PS & Staton ME (2019) Soil indigenous microbiome and plant genotypes cooperatively modify soybean rhizosphere microbiome assembly. *BMC Microbiology,* **19,** 201.

López-López A & Vogler AP (2017) The mitogenome phylogeny of Adephaga (Coleoptera). *Molecular Phylogenetics and Evolution,* **114,** 166-174.

Lovei GL & Sunderland KD (1996) Ecology and behavior of ground beetles (Coleoptera: Carabidae). *Annual Review of Entomology,* **41,** 231-256.

Lundgren JG & Lehman RM (2010) Bacterial gut symbionts contribute to seed digestion in an omnivorous beetle. *PloS One,* **5,** e10831.

Lundgren JG, Lehman RM & Chee-Sanford J (2007) Bacterial communities within digestive tracts of ground beetles (Coleoptera: Carabidae). *Annals of the Entomological Society of America,* **100,** 275-282.

Maurice N & Erdei L (2018) Termite gut microbiome. In: Khan MA & Ahmad W (eds.) *Termites and Sustainable Management. Sustainability in Plant and Crop Protection.* CHAM, Springer Nature, pgs. 69-99.

Michaud C, Hervé V, Dupont S, Dubreuil G, Bézier AM, Meunier J*, et al.* (2020) Efficient but occasionally imperfect vertical transmission of gut mutualistic protists in a wood-feeding termite. *Molecular Ecology,* **29,** 308-324.

Moldovan OT, Carrell AA, Bulzu P-A, Levei E, Bucur R, Sitar C*, et al.* (2023) The gut microbiome mediates adaptation to scarce food in Coleoptera. *Environmental Microbiome,* **18,** 80.

Nei M & Kumar S (2000) *Molecular Evolution and Phylogenetics,* New York, Oxford University Press.

Nikoh N, Hosokawa T, Moriyama M, Oshima K, Hattori M & Fukatsu T (2014) Evolutionary origin of insect–*Wolbachia* nutritional mutualism. *Proceedings of the National Academy of Sciences,* **111,** 10257-10262.

Nishino T, Tanahashi M, Lin C-P, Koga R & Fukatsu T (2016) Fungal and bacterial endosymbionts of eared leafhoppers of the subfamily Ledrinae (Hemiptera: Cicadellidae). *Applied Entomology and Zoology,* **51,** 465-477.

Ober KA & Maddison DR (2008) Phylogenetic relationships of tribes within Harpalinae (Coleoptera: Carabidae) as inferred from 28S ribosomal DNA and the wingless gene. *Journal of Insect Science,* **8,** 63.

Ohbayashi T, Takeshita K, Kitagawa W, Nikoh N, Koga R, Meng X*, et al.* (2015) Insect’s intestinal organ for symbiont sorting. *Proceedings of the National Academy of Sciences,* **112,** E5179-E5188.

Oren A (2024) On validly published names, correct names, and changes in the nomenclature of phyla and genera of prokaryotes: A guide for the perplexed. *NPJ Biofilms and Microbiomes,* **10,** 20.

Pita L, Rix L, Slaby BM, Franke A & Hentschel U (2018) The sponge holobiont in a changing ocean: From microbes to ecosystems. *Microbiome,* **6,** 46.

Porter J & Sullivan W (2023) The cellular lives of *Wolbachia*. *Nature Reviews Microbiology,* **21,** 750-766.

Rainio J & Niemela J (2003) Ground beetles (Coleoptera: Carabidae) as bioindicators. *Biodiversity and Conservation,* **12,** 487-506.

Ribera I, Mateu J & Bellés X (2005) Phylogenetic relationships of *Dalyat mirabilis* Mateu, 2002, with a revised molecular phylogeny of ground beetles (Coleoptera, Carabidae). *Journal of Zoological Systematics and Evolutionary Research,* **43,** 284-296.

Risely A & Tate A (2020) Applying the core microbiome to understand host–microbe systems. *The Journal of Animal Ecology,* **89,** 1549-1558.

Rocha FP, Ronque MU, Lyra ML, Bacci Jr M & Oliveira PS (2022) Habitat and host species drive the structure of bacterial communities of two neotropical trap-jaw *Odontomachus* ants. *Microbial Ecology,* **86,** 699-712.

Rosenberg E & Zilber-Rosenberg I (2018) The hologenome concept of evolution after 10 years. *Microbiome,* **6,** 78.

Ruxton GD & Beauchamp G (2008) Some suggestions about appropriate use of the Kruskal–Wallis test. *Animal Behaviour,* **76,** 1083-1087.

Satpathi CR (2021) *Ground Beetles and Their Role in Management of Crop Pests,* London, CRC Press.

Schmid RB, Lehman RM, Brözel VS & Lundgren JG (2014) An indigenous gut bacterium, *Enterococcus faecalis* (Lactobacillales: Enterococcaceae), increases seed consumption by *Harpalus pensylvanicus* (Coleoptera: Carabidae). *Florida Entomologist,* **97,** 575-584.

Shah D & Varahan S (2024) *Enterococcus faecalis*. *Trends in Microbiology*. 2024, ISSN 0966-842X

Silver A, Perez S, Gee M, Xu B, Garg S, Will K, *et al.* (2021) Persistence of the ground beetle (Coleoptera: Carabidae) microbiome to diet manipulation. *PloS One,* **16,** e0241529.

Smith AH, O'Connor MP, Deal B, Kotzer C, Lee A, Wagner B, *et al.* (2021) Does getting defensive get you anywhere?—Seasonal balancing selection, temperature, and parasitoids shape real-world, protective endosymbiont dynamics in the pea aphid. *Molecular Ecology,* **30,** 2449-2472.

Takano S-I, Gotoh Y & Hayashi T (2021) "*Candidatus Mesenet longicola*": Novel endosymbionts of *Brontispa longissima* that induce cytoplasmic incompatibility. *Microbial Ecology,* **82,** 512-522.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M & Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution,* **28,** 2731-2739.

Tamura K, Stecher G & Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution,* **38,** 3022-3027.

Vaughan AL, Altermann E, Glare TR & Hurst MRH (2022) Genome sequence of the entomopathogenic *Serratia entomophila* isolate 626 and characterisation of the species specific itaconate degradation pathway. *BMC Genomics,* **23,** 728.

Venn S (2016) To fly or not to fly: Factors influencing the flight capacity of carabid beetles (Coleoptera: Carabidae). *European Journal of Entomology,* **113,** 587-600.

Wang Y, Wu J, Lv M, Shao Z, Hungwe M, Wang J, *et al.* (2021) Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. *Frontiers in Bioengineering and Biotechnology,* **9,** 612285.

Wilches DM, Coghlin PC & Floate KD (2021) Next generation sequencing, insect microbiomes, and the confounding effect of *Wolbachia*: a case study using spotted-wing Drosophila (*Drosophila suzukii*) (Diptera: Drosophilidae). *Canadian Journal of Zoology,* **99,** 588-595.

Williamson DL, Whitcomb RF, Tully JG, Gasparich GE, Rose DL, Carle P, *et al.* (1998) Revised group classification of the genus *Spiroplasma*. *International Journal of Systematic Bacteriology,* **48 Pt 1,** 1-12.

Willis AD. (2019) Rarefaction, alpha diversity, and statistics. *Frontiers in Microbiology,* **10,** 2407.

Zhang Z, Mu X, Shi Y & Zheng H (2022) Distinct roles of honeybee gut bacteria on host metabolism and neurological processes. *Microbiology Spectrum,* **10,** e0243821.

Table 2.1. Counts of samples used to compare gut bacterial communities between taxonomic groups of beetles (subfamily, genera, species). Non-rarefied libraries were used for measures of relative abundance (RA) and differential abundance (DA). Rarefied libraries were used for measures of alpha diversity and principal coordinate analyses (PCoA). Sample counts that changed after rarefaction are bolded. Carabid species that had *Wolbachia* removed from the majority of their sequence libraries are marked with an asterisk *.

Subfamily	**Genus**	**Species**	**Non-rarefied (RA/DA)**	**Rarefied (Alpha diversity, PCoA)**
Carabinae	*Carabus*	*granulatus**	5	5
	Carabus	*nemoralis**	5	5
	Carabus	*taedatus*	5	5
	Scaphinotus	*marginatus*	**2**	**1**
	Scaphinotus	*relictus*	1	1
Cicindelinae	*Cicindela*	*oregona*	5	5
	Cicindela	*purpurea*	**5**	**3**
	Cicindela	*repanda*	5	5
	Cicindela	*lengi*	5	5
Elaphrinae	*Elaphrus*	*americanus**	5	5
Harpalinae	*Apristus*	*constrictus*	**3**	**1**
	Agonum	*piceolum**	5	5
	Agonum	*placidum*	5	5
	Agonum	*muelleri*	5	5
	Amara	*farcta*	5	5
	Amara	*littoralis*	5	5
	Amara	*quenseli**	**5**	**3**
	Amara	*tenax*	1	1
	Amara	*lacustris**	2	2
	Amara	*carinata*	5	5
	Badister	*neopulchellus**	**4**	**1**
	Calathus	*ingratus*	5	5
	Chlaenius	*sericeus*	5	5
	Harpalus	*affinis*	5	5
	Harpalus	*amputatus*	5	5
	Harpalus	*fuscipalpis*	5	5
	Harpalus	*pensylvanicus*	5	5
	Microlestes	*linearis*	5	2
	Pterostichus	*adstrictus*	5	5
	Pterostichus	*melanarius*	5	5
	Pterostichus	*oregonus**	5	5
	Pterostichus	*sphodrinus**	5	5

Subfamily	**Genus**	**Species**	**Non-rarefied (RA/DA)**	**Rarefied (Alpha diversity, PCoA)**
	Pterostichus	*testaceus*	5	5
	Syntomus	*americanus*	**2**	**0**
Nebriinae	*Nebria*	*crassicornis*	5	5
	Nebria	*hudsonica*	5	5
	Opisthius	*richardsoni*	**5**	**4**
Omophroninae	*Omophron*	*americanum*	5	5
	Omophron	*tesselatum*	5	5
Patrobinae	*Diplous*	*californicus**	5	5
Scaritinae	*Clivina*	*fossor**	5	5
	Dyschirius	*truncatus*	**2**	**1**
	Dyschirius	*sphaericollis*	**3**	**0**
	Pasimachus	*elongatus*	5	5
Trechinae	*Bembidion*	*carinula*	5	5
	Bembidion	*quadrimaculatum**	5	5
	Trechus	*oregonensis*	**5**	**4**
Totals			**210**	**189**

Table 2.2. Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in the gut bacterial community (GBC) of nine carabid subfamilies. Numbers in bold are the highest RA in each column. Only bacteria exceeding ≥ 0.5% RA are shown.

	Total (n = 210)	Carabinae (n = 18)	Cicindelinae (n = 20)	Elaphrinae (n = 5)	Harpalinae (n = 107)	Nebriinae (n = 15)	Omophroninae (n = 10)	Patrobinae (n = 5)	Scaritinae (n = 15)	Trechinae (n = 15)
Pseudomonadota	**43.9**	26.2[2]	32.2[1]	**54.8[1]**	**50.3***	**59.1[1]**	10.1[2]	38.1[2]	**40.0[2]**	**45.8[2]**
Bacillota	41	**58.1[1]**	**50.0[1]**	16.3[1]	28.7[2]	18.9[2]	**78.5[1]**	**40.7[2]**	32.6[2]	41.8[2]
Actinomycetota	5.1	6.8[2]	10.6[1]		9.5[2]	6.7	1.8[1]	9.5[1]	5.1[1]	7.0[2]
Bacteroidota	4.6	2.9	2.2	15.5[1]	2.7[2]	10.4[1]	7.9[1]	6.0	14.9	2.7[2]
Fusobacteriota	1.4	1.3	2.4	12.1	1.5	[2]	1.6	3.5		
Chloroflexota	1.2	2.1			2.1[1]				0.7	1.2
Planctomycetota	1.1	0.7	1.0		2.1[1]	2.7[1]			4.5	
Acidobacteriota	0.5				0.8			1.7		
Verrucomicrobiota		0.6		0.5	0.7				0.7	
Myxococcota		[2]	[2]		0.5	[2]			0.7	
total RA	98.7	98.7	98.4	99.2	98.9	97.8	99.9	99.5	99.2	98.5

[1] significantly high different abundance (DA) compared to other beetle groupings

[2] significantly low DA

*significant midrange DA

Table 2.3. Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera in the gut bacterial community (GBC) of nine carabid subfamilies. Numbers in bold are the highest RA in each column. Only bacteria ≥ 1.5% RA are shown.

	Total (n = 210)	Carabinae (n = 18)	Cicindelinae (n = 20)	Elaphrinae (n = 5)	Harpalinae (n = 107)	Nebriinae (n = 15)	Omophroninae (n = 10)	Patrobinae (n = 5)	Scaritinae (n = 15)	Trechinae (n = 15)
Enterococcus	**19.1**	**22.6***	**23.2***	10.3^{1}	**9.3***	6.3^{2}	**34.1^{1}**		13.7^{2}	
Gilliamella	8.1	2.7	9.5*	**44.7^{1}**	6.5*	14.0*	5.4^{1}	**30.3^{1}**	**^{2}**	6.1
f_Yersiniaceae	7.8				8.2^{1}	4.9			**13.9**	
Lactobacillus	5.7	5.5	6.5		4.1		27.3^{1}			
c_Alphaproteobacteria	4.3				4.1					
f_Enterobacteriaceae	3.7	6.3			5.1				3.0	
Spiroplasma	3.6				3.3	2.3*	5.3	27.7^{1}	^{2}	**12.3**
o_Enterobacterales	3.3				3.6^{1}					6.6
Leuconostoc	2.5		11.0		1.8					
Pseudomonas	2.0	2.0		1.6	1.9*	**14.6^{1}**	1.7^{1}			
Dysgonomonas	1.7				1.8	3.1	7.2^{1}	3.4	1.7	
f_Lachnospiraceae	1.6	7.7		3.4			8.2			
o_Lactobacillales	1.6	7.3		2.0		1.9		12.0		9.1
Lactococcus	1.5		3.1		2.1^{1}	4.4			2.4	4.4
Flavobacterium									6.6	
Serratia										5.6
Mesoplasma		5.7								
Sodalis		5.6								
f_Wohlfahrtiimonadaceae		4.1								
Lachnoclostridium		2.9								

	Total (n = 210)	Carabinae (n = 18)	Cicindelinae (n = 20)	Elaphrinae (n = 5)	Harpalinae (n = 107)	Nebriinae (n = 15)	Omophroninae (n = 10)	Patrobinae (n = 5)	Scaritinae (n = 15)	Trechinae (n = 15)
Carnobacterium		2.0				1.7				
JG30-KF-CM45		1.7								
c_Gammaproteobacteria			3.9					6.5		
Escherichia-Shigella			3.7[1]		4.3				5.7	3.6[1]
Burkholderia-Caballeronia-Paraburkholderia			3.1							
f_Entomoplasmataceae			2.5							
Fusobacterium			2.4					1.8		
o_Corynebacteriales			2.3							
Apibacter				14.8					3.2	
Sebaldella				11.6				1.7		
Pragia				4.3						
Weissella					2.2				1.7	4.9
Hafnia-Obesumbacterium					1.8					
Bacteroides						6.7				
f_Budviciaceae						5.5				
Aeromonas						4.7				
f__Rhodobacteraceae						1.5				
f_Micrococcaceae							1.8[1]			
f_Corynebacteriaceae								3.9		
Corynebacterium								1.8		
f_Blastocatellaceae								1.7		

	Total (n = 210)	Carabinae (n = 18)	Cicindelinae (n = 20)	Elaphrinae (n = 5)	Harpalinae (n = 107)	Nebriinae (n = 15)	Omophroninae (n = 10)	Patrobinae (n = 5)	Scaritinae (n = 15)	Trechinae (n = 15)
Arthrobacter								1.6		
Rhodococcus								1.5		
f_Orbaceae									4.8	6.9
f_Leuconostocaceae									4.0	
o_Burkholderiales									4.0	
o_Entomoplasmatales									3.8	
Pirellula									3.5	
Rickettsia									2.6	9.1
Bacillus									2.6	
Prevotella									1.8	
Candidatus_Bacilloplasma										4.5
Acinetobacter										3.9
Exiguobacterium										2.7
Nocardioides										1.8
Streptomyces										1.5
Total RA presented	66.5	76.1	71.2	92.7	60.1	71.6	91.0	93.9	79.0	83.0

[1] significantly high different abundance (DA) compared to other beetle groupings

[2] significantly low DA

*significant midrange DA

Table 2.4. Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in the gut bacterial community (GBC) of six Harpalinae genera. Numbers in bold are the highest RA in each column. Only bacteria ≥ 0.5% RA are shown.

	Total (n = 93)	*Agonum* (n = 15)	*Amara* (n = 23)	*Calathus* (n = 5)	*Chlaenius* (n = 5)	*Harpalus* (n = 20)	*Pterostichus* (n = 25)
Pseudomonadota	**49.7**	40.2[1]	**38.0[2]**	**69.5[1]**	38.1*	**58.2[1]**	**57.5[1]**
Bacillota	29.9	**43.3[1]**	23.6	20.2*	**48.1[1]**	20.6[2]	33.5[1]
Actinomycetota	8.2		19.1[1]	4.4	2.7*	10.7[1]	2.7*
Bacteroidota	3.1	10.9[1]	1.0	0.7	3.1[1]	1.2	2.4[1]
Chloroflexota	2.3		6.7[1]	1.0		2.3[1]	
Planctomycetota	1.9		3.9[1]	1.4	1.4	3.2	
Fusobacteriota	1.7	4.9[1]	[2]	[2]	1.0	*	3.0
Acidobacteriota	0.9		2.6	1.2		0.7	
Verrucomicrobiota	0.7		1.9	0.8		0.6	
Myxococcota	0.6		2.0				*
unidentified phylum						0.6	
Synergistota					3.9		
Cyanobacteria						0.8	
Campilobacterota					0.5		
Total	99.6	99.9	99.2	99.6	99.6	99.6	99.9

[1] significantly high different abundance (DA) compared to other beetle groups

[2] significantly low DA

*significant midrange DA

Table 2.5. Relative abundance (%RA) and significant differential abundance (DA) of bacterial genera in the GBC of six genera in the Harpalinae subfamily. Numbers in bold are the highest RA in each column. Only bacteria ≥ 1.5% RA are shown.

		Total (n = 93)	*Agonum* (n = 15)	*Amara* (n = 23)	*Calathus* (n = 5)	*Chlaenius* (n = 5)	*Harpalus* (n = 20)	*Pterostichus* (n = 25)
Actinomycetota;	*Cellulomonas*			1.6				
	f__Micrococcaceae			1.5				[2]
	Nocardioides			2.2				
Bacillota;	*Enterococcus*	**10.4**	15.8[1]	2.0		**32.8[1]**	1.6	19.8*
	Exiguobacterium			2.6				
	Lactobacillus	4.8		3.7	9.9[1]		8.0	5.5
	Lactococcus	2.5		5.8[1]		5.9	2.5	
	Leuconostoc	2.1	13.3					
	o__Lactobacillales			[2]	4.1[1]	[2]		3.4*
	Spiroplasma	3.8	12.5	1.8		[2]	5.8	
	Weissella				3.0	4.1		
Bacteroidota;	*Dysgonomonas*	2.1	11.4[1]	[2]		2.7		
Chloroflexota;	*KD4-96*			2.7				
	Gitt-GS-136			1.5				[2]
	JG30-KF-CM45			1.5				[2]
Fusobacteriota;	*Sebaldella*	1.5	4.8	[2]			[2]	2.7
Pseudomonadota;	*Acinetobacter*					1.7	1.6	
	c__Alphaproteobacteria	4.8					**21.9**	
	Escherichia-Shigella						2.4	
	f__Enterobacteriaceae	5.2	2.6	5.6	**29.8[1]**	8.1	3.3	2.4
	f__Rhodobacteraceae			1.8				0.0
	f__Yersiniaceae	9.5		2.8	9.9[1]	3.0	2.7	**27.6[1]**

	Total (n = 93)	*Agonum* (n = 15)	*Amara* (n = 23)	*Calathus* (n = 5)	*Chlaenius* (n = 5)	*Harpalus* (n = 20)	*Pterostichus* (n = 25)
Gilliamella	7.0	**24.8^{1}**	**^{2}**	4.5	12.8^{1}		8.3*
Hafnia-Obesumbacterium	2.1		2.5	14.7^{1}	**^{2}**		2.2
Klebsiella		3.5					
Methylobacterium-Methylorubrum					^{2}	5.6	
o__Enterobacterales	4.1		**6.2**			7.2^{1}	3.6
Pragia		3.5					2.8
Pseudomonas	2.2	2.7			4.2		3.9^{1}
Rickettsia			^{2}	2.4^{1}	^{2}		
Rickettsiella			3.3				
Serratia			4.2				
Synergistota; *Candidatus_Tammella*					3.0		
Verrucomicrobiota; *Chthoniobacter*			1.6		^{2}		
Total	62.1	95.0	54.9	78.3	78.2	62.6	82.2

^{1} significantly high different abundance (DA) compared to other beetle groupings,
^{2} significantly low DA, *significant midrange DA

Table 2.6. Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in the gut bacterial community (GBC) of three *Agonum* species. Numbers in bold are the highest RA in each *Agonum* species. Only bacteria ≥ 0.5% RA are shown.

	Total (n = 15)	*A. muelleri* (n = 5)	*A. piceolum* (n = 5)	*A. placidum* (n = 5)
Bacillota	**43.3**	23.2[2]	**59.4**[1]	**47.4***
Pseudomonadota	40.2	**55.9***	35.7[2]	29.1[1]
Bacteroidota	10.9	19.8[1]	1.1	11.9
Fusobacteriota	4.9	[2]	3.5	11.1
Actinomycetota		0.8	[2]	
Total RA	99.3	99.7	99.7	99.5

[1] significantly high different abundance (DA) compared to other beetle groupings

[2] significantly low DA

*significant midrange DA

Table 2.7. Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera in the gut bacterial community (GBC) of three *Agonum* species. Numbers in bold are the highest RA in each column. Only bacteria ≥ 1.5% RA are shown.

	Total (n = 15)	*A. muelleri* (n = 5)	*A. piceolum* (n = 5)	*A. placidum* (n = 5)
Gilliamella	**27.0**	**27.7**	34.3[1]	18.9[1]
Enterococcus	17.0	20.0[1]	18.5[1]	12.6[2]
Leuconostoc	12.1		**37.3[1]**	
Spiroplasma	11.7			**34.1[1]**
Dysgonomonas	10.6	19.3		12.0
Sebaldella	4.7		3.0	11.0
Pragia	3.3			9.9
Klebsiella	3.2	9.7		
Pseudomonas	2.5	7.6[1]		
f_Enterobacteriaceae	2.5	7.2		
Weissella			2.8	
ZOR0006		1.5		
f_Rhizobiaceae		1.5		
Total RA	94.6	94.5	95.9	98.5

[1] significantly high different abundance (DA) compared to other beetle groupings

Table 2.8. Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in the gut bacterial community (GBC) of four *Amara* species. Numbers in bold are the highest RA in each column. Only bacteria ≥ 0.5% RA are shown.

	Total (n = 20)	*A. carinata* (n = 5)	*A. farcta* (n = 5)	*A. littoralis* (n = 5)	*A. quenseli* (n = 5)
Pseudomonadota	**31.2**	25.4^1	27.7^2	28.3^2	**43.5^2**
Bacillota	25.8	24.5*	**30.9^1**	**35.8^1**	12.0^2
Actinomycetota	20.8	**25.8^1**	16.8^2	17.4^2	23.3^2
Chloroflexota	7.7	10.9^1	7.2	7.5^2	5.3
Planctomycetota	4.5	6.2^1	4.8	4.1^2	3.0
Acidobacteriota	3.0	1.8	5.6	2.9	1.7
Myxococcota	2.3	0.6	1.3	1.2	5.8
Verrucomicrobiota	2.2	2.6	1.1	0.5	4.6
Bacteroidota	1.2		2.9	1.3	
Bdellovibrionota			^{2}	0.7	0.5
WS2		0.8			
unidentified phylum		0.6	^{2}	^{2}	
Gemmatimonadota			0.6	^{2}	
Total	98.7	99.3	99.0	99.8	99.7

[1] significantly high different abundance (DA) compared to other beetle groupings

[2] significantly low DA

*significant midrange DA

Table 2.9. Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera in the gut bacterial community (GBC) of four *Amara* species. Numbers in bold are the highest RA in each column. Only bacteria exceeding ≥ 1.5% RA are shown.

	Total (n = 5)	*A. carinata* (n = 5)	*A. farcta* (n = 5)	*A. littoralis* (n = 5)	*A. quenseli* (n = 5)
Acidobacteriota;f__Blastocatellaceae			1.5^{1}		
Vicinamibacteraceae			1.5		
Actinomycetota;*Iamia*			1.6		
Corynebacterium		2.0			
Cellulomonas	1.9		^{2}		5.5
f__Intrasporangiaceae					3.2
f__Microbacteriaceae		2.7			
Schumannella				2.7	
f__Micrococcaceae	1.7	1.7		2.7^{1}	2.2
Nocardioides	2.3		*		5.8
Gaiella			1.8		
o__Gaiellales	1.6		*	2.2	2.7
67-14		2.2^{1}			
Solirubrobacter		2.3			
Chloroflexota;*JG30-KF-CM45*	1.7	2.3	2.5	1.6	
Gitt-GS-136	1.7	4.1^{1}	1.6*		
KD4-96	3.1	3.1	1.9^{1}	2.9	4.5
Bacillota;*Bacillus*		1.5		1.9	
f__Planococcaceae					1.5
Spiroplasma	2.0		2.5	5.6^{1}	
Exiguobacterium	2.2				7.2
Enterococcus	2.3		2.1	6.7	
Lactobacillus	4.2	**14.2**	1.8		
Weissella	1.5			4.4	
Lactococcus	**6.7**	6.8	6.9^{1}	**11.3^{1}**	1.9
f__Lachnospiraceae			5.6		
Tyzzerella			1.5		
Candidatus_Soleaferrea			1.9		
Myxococcota;c__Polyangia					4.8
Planctomycetota;*Gemmata*		1.5		2.0	
Pir4_lineage		1.7	^{1}		
Pirellula			1.5^{1}		
SH-PL14					1.5
Pseudomonadota;*Chelativorans*				2.5	
f__Rhodobacteraceae	2.0			7.5	

	Total (n = 5)	*A. carinata* (n = 5)	*A. farcta* (n = 5)	*A. littoralis* (n = 5)	*A. quenseli* (n = 5)
f__Rhodobacteraceae uncultured					1.6
Sphingomonas		1.9			
Rickettsiella	3.8		**13.9**		
o__Enterobacterales	2.0		2.9^{1}		4.5
f__Enterobacteriaceae	6.4				**25.1**
Escherichia-Shigella				4.3^{1}	
Hafnia-Obesumbacterium	2.8	10.0			
f__Yersiniaceae	3.2	5.4			7.3
Acinetobacter				2.4	
Stenotrophomonas					1.6
Verrucomicrobiota;*Chthoniobacter*	1.8	1.7	^{1}		4.6
Total	55.1	65.1	52.9	60.5	85.5

[1] significantly high different abundance (DA) compared to other beetle groupings

[2] significantly low DA

*significant midrange DA

Table 2.10. Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in the gut bacterial community (GBC) of four *Cicindela* species. Numbers in bold are the highest RA in each column. Only bacteria exceeding ≥ 0.5% RA are shown.

	Total (n = 20)	*C. lengi* (n = 5)	*C. oregona* (n = 5)	*C. purpurea* (n = 5)	*C. repanda* (n = 5)
Bacillota	**50.0**	**50.3**[1]	**70.2**[1]	10.5	**69.1**[1]
Pseudomonadota	32.2	28.3[2]	27.0[1]	**47.3***	26.4[1]
Actinomycetota	10.6	3.7	2.7[2]	33.3[1]	2.9*
Fusobacteriota	2.4	9.3		[2]	
Bacteroidota	2.2	7.7		[2]	1.0
Planctomycetota	1.0	[2]		3.7	[2]
Chloroflexota	0.5	[2]		1.9	[2]
Campilobacterota		0.7			
Verrucomicrobiota		[2]		1.2	
Acidobacteriota				0.9	
Armatimonadota				0.6	
Gemmatinonadota				0.5	
Total RA	98.9	100.0	99.9	99.9	99.4

[1] significantly high different abundance (DA) compared to other beetle groupings

[2] significantly low DA

*significant midrange DA

Table 2.11. Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera in the gut bacterial community (GBC) of four *Cicindela* species. Numbers in bold are the highest RA in each column. Only bacteria exceeding ≥ 1.5% RA are shown.

	Total (n = 20)	*C. lengi* (n = 5)	*C. oregona* (n = 5)	*C. purpurea* (n = 5)	*C. repanda* (n = 5)
Bacillota;*Enterococcus*	**23.2**		**55.5**[1]		**36.9**[1]
Bacillota;*Leuconostoc*	11.0		13.9		30.3[1]
Pseudomonadota;*Gilliamella*	9.5		22.4		15.5[1]
Bacillota;*Lactobacillus*	6.5	**17.1**		8.3	
Pseudomonadota;c_Gammaproteobacteria	3.9	15.6			
Pseudomonadota;*Escherichia-Shigella*	3.7	2.6		**10.8**	
Pseudomonadota;*Burkholderia-Caballeronia-Paraburkholderia*	3.1	2.4		10.0	
Bacillota;*Lactococcus*	3.1	12.3			
Bacillota;f_Entomoplasmataceae	2.5	9.1			
Fusobacteriota;*Fusobacterium*	2.4	9.3			
Actinomycetota;o_Corynebacteriales	2.3			9.1	
Bacillota;*Mesoplasma*		5.5			
Pseudomonadota;*Phreatobacter*				5.3	
Pseudomonadota;f_Caulobacteraceae				3.3	
Actinomycetota;f_Geodermatophilaceae				4.5	
Pseudomonadota;f_Caulobacteraceae				3.9	
Pseudomonadota;f_Acetobacteraceae				2.8	
Actinomycetota;*Tsukamurella*				3.7	
Pseudomonadota;f_Neisseriaceae		3.1			
Bacteroidota;f_Tannerellaceae		2.8			
Actinomycetota;*Williamsia*				2.9	
Actinomycetota;f_Microbacteriaceae				2.9	
Bacteroidota;o_Bacteroidales		2.7			
Actinomycetota;*Blastococcus*				2.4	
Pseudomonadota;*Acinetobacter*				1.9	
Pseudomonadota;*Serratia*			1.7		
Pseudomonadota;*Sphingomonas*				1.5	
Total	71.3	82.7	93.4	73.4	82.7

[1] significantly high different abundance (DA) compared to other beetle groupings

Table 2.12. Previous gut bacterial community (GBC) findings at the bacterial phyla taxonomic level in descending order of relative abundance (left to right). Sample sizes (n) are also included for each study.

species studied	**n**	**phylum 1**	**phylum 2**	**phylum 3**	**phylum 4**	**phylum 5**
Chlaenius pallipes (Harpalinae)[1]	32	Bacillota	Pseudomonadota	Bacteroidota	Actinomycetota	Mycoplasmatota
Pterostichus melas italicus (Harpalinae)[2]	30	Pseudomonadota	Bacillota	Fusobacteriota	Mycoplasmatota	Bacteroidota
Harpalus pensylvanicus (Harpalinae)[3]	1	Pseudomonadota	Mycoplasmatota	Actinomycetota	Bacillota	Bacteroidota
Pseudoophonus rufipes (Harpalinae)[4]	29	Mycoplasmatota	Pseudomonadota	Bacillota	Actinomycetota	Bacteroidota
Anisodactylus similis, *Pterostichus serripes*, and *Brachinus elongatulus* (2 Harpalinae, 1 Brachininae)[5]	(36) 12 each	Pseudomonadota	Bacteroidota	Mycoplasmatota	Bacillota	
Brachinus elongatulus (Brachininae)[6]	37	Pseudomonadota	Mycoplasmatota	Bacteroidota	Bacillota	Fusobacteriota
Calosoma peregrinator (Carabinae)[7]	3	Pseudomonadota	Bacillota			
Duvalius paroecus and *D. voitestii* (Trechinae), both from caves[8]	9 and 1					
Harpalus pensylvanicus and *Anisodactylus sanctaecrucis* (Harpalinae)[9]	4 and 6					
27 species (10 Harpalinae genera, 1 Cicindelinae genus, 2 Carabinae genera)[10]	85, 1, and 6					
Poecilus chalcites (Harpalinae)[11]	58					
Harpalus pensylvanicus (Harpalinae)[12]	75					

species studied	**n**	**phylum 1**	**phylum 2**	**phylum 3**	**phylum 4**	**phylum 5**
Bembidion decorum, *B. modestum*, *B. punctulatum*, *B. varicolor* (Trechinae)[13]	(39) ~10 each					

[1]Do *et al.*, 2022, [2]Giglio *et al.*, 2021, [3]Kieran 2020, [4]Magagnoli *et al.*, 2022, [5]Silver *et al.*, 2021, [6]McManus *et al.*, 2018, [7]Colman, 2012, [8]Moldovan *et al.*, 2022, [9]Lundgren *et al.*, 2007, [10]Kudo *et al.*, 2019, [11]Lehman *et al.*, 2009, [12]Lundgren & Lehman, 2010, [13]Kolasa *et al.*, 2019

Table 2.13. Previous gut bacterial community (GBC) findings at the bacterial genera taxonomic level in descending order of relative abundance (left to right).

species studied	**n =**	**genus 1**	**genus 2**	**genus 3**	**genus 4**	**genus 5**	**genus 6**
Duvalius paroecus and *D. voitestii* (Trechinae), both from caves[1]	9 and 1	*Carnobacterium*	*Enhydrobacter*	*Vagococcus*	*Acinetobacter*	*Lactococcus*	*Serratia*
Bembidion decorum, *B. modestum*, *B. punctulatum*, *B. varicolor* (Trechinae)[2]	~10 each (39)	*Orbus*	*Wolbachia*	*Rickettsia*	*Pseudomonas*	*Aeromonas*	*Enterococcus*
Chlaenius pallipes (Harpalinae)[3]	32	*Enterococcus*	*Gilliamella*	*Bacillus*	*Dysgonomonas*	*Pseudomonas*	*Aeromonas*
Pterostichus melas italicus (Harpalinae)[4]	30	*Serratia*	*Spiroplasma*	*Enterococcus*	*Dysgonomonas*	*Sebaldella*	*Gilliamella*
Harpalus pensylvanicus and *Anisodactylus sanctaecrucis* (Harpalinae)[5]	4 and 6	*Hafnia*	*Serratia*	*Weissella*	*Burkholderia*	*Enterobacter*	*Spiroplasma*
Poecilus chalcites (Harpalinae)[6]	58	*Enterococcus*	*Weissella*	*Lactobacillus*	*Lactococcus*	*Sebaldella*	*Dysgonomonas*
Harpalus pensylvanicus (Harpalinae)[7]	75	*Pantoea*	*Enterobacter*	*Spiroplasma*	*Wolbachia*	*Serratia*	*Acinetobacter*
Pseudoophonus rufipes (Harpalinae)[8]	29	*Spiroplasma*	*Bifidobacterium*	*Hepanticola*	*Enterococcus*	*Vagococcus*	*Wolbachia*
Anisodactylus similis, *Pterostichus serripes*, *Brachinus elongatulus* (2 Harpalinae, 1 Brachininae)[9]	12 each	*Acinetobacter*	*Spiroplasma*	*Yersinia*	*Flavobacterium*	*Pseudomonas*	*Enterococcus*
Brachinus elongatulus (Brachininae)[10]	37	*Enterococcus*	*Dysgonomonas*	*Spiroplasma*	*Candidatus Soleaferrea*	*Weissella*	
Calosoma peregrinator	3						

species studied	**n =**	**genus 1**	**genus 2**	**genus 3**	**genus 4**	**genus 5**	**genus 6**
(Carabinae)[11]							
Harpalus pensylvanicus (Harpalinae)[12]	1						
27 species (10 genera of Harpalinae, 1 genus of Cicindelinae, 2 genera of Carabinae)[13]	85, 1, and 6						

[1] Moldovan *et al.*, 2022, [2] Kolasa *et al.*, 2019, [3] Do *et al.*, 2022, [4] Giglio *et al.*, 2021, [5] Lundgren *et al.*, 2007, [6] Lehman *et al.*, 2009, [7] Lundgren & Lehman, 2010, [8] Magagnoli *et al.*, 2022, [9] Silver *et al.*, 2021, [10] McManus *et al.*, 2018, [11] Colman, 2012, [12] Kieran 2020, [13] Kudo *et al.*, 2019

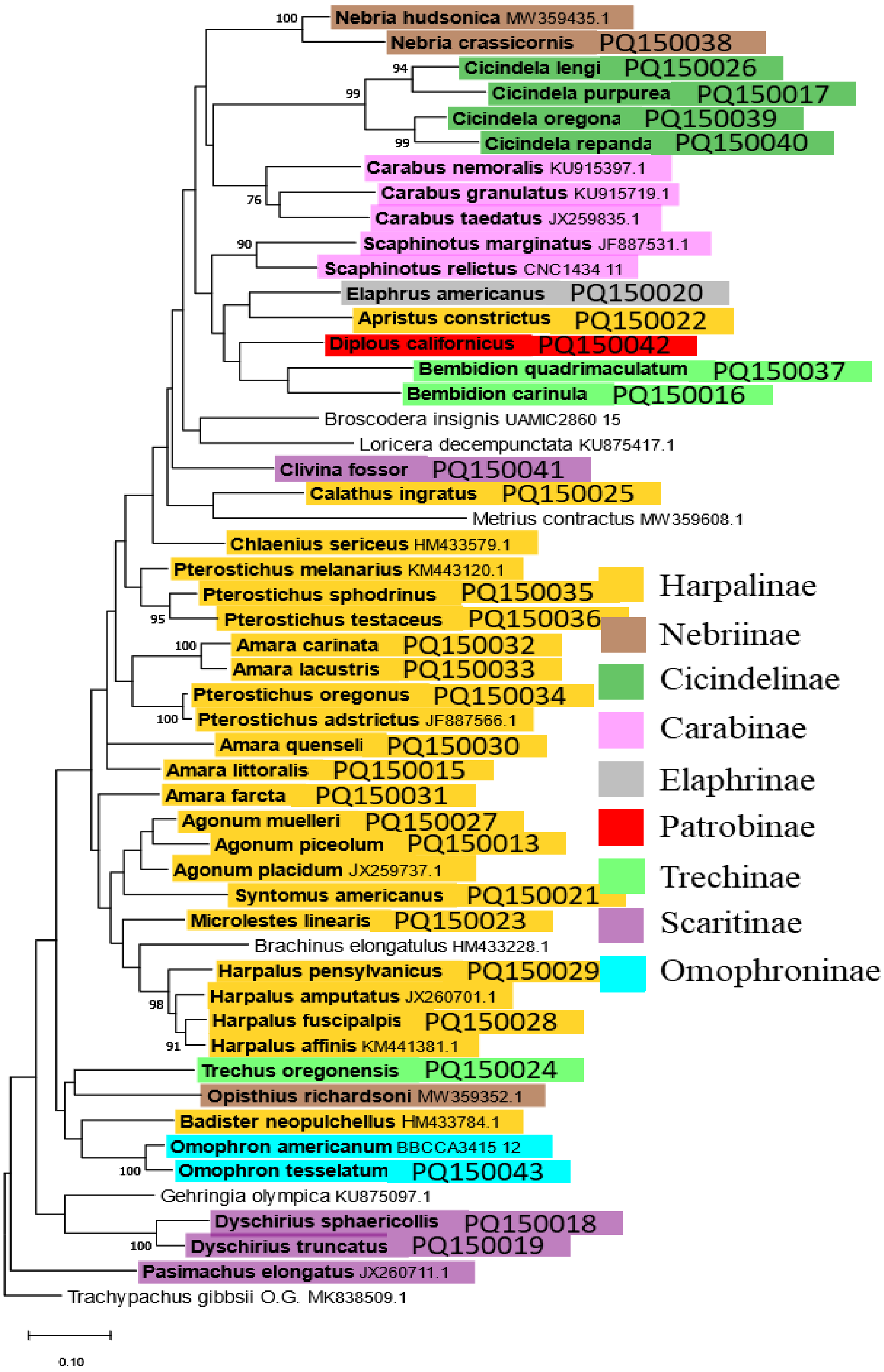

100
Nebria hudsonica MW359435.1
Nebria crassicornis PQ150038
94
Cicindela lengi PQ150026
99
Cicindela purpurea PQ150017
Cicindela oregona PQ150039
99
Cicindela repanda PQ150040
Carabus nemoralis KU915397.1
76
Carabus granulatus KU915719.1
Carabus taedatus JX259835.1
90
Scaphinotus marginatus JF887531.1
Scaphinotus relictus CNC1434 11
Elaphrus americanus PQ150020
Apristus constrictus PQ150022
Diplous californicus PQ150042
Bembidion quadrimaculatum PQ150037
Bembidion carinula PQ150016
Broscodera insignis UAMIC2860 15
Loricera decempunctata KU875417.1
Clivina fossor PQ150041
Calathus ingratus PQ150025
Metrius contractus MW359608.1
Chlaenius sericeus HM433579.1
Pterostichus melanarius KM443120.1
Pterostichus sphodrinus PQ150035
95
Pterostichus testaceus PQ150036
100
Amara carinata PQ150032
Amara lacustris PQ150033
Pterostichus oregonus PQ150034
100
Pterostichus adstrictus JF887566.1
Amara quenseli PQ150030
Amara littoralis PQ150015
Amara farcta PQ150031
Agonum muelleri PQ150027
Agonum piceolum PQ150013
Agonum placidum JX259737.1
Syntomus americanus PQ150021
Microlestes linearis PQ150023
Brachinus elongatulus HM433228.1
Harpalus pensylvanicus PQ150029
98
Harpalus amputatus JX260701.1
Harpalus fuscipalpis PQ150028
91
Harpalus affinis KM441381.1
Trechus oregonensis PQ150024
Opisthius richardsoni MW359352.1
Badister neopulchellus HM433784.1
Omophron americanum BBCCA3415 12
100
Omophron tesselatum PQ150043
Gehringia olympica KU875097.1
Dyschirius sphaericollis PQ150018
100
Dyschirius truncatus PQ150019
Pasimachus elongatus JX260711.1
Trachypachus gibbsii O.G. MK838509.1
0.10
Harpalinae
Nebriinae
Cicindelinae
Carabinae
Elaphrinae
Patrobinae
Trechinae
Scaritinae
Omophroninae

Figure 2.1. Phylogenetic tree based on 658 bp cytochrome oxidase subunit I (COI) data for carabid species from the 14 subfamilies in North America. The bacterial gut communities of species shown in bold font and colored boxes were analyzed in the current chapter. The COI sequences were obtained either in the current study (bold GenBank accession numbers) or from GenBank (non-bold accession numbers). Colors indicate the subfamily in which these species reside. The remaining species (not colored and in non-bold font) are members of subfamilies not collected in the current study. Bootstrap values over 70% are shown at the nodes and genetic distance (General Time Reversible) is at the bottom of the tree.

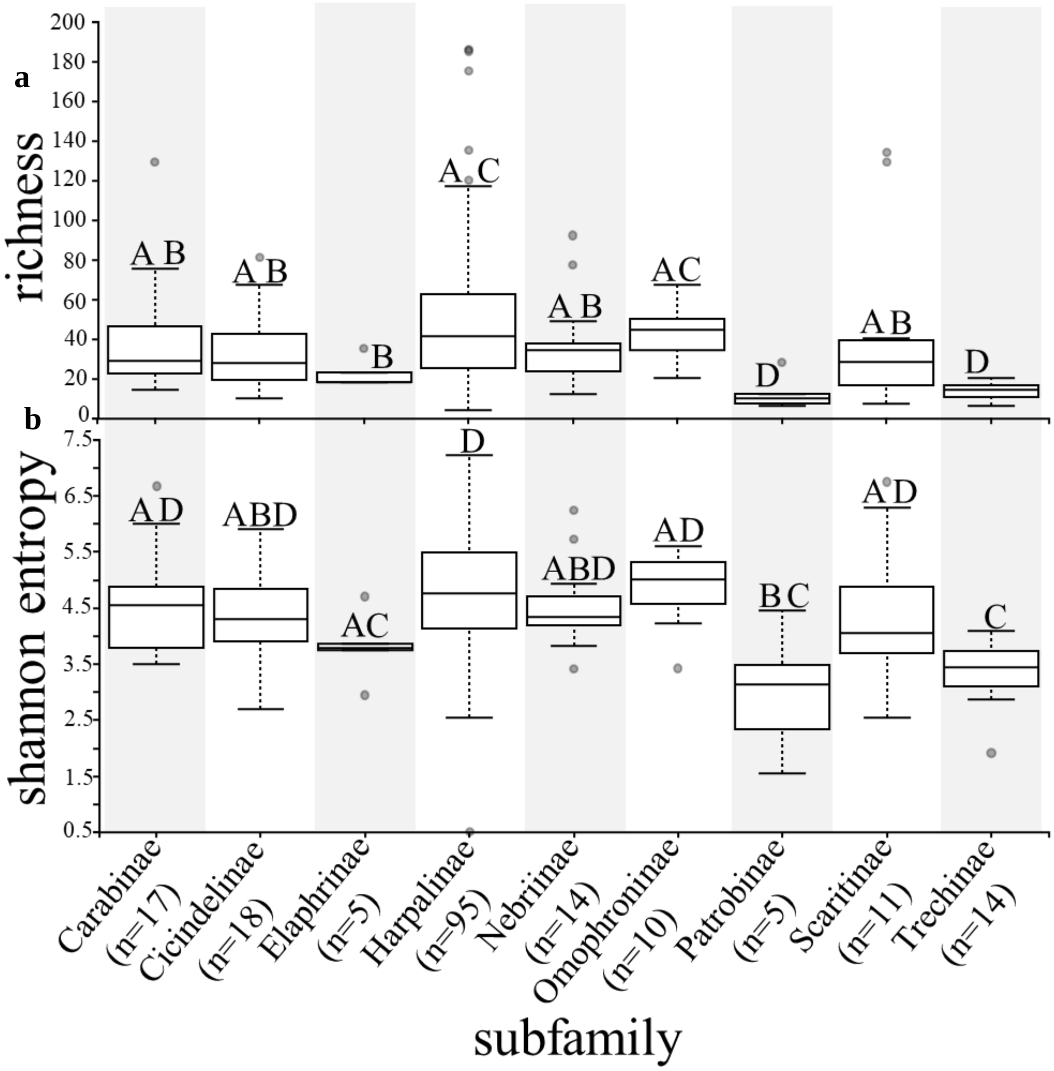


Figure 2.2. Alpha diversity measures of bacterial species a) richness and b) Shannon diversity in nine carabid subfamilies. Dots indicate outliers and error bars indicate highest and lowest values. The bottom and top edges of the boxes show quartile 1 and quartile 3 respectively and the middle line in the box indicates the median value. Letters above box plots indicate significance. Groups that share a letter are not significantly different from one another.

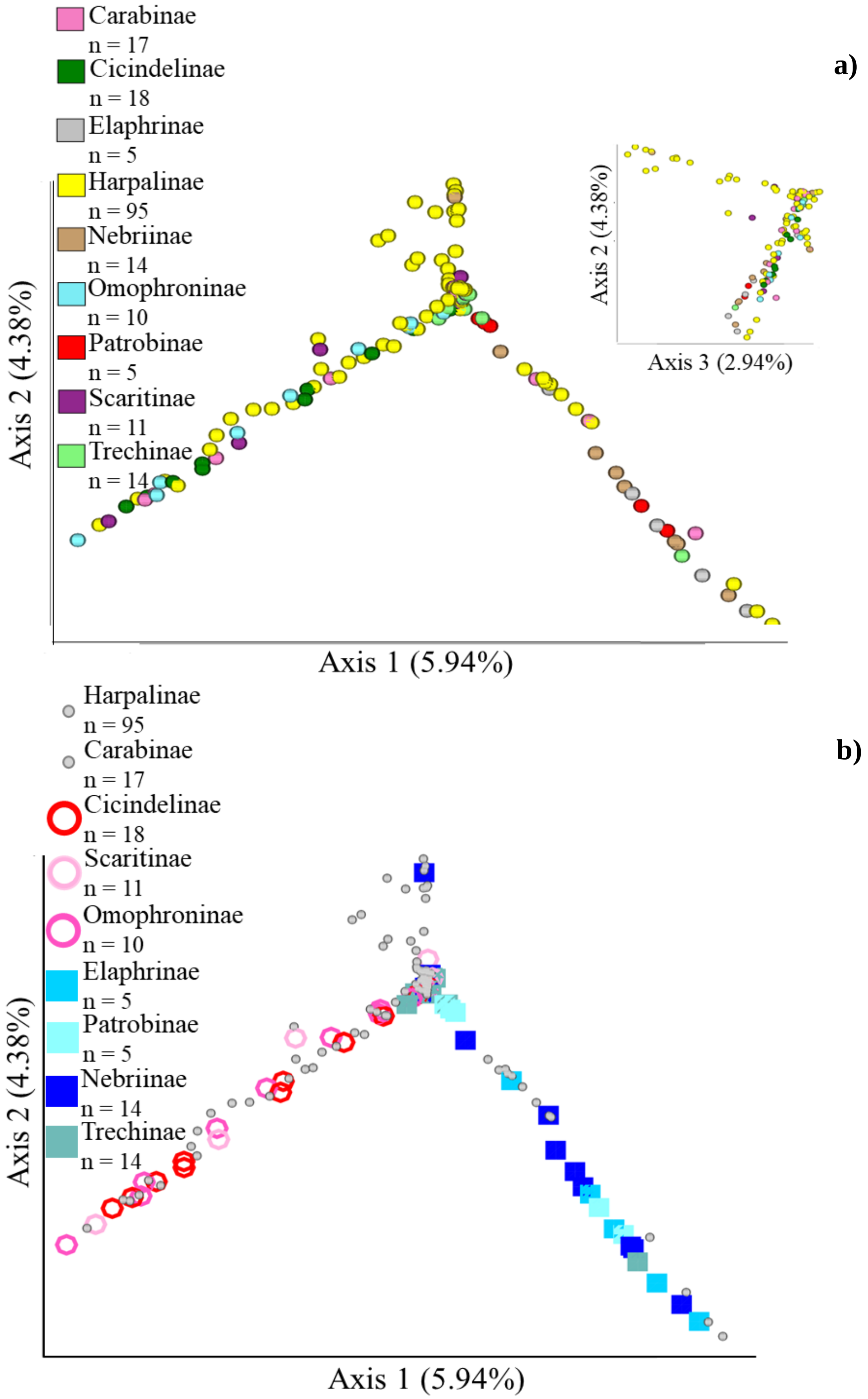
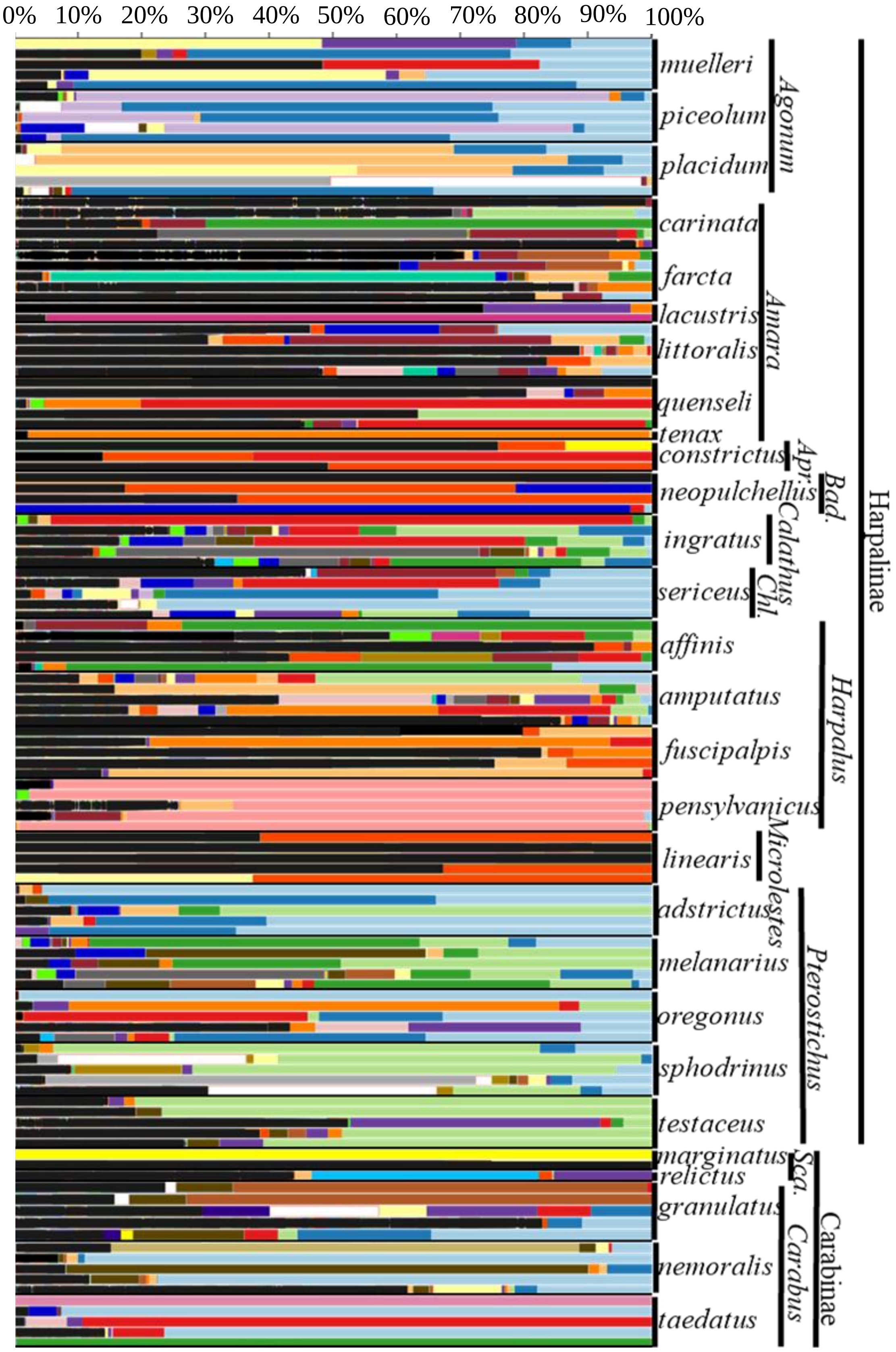
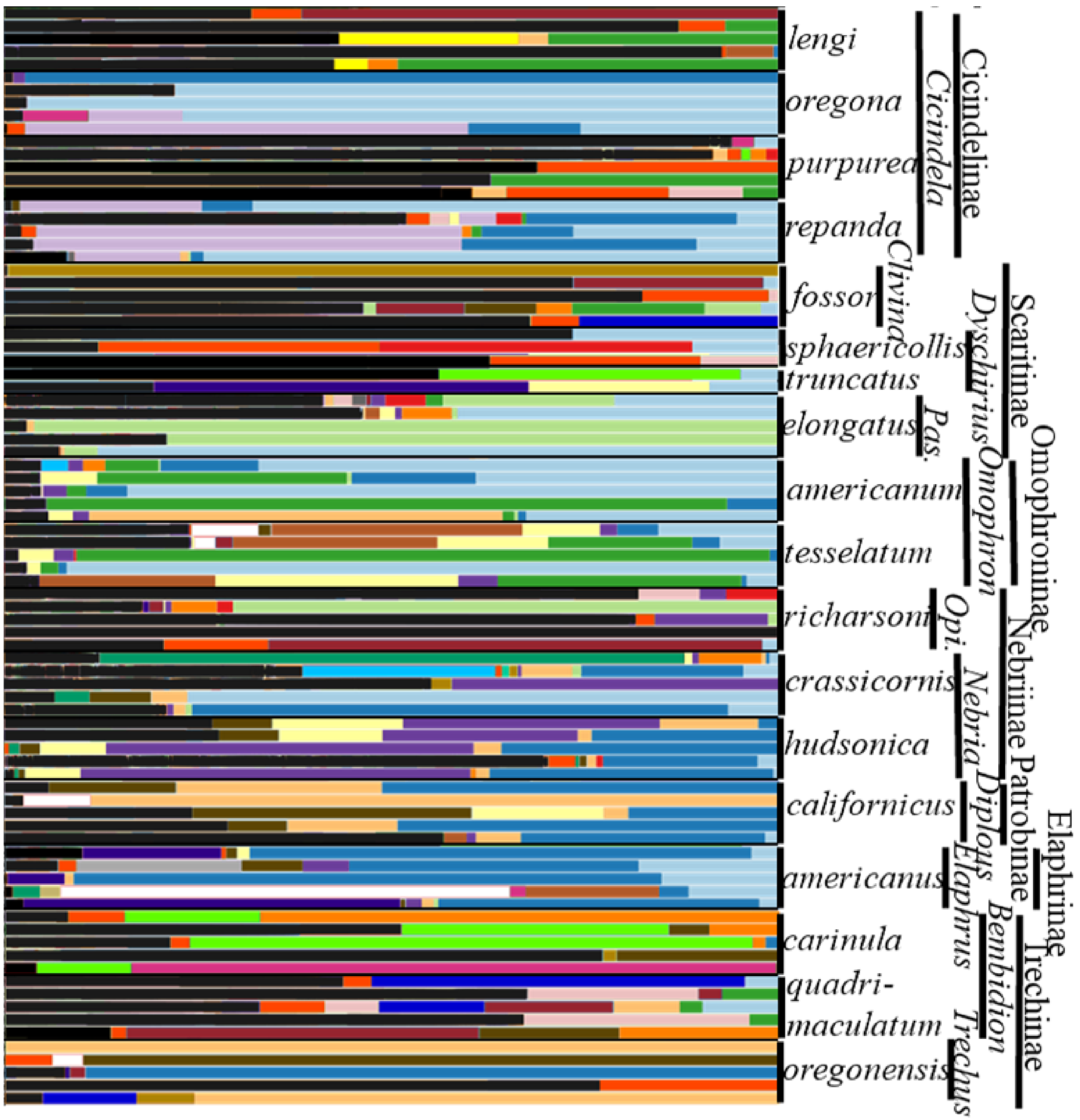


Figure 2.3. a) Bray-Curtis principal coordinate analysis (PCoA) for nine subfamilies within Carabidae. Inset shows PCoA with axes 2 and 3. The three axes combine to show 13.26% of the beta diversity. b) The same Bray-Curtis PCoA but with sample markers changed to better show groupings. Significant separation was shown among subfamilies (PERMANOVA: pseudo-F = 1.896, p = 0.001).


0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
muelleri
piceolum
placidum
Agonum
carinata
farcta
lacustris
littoralis
quenseli
tenax
Amara
constrictus
Apr.
neopulchellus
Bad.
ingratus
Calathus
sericeus
Chl.
affinis
amputatus
fuscipalpis
pensylvanicus
Harpalus
linearis
Microlestes
adstrictus
melanarius
oregonus
sphodrinus
testaceus
Pterostichus
Harpalinae
marginatus
Sca.
relictus
granulatus
nemoralis
taedatus
Carabus
Carabinae



Apr. = *Apristus*

Bad. = *Badister*

Chl. = *Chlaenius*

Sca. = *Scaphinotus*

Pas. = *Pasimachus*

Opi. = *Opisthius*

🟦 Bacillota; *Enterococcus*
🟦 Pseudomonadota; *Gilliamella*
🟩 Pseudomonadota;f_Yersiniaceae
🟩 Bacillota; *Lactobacillus*
🟥 Pseudomonadota;c_Alphaproteobacteria
🟥 Pseudomonadota;f_Enterobacteriaceae
🟧 Bacillota; *Spiroplasma*
🟧 Pseudomonadota;o_Enterobacterales
🟪 Bacillota; *Leuconostoc*
🟪 Pseudomonadota; *Pseudomonas*
🟨 Bacteroidota; *Dysgonomonas*
🟫 Bacillota;f_Lachnospiraceae
🟫 Bacillota;o_Lactobacillales
🟥 Bacillota; *Lactococcus*
🟨 Bacteroidota; *Flavobacterium*
🟪 Pseudomonadota; *Serratia*
⬛ Pseudomonadota; *Hafnia-Obesumbacterium*
⬜ Fusobacteriota; *Sebaldella*
🟪 Pseudomonadota; *Sodalis*
🟨 Bacillota; *Mesoplasma*
⬜ Pseudomonadota; *Pragia*
🟦 Bacillota; *Weissella*
🟨 Pseudomonadota;f_Wohlfahrtiimonadaceae
🟩 Pseudomonadota;f_Budviciaceae
🟩 Pseudomonadota; *Rickettsiella*
🟩 Pseudomonadota; *Rickettsia*
🟥 Pseudomonadota; *Acinetobacter*
🟧 Pseudomonadota; *Escherichia-Shigella*
🟪 Bacteroidota; *Apibacter*
🟦 Bacillota; *Carnobacterium*
⬛ *Others*

Figure 2.4. Bar-plot with legend showing the 30 bacterial genera with the highest relative abundance in 208 individual carabid samples used in Chapter 2.

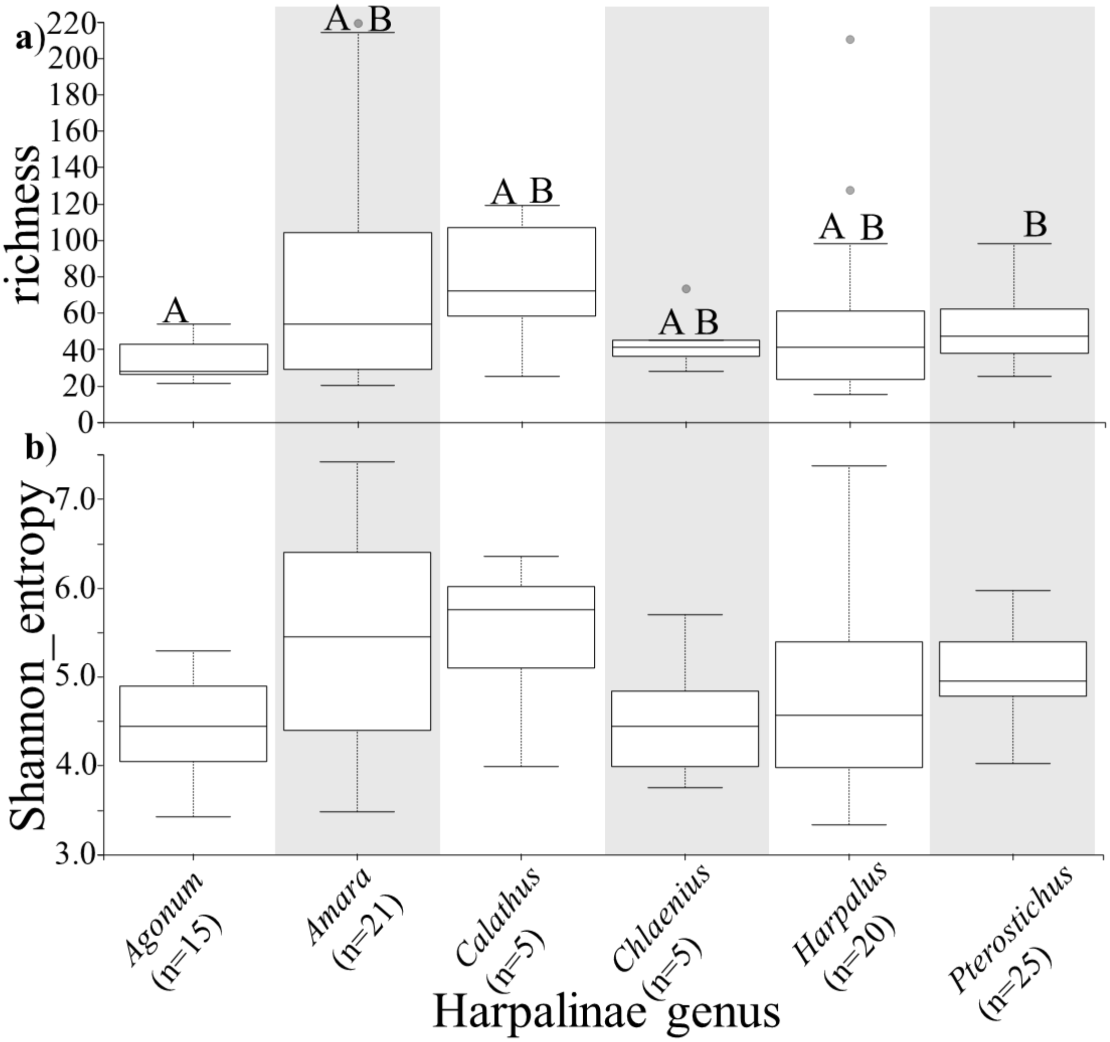


Figure 2.5. Alpha diversity measures of bacterial a) richness and b) Shannon diversity in six genera of Harpalinae. Dots indicate outliers and error bars indicate highest and lowest values. The bottom and top edges of the boxes show quartile 1 and quartile 3 respectively and the middle line in the box indicates the median value. Letters above box plots indicate significance. Groups that share a letter are not significantly different from one another.

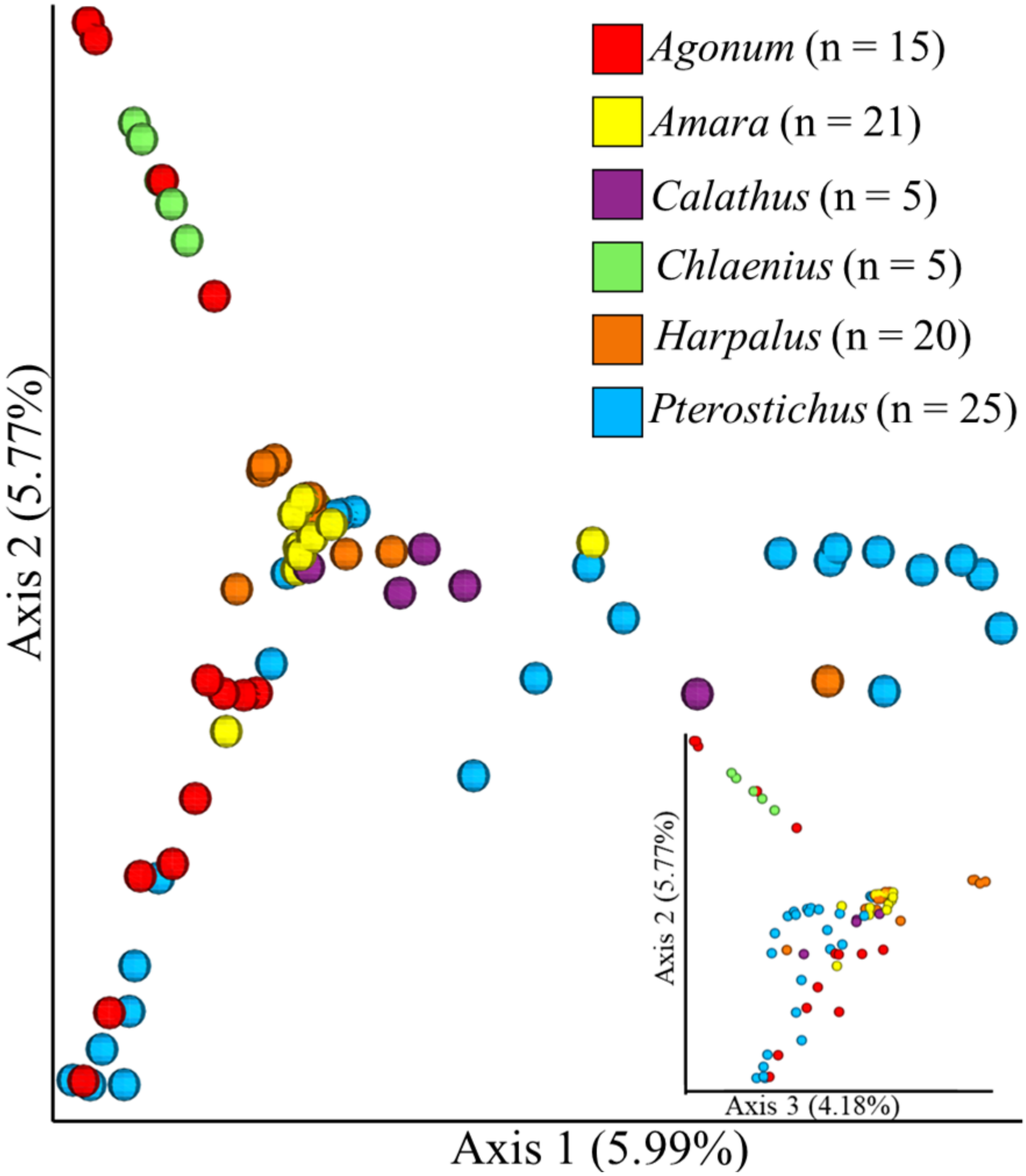


Figure 2.6. Bray-Curtis principal coordinate analysis (PCoA) for beetle genera within Harpalinae. Inset shows PCoA with axes 2 and 3. The three axes combine to show 15.94% of the beta diversity. Significant separation was shown among Harpalinae genera (PERMANOVA: pseudo-F = 2.204, p = 0.001).

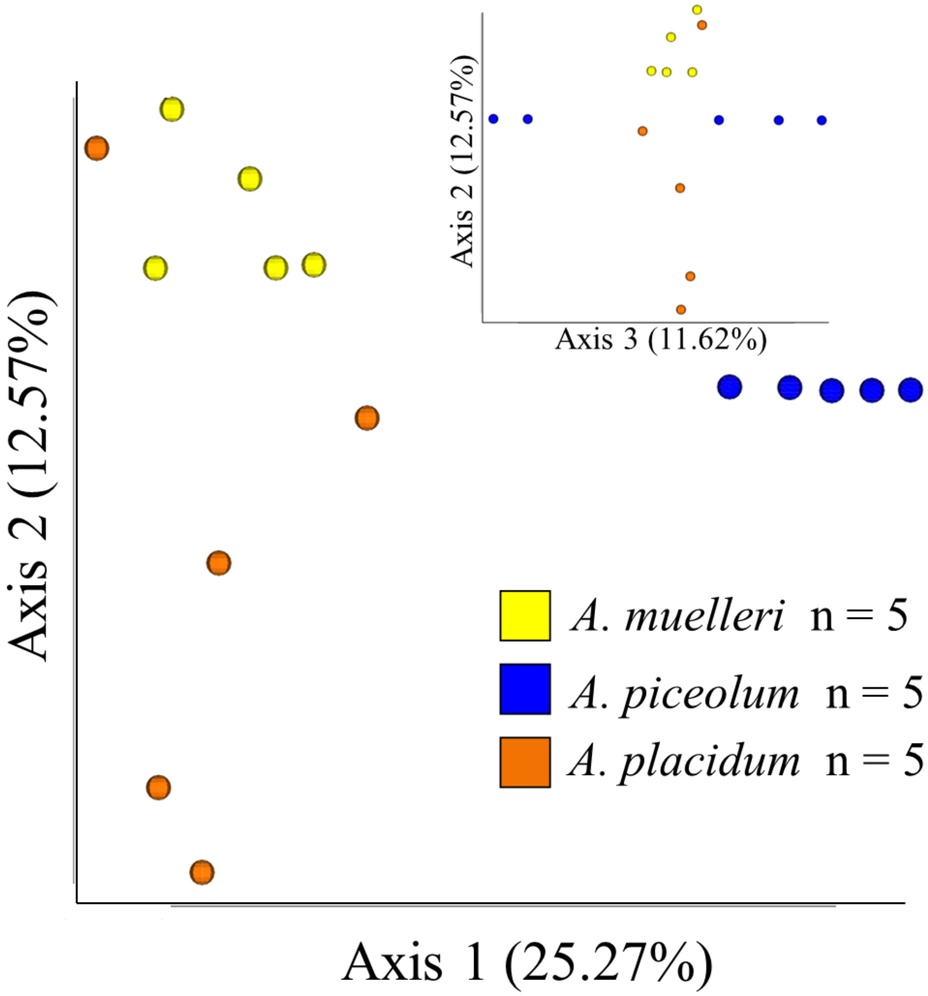


Figure 2.7. Bray-Curtis principal coordinate analysis (PCoA) for species of *Agonum*. Inset shows principal coordinate analysis (PCoA) with axes 2 and 3. The three axes combine to show 49.46% of the beta diversity. Significant separation was shown among *Agonum* species (PERMANOVA: pseudo-F = 2.886, p = 0.001).

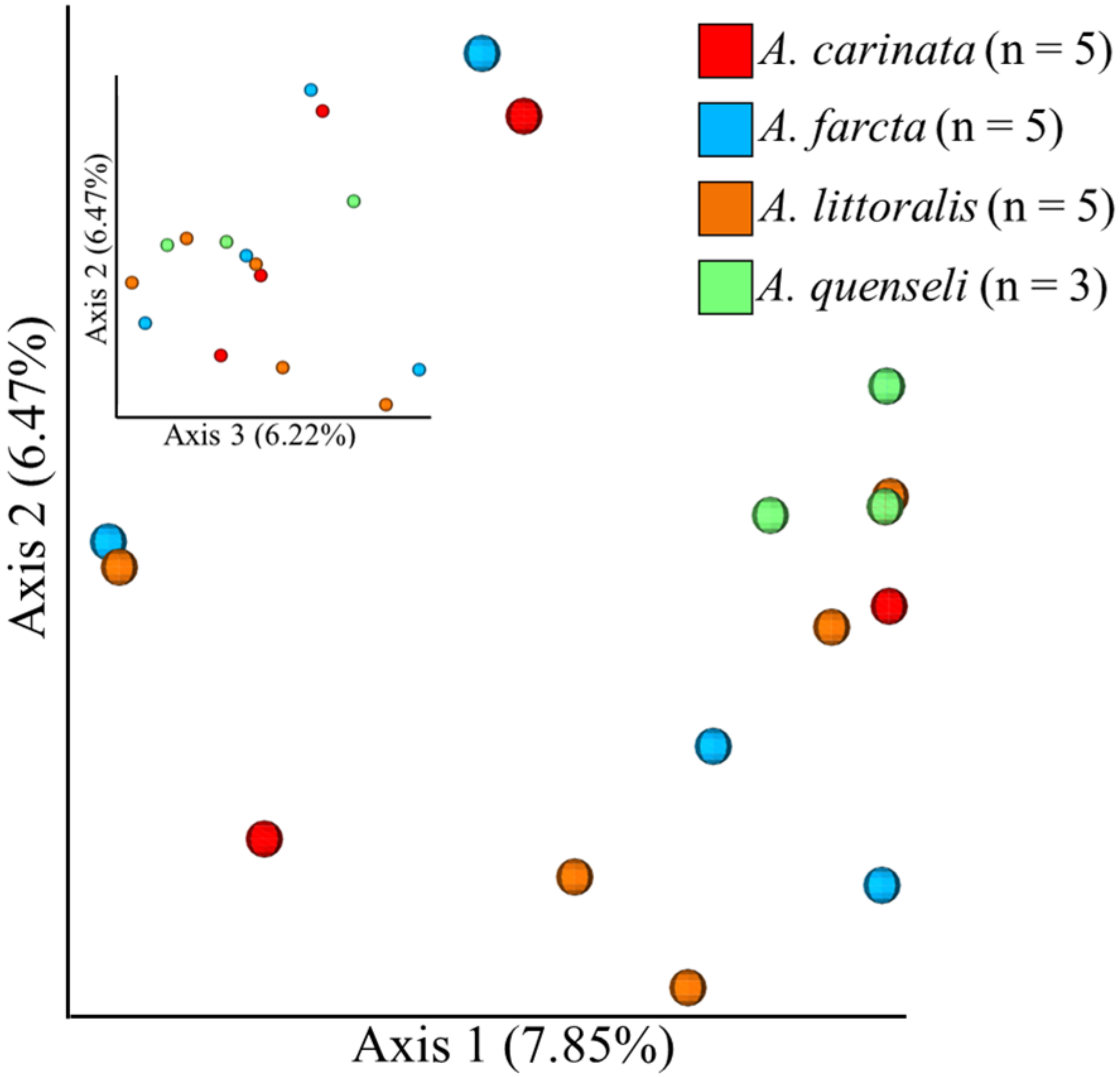


Figure 2.8. Bray-Curtis principal coordinate analysis (PCoA) for species of *Amara*. Inset shows PCoA with axes 2 and 3. The three axes combine to show 20.54% of the beta diversity. No significant separation was shown among *Amara* species (PERMANOVA: pseudo-F = 0.985, p = 0.706).

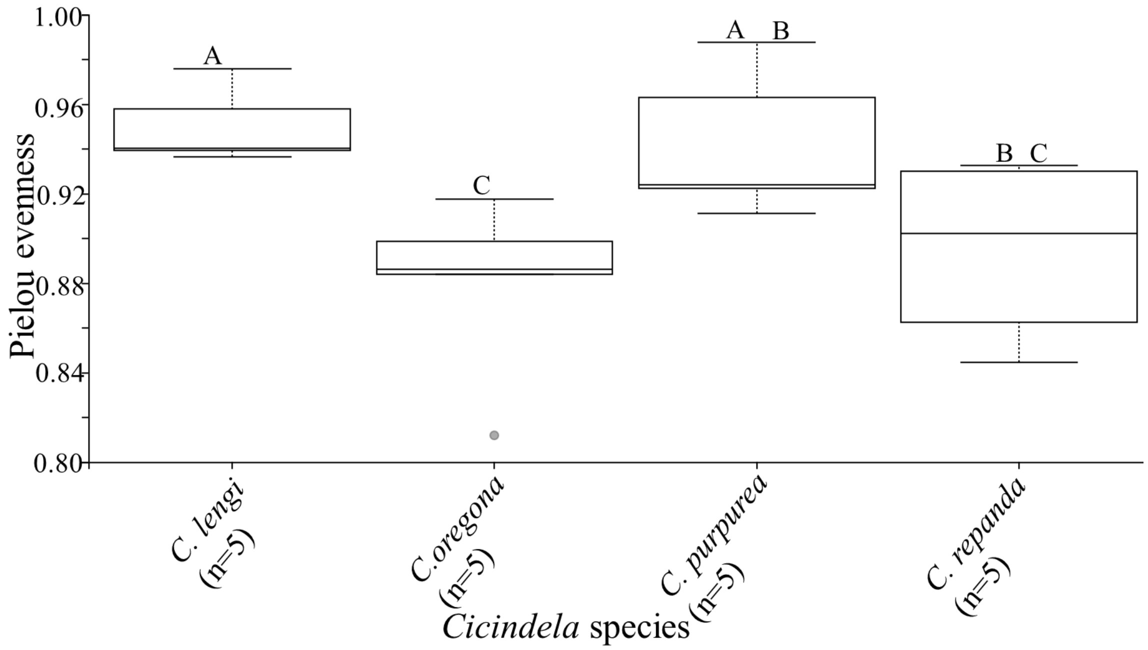


Figure 2.9. Alpha diversity measure of bacterial taxa evenness in four species of the *Cicindela* genus. Dots indicate outliers and error bars indicate highest and lowest values. The bottom and top edges of the boxes indicate quartile 1 and quartile 3 respectively and the middle line in the box indicates the median value. Letters above box plots indicate significance. Groups that share a letter are not significantly different from one another.

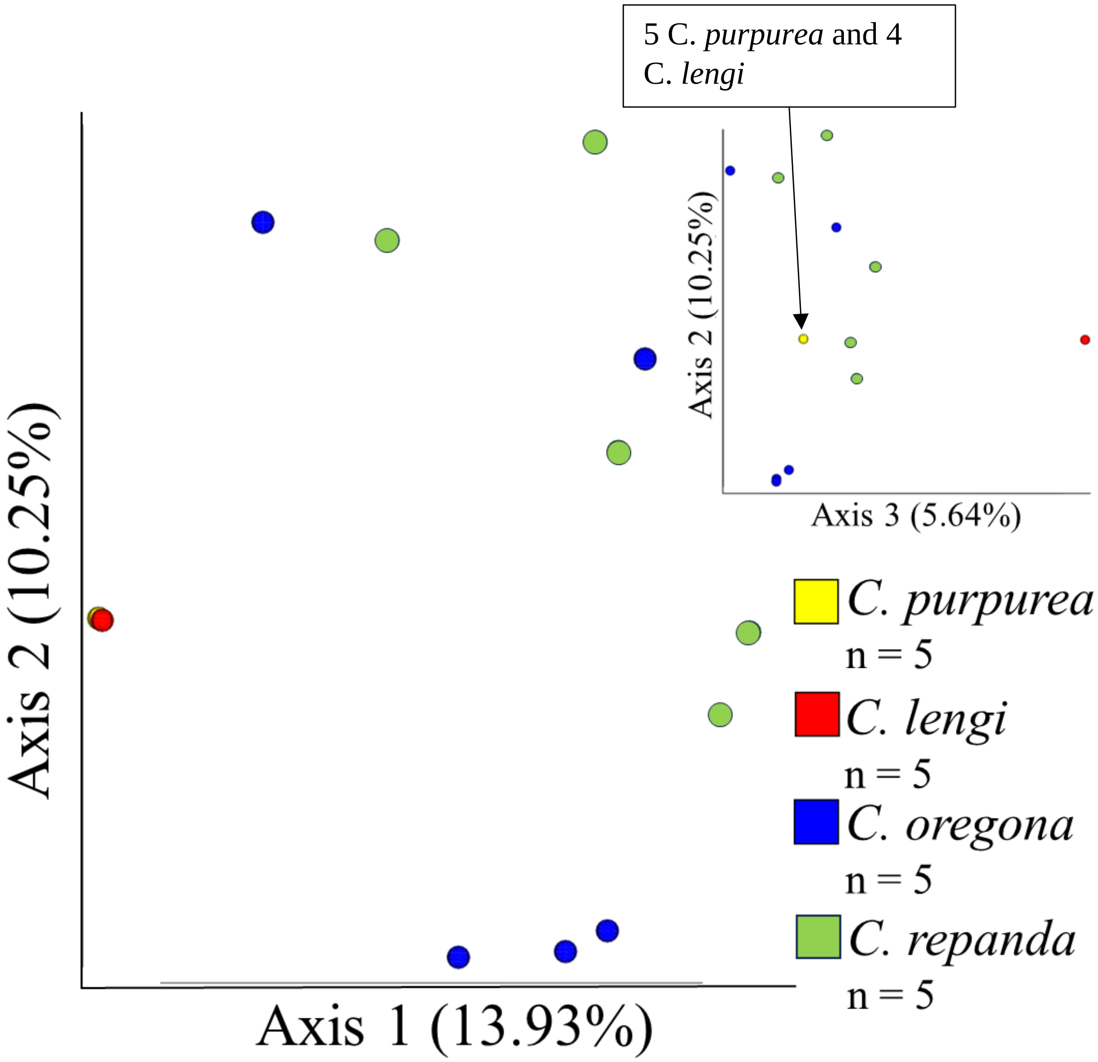


Figure 2.10. Bray-Curtis principal coordinate analysis (PCoA) for species of *Cicindela*. Inset shows PCoA with axes 2 and 3. The three axes combine to show 29.82% of the beta diversity. Significant separation was shown among *Cicindela* species (PERMANOVA: pseudo-F = 1.689, p = 0.001).

Chapter 3. Gut Bacterial Communities in Carabids: Effects of Extrinsic Factors and Feeding Habits of the Host

3.1 Abstract

Bacteria in animal guts (= the gut bacterial community, GBC) can be crucial to host survival. These gut bacteria may aid in digestion, synthesize vitamins and nutrients, or protect from pathogens. To fully understand the biology of the host, it is therefore necessary to study their gut bacteria. Ground beetles, or carabids (Coleoptera: Carabidae), are a diverse family of insects with ~40,000 species, 2500 of which are in North America. Ground beetles are found in many habitats and are generally considered beneficial in cropland where they can help control pest species of weeds and invertebrates. Few studies have examined how GBCs differ within a single carabid species collected in different extrinsic factors such as habitats or seasons. Previous studies have also done little to examine how feeding habit affects GBC diversity in cropland carabids. Here, next generation sequencing is used to determine how much GBC differentiation in carabids can be attributed to habitat, region, season, year, or feeding habit. The results show that GBCs can differ depending on all of those factors, especially habitat and feeding habit. Samples of the carabid *Pterostichus melanarius* found across vastly different habitats had different GBCs depending on where they were found. *Lactobacillus* bacteria was predominate in coniferous forest *P. melanarius* samples (n = 10), but found at much lower concentrations in other habitats (n = 13). *Gilliamella* was much more common in *P. melanarius* samples from canola cropland than samples of that carabid from other habitats. The bacterial genera *Enterococcus* and *Gilliamella* were associated with carnivorous carabid GBCs and the bacterial phyla Actinomycetota, Chloroflexota, and Planctomycetota associated with granivorous (seed feeding) carabid GBCs.

3.2 Introduction

Multicellular organisms (e.g., plants, animals, mushrooms) always form associations with microbes (e.g., fungi or bacteria). The various microbes in an environment are collectively termed a microbiome. Components of a host's microbiome may coevolve with the host. Under the holobiont concept, a host and its microbiome are viewed as a single evolutionary unit (Pita *et al.*, 2018). The health and reproduction of a host can be profoundly affected by its microbiome to the point that the host's survival depends on which kind of microbes are present. When a host's genomic functions are intertwined with the genomic functions of its microbiome, the genomes are considered a hologenome (Rosenberg & Zilber-Rosenberg, 2018). An example of a hologenome can be found in the honey bee *Apis* (Hymenoptera: Apidae) where bee species lacking some of their nuclear genes required to synthesize crucial amino acids rely on the genes of their gut bacteria (Zhang *et al.*, 2022b).

A microbiome can be located in, on, or around the host and may be relatively stable across generations depending upon the mode of transmission (Janke *et al.*, 2022, Michaud *et al.*, 2020, Robinson *et al.*, 2019). The microbes making up a microbiome can be grouped into three groups based on their location. The microbes within the contents of a gut lumen are termed exosymbionts because the lumen contents are considered to be connected directly to the outside of the host's body. The microbiome on or around the outermost layers of a host (e.g., skin or bark) are the ectosymbiont microbes. Endosymbiosis is when microbes are within the cells or body fluids (e.g., blood or sap) of a host. The mode of transmission can be either vertical or horizonal (Salem *et al.*, 2015). Cellular endosymbionts are usually vertically transmitted when the intracellular microbes infect gametes as they form within a parent (Durden *et al.*, 2019, Porter & Sullivan, 2023). Other microbiome components are acquired from the environment or application of microbiome material to the host's eggs, seeds, or offspring (Geerinck *et al.*, 2022, Grond *et al.*, 2017, Janke *et al.*, 2022, Liu *et al.*, 2019, Ohbayashi *et al.*, 2015). When offspring acquire their microbes directly from parents via vertical transmission, their microbiome is more likely to remain stable across generations. When offspring acquire their microbes from the environment, their microbiomes are more likely to reflect environmental conditions (Michaud *et al.*, 2020).

For animals, bacteria are the major constituent of nearly all gut microbiomes (Dias *et al.*, 2020). The gut bacterial community (GBC) can help the host with digestion, immune function, nutrient synthesis, and thermal tolerance (Maurice & Erdei, 2018, Blow & Douglas, 2019, Duron & Gottlieb, 2020, Smith *et al.*, 2021). Some bacteria in GBCs are transient and have varying timespans before they exit the digestive system. Some bacteria found in the upper digestive system (e.g., crop) cannot survive the conditions found further into the gut and are killed and digested as they reach the lower portions (e.g., midgut and hindgut) (Engel & Moran, 2013). Other bacteria can be permanent residents of the gut unless particular situations, such as the application of antibacterials, take place. Resident gut bacteria are most likely to affect host fitness; however, transient bacteria can also influence host fitness (Anderson *et al.*, 2013).

My MSc thesis focuses on ground beetles or carabids (Coleoptera: Carabidae). There are at least 40,000 carabid species worldwide (Lovei *et al.*, 1996) with about 2700 species found in North America (Larochelle & Larivière, 2003). They are generally considered beneficial to ecosystems and are natural enemies to numerous pest species

(Larochelle, 1990). Most carabids are predators, omnivores, or granivores, but a few are parasitoids, detritivores, or scavengers (Larochelle, 1990). The beneficial effects of carabids are most evident in cropland agroecosystems where they can eat close to their own body weight in pests every day during active seasons and can be a major part of a functioning ecosystem (Hill *et al.*, 2017, Satpathi, 2021). Many ground beetles are not avid fliers and others cannot fly at all (Venn, 2016). Winged species may only fly a couple times during their lives and this limited mobility can restrict them to a relatively small area and makes them relatively easy to catch by hand or with pitfall traps. Although multiple aspects of carabid biology and ecology have been well studied, little is known about the diversity of their GBCs.

Conclusions derived from the relatively few studies on GBC diversity in carabids are limited by the number and type of species studied, sample sizes, and methods. Kolasa *et al.* (2019) attempted to characterize the microbiome of Carabidae using four species (10 samples each) of *Bembidion* found in the riparian zone of the same river. They concluded that the GBCs have possible core bacteria such as the genus *Orbus*; however, it was not clear if that was the result of habitat or season. Other studies have found that habitat and feeding habit play a role in GBCs (Kudo *et al.*, 2019). Using 27 species from 12 carabid genera, Kudo *et al.* (2019) found feeding habit was a stronger determinant of GBCs than habitat albeit carabid GBCs differed significantly ($p = 0.001$) between forest and grassland habitats. Limitations of Kudo *et al.* (2019) include that the majority of species examined were represented by less than four specimens and that most species were limited to only one of the two habitats each of which contained mostly one feeding habit (e.g., forest had carnivores). Silver *et al.* (2021) compared carabid GBCs with and without diet manipulation of three species, *Pterostichus serripes*, *Anisodactylus similis*, and *Brachinus elongatulus* using 12 specimens per species. With increased sample sizes per species compared to previous studies (e.g., Kudo *et. al*., 2019), regardless of which sterile diet treatment the beetles were in, the GBC within a species were more similar. For example, the bacterial genus *Enterococcus* remained significantly more abundant in the two generalist species, *P. serripes* and *B. elongatus*, than in the granivorous species, *A. similis* (Silver *et al*., 2021). Other studies have concluded that GBC composition can affect diet. Two studies of GBCs in the granivorous carabid species, *Harpalus pensylvanicus*, reported a correlation between increased seed consumption and the presence of *Enterococcus faecalis* bacteria (Schmid *et al*., 2014, Lundgren & Lehman, 2010). Given the potential confounding effects of habitat and season, for example, examinations of diversity within a feeding habit (e.g., granivory) on host GBC may require specimens collected at the same time and site. If all the granivorous carabids are eating the same kinds of seeds, then differences among GBCs can more easily be attributed to differences in host taxonomy.

In Chapter 2 of this thesis, clear GBC differences across host taxonomy were demonstrated, but it was noted that other factors may affect GBC diversity. In this chapter, I address the following questions: Do GBCs differ for a given carabid species collected in (1) different habitats, (2) different seasons, (3) different years, and (4) different geographic regions? (5) Are there habitat or feeding habit GBC characteristics associated with multiple carabid species collected within the same localized habitat? And lastly, (6) are there core gut bacteria associated with each host or host scenario involved in answering these questions? I define core gut bacteria as bacteria that are found in nearly every sample (Risely & Tate, 2020) in a carabid group defined by a common characteristic; e.g., genus, species, habitat, or feeding habit.

To address limitations of previous studies, my study will use larger sample sizes, a greater diversity of taxonomic groups within a beetle family, and will try to control for confounding factors (e.g., season and habitat) (Kolasa *et al.*, 2019, Kudo *et al.*, 2019). The hypotheses were that GBC composition will remain similar within a species despite extrinsic factors and there will be GBC differentiation among and within feeding habits in the same habitat.

3.3 Methods

3.3.1 Beetle Collection

To address my research questions, I collected adult carabid beetles from different sites in southern Alberta (AB) and western Montana (MT) during the mid-spring and summer between June 2021 and July 2023. Sampled habitats included cropland, woodland, riparian zones, and urban settings. Samples that are compared between geographic regions are at least 325 km apart with samples from southern Alberta being from the plains or Cypress Hills region and samples from western Montana being from a mountainous region. Most samples for the analysis of cropland carabids were collected in an 11-day period (August 15th-26th) from two canola crops and a pea field in AB which were on a crop rotation of grain, pea, and canola ~7 km apart. Most samples were captured by pitfall trapping but some were collected by hand or butterfly nets. Beetles were captured alive and then placed in 70%-100% ethanol to preserve the DNA and bacterial diversity of a life-like state. Some samples were frozen for several weeks at -20°C before they were sorted and placed in ethanol.

3.3.2 Beetle Identifications

Beetles were first keyed to the species level using morphological traits (Lindroth, 1961) after which questionable identifications were verified through DNA barcoding. For barcoding, DNA extracted from gut tissues (see next section) was used to amplify a portion of the cytochrome oxidase I (COI) gene of mitochondrial DNA (mtDNA) with the universal primers HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') and LCO1490 (5'- GGT CAA CAA ATC ATA AAG ATA TTG G – 3') (Folmer *et al.*, 1994) and general methods described in Hebert *et al.* (2003). The amplified DNA was sent to Génome Québec Innovation Centre at McGill University (Montréal, Canada) (GQ) for sequencing. The returned COI sequences were edited in MEGA5 (Tamura *et al.*, 2011) and then compared with sequences in GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/) and BOLD (https://www.boldsystems.org/) to identify beetles to the species level. COI sequences were then uploaded to GenBank.

3.3.3 Beetle Dissections, DNA Extractions and NGS Sequencing

The GBC for each beetle specimen was characterized using next generation sequencing (NGS) to develop a sequence library for analyses. This was done by first extracting DNA from carabid gut samples removed through dissection and then sending the isolated DNA to Genome Quebec for NGS. The resulting DNA sequence files were then run through a series of quality control steps to produce the final sequence library which was analyzed to understand GBC diversity in carabids.

Sterile techniques were used for all dissections. Beetle samples first had their elytra carefully removed before being surface sterilized using a 0.6% solution of sodium hypochlorite (bleach) and finally being rinsed with sterile water. Beetle midguts and hindguts were dissected out using small scissors, tweezers, and a dissecting scope. After cutting and folding back the dorsal integument of the abdomen, the gut was cut just posterior to the thorax and again at the end of the abdomen. Specimens of *Bembidion quadrimaculatum* were too small to perform gut dissections on (< 3.5 mm), so whole abdomens were used after removal of legs and elytra. Specimens of *Carabus granulatus* and *Harpalus pensylvanicus* had guts that were too big for the extraction kit (> 2 mm^3), so their guts were split in two after dissection, DNA was extracted from each portion of the gut and DNA samples were recombined after extraction. Extractions were performed using DNeasy Blood and Tissue Kits (Qiagen) and DNA samples were kept frozen at -20°C until being sent to Genome Quebec (GQ) for NGS.

Two separate sets of extracted DNA samples were sent to GQ, one in fall 2022 and a second in fall 2023. Genome Quebec performed PCR amplifications of the V4 region of prokaryote 16S SSU rDNA using the 515FB-806RB primer pair and their amplification protocols (25 ul PCR, Roche FastStart High Fi Taq, 58^{o}C, 26 cycles) before sequencing on a MiSeq Illumina platform, following the manufacturer’s instructions (Illumina, 2019), to generate the requested PE 250 base pair reads and an average of 100k sequences per sample.

Demultiplexed sequence data were received from GQ and then quality filtering, denoising, paired-end merging (250 base pair PE reads merged to form ~292 base pair V4 hypervariable gene region), and feature-table construction steps were performed using the default settings of the dada2 (Callahan *et al.*, 2016) plug-in in Qiime 2 (version 2023.2) software (Estaki *et al.*, 2020). Trimming of sequences was done to any bases below a Phred score of 30, which was only one nucleotide on the 3' end of about 33% of the forward and reverse reads prior to paired-end merging. Before sequences are assigned a bacterial taxonomic identification, they are amplicon sequence variants (ASVs). An ASV is a single, unique DNA sequence from a high-throughput sequencing system. The ASVs were assigned taxonomic identity using the SILVA rRNA database (silva-138-99-515-806-nb-classifier) in Qiime 2. Given the large number of ASVs identified, those represented by fewer than 20 sequences (in one sample or combined across all samples) were considered trace levels and excluded to simplify analyses while not losing many samples due to small libraries. This threshold is consistent with other studies because if a sample with a small library (e.g., ~500 sequences) had 20 sequences for a rare ASV, then ~5% of the library would be removed (Kudo *et al*., 2019; Kolasa *et al*., 2019). Qiime 2 was also used for taxonomic filtering of all non-bacterial sequences (e.g., mitochondria) and statistical analyses. Bacterial sequences assigned to *Wolbachia,* an obligate intracellular endosymbiont, were removed after being documented because it has no known free-living extracellular stage (Nikoh *et al.*, 2014) and would only be found within the cells of the gut lining as opposed to within the gut lumen. Other potential endosymbionts such as *Spiroplasma* were retained in the analyses because of reports they can exist outside of cells (Gilad *et al.*, 2003) and were more likely to have originated within the gut lumen.

3.3.4 GBC Analyses

High levels of intraspecific and intra-treatment variation can make it so groups only represented by a few samples (e.g., n < 3) may not be good representatives of a species or treatment type. Carabid groups with only one or two samples are better suited when combined with other treatment types. For example, a single sample from a cattle pasture may not be adequate to represent a habitat, but that sample may be grouped with samples from other habitats if they are used to represent different treatment type (e.g., region).

To address my research questions using the beetles available, I compared the diversity of bacterial taxa in sequence libraries using Qiime 2. This was done for bacteria classified to phylum and repeated for bacteria classified to genus when possible. The phylum and genus levels of bacteria are presented here so results are most comparable to other studies and because identification to the bacterial species level is not possible in most cases when using only the V4 region of 16S rDNA. When identification to genus was not possible, Class (c_), Order (o_) or Family (f_) was used. The thresholds for including bacterial taxa in tables of relative abundance (RA) were set to capture most differentially abundant (DA) bacteria. For bacterial phyla, a cutoff of 0.5% RA was applied and for bacterial genera, where there is far more diversity, a cutoff of 1.5% RA was chosen to represent which bacterial taxa are prominent. Cutoff thresholds for this study are consistent with those of other studies (Silver *et al.*, 2021, Geerinck *et al.*, 2022, Rocha *et al.*, 2022).

An analysis of differential abundances was used to statistically test the abundance of bacteria among sample groups to help identify which bacteria are driving separation in the PCoA. To examine which bacteria at the phylum and genus level have significant DA amongst carabid groups, an analysis of composition with bias correction (ANCOM-BC) was performed (Lin & Peddada, 2020). An ANCOM-BC test takes into account not only how many samples the bacterial taxa appeared in, but also measures the true absolute abundances assigned to an ASV thereby reducing the impact of predominate bacterial taxa sequences on less abundant taxa (Cappellato *et al.*, 2022). The results of an ANCOM-BC can generate a significant value that is different than what is seen in the pooled RA, partly because how bacteria are distributed across samples is taken into account but also because of the size of the sequence library. Due to possible variance in sequence counts created by inconsistencies in DNA extraction and amplification, the sequence library size is not always an indication of the original bacterial load in a sample. For example, if you generate one sequence library with 1000 sequences and another with 500 sequences it does not always mean that one of those sample had twice as much bacteria as the other, but it is evidence that one was more robust than the other. A strict Bonferroni test was used to correct the p-values (p) and generate the q-values (q) (Armstrong, 2014). Critical p-values and q-values in this study were set to 0.05.

Three measures of alpha diversity were calculated: richness quantifies how many ASVs are found in a sample group, evenness measures how evenly the relative abundances (RA) of the ASVs are spread through the sample group, and a Shannon index incorporates both richness and evenness (Kers & Saccenti, 2022). For all three measures, Kruskal-Wallis (all groups and pairwise) tests were used to determine significance

(Ruxton & Beauchamp, 2008). Pairwise Kruskal-Wallis measures were performed if the Kruskal-Wallis all-groups measure was significant. A Benjamini-Hochberg correction, the Qiime 2 default method for correcting pairwise p-values to generate q-values, was applied to all p-values to account for non-independence and determine significance (Ferreira & Zwinderman, 2006).

To visualize the beta diversity present between carabid samples, Bray-Curtis dissimilarities were calculated then plotted in a principal coordinate analyses (PCoA) (Kers & Saccenti, 2022). Bray-Curtis measures the presence and absence of ASVs while also weighing the ASVs at higher RA. The PCoA places samples with more similar GBCs closer together in multivariate space. Permutational multivariate analysis of variance (PERMANOVA) was performed on each PCoA to determine if there was statistical significance to how the samples grouped.

Sequence counts varied from sample to sample and standardization was needed for analyses of richness, evenness, and Shannon (alpha diversity) and Bray-Curtis (beta diversity) (Willis, 2019). For each group examined, the optimal rarefaction level was set according to sequence counts within the group of samples being analyzed and leveling of rarefaction curves. This allowed me to retain the most ASVs as possible while only losing a few samples with smaller sequence libraries from some analyses.

3.4 Results

I obtained 16S rDNA sequences to characterize the gut bacterial community of 282 beetles. These 282 sequence libraries included a total of 25,714,548 sequences and had an average Phred score of 37; i.e., they were of high quality (Ewing *et al.*, 1998). After quality filtering and removal of non-bacterial and *Wolbachia* sequences, 2,492,935 total sequences remained for 280 beetles. For these beetles, the average sequence library contained 8,903 sequences (range: 20 to 39,741; median: 8,535) representing a total of 7,358 ASVs. For comparisons in the current chapter, a subset of the 280 beetles were selected depending on the research question.

3.4.1 Habitat

Two species (*Pterostichus melanarius* and *Amara littoralis*) were used to test for the effect of habitat on GBC.

3.4.1.1 *Pterostichus melanarius*

I compared the GBCs for 23 beetles (= samples) recovered in four different habitats: coniferous forest (n=10), canola (n=5), riparian zone (n=5), and timothy hay (n=3) (Table 3.1). Prior to analyses of Bray-Curtis (beta diversity) and richness, evenness, and Shannon diversity (alpha diversity); libraries were rarefied to a standard size of 6000 sequences per sample while retaining all samples. Unrarefied data were used to generate the tables of RA and significant DA for bacterial phyla and genera (Tables 3.2 and 3.3).

Alpha Diversity among habitats of P. melanarius

No significant difference was detected in the alpha diversity of GBCs for *P. melanarius* from different habitats (Kruskal-Wallis test). Results (mean ± SD (min.-max)) for individual measures were as follows: richness (53.7 ± 16.4 (22 - 84), K-W test: H = 3.508, p = 0.320), evenness (0.896 ± 0.050 (0.779 – 0.965), K-W test: H = 0.209, p = 0.976), Shannon diversity (5.094 ± 0.594 (3.623 – 5.832), K-W test: H = 2.505, p = 0.474).

Beta diversity among habitats of P. melanarius

In the Bray-Curtis PCoA (Fig. 3.1), the first three axes combined to show 42.94% of the beta diversity present in *P. melanarius* samples. Results of the PERMANOVA test showed significant separation of GBCs for *P. melanarius* from different habitats (pseudo-F = 1.865, p = 0.004). Most of the separation was associated with four of ten samples from the coniferous forest site in Cypress Hills Interprovincial Park in Alberta that separated on axis 1 (21.70%). The other six samples from the same coniferous forest grouped more closely with samples collected in other habitats (Fig. 3.1). A second PERMANOVA test was performed after removing the four coniferous forest outliers to determine if they were causing the significance. The second PERMANOVA test still showed separation by habitat type (pseudo-F = 1.460, p = 0.008).

Bacterial phyla: For *P. melanarius* samples combined across the four habitats examined here, five bacterial phyla above 0.5% RA represented 99.6% of the GBC (Table 3.2). The two predominate phyla were Bacillota (54.2%) and Pseudomonadota (42.1%); however, which of the two phyla had the highest RA differed among habitats. Within each individual habitat, two to four bacterial phyla had RA above 0.5% with Bacillota and Pseudomonadota being the only two phyla found in all habitats.

Bacterial genera: For *P. melanarius* samples combined across all habitats examined here, the ten bacterial genera above 1.5% RA totaled 91.5% (Table 3.3). *Weissela* and f_Yersiniaceae were above the threshold in all habitats. In the PCoA, low RA of *Enterococcus* and significantly high DA of *Pseudomonas* were major contributors to the separation of samples from timothy hay and other habitats such as canola. There is an association between *P. melanarius*, the bacterial genus *Gilliamella*, and the canola habitat. Some coniferous forest samples separated from other habitats in the PCoA (Fig. 3.1) due to *Lactobacillus* (47.8% RA), which had a significantly high DA ($q < 0.001$). Comparisons among the ten coniferous forest *P. melanarius* samples are examined in section 3.4.2.1.

3.4.1.2 *Amara littoralis*, habitats/seasons

The second carabid species used to study habitat effects on GBC was *A. littoralis*; however, due to how the samples were collected, I was unable to remove the effect of season. The 13 samples were grouped into three habitats/seasons: canola/late-summer (n=5), riparian-zone/early-summer (n=3), and urban-yard/mid-spring (n=5) (Table 3.1). A rarefaction curve set to 431 sequences per specimen reached a plateau for all samples except one from canola/late-summer, but the slope for that sample was close to zero. It was decided that it was better not to lose one of three riparian/early-summer samples and have one canola/late-summer sample that did not quite capture 100% of its taxa in subsampling.

Alpha diversity among habitats/seasons of A. littoralis

Significant differences in alpha diversity for *A. littoralis* habitat groupings were found in measures of richness and Shannon diversity (Kruskal-Wallis, $p < 0.05$) (Fig. 3.2), but not for evenness ($H = 0.457$, $p = 0.796$). Results (mean ± SD (min.-max)) for a measure of evenness were as follows: (0.913 ± 0.072 ($0.742 – 0.972$)). A Kruskal-Wallis (pairwise) comparison showed a significantly higher richness and Shannon diversity ($q < 0.05$) in canola/late-summer samples than urban/mid-spring samples (Fig. 3.2).

Beta diversity among habitats/seasons of A. littoralis

The three axes shown in the Bray-Curtis PCoA combine to represent 27.1% of the beta diversity in *A. littoralis* samples. Results of the PERMANOVA test showed significant separation (pseudo-F = 1.056, $p = 0.023$) among samples by habitat/season. For example, four of five samples from canola/late-summer showed separation along axis 1 from samples associated with riparian and urban habitats (Fig. 3.3). A few samples from each of the three habitats/seasons formed a tight cluster in the PCoA, indicating some commonalities among the habitats/seasons. The urban-yard/mid-spring samples had the most separation among themselves. Samples from canola/late-summer spread on axis 1

(9.6%) and axis 3 (8.6%) but did not spread on axis 2 (8.9%) at all. Samples from the urban-yard/mid-spring and riparian-zone/early-summer spread on axis 2 and 3, but barely spread at all on axis 1 (Fig 3.3).

Bacterial phyla: For *A. littoralis* samples combined across all habitats/seasons examined here, nine bacterial phyla above 0.5% RA totaled 99.2% of the GBC (Table 3.4). The three predominant RA bacterial phyla in all habitats/seasons were Pseudomonadota (43.6%), Bacillota (23.4%), and Actinomycetota (17.9%). The five bacterial phyla consistently found in all three habitats/seasons above 0.5% RA were: Actinomycetota, Bacteroidota, Bacillota, Planctomycetota, and Pseudomonadota; however, the RA differed among the three habitats/seasons especially for Bacillota and Pseudomonadota. A significantly high DA (q-value < 0.05) of Actinomycetota was found in the two later-season samples, canola/late-summer and riparian/early-summer (Table 3.4).

Bacterial genera: For *A. littoralis* samples combined across all habitats/seasons examined here, the 13 bacterial genera above 1.5% RA totaled 64.4% (Table 3.5). The only bacterial genus found consistently across all habitats/seasons in *A. littoralis* was *Spiroplasma* (5.5-15.7% RA), which had a significantly high DA in riparian/early-summer samples. The DA of *Arthrobacter, Brevundimonas,* and *Devosia* were significantly low and *Escherichia-Shigella* was significantly high in canola/late-summer samples (Table 3.5).

3.4.2 One species, multiple years

3.4.2.1 *Pterostichus melanarius*

To examine how patterns of GBC diversity may change from year to year within the carabid species *Pterostichus melanarius*, ten samples collected in the late summer from a coniferous forest in southeast AB were grouped into the two years they were found: 2021 (n = 5) and 2022 (n = 5). Rarefication was set to 7,000 sequences per sample while retaining all samples.

Alpha diversity among years for P. melanarius

Alpha diversity for the GBC of *P. melanarius* collected in 2022 was significantly higher (Kruskal-Wallis test, $p \leq 0.028$) than that of *P. melanarius* collected in 2021 for measures of richness (H = 5.771, p = 0.016), evenness (H = 4.811, p = 0.028), and Shannon diversity (H = 6.818, p = 0.009) (Fig. 3.4).

Beta diversity among years for P. melanarius

The first three axes of the Bray-Curtis PCoA represent 69.0% of the total variation (Fig. 3.5). Results of the PERMANOVA test showed significant separation (pseudo-F = 4.417, p = 0.008) among samples by year. Primary separation between years is on axes 1 (40.3%) and 3 (13.5%), but one sample from 2021 is an outlier on axis 2 (15.2%).

Bacterial phyla: For *P. melanarius* samples combined across both years examined here, the two most common bacterial phyla were: Bacillota (70.0%), and Pseudomonadota

(28.9%) (Table 3.6). Only Bacillota (55.9-84.5%) and Pseudomonadota (15.1-42.7%) were above the 0.5% RA threshold in both years; the RA was more similar for the two bacteria phyla in 2022 whereas in 2021 Bacillota had the highest RA (84.0%). Samples from 2022 had significantly high DA ($q < 0.05$) of both phyla while in 2021 Bacillota had a significantly low DA (Table 3.6).

<u>Bacterial genera</u>: For *P. melanarius* samples combined across years, there were eight bacterial genera above 1.5% RA (totaling 92.6%) (Table 3.7). While *Lactobacillus* (47.8% RA) was predominant for all samples combined and the highest RA in each individual year, DA was significantly high for 2021 and low for 2022. With the exceptions of an unknown bacterial genus of the order Lactobacillales and the genus *Hafnia-Obesumbacterium* which were only present in the 2022 samples above the RA threshold, the other bacterial genera with an RA > 5% were present in both years. In at least three instances, the RA between years showed large differences. For example, *Lactobacillus* and *Gilliamella* were more abundant in 2021 than 2022, 70.0% vs 25.5% and 10.1% and 2.4%, respectively. In contrast, f_Yersiniaceae had 8x higher RA in 2022 than in 2021. A lot of the separation of samples in the PCoA between years (Fig. 3.5) and within the coniferous habitat (Fig. 3.1) was driven by differences in *Lactobacillus* and f_Yersiniaceae (Table 3.7) while the significantly high DA of o_Lactobacillales (13.2% RA) in samples from 2022 also contributed to separation between years.

3.4.2.2 *Carabus granulatus*

To elucidate how patterns of GBC diversity may change from year to year within the carabid species *Carabus granulatus*, eight samples from a riparian zone in southern AB (Lethbridge, AB) were grouped into the years they were found: 2021 (n = 5) and 2022 (n = 3). Prior to analyses for Bray-Curtis (beta diversity) and richness, evenness, and Shannon (alpha diversity), libraries were rarefied to 3,000 sequences per sample while retaining all samples.

Alpha diversity among years for C. granulatus

Kruskal-Wallis tests did not detect a significant effect of year on the GBC of *C. granulatus* for measures of richness (38.0 ± 17.3 (24 - 75), H = 1.822, p = 0.177), evenness (0.870 ± 0.066 (0.740 – 0.940), H = 0.556, p = 0.456), or Shannon diversity (4.477 ± 0.620 (3.393 – 5.330), H = 1.089, p = 0.297).

Beta diversity among years for C. granulatus

The three axes shown in the Bray-Curtis PCoA represent 64.88% of the total beta diversity (Fig. 3.6). Results of the PERMANOVA test showed significant separation (pseudo-F = 1.738, p = 0.015) among samples by year. Most of the separation between samples by year was on axis 1 (25.96%) and axis 3 (16.20%). The samples spread out along axis 2 (22.72%), but it did not separate samples by year.

Bacterial phyla: For *C. granulatus* samples combined across the three years examined here, the six bacterial phyla above 0.5% RA totaled 99.4% (Table 3.8). The two predominate bacterial phyla were Bacillota (66.2%) and Pseudomonadota (22.1%). The RA for Pseudomonadota was consistent among years (22.1%). Five bacterial phyla were above the 0.5% RA threshold in both years (Table 3.8). Actinomycetota had a higher RA in 2021 (4.1%) than in 2022 (0.7%). In contrast, Bacteroidota had a high RA in 2022 (4.9%) compared to 2021 (0.9%).

Bacterial genera: For *C. granulatus* samples combined across years, the eleven bacterial genera above 1.5% RA totaled 85.7% (Table 3.9). *Enterococcus* was the predominate bacterium in samples from 2021 (61.0%) but was below the threshold in 2022. A bacterium from f_Lachnospiraceae (47.8%) was the predominate bacterial genus in 2022 but was below the threshold in 2021. The only bacterial genus found in both years above 1.5% RA was *Sebaldella* (3.9-5.8%), which had significantly high DA in 2022, and *Gilliamella* (2.5-3.0%) (Table 3.9).

3.4.3 One species, multiple regions

3.4.3.1 *Pterostichus melanarius*

To elucidate how patterns of GBC diversity may change between regions within the carabid species *Pterostichus melanarius,* 25 samples were grouped into the two geographic regions where they were found: southern Alberta (n = 20) and western Montana (n = 5) (Table 3.1). Prior to analyses for Bray-Curtis (beta diversity) and richness, evenness, and Shannon diversity (alpha diversity), all libraries were rarefied to a standard size of 6,000 sequences per sample.

Alpha diversity among P. melanarius *by region*

Kruskal-Wallis tests did not detect a significant effect of region on the GBC of *P. melanarius* for measures of richness (54.8 ± 18.1 (22 - 96), H = 0.611, p = 0.434), evenness (0.890 ± 0.052 (0.777 – 0.965), H = 1.038, p = 0.308), or Shannon diversity (5.079 ± 0.587 (3.616 – 5.831), H = 1.666, p = 0.197).

Beta diversity among P. melanarius *by region*

The Bray-Curtis PCoA (Fig. 3.7) shows the three axes combine to represent 39.3% of the variation. The PERMANOVA test did not show significant separation (pseudo-F = 1.365, p = 0.099) among samples by region.

Bacterial phyla: Two bacteria phyla were most abundant in each of the two areas; however, the most abundant phyla in one area, was the second most abundant in the other. Bacillota was predominate in southern Alberta (61.9%) while Pseudomonadota was the predominate bacterial phylum in western Montana (68.9%) (Table 3.10). The three other bacterial phyla above the threshold, were present at low levels (RA < 4.6%). Alberta samples had a significantly high DA of Bacteroidota (1.4% RA) and Bacillota (61.9% RA) and a significantly low DA of Actinomycetota (0.3% RA) and Pseudomonadota (35.5% RA). Samples from MT had a significantly high DA of

Pseudomonadota (68.9% RA) and a significantly low DA of Bacillota (23.5% RA) (Table 3.10).

Bacterial genera: For *P. melanarius* samples combined across both regions examined here, the ten RA bacterial genera above 1.5% RA totaled 88.9% (Table 3.11). Early in the analyses, ASVs assigned to the obligate endosymbiont *Wolbachia* (only found in the five MT samples for this analysis) were removed. Although both regions had a lot of the same bacterial genera there were significant differences in RA and DA between them. Notable differences in bacterial genera were the significantly high DA and RA > 1.5% of f_Enterobacteriaceae, *Acinetobacter,* and *Pseudomonas* in MT samples and the significantly high DA and RA > 1.5% of *Gilliamella* in AB samples (Table 3.11).

3.4.3.2 *Opisthius richardsoni*

Ten samples of *O. richardsoni* were grouped according to region, AB (n=5) and MT (n=5). Libraries were rarefied to 419 sequences per specimen and two specimens from AB were removed from measures of alpha diversity and Bray-Curtis beta diversity because they were below the required sequence library size.

Alpha diversity among O. richardsoni _by region_

Kruskal-Wallis tests did not detect a significant effect of region on the GBC of *O. richardsoni* measures of richness (25.6 ± 17.9 (4 - 54), H = 0.200, p = 0.655), evenness

(0.909 ± 0.049 (0.836 – 0.972), H = 0.200, p = 0.655), or Shannon diversity (3.827 ± 1.187 (1.898 – 4.859), H = 0.556, p = 0.456).

Beta diversity among O. richardsoni *by region*

The three axes in the Bray-Curtis PCoA combined to represent 46.8% of the beta diversity (Fig. 3.8). Results of the PERMANOVA test did not show significant separation (pseudo-F = 0.951, p = 0.825) among samples by region.

Bacterial phyla: For *O. richardsoni* samples combined across both regions examined here, the two predominantly RA bacterial phyla were Pseudomonadota (46.0%) and Bacillota (34.8%) (Table 3.12). Samples from western MT had significantly low DA of Bacillota (16.4% RA) and Pseudomonadota (50.4% RA), but had six more bacterial phyla above the RA threshold than southern AB samples (Table 3.12). In addition to a higher RA for Bacillota in AB (53.2%) compared to MT (16.4%), the other notable difference was a 20.2% RA for Bacteroidota in MT for a bacteria phylum below the 0.5% RA threshold in AB.

Bacterial genera: The 14 bacterial genera in *O. richardsoni* above 1.5% RA totaled 89.3% (Table 3.13). The three bacterial genera found across both regions were *Lactococcus*, f_Enterobacteriaceae, and f_Yersiniaceae. At least seven bacteria genera had >10% difference in RA between the two areas. The only significant DA bacterial genus between the regions was a high DA of *Lactococcus* (41.7% RA) in AB (Table 3.13).

3.4.4 One Location, Multiple Species

To examine how patterns of GBC diversity may be similar if sampling ground beetles with similar feeding habits from the same habitat type in the same region, samples collected within an 11-day period (August 15-26, 2022) from two canola crops and a pea crop which were on a grain, canola, pea crop rotation ~7 km apart in southern AB cropland were analyzed. The samples contained 11 carabid species including two carnivores: *Agonum placidum* (n = 6) and *Pterostichus melanarius* (n=5); two omnivores: *Bembidion quadrimaculatum* (n=8) and *Clivina fossor* (n=5); and seven granivores: *Amara carinata* (n=7), *Amara farcta* (n=5), *Amara littoralis* (n=5), *Amara quenseli* (n=10), *Harpalus affinis* (n=5), *Harpalus amputatus* (n=5), and *Harpalus pensylvanicus* (n=5) (Table 3.1). Feeding habits were determined using published material and in some cases carabid genera generalizations while remembering that feeding habits are rarely absolute, meaning that there is overlap in what food items they will eat (Larochelle, 1990). Prior to analyses for Bray-Curtis (beta diversity) and richness, evenness, and Shannon (alpha diversity), libraries were rarefied to a standard size of 508 sequences per sample. Six samples with less than 508 sequences were removed: three individuals from each of *A. quenseli* and *B. quadrimaculatum* (Table 3.1).

Alpha diversity among carabid species in cropland

Kruskal-Wallis tests detected significant differences in the GBCs of carabid cropland species for measures of richness (H = 29.253, p = 0.001), evenness (H = 21.525, p = 0.018), and Shannon diversity (H = 27.708, p = 0.002) (Fig. 3.9). However, Kruskal-Wallis (pairwise) comparisons did not detect significant differences between carabid species for richness (q > 0.056), evenness (q > 0.083), or Shannon diversity (q > 0.111).

Beta diversity among carabid species in cropland

The three PCoA axes combined represent 14.08% of the beta diversity between cropland samples (Fig. 3.10). Results of the PERMANOVA tests showed significant separation among samples by species (pseudo-F = 1.453, p = 0.001). For example, *P. melanarius* and most *A. placidum* samples (both carnivores) cluster with one another and separate from samples of other species on axis 1 (5.51%) and axis 2 (4.55%). Four of five *H. pensylvanicus* cluster together and separate from samples of all other species on axes 1 and 2 (Fig. 3.10).

Bacterial phyla: Bacillota and Pseudomonadota were the only two bacteria phyla found in all carabid species above 0.5% and the RA varied among species (Table 3.14). Bacillota had significantly high DA in *A. placidum* (45.0% RA), *Clivina fossor* (52.5% RA), and *P. melanarius* (33.1% RA) (Table 3.14). Actinomycetota was > 0.5% RA in all but the *Agonum* and *Pterostichus* species and DA and RA was highest in *Amara*. An unknown phylum, Acidobacteriota, and Verrucomicrobiota were only above the threshold in species of *Amara* and *Harpalus.*

Bacterial genera: The genus *Enterococcus* had significantly high DA in the carnivores *A. placidum* (13.6% RA) and *P. melanarius* (27.3% RA) but was also found in six other species above the threshold for RA (Table 3.15). *Lactococcus* was in eight of 11 species, and DA was significantly high in three *Amara* species. Two of the three species without Lactococcus were the carnivores *A. placidum* and *P. melanarius*. The DA of *Gilliamella* was significantly high in *A. placidum* (16.6% RA) and *P. melanarius* (29.1% RA) but was not above the 1.5% threshold in any other cropland carabid species. The endosymbiont *Spiroplasma* had a significantly high DA in *A. placidum* (30.4% RA), *Amara littoralis* (5.4% RA), and *H. pensylvanicus* (1.8% RA) (Table 3.15).

3.4.5 Feeding Habit and GBC

Using the same samples as the previous section (3.4.4 One location, multiple species), I examined the data from the perspective of feeding habits and found several key differences. Granivores and omnivores were generally more similar than either group was to carnivores. Carnivores had less bacterial diversity, and bacterial diversity differed among feeding habits. Note, however, the discrepancy in the number of species and individuals representing the different feeding guilds, i.e., seven granivorous species (n = 42 beetles), two carnivorous species (n = 11), and two omnivorous species (n = 13).

Alpha diversity among feeding habits in cropland

Kruskal-Wallis tests detected significant differences in the GBCs of carabid feeding habits in cropland for measures of richness (H = 14.290, p = 0.001), evenness (H = 6.074, p = 0.048), and Shannon diversity (H = 10.454, p = 0.005) (Fig. 3.11). Kruskal-Wallis (pairwise) tests showed the omnivores had significantly lower richness (H > 12.156, q = 0.001 (Fig. 3.11a)) and Shannon diversity (H > 8.138, q < 0.006 (Fig. 3.11c)) than carnivores and granivores. The carnivores had significantly lower evenness than omnivores (K-W test: H = 7.160, q = 0.022 (Fig. 3.11b)).

Beta diversity among feeding habits in cropland

In the PCoA there was significant separation among samples by feeding habit (pseudo-F = 1.909 p = 0.001) The carnivores mainly form one cluster separate from other non-carnivores on axis 1 (5.51%) and axis 2 (4.55%). The omnivores formed a tight cluster within a larger cluster of granivores (Fig. 3.10). The exception to the granivore cluster is four of five *H. pensylvanicus* which cluster together and separate from samples of all other species on axis 1 and 2 (Fig. 3.10), showing some of the diversity within granivores.

Bacterial phyla: Clear differences were found among the different feeding habits with regards to number of bacteria phyla as well as what phyla were present (Table 3.16). While Actinomycetota was absent (RA < 0.5%) in the carnivores, it was found in both non-carnivore groups (RA 10.3-14.7%) where it was also at significantly high DA.

Similarly, Chloroflexota and Planctomycetota were present in the non-carnivore groups (1.9-5.3% and 0.7-2.9% respectively) and at significantly high DA in granivores while being below the threshold in carnivores. In contrast, Fusobacteriota was found in carnivores (7.8% RA) at a significantly high DA and was not found above the 0.5% RA threshold in the non-carnivores while also having significantly low DA in the granivores. Acidobacteriota, Myxococcota, and Verrucomicrobiota were only above the threshold in the granivores. Bacillota and Pseudomonadota had a significantly high DA ($q < 0.05$) in the carnivores while being at significantly low DA in the non-carnivores. Bacteroidota had significantly high DA in both carnivores and omnivores (Table 3.16). Granivores had more bacterial phyla above the threshold (nine phyla) while carnivores had the lowest amount of bacterial phyla above the threshold (four phyla).

Bacterial genera: The total RA for bacterial genera above the 1.5% RA threshold was much higher in carnivores (96.1%) and omnivores (79.1%) than in granivores (56.1%) (Table 3.17). Less bacterial genera were found in carnivores (9 genera) than granivores (16 genera) or omnivores (14 genera). The genus *Enterococcus* had significantly high DA in the carnivores (19.8% RA), but was also found at low RA (2.7%) in the granivores. *Gilliamella* was only found in the carnivores (22.3% RA) where the DA was also significantly high for that bacterium. In contrast, *Lactococcus* was present in the non-carnivores (5.0-7.9% RA), at significantly high DA in granivores, and absent or below the RA threshold in the carnivores (Table 3.17).

Venn diagram

Key shared and unique bacterial taxa among feeding habits in cropland were displayed in a Venn diagram (Fig. 3.12)

3.4.6 Core Gut Bacteria

Examining which bacterial taxa remained present across relatively large sample sizes allowed for a more comprehensive examination of core gut bacteria, although a deeper look into individual carabid taxa or scenarios may be warranted.

There appears to be core GBC members in some individual host species used in this thesis chapter. The bacterium *Weissela* was detected in 22 of 25 samples of *P. melanarius* (0.3 to 66.9% RA) and was not found in enough samples of other carabid species used in this thesis chapter to give evidence of close associations. A member of the Yersiniaceae family was detected in 21 of 25 samples of *P. melanarius* (0.4 to 62.4% RA) (Tables 3.3, 3.7, 3.11, 3.15, and github Carabid GBC) and was not found in enough samples of other carabid species used in this chapter to give evidence of close associations. *Lactococcus* was in seven of ten *O. richardsoni* (1.7 to 100.0% RA) across regions, five of seven *A. carinula* (1.1 to 24.6% RA), and five of five *A. farcta* (0.9 to 20.9% RA) (Tables 3.13, 3.15 and github Carabid GBC) and was not found in enough samples of other carabid species used in this chapter to give evidence of close associations. A member of Alphaproteobacteria was predominant in five of five *H. pensylvanicus* (65.9 to 98.7% RA) samples, but absent from the other carabids studied here, and has also been reported in previous studies of *H. pensylvanicus* (Fig. 2.4, Table

3.15 and github Carabid GBC). In *Amara littoralis* there was *Spiroplasma* in eight of 13 samples (0.5 to 78.5% RA) and it was above the 1.5% threshold in all three habitats and different seasons (Table 3.5 and github Carabid GBC). *Agonum placidum* also had *Spiroplasma* in five of six samples (0.8 to 83.1% RA) and had some of the highest RA for that bacterium (30.4% combined) of any carabid species studied in this thesis (Table 3.15 and github Carabid GBC).

3.5 Discussion

3.5.1 Core Gut Bacteria

Some bacteria appear to occur in certain species of carabids more often than expected by chance, suggesting that these bacteria may be members of a core GMB. An unidentified member of the bacterial family Yersiniaeae was found in almost all *P. melanarius* samples (Fig. 2.4; Tables 3.3, 3.7, 3.11, 3.15; github Carabid GBC). Yersiniaceae bacteria is often pathogenic, harming either the host or a parasitoid larvae within the host, thereby protecting the host (Boyd *et al.*, 2021, Moxley, 2022, Vaughan *et al.*, 2022). *Weissella*, a LAB, was also found in almost all *P. melanarius* samples. *Weissella* and other LAB are often helpful to the host for digestion and other functions and can usually be picked up from the host's environment (Kim *et al.*, 2023, Endo *et al.*, 2010). Lactic acid bacteria are found in almost all carabid groupings throughout this thesis regardless of feeding habits but particular types of LAB are more likely among feeding habits; i.e., *Enterococcus* in carnivores (Silver *et al.*, 2021, Endo *et al.*, 2010). Finding the LAB *Lactococcus* in almost all samples of *O. richardsoni* is evidence of either vertical transmission or consistently ingesting the bacterium from the riparian habitat this carabid species is usually restricted to.

Some bacteria appearing to be core members of the GMB may actually be intracellular endosymbionts found in the cells that line the gut and not in the gut lumen. An unidentified member of Alphaproteobacteria occurred in all specimens of *Harpalus pensylvanicus* and no other carabids examined in this thesis (Fig. 2.4; Table 3.15; github Carabid GBC) and was also found in *H. pensylvanicus* by Lundgren and Lehman (2010). The closest sequence match (97.3%) in GenBank to this unidentified Alphaproteobacteria is with the bacteria *Candidatus Mesenet longicola*, which causes cytoplasmic incompatibility in its host the coconut beetle *Brontispa longissimi* (Takano *et al.*, 2021). I speculate that the unidentified Alphaproteobacteria is also an endosymbiont and possibly with a similar host effect. The effect of endosymbiotic bacteria on the host’s GBC requires further study. The endosymbiont *Spiroplasma* was abundant in most samples of *Amara littoralis* (Table 3.5 and github Carabid GBC) and also of *Agonum placidum* (Table 3.15 and github Carabid GBC) for which its RA was particularly high. Some species of *Spiroplasma* have been reported as both an endosymbiont and an exosymbiont depending on circumstances (Anbutsu & Fukatsu, 2003).

3.5.2 Effects of Extrinsic Factors and Feeding Habits

I found evidence of spatial and temporal factors affecting GBC diversity including habitat and year. In addition, I found differences in GBC corresponding to feeding habit within croplands.

3.5.2.1 Habitats

The GBCs in *Pterostichus melanarius* sampled across habitats (coniferous forest, canola, timothy hay, and riparian) were stable in alpha diversity but had significant differences in beta diversity (Fig. 3.2), RA, and DA (Table 3.3) suggesting some underlying uniformity, or regulation, of the GBC within the carabid species. Four of the bacterial genera in the order Lactobacillales, also known as lactic acid bacteria (LAB), were found including *Lactobacillus, Enterococcus, Weisella,* and an unidentified genus. Lactic acid bacteria are often acquired from the environment, some may be transient members of the GBC while others may be resident in the digestive tract of the host (Endo *et al.*, 2010). *Enterococcus* and *Weissella* are predominant across most habitats in *P. melanarius* but in coniferous forest samples *Lactobacillus* were more prevalent (47.8% RA, Table 3.3). It and other LAB have been linked to good health in insects and other animals (Foysal *et al.*, 2020, Zhang *et al.*, 2022a). Different habitats may have different prey items or environmental exposures (e.g., soil) which provide different LAB. A few of the primary food sources of *P. melanarius* are slugs, caterpillars, and earthworms (King *et al.*, 2010, Larochelle, 1990) and some of those prey have *Lactobacillus*, a LAB (Bai *et al*., 2023). The bacterial genus *Gilliamella* had high RA (29.1%) and significantly high DA in samples from canola (Table 3.3) and has only been studied in the GBC of bees (Hymenoptera) as a digester of carbohydrates.

Although seasonality was a confounding factor, habitat may have still affected the GBC diversity of *A. littoralis*. Only samples from canola cropland/late-summer had the LAB genera *Lactococcus*, *Enterococcus*, and *Weissella* above the threshold (four of five samples). In the comparison of all cropland carabids used in this study only the carnivores had *Enterococcus* above 10% RA and with a significantly high DA. It being at 7% RA in samples of *A. littoralis* (highest of the granivores studied here) could be an indication of scavenging or other carnivorous behavior by some *A. littoralis* in cropland.

3.5.2.2 Regional Variation

Results of *P. melanarius* and *O. richardsoni* examined across different regions did not show major significant differences in the GBC. The stability of GBCs across regions in these two carabid species could be because of similar food items or vertically transmitted microbes. For *P. melanarius, Wolbachia* was only detected in specimens from MT (n=5) with no trace of this endosymbiont detected in specimens from AB (n=20). This finding indicates that infections of *Wolbachia* are not yet fixed in the species such that some populations remain uninfected. *Wolbachia*-infected and uninfected populations of the same host species have previously been reported for other species of beetles (Li *et al.*, 2015).

3.5.2.3 Seasonal Variation

The GBC diversity in *Amara littoralis* samples had distinct patterns depending on either habitat or time of year the samples were collected. As samples from different habitats were also collected at different times of the year, it is difficult to determine if time of year, habitat, or both contributed to the differences. For example, spring-collected specimens had overwintered as adults, whereas late summer-collected specimens had developed from eggs laid in spring/early summer. Li *et al.* (2023) found bacterial growth is profoundly affected by temperature and, as insects are ectothermic, the time of year samples were collected likely contributed to differences in GBC diversity. The number of bacterial taxa increased sequentially as temperatures increased and seasons changed from midspring to early summer to late summer (Fig. 3.2a) the low diversity early in the year may be due to overwintering without exposure to food or foodborne bacteria (Ferguson *et al.*, 2018).

3.5.2.4 Year to Year Variation

There was evidence of change in the GBCs of *Pterostichus melanarius* and *Carabus granulatus* across years; however, storage may have been a confounding factor. Both species showed lower GBC diversity when stored in 70% ethanol (2021) compared to 95-100% ethanol (2022, 2023) similar to Song *et al.* (2016). With 70% ethanol, samples are not preserved as well, therefore RA is a less reliable metric. As such, I focused on the presence and absence of bacterial taxa rather than their relative abundances.

Pterostichus melanarius samples collected at the same coniferous site in 2021 versus 2022 had a lot of the same bacteria present, but in different relative abundances (Tables 3.6 and 3.7) which could be an artifact of preservation methods. Among the very few differences in bacterial taxa present or absent between years in *P. melanarius*, the bacterium *Rickettsia* was only found in samples from 2022 (three of five samples), albeit at low RA (github Carabid GBC). The symbiont of arthropods and other invertebrates, *Rickettsia,* could be an infection in some *P. melanarius* but because it was not found in both years, it may have been in a prey whose abundance fluctuates from year to year (Perlman & Zchori-Fein, 2006).

The GBC of *Carabus granulatus* appeared more stable across years based on measures of Bray-Curtis beta diversity and alpha diversity. One major difference between years was the high RA and significantly high DA of Actinomycetota in samples from 2021 and 2023 but not 2022, meaning *C. granulatus* may have had a more carnivorous diet in 2022. In the analysis of all cropland samples for this study, Actinomycetota was most often found in granivores (Tables 3.14 and 3.15) which is consistent with García-Amado *et al.* (2018). The genus *Carabus* is often referred to as a group of caterpillar eaters and the abundance of that and other food sources can fluctuate drastically from year to year (Owen & Owen, 1990).

3.5.2.5 Feeding Habits

The differences in GBCs between the three feeding habits were distinct. Those of granivores tended to have higher richness and higher variance of richness than those of carnivores or omnivores (Fig. 3.9). This may be because granivores encounter more contaminants by eating seeds that have been laying on, or in, the soil for a while or because carnivores and omnivores have GBCs regulated by antimicrobials. The GBC pattern of high richness and variance in granivores and lower richness and stability in carnivores was also found in previous studies (Kudo *et al.*, 2019). The stability of some bacterial taxa in carnivores could be an indication of core GMB components producing antimicrobials and regulating the bacterial taxa and their abundances in the GBC (Chanthasena *et al.*, 2021, Kudo *et al.*, 2019).

According to results of this and previous studies, core gut bacteria may exist for feeding habits of carabids. Core gut bacteria of carnivorous carabids may include *Enterococcus, Sebaldella*, *Gilliamella*, the phylum Fusobacteriota, and a member of the Yersiniaceae family (Tables 3.14 and 3.15). The core gut bacteria of granivores may include the six phyla Actinomycetota, Acidobacteriota, Chloroflexota, Planctomycetota, an unknown phylum, and Verrucomicrobiota as well as the bacterial genera *Lactobacillus, Lactococcus,* and *Escherichia-Shigella* found in two other bacteria phyla. Most results in this study as they relate to feeding habits are consistent with the results of previous studies (Kudo *et al.*, 2019, García-Amado *et al.*, 2018, Silver *et al.*, 2021)

Variance among granivorous carabids were also found. For example, the phylum Myxococcota and the Chloroflexota genus *KD4-96* may be part of a core GBC in species of the genus *Amara* but not in the other granivorous genus studied here, *Harpalus* (Tables 3.14 and 3.15). Although species showed similar bacterial phyla in their GBCs there was little overlap of bacterial genera among the eight granivorous carabid species. This could be an indication of GBCs being more closely related within closely related carabid taxa or granivore species may prefer to eat different types of seeds containing different bacteria (Foffová *et al.*, 2020). Another explanation for high diversity of bacterial genera (especially in the bacterial phylum Actinomycetota) is exposure to soil microbes. The diversity of bacteria in soil can be vast and often contains various genera in the Actinomycetota phylum (Liu *et al.*, 2022, Liu *et al.*, 2023).

3.5.3 Conclusions

Some of the questions asked in this thesis chapter are answered through controlled sampling and large sample sizes such that I was able to make firm conclusions. For example, we have a good idea of the GBC diversity in southern AB cropland granivores in the late summer as 42 specimens across seven species are examined. The bacterium *Gilliamella*, which is a known producer of antimicrobials that protect against entomopathogenic bacteria (Lang et al., 2023), is not found in cropland granivores of the *Harpalus* or *Amara* genera although it can be found in many other omnivores and carnivores, including those in cropland, studied here. Another example of controlled observation combined with substantial sample sizes is the analysis of 23 specimens of one species, *Pterostichus melanarius*, across four different habitats (coniferous forest, canola, timothy hay, and a riparian zone). Fifteen of those samples were collected in a 30 day period in 2022 across three different habitats. An additional five *P. melanarius* samples were collected at exactly the same time of year (August) but the previous year. It is clear that habitat has a significant effect on the GBC of this carabid species. Extrinsic factors, feeding habit, and taxonomy of the host can all influence the diversity of GBCs in carabids.

Some GBC characteristics examined in this study appeared to have more variance across hosts, extrinsic factors, or feeding habits than others. In some cases, different bacterial genera may serve the same purpose in the GBC depending on their metabolic systems (e.g., LAB). There are different LAB linked to particular feeding habits and habitats or seasons. Of the extrinsic factors examined here, region and year-to-year differences appeared to make the least amount of difference for GBC diversity.

According to this study, when confounding factors are controlled, the feeding habit and taxonomy of the host create the clearest patterns in GBC diversity.

I detected changes in the GBC of carabid beetles associated with different habitats, seasons, years, and regions. When these factors were controlled by examining the GBC of different carabid species collected at the same time and site, I also detected evidence of a core GBC associated with a given carabid species or with feeding habit (carnivore or granivore).

3.6 References

Anbutsu H & Fukatsu T (2003) Population dynamics of male-killing and non-male-killing Spiroplasmas in *Drosophila melanogaster*. *Applied and Environmental Microbiology,* **69,** 1428-1434.

Anderson KE, Sheehan TH, Mott BM, Maes P, Snyder L, Schwan MR*, et al.* (2013) Microbial ecology of the hive and pollination landscape: bacterial associates from floral nectar, the alimentary tract and stored food of honey bees (*Apis mellifera*). *PloS One,* **8,** e83125.

Armstrong RA (2014) When to use the Bonferroni correction. *Ophthalmic & Physiological Optics,* **34,** 502-508.

Bai J, Xu Z, Li L, Zhang Y, Diao J, Cao J*, et al.* (2023) Gut bacterial microbiota of *Lymantria dispar asiatica* and its involvement in *Beauveria bassiana* infection. *Journal of Invertebrate Pathology,* **197,** 107897.

Blow F & Douglas AE (2019) The hemolymph microbiome of insects. *Journal of Insect Physiology,* **115,** 33-39.

Boyd BM, Chevignon G, Patel V, Oliver KM & Strand MR (2021) Evolutionary genomics of APSE: a tailed phage that lysogenically converts the bacterium *Hamiltonella defensa* into a heritable protective symbiont of aphids. *Virology Journal,* **18,** 219.

Callahan BJ, Mcmurdie PJ, Rosen MJ, Han AW, Johnson AJ & Holmes SP (2016) DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods,* **13,** 581-583.

Cappellato M, Baruzzo G & Di Camillo B (2022) Investigating differential abundance methods in microbiome data: A benchmark study. *PLoS Computational Biology,* **18,** e1010467.

Chanthasena P, Penkhrue W, Khowangklang P, Sritangos P & Nantapong N (2021) Antimicrobial potential of fungi isolated from soils of dry dipterocarp forest in northeast Thailand. *Chiang Mai Journal of Science,* **48,** 793-807.

Dias AM, Cordeiro G, Estevinho MM, Veiga R, Figueira L, Reina-Couto M*, et al.* (2020) Gut bacterial microbiome composition and statin intake—A systematic review. *Pharmacology Research & Perspectives,* **8,** e00601.

Durden L, Wang D, Panaccione D & Clay K (2019) Decreased root-knot nematode gall formation in roots of the morning glory *Ipomoea tricolor* symbiotic with ergot alkaloid-producing fungal *Periglandula Sp. Journal of Chemical Ecology,* **45,** 879-887.

Duron O & Gottlieb Y (2020) Convergence of nutritional symbioses in obligate blood feeders. *Trends in Parasitology,* **36,** 816-825.

Endo A, Futagawa-Endo Y & Dicks LMT (2010) Diversity of *Lactobacillus* and *Bifidobacterium* in feces of herbivores, omnivores and carnivores. *Anaerobe,* **16,** 590-596.

Estaki M, Jiang L, Bokulich NA, Mcdonald D, González A, Kosciolek T, *et al.* (2020) QIIME 2 enables comprehensive end-to-end analysis of diverse microbiome data and comparative studies with publicly available data. *Current Protocols in Bioinformatics,* **70,** e100.

Ewing B, Hillier L, Wendl MC & Green P (1998) Base-calling of automated sequencer traces using Phred. I. accuracy assessment. *Genome Research,* **8,** 175-185.

Ferguson LV, Dhakal P, Lebenzon JE, Heinrichs DE, Bucking C & Sinclair BJ (2018) Seasonal shifts in the insect gut microbiome are concurrent with changes in cold tolerance and immunity. *Functional Ecology,* **32,** 2357-2368.

Ferreira JA & Zwinderman AH (2006) On the Benjamini-Hochberg method. *The Annals of Statistics,* **34,** 1827-1849.

Foffová H, Ćavar Zeljković S, Honěk A, Martinková Z, Tarkowski P & Saska P (2020) Which seed properties determine the preferences of carabid beetle seed predators? *Insects,* **11,** 757.

Folmer O, Black M, Hoeh W, Lutz R & Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology,* **3,** 294.

Foysal MJ, Fotedar R, Siddik MaB & Tay A (2020) *Lactobacillus acidophilus* and *L. plantarum* improve health status, modulate gut microbiota and innate immune response of marron (*Cherax cainii*). *Scientific Reports,* **10,** 5916.

García-Amado MA, Shin H, Sanz V, Lentino M, Martínez LM, Contreras M, *et al.* (2018) Comparison of gizzard and intestinal microbiota of wild neotropical birds. *PloS One,* **13,** e0194857.

Geerinck MWJ, Van Hee S, Gloder G, Crauwels S, Colazza S, Jacquemyn H, *et al.* (2022) Diversity and composition of the microbiome associated with eggs of the southern green stinkbug, *Nezara viridula* (Hemiptera: Pentatomidae). *Microbiology Open (Weinheim),* **11,** e1337.

Gilad R, Porat A & Trachtenberg S (2003) Motility modes of *Spiroplasma melliferum BC3*: a helical, wall-less bacterium driven by a linear motor. *Molecular Microbiology,* **47,** 657-669.

github Carabid GBC: https://github.com/kingfishertile/Carabid-gut-bacterial-communities.git Archived supplemental data from this thesis.

Grond K, Lanctot RB, Jumpponen A & Sandercock BK (2017) Recruitment and establishment of the gut microbiome in arctic shorebirds. *FEMS Microbiology Ecology,* **93,** 12, 1-9.

Hebert PDN, Cywinska A, Ball SL & Dewaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society. B, Biological Sciences,* **270,** 313-321.

Hill MP, Macfadyen S & Nash MA (2017) Broad spectrum pesticide application alters natural enemy communities and may facilitate secondary pest outbreaks. *PeerJ,* **5,** e4179.

Janke RS, Moog S, Weiss B, Kaltenpoth M & Florez LV (2022) Morphological adaptation for ectosymbiont maintenance and transmission during metamorphosis in *Lagria* beetles. *Frontiers in Physiology,* **13,** 979200.

Kers JG & Saccenti E (2022) The power of microbiome studies: Some considerations on which alpha and beta metrics to use and how to report results. *Frontiers in Microbiology,* **12,** 796025.

Kim E, Yang S-M & Kim H-Y (2023) Weissella and the two Janus faces of the genus. *Applied Microbiology and Biotechnology,* **107,** 1119-1127.

King RA, Vaughan IP, Bell JR, Bohan DA & Symondson WOC (2010) Prey choice by carabid beetles feeding on an earthworm community analysed using species- and lineage-specific PCR primers. *Molecular Ecology,* **19,** 1721-1732.

Kolasa M, Scibior R, Mazur MA, Kubisz D, Dudek K & Kajtoch Ł (2019) How hosts taxonomy, trophy, and endosymbionts shape microbiome diversity in beetles. *Microbial Ecology,* **78,** 995-1013.

Kudo R, Masuya H, Endoh R, Kikuchi T & Ikeda H (2019) Gut bacterial and fungal communities in ground-dwelling beetles are associated with host food habit and habitat. *The ISME Journal,* **13,** 676-685.

Lang H, Liu Y, Duan H, Zhang W, Hu X, & Zheng H (2023). Identification of peptides from honeybee gut symbionts as potential antimicrobial agents against *Melissococcus plutonius*. *Nature Communications*. **14**(1), 7650-7661

Larochelle A (1990) *Food of Carabid Beetles (The):(Coleoptera: Carabidae, Including Cicindelinae),* Quebec, QC, Canada., Association des Entomologistes du Québec.

Larochelle A & Larivière M (2003) *A Natural History of the Ground-Beetles (Coleoptera: Carabidae) of America North of Mexico,* Sofia, Bulgaria, Pensoft.

Li YY, Fields PG, Pang BP, Coghlin PC & Floate KD (2015) Prevalence and diversity of *Wolbachia* bacteria infecting insect pests of stored products. *Journal of Stored Products Research,* **62,** 93-100.

Lin H & Peddada SD (2020) Analysis of compositions of microbiomes with bias correction. *Nature Communications,* **11,** 3514.

Lindroth CH (1961) The ground beetles (Carabidae, excl. Cicindelinae) of Canada and Alaska, Part 2-6. *Opuscula Entomologica***,** 1-1192.

Liu F, Hewezi T, Lebeis SL, Pantalone V, Grewal PS & Staton ME (2019) Soil indigenous microbiome and plant genotypes cooperatively modify soybean rhizosphere microbiome assembly. *BMC Microbiology,* **19,** 201.

Liu L, Wang Z, Ma D, Zhang M & Fu L (2022) Diversity and distribution characteristics of soil microbes across forest-peatland ecotones in the permafrost regions. *International Journal of Environmental Research and Public Health,* **19,** 14782.

Liu X, Floate KD, Gorzelak MA, Holman DB, Hrycauk S, Kubota H, *et al.* (2023) Prairie agroecosystems: Interconnected microbiomes of livestock, soil and insects. *Agriculture (Basel),* **13,** 326.

Lovei GL & Sunderland KD (1996) Ecology and behavior of ground beetles (Coleoptera: Carabidae). *Annual Review of Entomology,* **41,** 231-256.

Lundgren JG & Lehman RM (2010) Bacterial gut symbionts contribute to seed digestion in an omnivorous beetle. *PloS One,* **5,** e10831.

Maurice N & Erdei L (2018) Termite gut microbiome. In: Khan MA & Ahmad W (eds.) *Termites and Sustainable Management. Sustainability in Plant and Crop Protection.* CHAM, Springer Nature, pgs. 69-99.

Michaud C, Hervé V, Dupont S, Dubreuil G, Bézier AM, Meunier J*, et al.* (2020) Efficient but occasionally imperfect vertical transmission of gut mutualistic protists in a wood-feeding termite. *Molecular Ecology,* **29,** 308-324.

Moxley RA (2022) Family Yersiniaceae. *Veterinary Microbiology***,** 88-99.

Nikoh N, Hosokawa T, Moriyama M, Oshima K, Hattori M & Fukatsu T (2014) Evolutionary origin of insect–*Wolbachia* nutritional mutualism. *Proceedings of the National Academy of Sciences,* **111,** 10257-10262.

Ohbayashi T, Takeshita K, Kitagawa W, Nikoh N, Koga R, Meng X, *et al.* (2015) Insect's intestinal organ for symbiont sorting. *Proceedings of the National Academy of Sciences*, **112,** E5179-E5188.

Owen DF & Owen J (1990) Assessing insect species-richness at a single site. *Environmental Conservation,* **17,** 362-364.

Perlman SJ & Zchori-Fein E (2006) Emerging diversity of *Rickettsia*. *Proceedings of the Royal Society. B, Biological Sciences,* **273,** 2097-2106.

Pita L, Rix L, Slaby BM, Franke A & Hentschel U (2018) The sponge holobiont in a changing ocean: From microbes to ecosystems. *Microbiome,* **6,** 1-18.

Porter J & Sullivan W (2023) The cellular lives of *Wolbachia*. *Nature Reviews Microbiology,* **21,** 750-766.

Robinson CD, Bohannan BJ & Britton RA (2019) Scales of persistence: transmission and the microbiome. *Current Opinion in Microbiology,* **50,** 42-49.

Rocha FP, Ronque MU, Lyra ML, Bacci Jr M & Oliveira PS (2022) Habitat and host species drive the structure of bacterial communities of two neotropical trap-jaw *Odontomachus* ants. *Microbial Ecology,* **86,** 699–712.

Rosenberg E & Zilber-Rosenberg I (2018) The hologenome concept of evolution after 10 years. *Microbiome,* **6,** 78.

Ruxton GD & Beauchamp G (2008) Some suggestions about appropriate use of the Kruskal–Wallis test. *Animal Behaviour,* **76,** 1083-1087.

Salem H, Florez L, Gerardo N & Kaltenpoth M (2015) An out-of-body experience: the extracellular dimension for the transmission of mutualistic bacteria in insects. *Proceedings of the Royal Society. B, Biological sciences,* **282,** 20142957.

Satpathi CR (2021) *Ground Beetles and Their Role in Management of Crop Pests,* London, CRC Press.

Schmid RB, Lehman RM, Brözel VS & Lundgren JG (2014) An indigenous gut bacterium, *Enterococcus faecalis* (Lactobacillales: Enterococcaceae), increases seed consumption by *Harpalus pensylvanicus* (Coleoptera: Carabidae). *Florida Entomologist,* **97,** 575-584.

Silver A, Perez S, Gee M, Xu B, Garg S, Will K, *et al.* (2021) Persistence of the ground beetle (Coleoptera: Carabidae) microbiome to diet manipulation. *PloS One,* **16,** e0241529.

Smith AH, O'connor MP, Deal B, Kotzer C, Lee A, Wagner B*, et al.* (2021) Does getting defensive get you anywhere?—Seasonal balancing selection, temperature, and parasitoids shape real-world, protective endosymbiont dynamics in the pea aphid. *Molecular Ecology,* **30,** 2449-2472.

Song SJ, Amir A, Metcalf JL, Amato KR, Xu ZZ, Humphrey G, *et al.* (2016) Preservation methods differ in fecal microbiome stability, affecting suitability for field studies. *mSystems,* **1**, e00021-16.

Takano S-I, Gotoh Y & Hayashi T (2021) "*Candidatus Mesenet longicola*": Novel endosymbionts of *Brontispa longissima* that induce cytoplasmic incompatibility. *Microbial Ecology,* **82,** 512-522.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M & Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution,* **28,** 2731-2739.

Vaughan AL, Altermann E, Glare TR & Hurst MRH (2022) Genome sequence of the entomopathogenic *Serratia entomophila* isolate 626 and characterisation of the species specific itaconate degradation pathway. *BMC Genomics,* **23,** 728.

Venn S (2016) To fly or not to fly: Factors influencing the flight capacity of carabid beetles (Coleoptera: Carabidae). *European Journal of Entomology,* **113,** 587-600.

Willis AD. (2019) Rarefaction, alpha diversity, and statistics. *Frontiers in Microbiology.* **10,** 2407.

Zhang Z, Mu X, Cao Q, Shi Y, Hu X & Zheng H (2022a) Honeybee gut *Lactobacillus* modulates host learning and memory behaviors via regulating tryptophan metabolism. *Nature Communications,* **13,** 2037.

Zhang Z, Mu X, Shi Y & Zheng H (2022b) Distinct roles of honeybee gut bacteria on host metabolism and neurological processes. *Microbiology Spectrum,* **10,** e0243821.

Table 3.1. Sample counts of carabid species used in this chapter with sample sizes and taxonomic designation. Rarefaction was needed for analyses of alpha diversity and the Bray-Curtis principal coordinate analysis (PCoA) (beta diversity). Non-rarefied data were used for the differential abundance (DA) and relative abundance (RA) shown in tables. Sample counts that changed after rarefaction are bolded.

Subfamily	**Genus**	**Species**	**Unrarefied (RA% and DA tables)**	**Rarefied (Alpha diversity and PCoA)**
Carabinae	*Carabus*	*granulatus*	8	8
Harpalinae	*Agonum*	*placidum*	6	6
	Amara	*farcta*	5	5
	Amara	*littoralis*	13	13
	Amara	*quenseli*	**10**	**7**
	Amara	*carinata*	7	7
	Harpalus	*affinis*	5	5
	Harpalus	*amputatus*	5	5
	Harpalus	*pensylvanicus*	5	5
	Pterostichus	*melanarius*	25	25
Nebriinae	*Opisthius*	*richardsoni*	**10**	**8**
Scaritinae	*Clivina*	*fossor*	5	5
Trechinae	*Bembidion*	*quadrimaculatum*	**8**	**5**
Totals			**112**	**104**

Table 3.2. Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in *Pterostichus melanarius* samples by habitat. Numbers in bold are the highest RA in each column. Only bacteria exceeding a threshold ≥ 0.5% RA are shown.

	Total (n = 23)	canola (n = 5)	coniferous forest (n = 10)	riparian (n = 5)	hay (n = 3)
Bacillota	**54.2**	33.1[2]	**70.0[1]**	**74.6***	15.9[2]
Pseudomonadota	42.1	**61.6***	28.9[2]	22.4[2]	**83.9[1]**
Bacteroidota	1.5	2.0[1]	0.8	2.1[1]	
Actinomycetota	1.2				
Fusobacteriota	0.6	2.6			
Total	99.6	99.3	99.7	99.1	99.8

[1] significantly high differential abundance (DA) compared to other beetle groupings
[2] significantly low DA
*significant midrange DA

Table 3.3. Relative abundance (RA%) and significant differential abundance (DA) of bacterial genera in *Pterostichus melanarius* samples by habitat. Numbers in bold are the highest RA in each column. Only bacteria exceeding a threshold ≥ 1.5% RA are shown.

	Total (n = 23)	canola (n = 5)	coniferous forest (n = 10)	riparian (n = 5)	hay (n = 3)
Bacillota;*Enterococcus*	14.0	27.3^{1}	6.0	25.4^{1}	^{2}
f__Lachnospiraceae	3.5		1.7	12.6	^{2}
f__Ruminococcaceae				1.5	^{2}
Lactobacillus	**21.3**		**47.8**^{1}		2.8
o__Lactobacillales	4.0		6.9*	2.9	2.5^{1}
Weissella	10.5	4.9^{1}	4.8^{2}	**27.6**^{1}	10.6^{1}
Bacteroidota;*Dysgonomonas*		2.0^{1}		2.1^{1}	
Fusobacteriota;*Sebaldella*		2.6			^{2}
Pseudomonadota;*Acinetobacter*					1.7^{1}
f__Enterobacteriaceae					3.2^{1}
f__Morganellaceae	3.2	1.8			17.4
f__Yersiniaceae	20.8	27.7*	13.8*	13.8	**44.5**^{1}
Gilliamella	9.1	**29.1**^{1}	6.3		^{2}
Hafnia-Obesumbacterium	2.6		5.4		^{2}
Pseudomonas	2.5				16.3^{1}
Total	91.5	95.4	92.6	85.8	99.1

[1] significantly high DA compared to other beetle groupings.

[2] significantly low DA.

*significant midrange DA

Table 3.4. Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in *Amara littoralis* samples by habitat/season. Numbers in bold are the highest RA in each column. Only bacteria exceeding a threshold ≥ 0.5% RA are shown.

	total (n=13)	canola/late-summer (n=5)	riparian/early-summer (n=3)	urban-yard/mid-spring (n=5)
Unknown phylum	5.1			13.3
Acidobacteriota	1.2	2.8		
Actinomycetota	17.9	16.6^{1}	15.7^{1}	20.4
Bacteroidota	1.9	1.3	4.8	0.7
Bdellovibrionota		0.7		
Chloroflexota	3.5	7.5	2.8^{1}	
Bacillota	23.4	**36.9**	9.1^{2}	18.6
Myxococcota	0.5	1.2		
Patescibacteria		^{2}	0.5	
Planctomycetota	2.1	4.1	0.8	0.8
Pseudomonadota	**43.6**	28.1^{2}	**65.3^{1}**	**46.2^{2}**
Verrucomicrobiota		0.5^{1}		
WPS-2			0.7	
Total	99.2	99.8	99.7	100.0

[1] significantly high differential abundance (DA) compared to other beetle groupings
[2] significantly low DA

Table 3.5. Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera in *Amara littoralis* samples by habitat/season. Numbers in bold are the highest RA in each column. Only bacteria exceeding a threshold ≥ 1.5% RA are shown.

	total (n=13)	canola/late-summer (n=5)	riparian/early-summer (n=3)	urban-yard/mid-spring (n=5)
unknown_phylum	5.1			13.3
Actinomycetota;*Actinomycetospora*			1.7	
Agromyces				1.5
Arthrobacter		[2]		1.6
f_Microbacteriaceae				1.8
f_Micrococcaceae		2.6		
Gaiella			2.0	
Klenkia				2.0
Nocardioides	4.4		6.4	6.4
o_Gaiellales		2.2		
Schumannella		2.1		
Bacteroidota;*Dyadobacter*			1.9	
Chloroflexota;*JG30-KF-CM45*		1.6		
KD4-96	1.5	2.9		
Bacillota;*Bacillus*		1.9		
Carnobacterium				2.1
Enterococcus	2.7	7.0		
Lactococcus	4.8	**12.6**		
Spiroplasma	**10.2**	5.5	9.1[1]	15.7
Weissella	1.9	4.9		
Planctomycetota;*Gemmata*		2.0		
Pseudomonadota;*Acinetobacter*		2.2		
Brevundimonas		[2]		1.5
Chelativorans		2.5		
Devosia		[2]		1.9
Escherichia-Shigella	8.6	4.2[1]		18.2
f_Caulobacteraceae	5.1		15.1	3.9
f_Enterobacteriaceae	7.3		**31.4**	
f_Rhodobacteraceae	3.0	7.7		
Methylobacterium-Methylorubrum	1.8		6.6	
o_Enterobacterales	7.9			**20.0**
Sphingomonas			4.1	
Total	64.4	62.0	78.2	90.0

[1] significantly high differential abundance (DA) compared to other beetle groupings
[2] significantly low DA

Table 3.6. Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in *Pterostichus melanarius* samples by year. Numbers in bold are the highest RA in each column. Only bacteria exceeding a threshold ≥ 0.5% RA are shown.

	Total (n=10)	2021 (n=5)	2022 (n=5)
Bacteroidota	0.8		1.3
Bacillota	**70.0**	**84.0**^{2}	**55.9**^{1}
Pseudomonadota	28.9	15.1	42.7^{1}
Total	99.7	99.1	99.9

^{1} significantly high differential abundance (DA) compared to other beetle groupings
^{2} significantly low DA

Table 3.7. Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera in *Pterostichus melanarius* samples by year. Numbers in bold are the highest RA in each column. Only bacteria exceeding a threshold ≥ 1.5% RA are shown.

		Total (n=10)	2021 (n=5)	2022 (n=5)
Bacillota;	*Enterococcus*	6.0	7.2	4.7
	f_Lachnospiraceae	1.7		3.4
	Lactobacillus	**47.8**	**70.0**^{1}	**25.5**^{2}
	o_Lactobacillales	6.9		13.2^{1}
	Weissella	4.8	4.0^{1}	5.5^{2}
Pseudomonadota;	f_Morganellaceae		1.8	
	f_Yersiniaceae	13.8	3.0	24.6^{1}
	Gilliamella	6.3	10.1	2.4
	Hafnia-Obesumbacterium	5.4		10.8
	Total	92.6	96.1	90.0

^{1} significantly high differential abundance (DA) compared to other beetle groupings
^{2} significantly low DA

Table 3.8. Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in *Carabus granulatus* samples by year. Numbers in bold are the highest RA in each column. Only bacteria exceeding a threshold ≥ 0.5% RA are shown.

	Total (n = 8)	2021 (n = 5)	2022 (n = 3)
Bacillota	**66.2**	**67.8**[1]	**63.5**[2]
Pseudomonadota	22.1	22.1[2]	22.1[1]
Actinomycetota	2.8	4.1[1]	0.7
Fusobacteriota	4.6	3.9	5.8[1]
Bacteroidota	2.4	0.9	4.9
unidentified phylum	1.2	[2]	2.8
Total	99.4	99.1	99.8

[1] significantly high differential abundance (DA) compared to other beetle groupings
[2] significantly low DA

Table 3.9. Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera in *Carabus granulatus* samples by year. Numbers in bold are the highest RA in each column. Only bacteria exceeding a threshold ≥ 1.5% RA are shown.

	Total (n = 8)	2021 (n = 5)	2022 (n = 3)
unidentified phylum			2.8
Bacteroidota;*Dysgonomonas*		[1]	2.0
Apibacter		[2]	2.9
Bacillota;c__Bacilli			3.5[1]
Breznakia		[2]	2.2[1]
o__Lactobacillales	3.1		6.7[1]
Enterococcus	**38.1**	**61.0**[1]	
Lactobacillus	1.7	2.7	
f__Lachnospiraceae	18.5		**47.8**[1]
f__Ruminococcaceae			2.8
Fusobacteriota;*Sebaldella*	4.6	3.9	5.8[1]
Pseudomonadota;f__Rhizobiaceae	2.6		5.6[1]
o__Enterobacterales	8.6	13.8	
f__Enterobacteriaceae	1.5		2.8[1]
o__Enterobacterales uncultured	2.2	[2]	5.9
Gilliamella	2.7	2.5	3.0
Pseudomonas	2.2	[1]	3.5
Total	85.7	83.9	97.4

[1] significantly high differential abundance (DA) compared to other beetle groupings
[2] significantly low DA

Table 3.10. Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in *Pterostichus melanarius* samples by region. Numbers in bold are the highest RA in each column. Only bacteria exceeding a threshold ≥ 0.5% RA are shown.

	Total (n=25)	Alberta (n=20)	Montana (n=5)
Actinomycetota	1.2	^{2}	4.6
Bacteroidota	1.5	1.4^{1}	1.8
Bacillota	**54.2**	**61.9^{1}**	23.5^{2}
Fusobacteriota	0.6	0.8	
Pseudomonadota	42.1	35.5^{2}	**68.9^{1}**
Total	99.6	99.6	98.9

[1] significantly high differential abundance (DA) compared to other beetle groupings
[2] significantly low DA

Table 3.11. Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera in *Pterostichus melanarius* samples by region. Numbers in bold are the highest RA in each column. Only bacteria exceeding a threshold ≥ 1.5% RA are shown.

		Total (n = 25)	Alberta (n = 20)	Montana (n = 5)
Bacillota;o_Lactobacillales		3.7	4.2^{2}	1.9^{1}
Enterococcus		15.2	16.1^{1}	11.6
Lactobacillus		19.6	**24.1^{1}**	1.7
Weissella		9.7	10.5^{1}	6.4
f_Lachnospiraceae		3.2	4.0	
Pseudomonadota;f_Enterobacteriaceae				3.2^{1}
Hafnia-Obesumbacterium		2.4	3.0	
f_Morganellaceae		2.9		10.5
f_Yersiniaceae		**21.3**	17.3^{2}	**37.3^{1}**
Gilliamella		8.4	10.5^{1}	
Acinetobacter				1.5^{1}
Pseudomonas		2.5		10.7^{1}
Total		88.9	89.6	84.7

[1] significantly high differential abundance (DA) compared to other beetle groupings
[2] significantly low DA

Table 3.12. Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in *Opisthius richardsoni* samples by region. Numbers in bold are the highest RA in each column. Only bacteria exceeding a threshold ≥ 0.5% RA are shown.

	Total (n=10)	W. Montana (n=5)	S. Alberta (n=5)
Acidobacteriota		0.6	
Actinomycetota	4.1	7.9	
Bacteroidota	10.1	20.2	
Bdellovibrionota		0.6	
Chloroflexota		0.9	
Cyanobacteria	0.7	1.4	
Desulfobacterota	2.4		4.9
Bacillota	34.8	16.4[2]	**53.2[1]**
Planctomycetota	0.7	1.5	
Pseudomonadota	**46.0**	**50.4[2]**	41.5[1]
Total	98.9	99.8	99.6

[1] significantly high differential abundance (DA) compared to other beetle groupings
[2] significantly low DA

Table 3.13. Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera in *Opisthius richardsoni* samples by region. Numbers in bold are the highest RA in each column. Only bacteria exceeding a threshold ≥ 1.5% RA are shown.

	Total (n=10)	w. Montana (n=5)	s. Alberta (n=5)
Actinomycetota;f_Microbacteriaceae		1.8	
f_Nocardioidaceae	1.6	3.1	
Bacteroidota;*Bacteroides*	10.0	**20.0**	
Bacillota;*Exiguobacterium*		1.6	
Lactobacillus	5.4		10.9
Lactococcus	**27.8**	13.9	**41.7**[1]
Desulfobacterota;*Bilophila*	2.4		4.9
Pseudomonadota;*Acinetobacter*		1.6	
Aeromonas	6.7	13.5	
Asaia	1.6	3.2	
Escherichia-Shigella	2.0	2.9	
f_Enterobacteriaceae	12.2	1.7	22.7
f_Morganellaceae_	5.3		10.6
f_Yersiniaceae	8.3	14.8	1.9
Pseudomonas	1.9	3.8	
Shewanella	1.9	3.8	
Xanthomonas	2.0		3.9
Total	89.3	85.7	96.6

[1] significantly high differential abundance (DA) compared to other beetle groupings

Table 3.14. Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in cropland samples by species and colored by feeding habits (pink = carnivores, gold = granivores, blue = omnivores). Numbers in bold are the highest RA in each column. Only bacteria exceeding a threshold ≥ 0.5% RA are shown.

	Total (n = 66)	*P. melanarius* (n = 5)	*Ag. Placidum* (n = 6)	*Am. carinata* (n = 7)	*Am. farcta* (n = 5)	*Am. littoralis* (n = 5)	*Am. Quenseli* (n = 10)	*H. affinis* (n = 5)	*H. amputates* (n = 5)	*H. pensylvanicus* (n = 5)	*B. quadrimaculatum* (n = 8)	*C. fossor* (n = 5)
Pseudomonadota	**43.2**	**61.6^{1}**	34.8^{1}	**39.3^{2}**	27.4^{2}	28.1^{2}	**38.7^{2}**	36.1*	**68.6^{1}**	**88.7^{1}**	**49.3^{2}**	7.3^{2}
Bacillota	29.6	33.1^{1}	**45.0^{1}**	23.6^{2}	**31.4^{2}**	**36.9***	22.0^{2}	**42.0***	9.6^{2}	6.0^{2}	30.4^{2}	**52.5^{1}**
Actinomycetota	11.4			19.4^{1}	16.7*	16.6^{1}	21.2^{1}	6.2*	12.5^{1}	1.8	9.7*	11.1^{1}
Bacteroidota	4.3	2.0^{1}	7.7^{1}		2.9	1.3	0.7	1.4	1.7^{1}	0.9	6.4*	25.9
Chloroflexota	3.7			8.0^{1}	7.2	7.5	5.0^{1}	4.7	3.2*		2.3	1.3
Planctomycetota	2.0			4.6	4.8	4.1	2.7	1.1	2.0			1.8
Fusobacteriota	1.4	2.6	12.2^{1}	^{2}	^{2}	^{2}		1.0				
Acidobacteriota	1.4			1.4	5.6	2.8	2.4	1.2	1.5			
Myxococcota	1.1			0.5	1.3	1.2	4.7				0.6	
Verrucomicrobiota	0.8			1.9	1.1	0.5	2.3	1.6	0.5			
Cyanobacteria								2.9				
unidentified phylum				0.5	^{2}	^{2}		0.7		1.7		
Total	99.0	99.3	99.7	98.9	98.4	99.1	99.7	99.0	99.4	99.1	98.7	100.0

[1] significantly high differential abundance (DA) compared to other beetle groupings

[2] significantly low DA

*significant midrange DA

Table 3.15. Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera in cropland samples by species and colored by feeding habits (pink = carnivores, gold = granivores, blue = omnivores). Numbers in bold are the highest RA in each column. Only bacteria exceeding a threshold ≥ 1.5% RA are shown.

	Total (n = 66)	*P. melanarius* (n = 5)	*Ag. placidum* (n = 6)	*Am. carinata* (n = 7)	*Am. farcta* (n = 5)	*Am. littoralis* (n = 5)	*Am. quenseli* (n = 10)	*H. affinis* (n = 5)	*H. amputatus* (n = 5)	*H. pensylvanicus* (n = 5)	*B. quadrimaculatum* (n = 8)	*C. fossor* (n = 5)
unidentified phylum					[2]					1.7		
Acidobacteriota;o_Vicinamibacterales							2.2					
Vicinamibacteraceae					1.5							
Actinomycetota; *Iamia*					1.6							
Corynebacterium												2.0
Blastococcus												2.1
Cellulomonas							3.6					
f_Intrasporangiaceae							1.8					
Ornithinimicrobium												1.7
f_Microbacteriaceae				2.0			1.9	2.0				
Schumannella						2.1						
f_Micrococcaceae						2.6[1]		1.6				
Allocatelliglobosispora												3.2
Nocardioides				[1]			4.0				1.8	
Streptomyces					[2]						2.8	
o_Gaiellales							4.8					
Gaiella					1.8							
o_Gaiellales						2.2						

	Total (n = 66)	*P. melanarius* (n = 5)	*Ag. placidum* (n = 6)	*Am. carinata* (n = 7)	*Am. farcta* (n = 5)	*Am. littoralis* (n = 5)	*Am. quenseli* (n = 10)	*H. affinis* (n = 5)	*H. amputatus* (n = 5)	*H. pensylvanicus* (n = 5)	*B. quadrimaculatum* (n = 8)	*C. fossor* (n = 5)
uncultured												
67-14				1.6								
Solirubrobacter				1.6								
Bacteroidota; *Bacteroides*											1.5	
Dysgonomonas		2.0[1]	7.7[1]									
Prevotella												5.5
Flavobacterium	1.9										2.9	**19.9**
Chloroflexota; *JG30-KF-CM45*				1.8[1]	2.4	1.6		2.2				
Gitt-GS-136				2.9[1]	1.6[1]							
KD4-96				2.4[1]	1.8[1]	2.9	2.2					
Cyanobacteria;*Tychonema_CCAP_1459-11B*								2.9				
Bacillota; *Bacillus*	2.4					1.9	10.3					6.7
f_Planococcaceae											2.3	
o_Entomoplasmatales												11.5
Spiroplasma	3.7		**30.4**[1]		2.4	5.5[1]				1.8[1]		
Exiguobacterium	1.5						3.7				5.1	2.5
o_Lactobacillales											2.2	2.0
Enterococcus	5.3	27.3[1]	13.6[1]	5.2	1.8	7.0		2.2	2.6		1.7	
Lactobacillus	4.8			9.8	2.2		4.0	**32.5**[1]				4.3
f_Leuconostocaceae												11.9
Weissella	2.4	4.9[1]				4.9			1.5*		8.7	5.4

	Total (n = 66)	*P. melanarius* (n = 5)	*Ag. placidum* (n = 6)	*Am. carinata* (n = 7)	*Am. farcta* (n = 5)	*Am. littoralis* (n = 5)	*Am. quenseli* (n = 10)	*H. affinis* (n = 5)	*H. amputatus* (n = 5)	*H. pensylvanicus* (n = 5)	*B. quadrimaculatum* (n = 8)	*C. fossor* (n = 5)
Lactococcus	4.8			6.1^1	7.5^1	**12.6^1**		6.2	1.8	2.7	8.3	7.3
f_Lachnospiraceae					5.6							
Tyzzerella					1.5							
Candidatus_Soleaferrea					1.8							
Fusobacteriota;*Sebaldella*		2.6	12.1									
Myxococcota;*Cystobacter*							1.8					
c_Polyangia							2.4					
Planctomycetota;*Gemmata*						2.0						
Pirellula					1.5^1							
Pseudomonadota; c_Alphaproteobacteria	**6.6**									**86.9**		
Roseomonas							2.0					
Methylobacterium-Methylorubrum									**16.2**			
Microvirga							1.6	3.9				
Chelativorans						2.5						
f_Rhodobacteraceae						7.7						
Sphingomonas				1.6^1								
f_Comamonadaceae								2.1				
Rickettsiella					**13.8**							
o_Enterobacterales	2.3				2.9		4.7	2.4	8.0^1		2.5	
Pragia			7.6									

	Total (n = 66)	*P. melanarius* (n = 5)	*Ag. placidum* (n = 6)	*Am. carinata* (n = 7)	*Am. farcta* (n = 5)	*Am. littoralis* (n = 5)	*Am. quenseli* (n = 10)	*H. affinis* (n = 5)	*H. amputatus* (n = 5)	*H. pensylvanicus* (n = 5)	*B. quadrimaculatum* (n = 8)	*C. fossor* (n = 5)
f_Enterobacteriaceae	6.2		9.2	**19.6**	^{2}		**14.4**	5.5	8.5			
Escherichia-Shigella	5.8					4.2^{1}	2.7	2.7	2.4		**36.2^{1}**	3.8
Hafnia-Obesumbacterium				7.1	^{2}				1.7			
f_Morganellaceae		1.8										
Proteus							1.8	2.2				
f_Yersiniaceae	4.2	27.7^{1}		4.5	^{2}		3.8		12.3			
Serratia								5.6				
Gilliamella	3.7	**29.1^{1}**	16.6^{1}		^{2}							
Acinetobacter	2.1					2.2	3.0		6.4		7.3	
Pseudomonas					^{2}				2.1			
Stenotrophomonas					^{2}				2.1^{1}		1.7	
Verrucomicrobiota;*Chthoniobacter*							2.3					
Total	57.6	95.4	97.2	66.1	51.8	63.4	78.6	74.1	65.6	93.1	84.9	89.8

[1] significantly high differential abundance (DA) compared to other beetle groupings

[2] significantly low DA

*significant midrange DA

Table 3.16. Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in cropland samples by feeding habits. Numbers in bold are the highest RA in each column. Only bacteria exceeding a threshold ≥ 0.5% RA are shown.

	Total (n = 66)	granivore (n = 42)	omnivore (n = 13)	carnivore (n = 11)
Pseudomonadota	**43.2**	**45.4**[2]	33.1[2]	**47.0**[1]
Bacillota	29.6	24.2[2]	**38.9**[2]	39.6[1]
Actinomycetota	11.4	14.7[1]	10.3[1]	
Bacteroidota	4.3	1.2	13.9[1]	5.1[1]
Chloroflexota	3.7	5.3[1]	1.9	
Planctomycetota	2.0	2.9[1]	0.7	
Fusobacteriota	1.4	[2]		7.8[1]
Acidobacteriota	1.4	2.1		
Myxococcota	1.1	1.6		
Verrucomicrobiota	0.8	1.3		
Total	99.0	98.5	98.8	99.5

[1] significantly high differential abundance (DA) compared to other beetle groupings

[2] significantly low DA

Table 3.17. Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera in cropland samples by feeding habits. Numbers in bold are the highest RA in each column. Only bacteria exceeding a threshold ≥ 1.5% RA are shown.

	Total (n = 66)	granivore (n = 42)	omnivore (n = 13)	carnivore (n = 11)
Actinomycetota;*Nocardioides*		1.5[1]		
Streptomyces			1.7	
Bacteroidota;*Dysgonomonas*				5.1[1]
Prevotella			2.1	
Flavobacterium	1.9		9.4	
Chloroflexota;*JG30-KF-CM45*		1.5		
KD4-96		1.8[1]		
Bacillota;*Bacillus*	2.4	3.0	2.6	
o_Entomoplasmatales			4.4	
Spiroplasma	3.7			16.7
Exiguobacterium	1.5		4.1[1]	
o_Lactobacillales			2.1	
Enterococcus	5.3	2.7		19.8[1]
Lactobacillus	4.8	6.8	2.4	
f_Leuconostocaceae			4.6	
Weissella	2.4		7.4	2.2
Lactococcus	4.8	5.0[1]	7.9	
Fusobacteriota;*Sebaldella*				7.8
Pseudomonadota; c_Alphaproteobacteria	**6.6**	**10.3**		
Methylobacterium-Methylorubrum		2.1		
Rickettsiella		1.8		
o_Enterobacterales	2.3	2.8	1.9	
Pragia				4.2
f_Enterobacteriaceae	6.2	8.4		5.4
Escherichia-Shigella	5.8	1.8	**23.8[1]**	
Hafnia-Obesumbacterium		1.7		
f_Yersiniaceae	4.2	3.2		12.6
Gilliamella	3.7	[2]		**22.3[1]**
Acinetobacter	2.1	1.8	4.7	
Total	57.6	56.1	79.1	96.1

[1] significantly high differential abundance (DA) compared to other beetle groupings
[2] significantly low DA

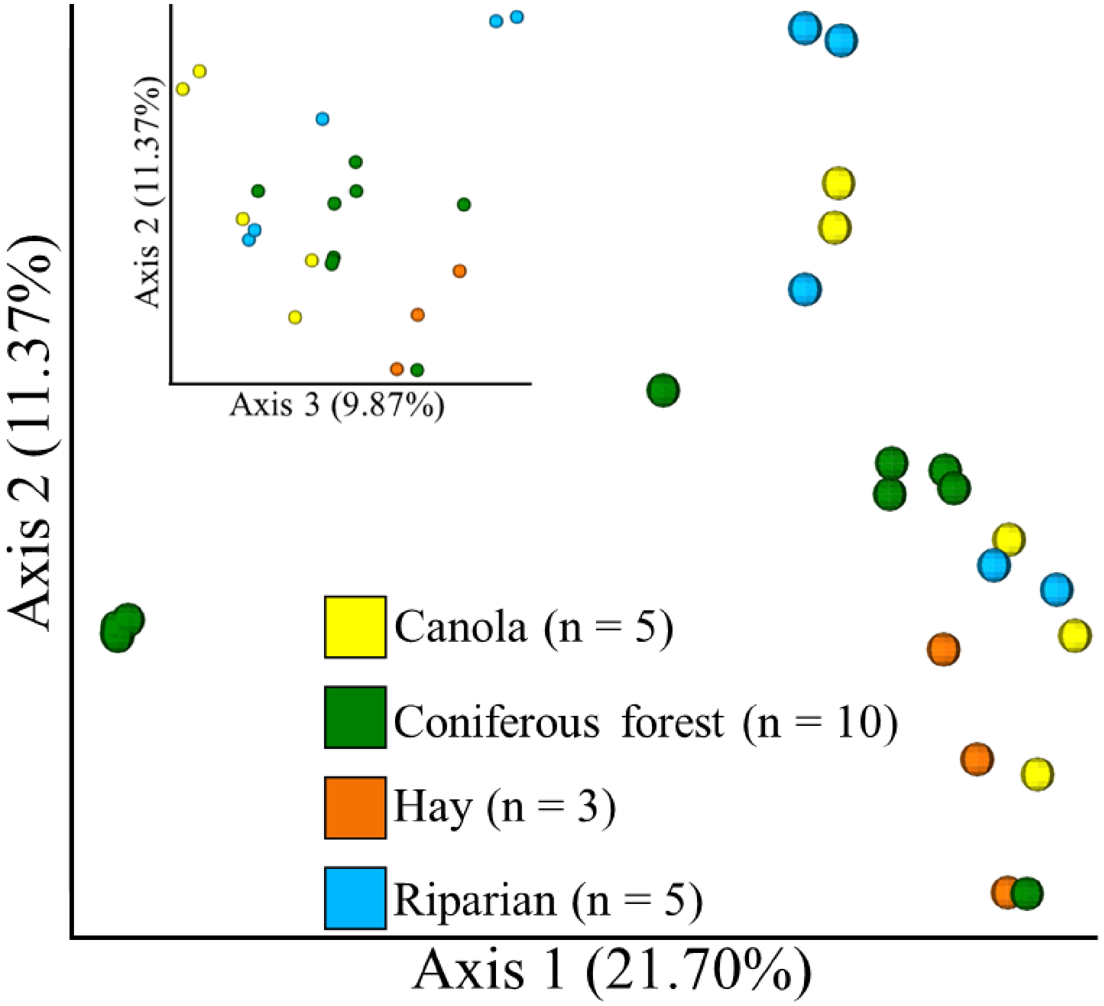


Figure 3.1. Bray-Curtis principal coordinate analysis (PCoA) for *Pterostichus melanarius* samples colored by habitat. Inset shows PCoA with axes 2 and 3. The three axes combine to show 42.94% of the beta diversity and significant separation in GBC among habitats (PERMANOVA test: (pseudo-F = 1.865, p = 0.004)). A second PERMANOVA test removing the four coniferous forest outliers still showed significant separation by habitat type (pseudo-F = 1.460, p = 0.008).

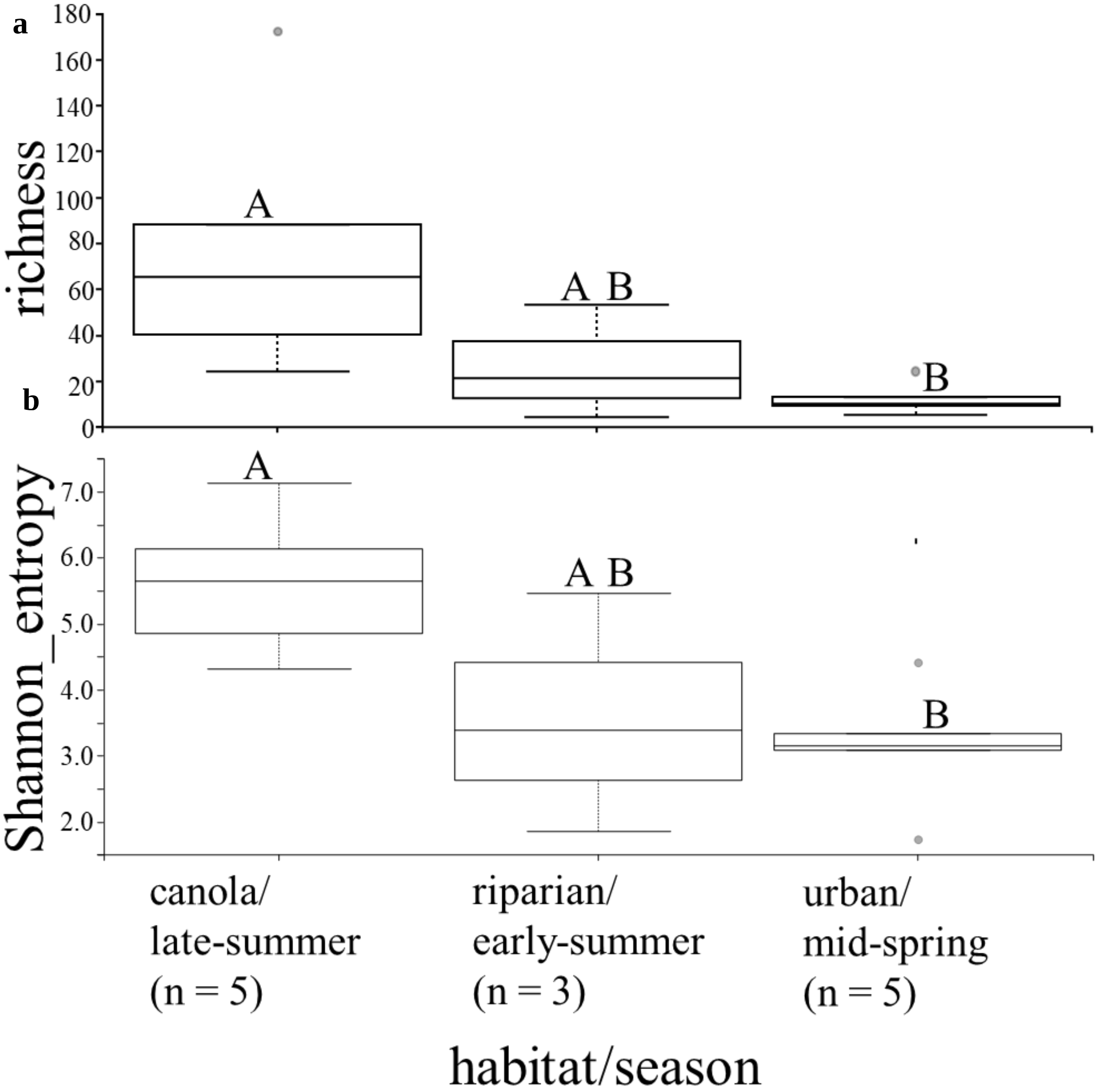


Figure 3.2. a) Richness and b) Shannon diversity box-plots for *Amara littoralis* to compare habitat/season alpha diversity. Dots indicate outliers and error bars indicate highest and lowest values. The bottom and top edges of the boxes indicate quartile 1 and quartile 3 respectively and the middle line in the box indicates the median value. Letters above box plots indicate significance. Groups that share a letter are not significantly different from one another.

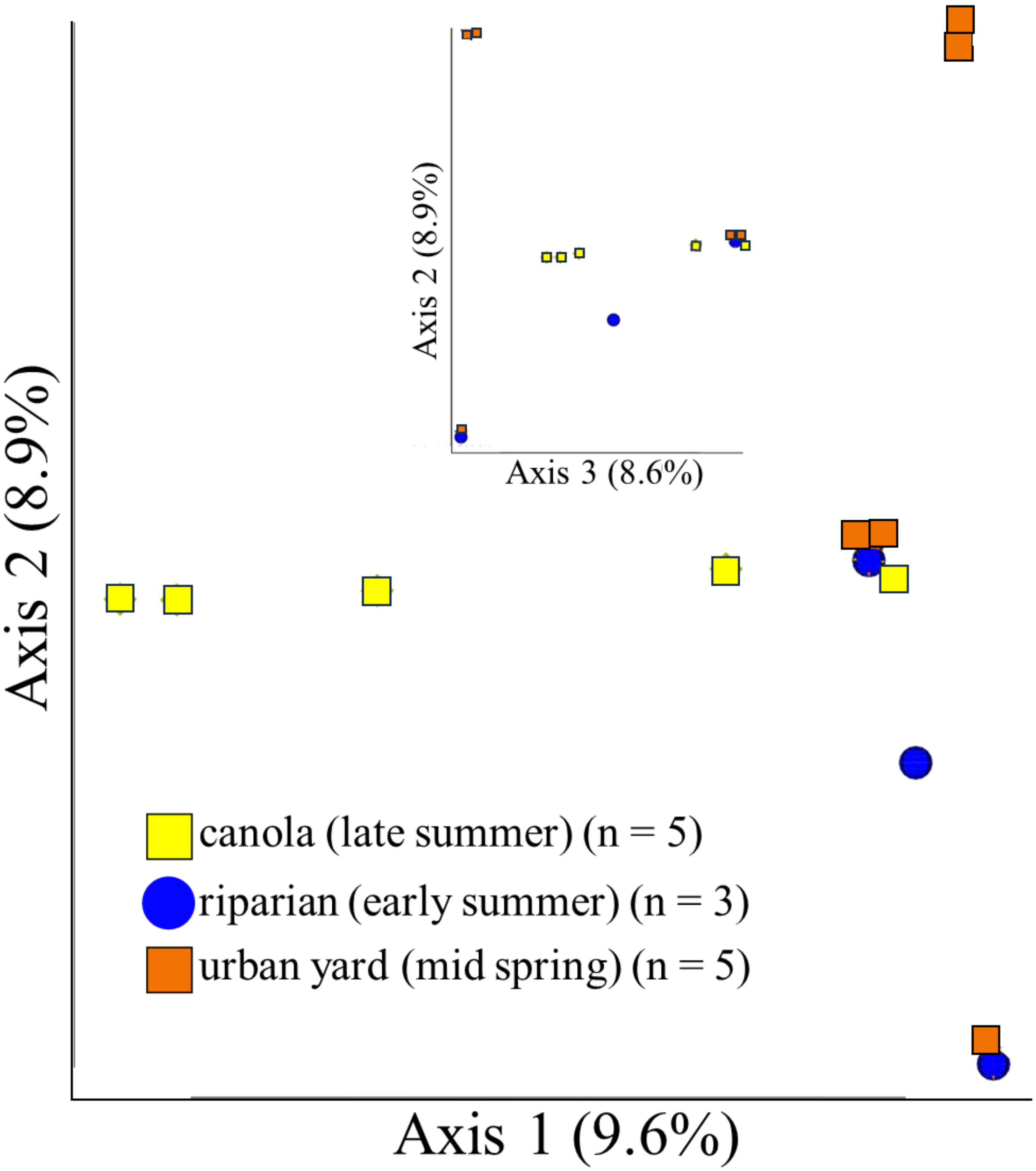


Figure 3.3. Bray-Curtis principal coordinate analysis (PCoA) of *Amara littoralis* samples from different habitats/seasons. Inset shows PCoA with axes 2 and 3. The three axes combine to show 27.1% of the beta diversity and significant separation in GBC among habitats/seasons (PERMANOVA test: pseudo-F = 1.056, p = 0.023).

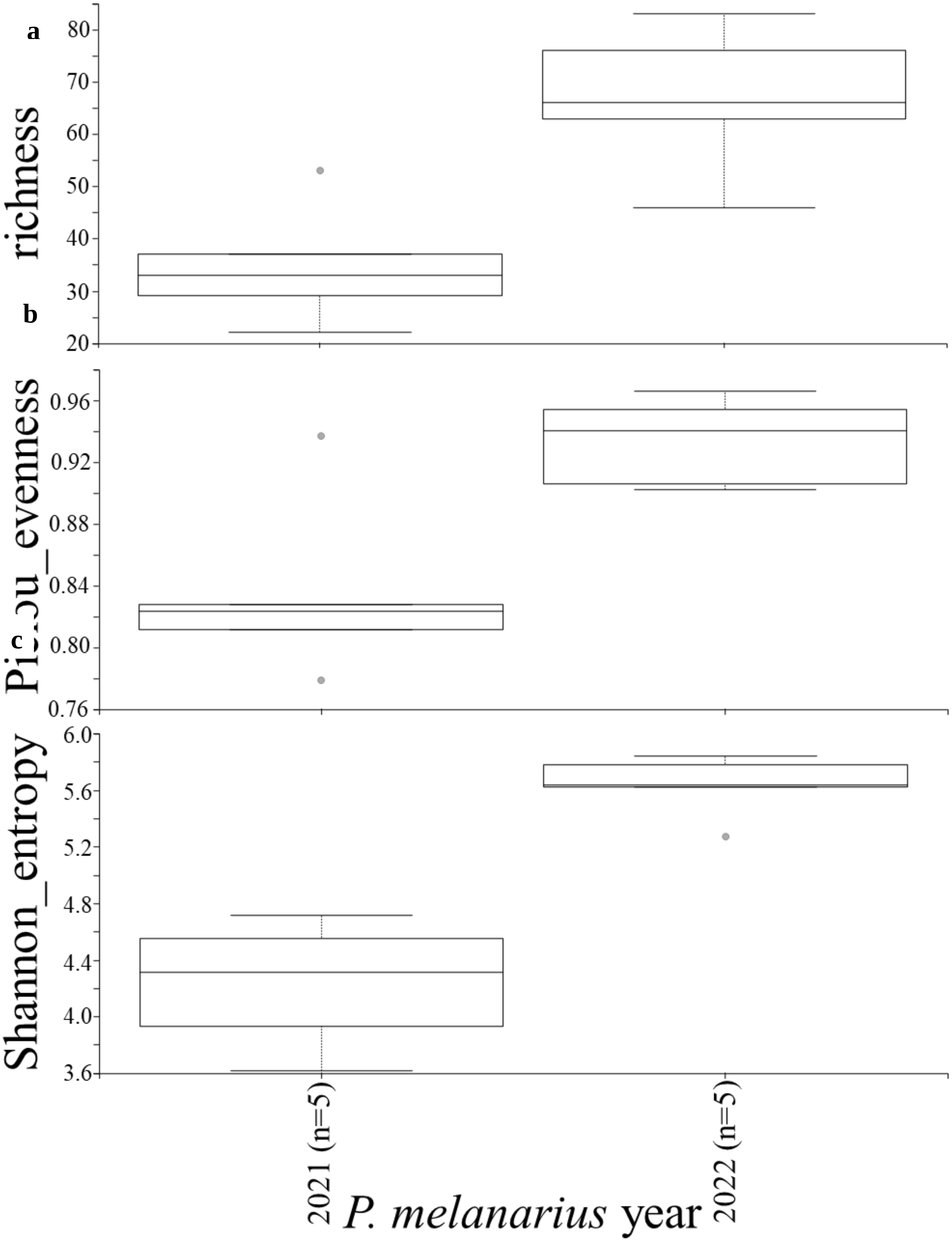


Figure 3.4. Alpha diversity measures of bacterial a) richness, b) evenness, and c) Shannon diversity in *Pterostichus melanarius* samples from different years at the same coniferous forest. Dots indicate outliers and error bars indicate highest and lowest values. The bottom and top edges of the boxes show quartile 1 and quartile 3 respectively and the middle line in the box indicates the median value.

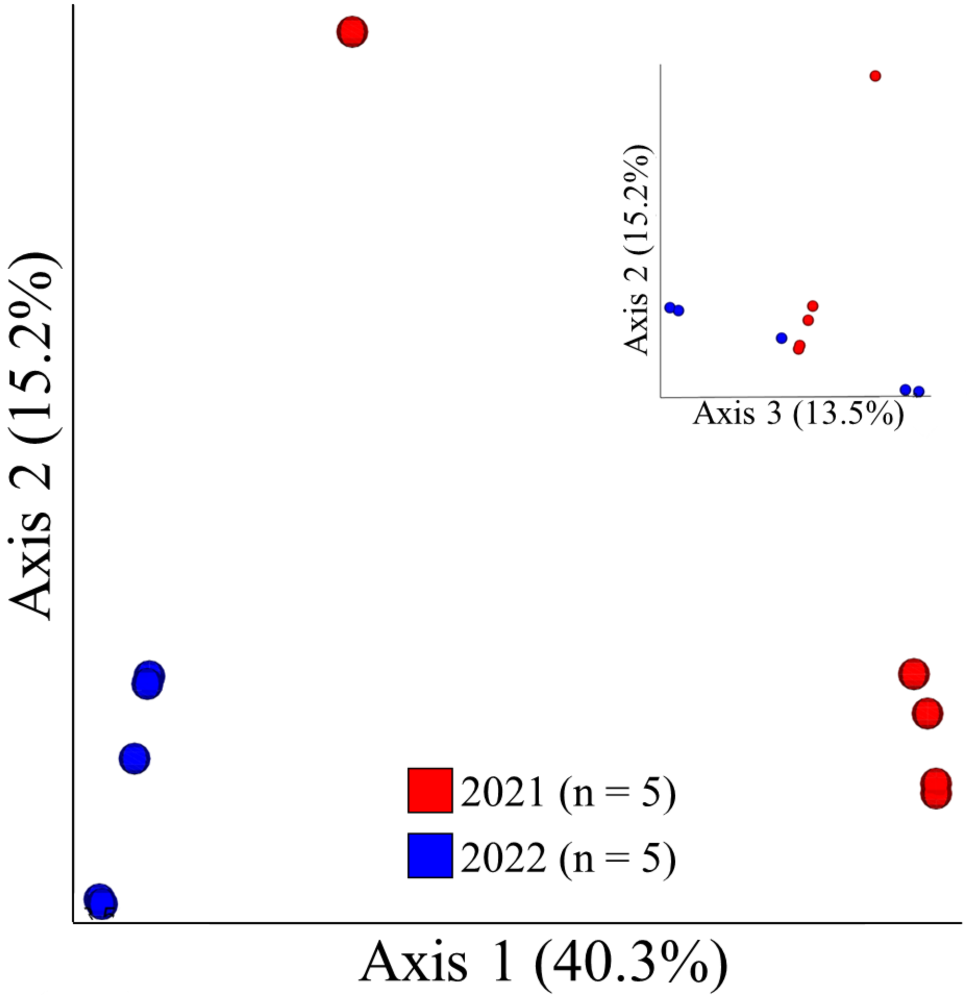


Figure 3.5. Bray-Curtis principal coordinate analysis (PCoA) for samples of *Pterostichus melanarius* from different years in the same coniferous forest. Inset shows PCoA with Axes 2 and 3. The three axes combine to show 69.0% of the beta diversity and significant separation in GBC between years (PERMANOVA test: pseudo-F = 4.417, p = 0.008).

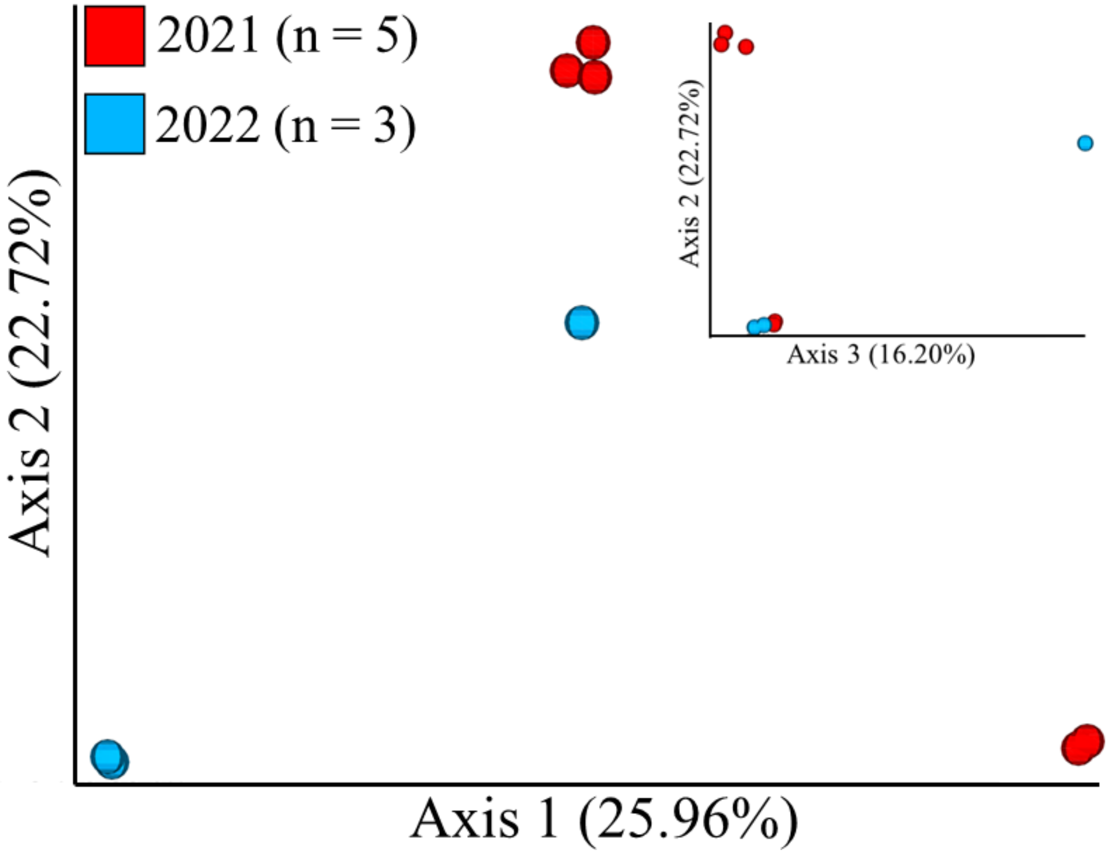


Figure 3.6. Bray-Curtis principal coordinate analysis (PCoA) for samples of *Carabus granulatus* from different years in the same riparian location. Inset shows PCoA with axes 2 and 3. The three axes combine to show 64.88% of the beta diversity and significant separation in GBC among years (PERMANOVA test: pseudo-F = 1.738, p = 0.015).

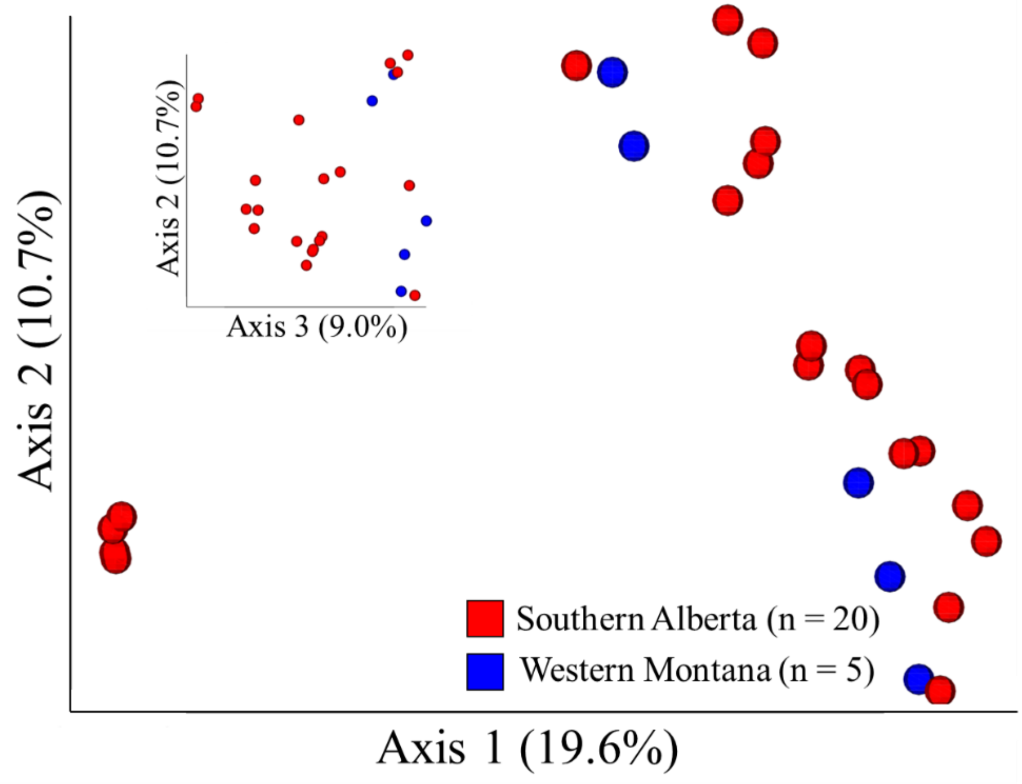


Figure 3.7. Bray-Curtis principal coordinate analysis (PCoA) for samples of *Pterostichus melanarius* from different regions. Inset shows PCoA with axes 2 and 3. The three axes combine to show 39.3% of the beta diversity and no significant separation in GBC between regions (PERMANOVA test: pseudo-F = 1.365, p = 0.099).

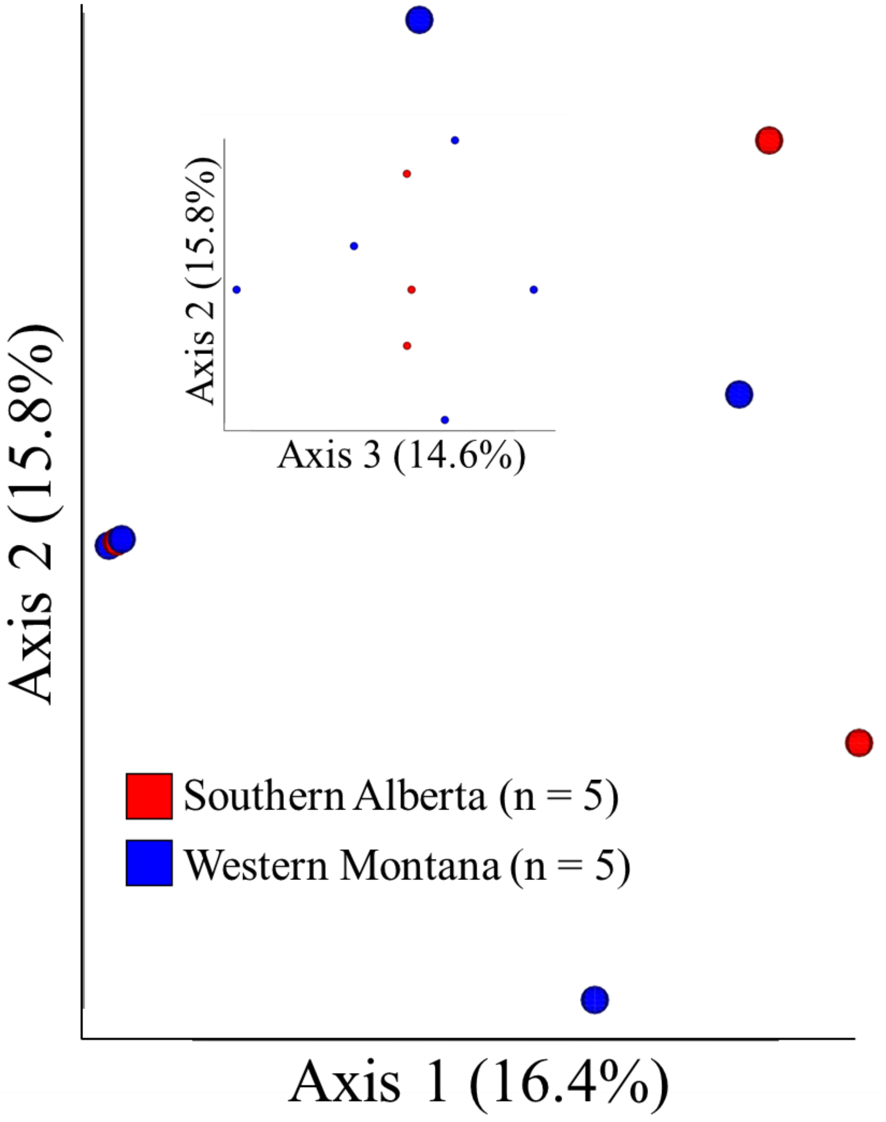


Figure 3.8. Bray-Curtis principal coordinate analysis (PCoA) for samples of *Opisthius richardsoni* from different regions. Inset shows PCoA with axes 2 and 3. The three axes combine to show 46.8% of the beta diversity and no significant separation in GBC between regions (PERMANOVA test: pseudo-F = 0.951, p = 0.825).

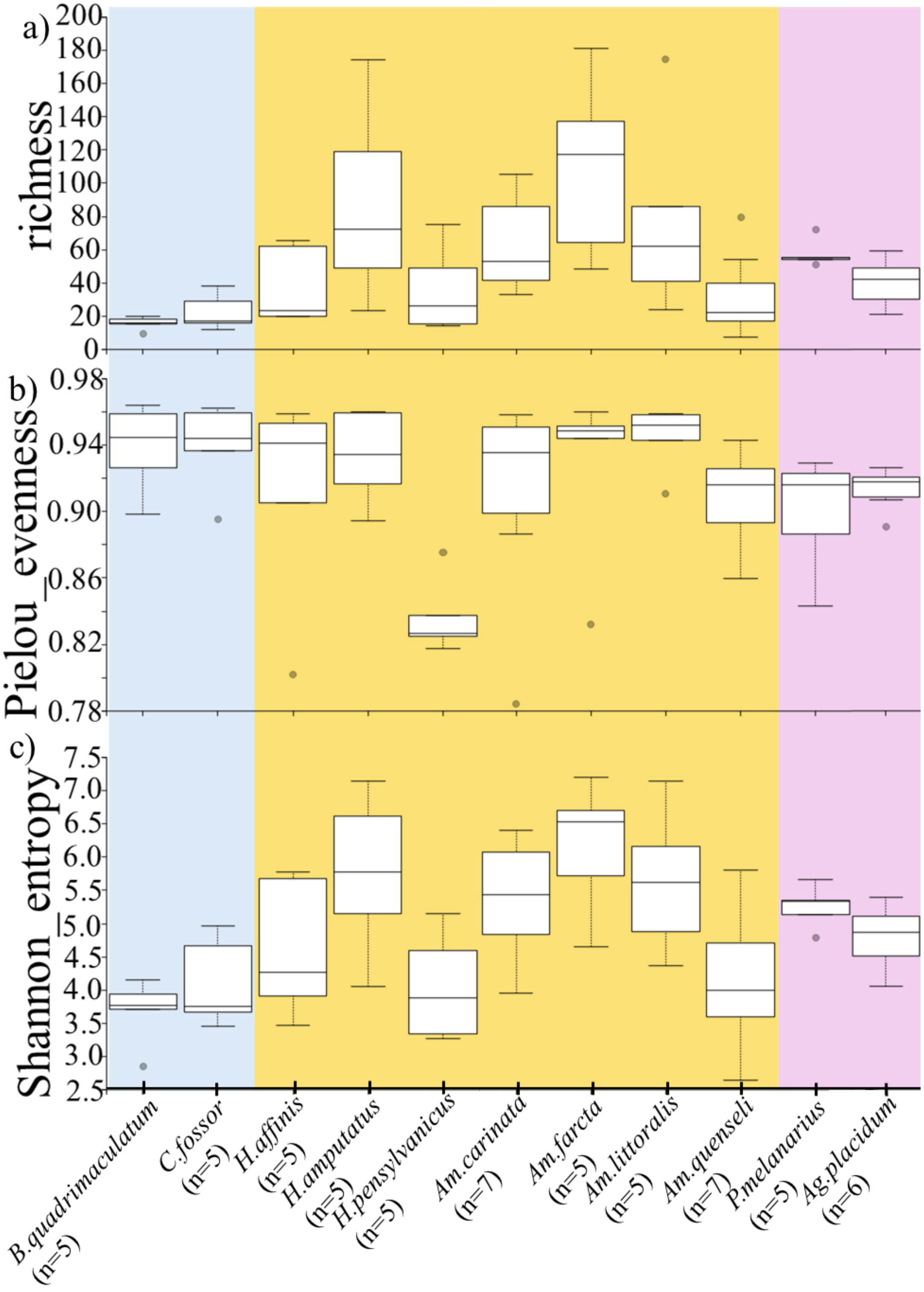


Figure 3.9. Alpha diversity measures of bacterial a) richness, b) evenness, and c) Shannon diversity in AB cropland samples grouped by carabid species and colored by feeding habit (blue = omnivore, gold = granivore, pink = carnivore,). Dots indicate outliers and error bars indicate highest and lowest values. The bottom and top edges of the boxes show quartile 1 and quartile 3 respectively and the middle line in the box indicates the median value.

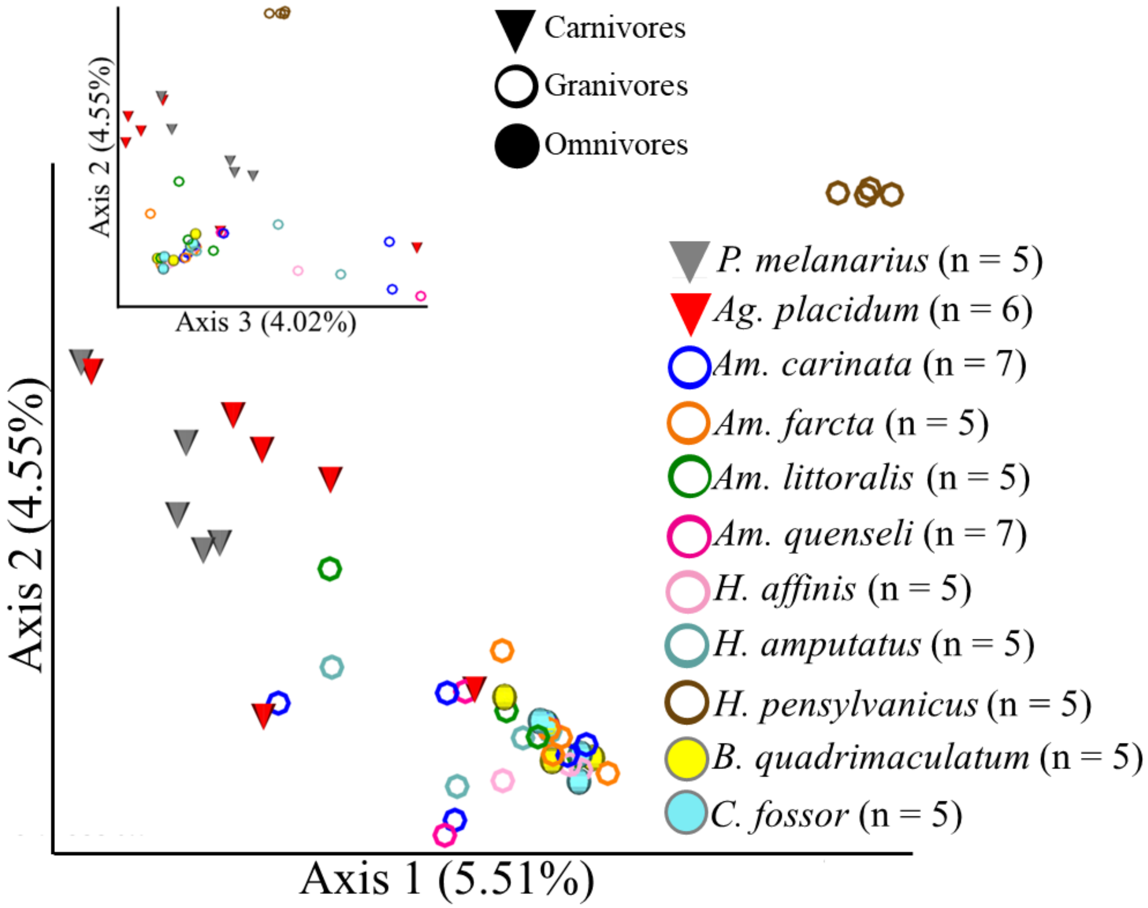


Figure 3.10. Bray-Curtis principal coordinate analysis (PCoA) for Alberta (AB) cropland samples colored by species. Shapes indicate feeding habits (triangles = carnivores, dots = omnivores, rings = granivores). Inset shows PCoA with axes 2 and 3. The three axes combine to show 14.08% of the beta diversity. Results of two different PERMANOVA tests showed significant separation among samples by species (pseudo-F = 1.453, p = 0.001) and among samples by feeding habit (pseudo-F = 1.909, p = 0.001).

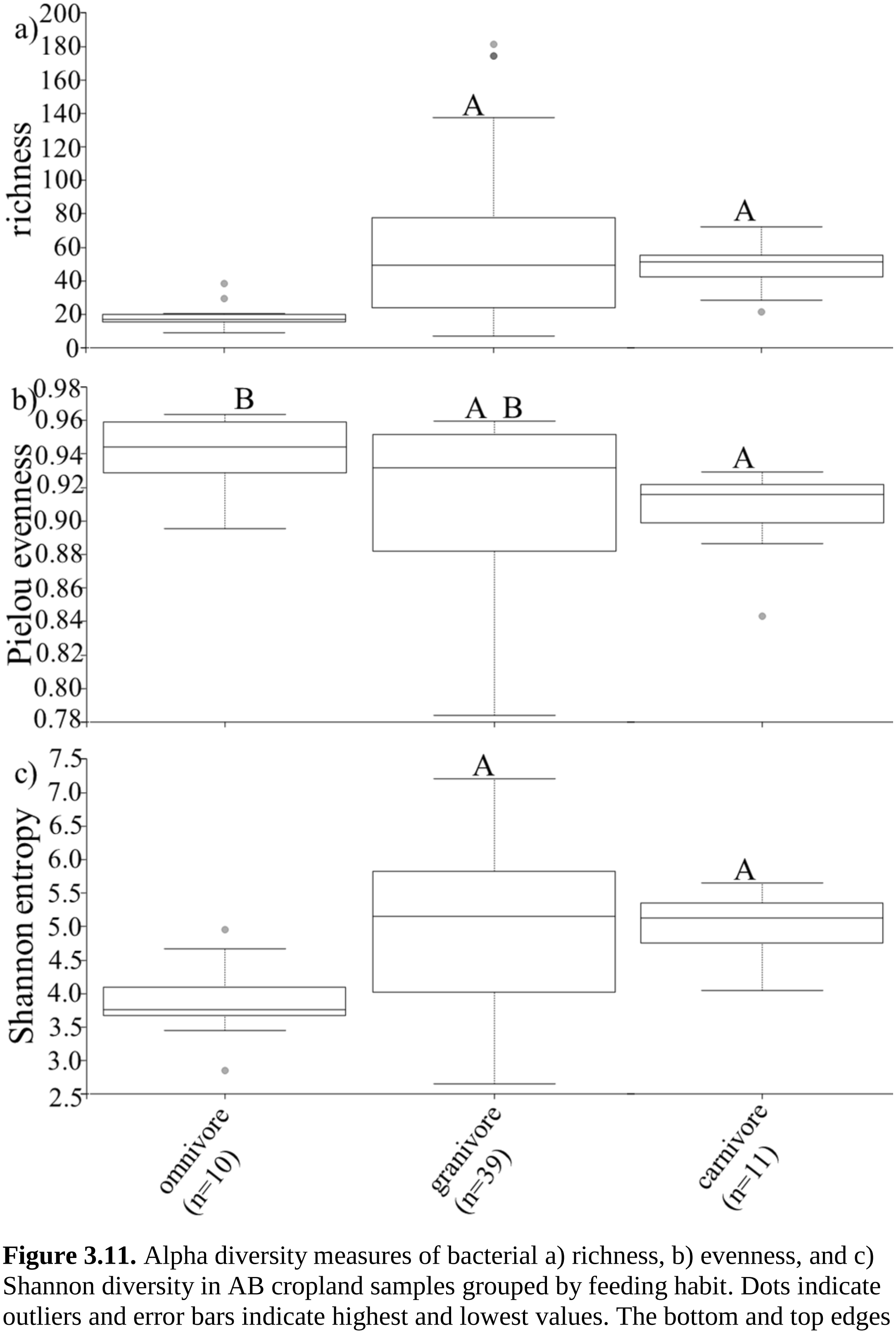


Figure 3.11. Alpha diversity measures of bacterial a) richness, b) evenness, and c) Shannon diversity in AB cropland samples grouped by feeding habit. Dots indicate outliers and error bars indicate highest and lowest values. The bottom and top edges of the boxes show quartile 1 and quartile 3 respectively and the middle line in the box indicates the median value. Letters above box plots indicate significance. Groups that share a letter are not significantly different from one another.

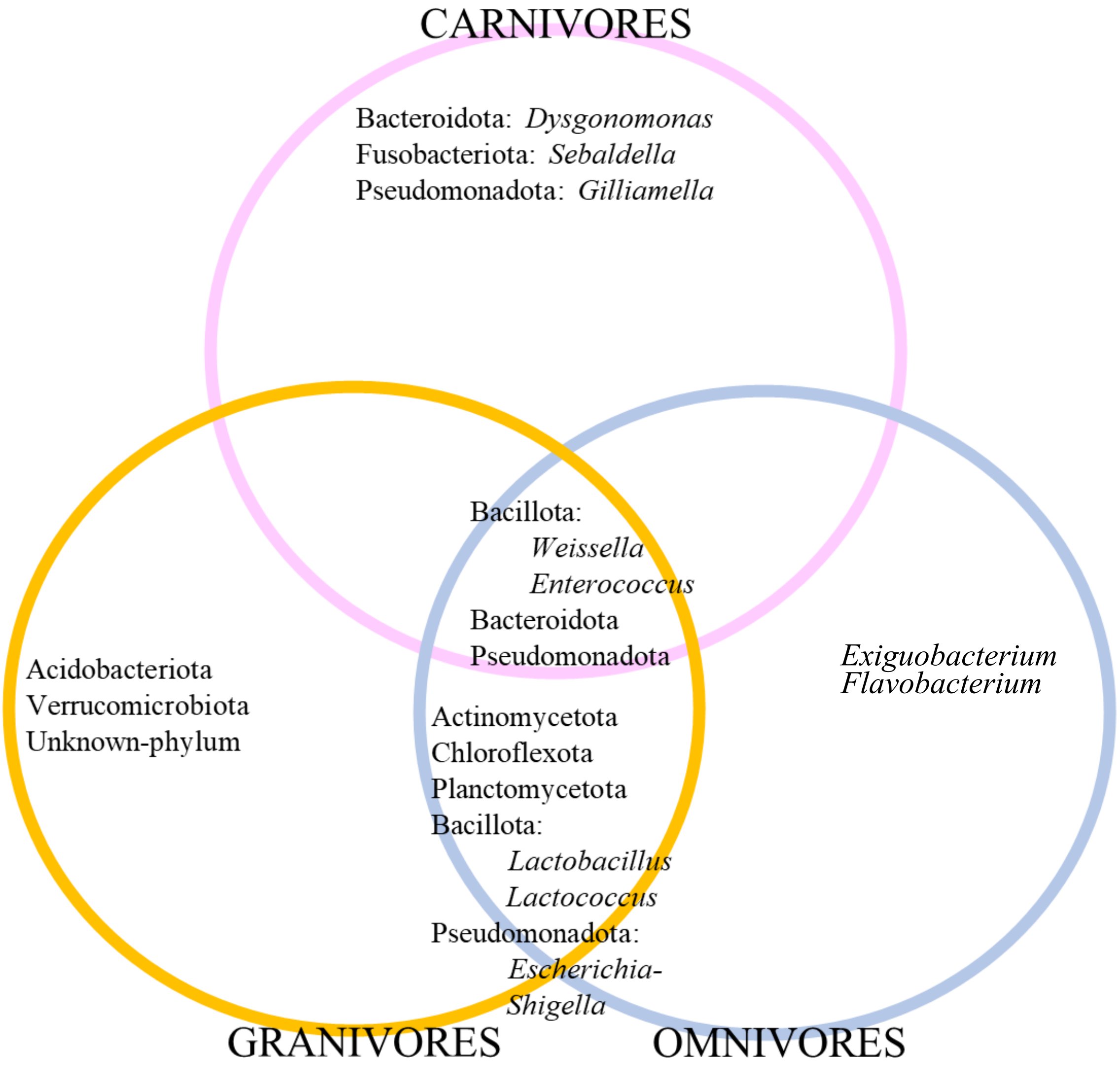


Figure 3.12. Venn diagram showing shared and unique bacterial taxa among feeding habits in AB cropland.

Chapter 4: Conclusions

4.1 General Discussion

I found that most extrinsic factors examined here affect the GBCs and the bacteria present can differ from one carabid taxon to the next. I also found GBC differences among carabid feeding groups (carnivore, omnivore, and granivore), including high diversity within the granivore feeding group.

4.1.1 Host Phylogenetics, Host Taxonomy, and GBC Diversity

In Chapter 2, I detected associations between host taxonomy, and GBC diversity. The GBC of Cicindelinae did not stand out much from that of other carabid subfamilies but the four species of the *Cicindela* genus used here were two sets of sister species (Fig 2.1) (Gough *et al.*, 2019) each with distinct GBC composition. Elaphrinae, Patrobinae, and Trechinae form clades together in phylogenetic analyses from other studies (Ribera *et al.*, 2005), and somewhat in this one (Fig. 2.1). These three carabid subfamilies had similar GBC results for both alpha and beta diversity measures (Tables 2.2 and 2.3; Figs. 2.2 and 2.3b). Within the genus *Pterostichus*, *P. oregona* and *P. adstrictus* (subgenus *Brothriopterus*) share similarities in their GBCs that are distinct from the other three *Pterostichus* species, which are each in different subgenera (Fig. 2.1; Fig. 2.4; Appendix 1.2.6 - Fig. S8). Each species of *Agonum* used in this study had a distinctly different GBC. This pattern of GBC diversity among congeneric carabids shows that even a well studied species may not accurately represent the GBC of the genus. The GBC of Omophroninae (n = 10) stood out from that of other subfamilies because of a low number of bacterial genera above the threshold (≥ 1.5% RA = 8, Table 2.3), this was consistent within both species of Omophroninae (*Omophron americanum*, *O. tesselatum*) who had similar GBCs (Appendix 1.2.5).

The diversity patterns of GBCs within Carabidae genera in this study mean the GBC of a single carabid species may not be an accurate representation of the whole genus. For instance, the current study shows that the granivorous carabid *Harpalus pensylvanicus'* GBC is unique among carabids. Several previous GBC studies have been performed on *H. pensylvanicus* for which the results of may not be applicable to other *Harpalus* or carabid species. Other *Harpalus* species have GBCs that are somewhat similar to one another and other granivorous carabids but without a study of this nature it would be hard to determine that.

There was no evidence of core GBC members common to all carabid taxa, albeit evidence of core GBC members were detected for individual carabid genera and species. A member of Yersiniaceaea was present in all five species of *Pterostichus* examined (35 of 45 specimens), plus all specimens of *Calathus ingratus* and *Pasimachus elongatus* (Fig. 2.4). This bacterium was at relatively high RA in many of those samples to the extent that it is likely not there by chance. The effect of Yersiniaceae in the host's GBC is unknown but, if the bacteria present are of the *Yersinia* or *Serratia* genera of Yersiniaceae, then it is likely that either many carabids are infected by something pathogenic to them or infected by a symbiont that helps protect the host from parasites and parasitoid larvae (Moxley, 2022, Boyd *et al.*, 2021).

Some bacteria found in the GBC may protect the host from parasitism. All samples used to analyze the GBC of *H. fuscipalpis* in this study had parasitoid Dipteran larvae within their coelom. It is not clear if the larvae affected the composition of the

GBC but *Spiroplasma*, which can confer protection from parasites and parasitoid larvae (Jones & Hurst, 2020), was found in two of five samples (Fig. 2.4). Although it was not in the majority of *H. fuscipalpis* samples, *Spiroplasma* was at 17.6% and 83.6% RA (Fig. 2.4). No other samples in this study, with or without *Spiroplasma*, had noticeable parasites or parasitoid larvae within them during dissections.

4.1.2 Feeding Habits

In Chapter 3, I showed that carabids with different feeding habits (carnivory, omnivory, and granivory) had distinct GBCs, although there were overlapping members. The most noticeable differences were between carnivores and granivores. Many carnivores both in the cropland (*Agonum* and *Pterostichus*) and non-cropland habitats (e.g., the genera *Cicindela*, *Carabus*, *Chlaenius*, *Omophron*, and *Elaphrus* (Larochelle, 1990)), had a close association with particular bacteria including *Enterococcus* (Table 3.15; Fig. 2.4). This bacterium was only found in a few granivore specimens (*Amara* and *Harpalus*) and usually at low RA (Table 3.15; Fig. 2.4). *Enterococcus* often co-occurred with high RA of *Gilliamella* bacteria, which was absent in *Amara* and *Harpalus*. Granivores had a consistent association with the bacterial phyla Actinomycetota, Planctomycetota, Chloroflexota, and Verrucomicrobiota (Table 3.14). Due to nutritional requirements, there likely are no pure granivores or carnivores in carabids (Kulkarni *et al.*, 2015, Ali & Willenborg, 2021, Larochelle, 1990, Lovei *et al.*, 1996), which may explain some of the overlap in GBCs among feeding habits, i.e., *Enterococcus* in three of 13 *A. littoralis* samples (1.2-25.4% RA). It seems that a diet that includes a small amount of omnivory would have traces of bacteria that can be strongly linked to eating other diets (e.g., carnivorous or granivory) and that is what is seen here.

4.1.3 Extrinsic Factors

Food items and other exposures may differ between habits to affect the GBCs of a given carabid species. I examined this possibility in Chapter 3 for *P. melanarius* (coniferous forest, canola, riparian zone, timothy hay, cattle pasture, urban yard) and *Amara littoralis* (canola/late-summer, riparian-zone/early-summer, urban-yard/mid-spring). The differences in GBC diversity across habitats (mostly different lactic acid bacteria (LABs)) was clear for *P. melanarius* but the results for *A. littoralis* were confounded by different seasons of collection. For *A. littoralis*, adults collected in spring (April 29 AB, Canada) had overwintered whereas adults collected in late summer had developed from eggs laid in late spring and early summer (Kulkarni *et al.*, 2015). The long period of time without food for overwintered adults may have contributed to some of the observed differences. Without the input of food and excretion of waste, the gut of a host becomes a, more or less, closed system for bacteria which could go through successional changes over the winter.

In Chapter 3, I also found evidence of shifts in GBC diversity between years in *P. melanarius* and *Carabus granulatus*. Due to interannual variance in food items, precipitation, and temperature, some differences can be expected in GBC diversity depending on year (Owen & Owen, 1990). Most major GBC differences for *P. melanarius* samples from one year to the next were limited to RA and significantly different DA of bacterial taxa shared between years, and the differences in bacterial taxa present/absent were mostly limited to *Rickettsia*. *Rickettsia* being found one year but not the other could be caused by *P. melanarius* eating a prey item such as ticks (Arachnida: Ixodida) one year but not the other, although other invertebrates are also known to carry *Rickettsia* bacteria (Perlman & Zchori-Fein, 2006). The year-to-year GBC differences in *C. granulatus* resembled what may be a fluctuating GBC configuration that more closely resembles granivory during some periods and carnivory for other periodss.

While regional differences in the GBC between samples of *P. melanarius* and *O. richardsoni* collected in either Montana (MT) or Alberta (AB) barely showed any significant differences, there were some differences. A surprising discovery in *P. melanarius* was the presence of *Wolbachia* in all five of the MT samples and none of the 20 AB samples, showing uneven infection rates for *P. melanarius* depending upon where it is found.

4.1.4 Relation to Previous Studies

My thesis expands and complements previous studies describing the GBCs of carabid beetles. My research shows patterns of GBC diversity in some carnivores (e.g., *Agonum*, *Pterostichus*, *Omophron*) that match the patterns of GBC diversity seen by Kudo *et al.* (2019) for carnivores that had associations with fungal GMB members that may regulate the GBC. Compared to Kudo *et al.* (2019), I presented the results of the overall bacterial diversity in the guts of carabid samples to a higher taxonomic resolution of bacteria (i.e., genera) and provided tables of their RA and DA. Conversely, Kudo *et al.* (2019) examined the possible interactions between bacteria and fungi in the guts of multiple carabid species, albeit mainly using fewer beetles per species than used here. My research also adds to the report of the GBC in four species of riparian *Bembidion* by Kolasa *et al.* (2019), who hinted at *Orbus* as possible core gut bacteria for this carabid genus. I detected *Orbus* in a riparian zone *Bembidion*, but not in a cropland *Bembidion*, showing that the bacterium *Orbus* may be associated with the habitat where the samples were collected.

4.2 Future Directions

My study can serve as an evidence-supported starting point and a model for methodology for future GMB studies examining individual carabid groups, extrinsic factors, or microbial taxa of interest. It shows the importance of reporting as many conditions of sample collection and preservation as is reasonably possible (i.e., ethanol concentration, time of year, habitat).

The large scale and scope of this study led to some limitations imposed by time and budget. Trying to include as much diversity of carabid taxa as possible meant that a few species, genera, or subfamilies, were represented by small sample sizes. For example, in this study, the subfamily Harpalinae is represented by ten genera and many species while some other carabid subfamilies are represented by only a single species or genus. A single carabid genus or species can give a glimpse of what GBCs in the subfamily or genus may all look like but more diversity would be needed to confidently say that a carabid subfamily or genus’s GBC has certain characteristics across it. The GBCs of Harpalinae have been studied more than any other carabid subfamily, by far. Some of the other carabid subfamilies used in this study could be studied more thoroughly. If more genera within them were included, someone could see if patterns found here continued through samples of other host taxa (e.g., Patrobinae: *Patrobus* to see if that generus also has associations with *Spiroplasma* as I found with Patrobinae: *Diplous*).

Levels of intraspecific GBC variation mean that relatively large sample sizes are needed to determine if patterns are consistent. For specimens collected at the same site and roughly the same time, a sample size of ≥ 5 may be sufficient (Fig. 2.4). To fully document the GBC of a carabid species found in multiple habitats, it may take upwards of 50 specimens to cover the major extrinsic factors and life stages involved in GBC diversity depending on the host carabid being studied. For instance, this study used 25 *Pterostichus melanarius* specimens to show how GBCs vary across two years, two regions, and four habitats. *Pterostichus melanarius* is also found in other habitats such as cattle pastures and urban settings, has adult and larval stages, and has two sexes. It would not be difficult to add another 25 samples to examine all aspects associated with this species' GBC. Previous studies have used as few as one specimen to characterize the GBC of a species (Supplementary Table S3 in Kudo *et al*., 2019), but I found that even use of three or four specimens does not sample the variation found within some species such as *Apristus constrictus* (n = 3) and *Badister neopulchelus* (n = 3) (Fig. 2.4).

If a "healthy GBC" can be determined for a carabid group by finding which bacteria are crucial, beneficial, or pathogenic, then it can help see which conditions affect host health and fitness from a microbiome perspective. If a core bacterium was found to be crucial to the health of a carabid found in cropland, then a study could see if that gut bacterium is affected by practices such as the application of fertilizers or pesticides.

Some carabid species seem to be especially adept to colonizing new land and expanding their distributions (e.g., the European species *P. melanarius*) (Holliday *et al.*, 2014). A topic of future research could be to determine if features of the GMB contribute to the adaptability of these species to multiple environments. During this study I recovered the riparian carabid, *Agonum extensicolle*, which I believe to be a new provincial record for Alberta. It is not reported from Alberta in Bousquet *et al.* (2013) and there are no other reports of the species in iNaturalist excluding my own (https://www.inaturalist.org/observations/191560161). A study of this species and others could look for similar patterns in GBCs.

Future studies could expand on my research to examine how feeding habits and GBCs are related. This study investigated the GBCs of seven granivores (n = 42) from the same site and same time, but was not able to characterize the GBC of carnivores as well due to only having two carnivorous species (n = 11). The results of my thesis and previous studies show that feeding habits of a carabid may determine where it is most likely to be found (Thiele, 1977, Kudo *et al.*, 2019). The cropland/open-land habitat contains many granivorous carabids but other habitats such as riparian zones or forests have more carnivores than granivores (Thiele, 1977, Kudo *et al.*, 2019). A future study examining all carabid species from a forest or riparian zone could help characterize the GBCs of carnivorous carabids more thoroughly, while simultaneously seeing if there are riparian zone or forest associated bacteria found in all carabids regardless of feeding habits. Other habitats that could be characterized more thoroughly include various forests, prairies, pastures, and urban yards.

A study on different life stages of carabids, in the field or a lab, could determine if there are core GMB members associated with age. All carabids are holometabolous, there may be substantial differences between the lifestyles of larvae and adults. *Amara littoralis* females may have higher abundances of the endosymbiont *Spiroplasma* in the spring during egg formation and transovarial infection of the eggs. This study only captured males of that species during the mid-spring season.

Experiments could identify the role of microbes of particular interest on host biology. Antimicrobials fed to carabids might see how their GMB responds to the removal of certain members (e.g., fungi). Cropland carabids could be tested for patterns of insecticide degrading bacteria (de Almeida *et al.*, 2017). More could be done to assess the role of endosymbionts in the biology of GMBs (Wilches *et al.*, 2021). We do not know which of them are only in the lining of the gut as opposed to the lumen of the gut. We also don’t know if the endosymbionts in the lumen or epithelial cells of the gut can affect the GMB by regulating it somehow. A high abundance of endosymbionts in the gut lining may also affect how the genetic material is extracted and therefore affect only the analysis of the GBC and not the gut lumen GBC. During the extraction process lysing chemicals will breakdown cells that lack a cell wall the easiest. If the only bacterial cells without a cell wall are from animals or *Wolbachia* bacteria then their genetic material will be most likely to be lysed and subsequently extracted.

Regardless of the questions being asked, the experimental design is also important. Because the gut lumen of a carabid host is not a closed system and is influenced by feeding and defecation, it is difficult to tell which bacteria are transient and which are resident. A starvation period of ~24 hours might allow some of the transient bacteria to pass through the host and create less sparsity in the data to allow a core GBC to be determined. Some of the bacterial taxa examined in this study could only be recognized to a certain taxonomic level due to limitations of the bacterial gene that was amplified (V4 region of 16S rDNA) or the database used for identification. Future studies could try different databases or amplifying different gene sequences for identification. Examples of other genetic sequences that could be amplified include whole genome, different V region, or "housekeeping" genes (La Rosa et al., 2011). Other gene sequences may not identify as many bacterial taxa as the V4 region, but they can achieve better taxonomic resolution of certain bacteria or clarify their role in the GBC. Examples of bacteria that could be more closely examined are f_Yersiniaceae in various species, especially the *Pterostichus* genus, and c_Alphaproteobacteria in *Harpalus pensylvanicus*.

4.3 References

Ali KA & Willenborg CJ (2021) The biology of seed discrimination and its role in shaping the foraging ecology of carabids: A review. *Ecology and Evolution,* **11,** 13702-13722.

Baulechner D, Jauker F, Neubauer TA & Wolters V (2020) Convergent evolution of specialized generalists: Implications for phylogenetic and functional diversity of carabid feeding groups. *Ecology and Evolution,* **10,** 11100-11110.

Bousquet Y, Bouchard P, Davies AE & Sikes DS (2013) Checklist of beetles (Coleoptera) of Canada and Alaska. Second edition. *ZooKeys,* **360,** 1-44.

Boyd BM, Chevignon G, Patel V, Oliver KM & Strand MR (2021) Evolutionary genomics of APSE: a tailed phage that lysogenically converts the bacterium *Hamiltonella defensa* into a heritable protective symbiont of aphids. *Virology Journal,* **18,** 219.

De Almeida LG, Beraldo De Moraes LA, Trigo JR, Omoto C & Consoli FL (2017) The gut microbiota of insecticide-resistant insects houses insecticide-degrading bacteria: A potential source for biotechnological exploitation. *PloS One,* **12,** e0174754.

Gough HM, Duran DP, Kawahara AY & Toussaint EFA (2019) A comprehensive molecular phylogeny of tiger beetles (Coleoptera, Carabidae, Cicindelinae). *Systematic Entomology,* **44,** 305-321.

Holliday NJ, Floate KD, Cárcamo H, Pollock DA, Stjernberg A & Roughley RE (2014) Ground beetles (Coleoptera: Carabidae) of the prairie grasslands of Canada. *Arthropods of Canadian Grasslands,* **4,** 1-85.

Jones JE & Hurst GDD (2020) Symbiont-mediated fly survival is independent of defensive symbiont genotype in the *Drosophila melanogaster–Spiroplasma*–wasp interaction. *Journal of Evolutionary Biology,* **33,** 1625-1633.

Kolasa M, Scibior R, Mazur MA, Kubisz D, Dudek K & Kajtoch Ł (2019) How hosts taxonomy, trophy, and endosymbionts shape microbiome diversity in beetles. *Microbial Ecology,* **78,** 995-1013.

Kudo R, Masuya H, Endoh R, Kikuchi T & Ikeda H (2019) Gut bacterial and fungal communities in ground-dwelling beetles are associated with host food habit and habitat. *The ISME Journal,* **13,** 676-685.

Kulkarni SS, Dosdall LM, Spence JR & Willenborg CJ (2015) Depth of seed burial and gender influence weed seed predation by three species of ground beetle (Coleoptera: Carabidae). *Weed Science,* **63,** 910-915.

Larochelle A (1990) *Food of Carabid Beetles (The):(Coleoptera: Carabidae, Including Cicindelinae),* Quebec, QC, Canada., Association des Entomologistes du Québec.

La Rosa M, Rizzo R & Urso A (2011) Soft topographic maps for clustering and classifying bacteria using housekeeping genes. *Advances in Artificial Neural Systems*, **2011,** ID 617427, 1-8.

Lovei GL & Sunderland KD (1996) Ecology and behavior of ground beetles (Coleoptera: Carabidae). *Annual Review of Entomology,* **41,** 231-256.

Moxley RA (2022) Family Yersiniaceae. *Veterinary Microbiology*, **4,** 88-99.

Owen DF & Owen J (1990) Assessing insect species-richness at a single site. *Environmental Conservation,* **17,** 362-364.

Perlman SJ & Zchori-Fein E (2006) Emerging diversity of *Rickettsia*. *Proceedings of the Royal Society. B, Biological Sciences,* **273,** 2097-2106.

Ribera I, Mateu J & Bellés X (2005) Phylogenetic relationships of *Dalyat mirabilis* Mateu, 2002, with a revised molecular phylogeny of ground beetles (Coleoptera, Carabidae). *Journal of Zoological Systematics and Evolutionary Research,* **43,** 284-296.

Thiele HU (1977) *Carabid Beetles in Their Environments: A Study on Habitat Selection By Adaptations in Physiology and Behaviour,* New York; Berlin, Springer-Verlag.

Wilches DM, Coghlin PC & Floate KD (2021) Next generation sequencing, insect microbiomes, and the confounding effect of *Wolbachia*: a case study using spotted-wing Drosophila (*Drosophila suzukii*) (Diptera: Drosophilidae). *Canadian Journal of Zoology,* **99,** 588-595.

Appendix 1 Supplemental Data for Chapter 2

Appendix 1.1 Rarefaction of All Chapter 2 samples

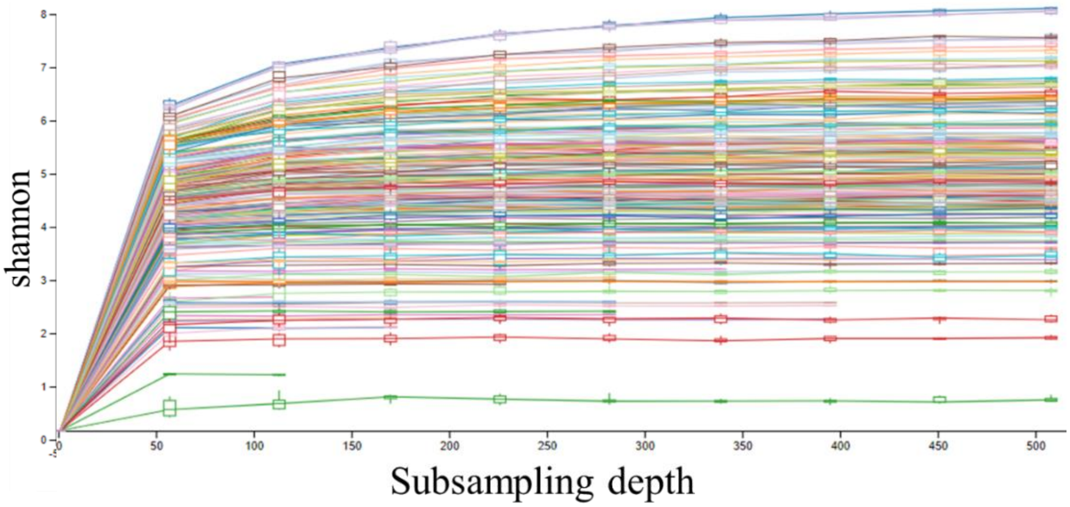


Figure S1: Rarefaction chart for analysis of nine carabid subfamilies. Samples are subsampled to 508 sequences each.

Appendix 1.2 GBC Analyses For Carabid Species in Genera

This section contains analyses performed for Chapter 2

1.2.1 *Bembidion*

Beta diversity

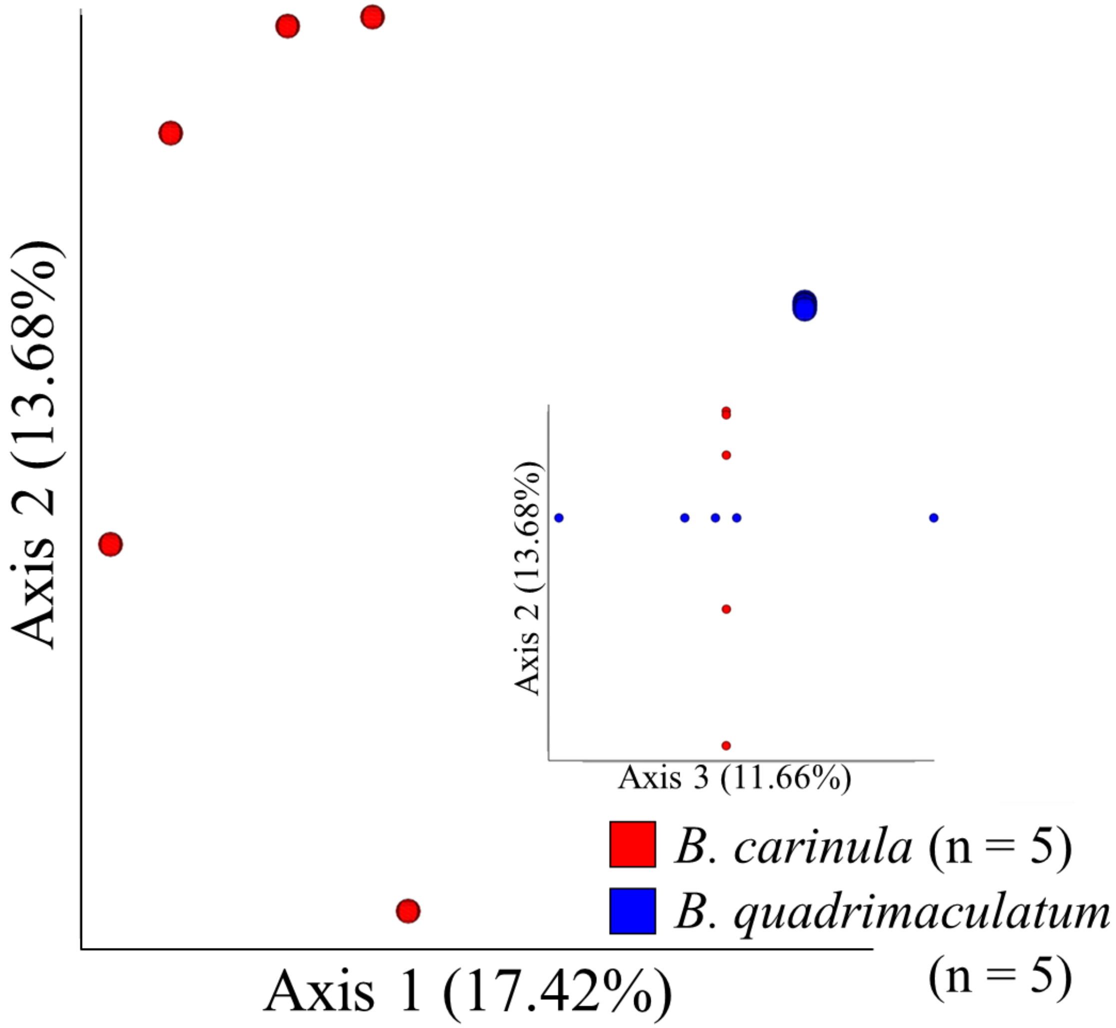


Figure S2: Bray-Curtis principal coordinate analysis (PCoA) for species of *Bembidion.* Inset shows PCoA with axes 2 and 3. The three axes combine to show 42.76% of the beta diversity. Results of the permutational analysis of variance (PERMANOVA) show significant separation (pseudo-F = 1.594, p = 0.01) among samples by species.

Table S1: Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla found in the gut bacterial community (GBC) of two species of *Bembidion*. Numbers in bold are the highest RA in each column.

	Total (n = 10)	*B. carinula* (n = 5)	*B. quadrimaculatum* (n = 5)
Pseudomonadota	**56.1**	**88.5**[1]	23.7[2]
Bacillota	28.0	7.7[2]	**48.3**[1]
Actinomycetota	9.0	2.6	15.4[1]
Bacteroidota	3.2	0.7	5.7
Chloroflexota	1.9	[1]	3.7
Deinococcota	1.1	[2]	2.2
Myxococcota	0.5	[1]	1.0
Total RA	99.8	99.5	100.0

[1] significantly high differential abundance (DA) compared to other beetle groupings

[2] significantly low DA

Table S2: Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera found in the gut bacterial community (GBC) of two species of *Bembidion*. Numbers in bold are the highest RA in each column.

	Total (n = 10)	*B. carinula* (n = 5)	*B. quadrimaculatum* (n = 5)
Pseudomonadota;*Rickettsia*	**13.7**	**27.4**[1]	
Pseudomonadota;f_Orbaceae	10.3	20.7	
Pseudomonadota;o_Enterobacterales	9.8	15.6	4.1
Pseudomonadota;*Serratia*	8.3	16.7	
Bacillota;*Lactococcus*	6.5		**13.1**
Bacillota;*Weissella*	6.2		12.4
Pseudomonadota;*Acinetobacter*	5.8		11.6
Bacillota;o_Lactobacillales	4.4	5.3	3.6
Bacillota;*Exiguobacterium*	4.0		8.1[1]
Actinomycetota;*Nocardioides*	2.8	2.6	2.9
Pseudomonadota;*Escherichia-Shigella*	2.4	2.0	2.8
Actinomycetota;*Streptomyces*	2.2		4.5
Bacillota;f_Planococcaceae	1.9		3.8
Bacillota;*Enterococcus*			2.9
Bacillota;*Lactobacillus*			2.8
Pseudomonadota;*Stenotrophomonas*			2.7
Bacteroidota;*Bacteroides*			2.6
Actinomycetota;*Leucobacter*			2.2
Actinomycetota;f_Micrococcaceae			2.2
Deinococcota;*Deinococcus*			2.2
Pseudomonadota;*Arenimonas*		2.0	
Chloroflexota;*KD4-96*			1.9
Chloroflexota;*JG30-KF-CM45*			1.8
Bacillota;*Spiroplasma*			1.7
Pseudomonadota;*Paracoccus*			1.7
Total	78.5	92.2	91.5

[1] significantly high differential abundance (DA) compared to other beetle groupings

1.2.2 *Carabus*

Beta diversity

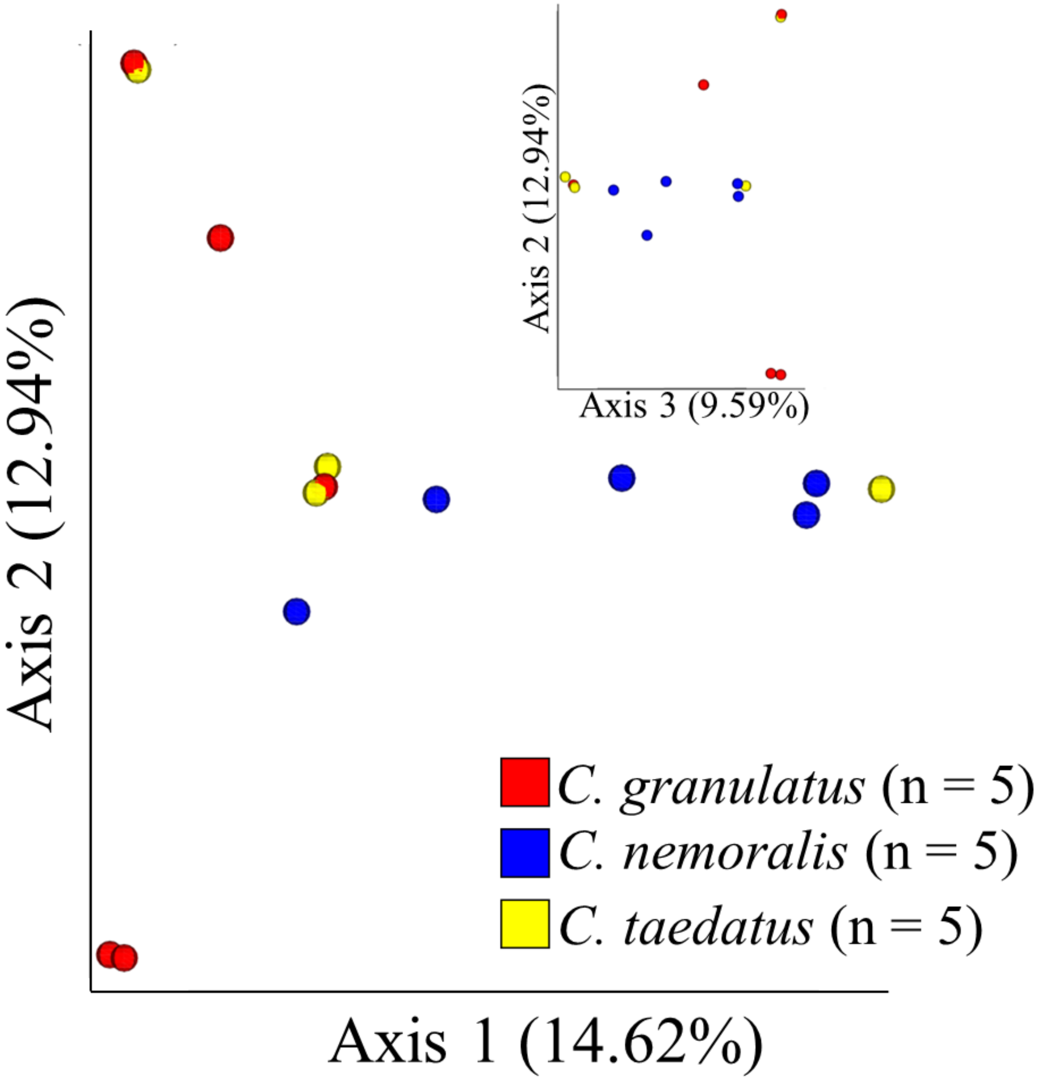


Figure S3: Bray-Curtis principal coordinate analysis (PCoA) for species of *Carabus*. Inset shows PCoA with axes 2 and 3. The three axes combine to show 37.15% of the beta diversity. Results of the permutational analysis of variance (PERMANOVA) did not show significant separation (pseudo-F = 1.173, p = 0.124) among samples by species.

Table S3: Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla found in the gut bacterial community (GBC) of three species of *Carabus*. Numbers in bold are the highest RA in each column.

	Total (n = 15)	*C. granulatus* (n = 5)	*C. nemoralis* (*n* = 5)	*C. taedatus* (n = 5)
Bacillota	**56.9**	**51.9***	**63.8**[1]	**54.9**
Pseudomonadota	29.1	22.2*	23.6[1]	41.6[2]
Actinomycetota	6.2	10.7*	5.5[1]	2.4
Bacteroidota	3.1	4.6	4.4[1]	
Fusobacteriota	1.6	4.2	0.7	
Planctomycetota	0.8	1.7		
unknown phylum	0.8	1.6	0.7	
Chloroflexota	0.6	1.6		
Verrucomicrobiota	0.5	0.7	0.7	
Total RA	99.6	99.2	99.4	98.9

[1] significantly high differential abundance (DA) compared to other beetle groupings

[2] significantly low DA

*significant midrange DA

Table S4: Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera in the gut bacterial community (GBC) of three species of *Carabus*. Numbers in bold are the highest RA in each column.

	Total (n = 15)	*C. granulatus* (n = 5)	*C. nemoralis* (n = 5)	*C. taedatus* (n = 5)
Bacillota;*Enterococcus*	**19.7**	2.0	**19.5**[1]	**37.7**
Bacillota;o_Lactobacillales	9.8	7.2	3.7[1]	18.4
Bacillota;f_Lachnospiraceae	9.3	**27.4**		
Pseudomonadota;*Sodalis*	6.7			20.0
Pseudomonadota;f_Wohlfahrtiimonadaceae	4.9		14.7	
Pseudomonadota;*Hafnia-Obesumbacterium*	4.8		14.3	
Bacillota;*Lactococcus*	4.4	12.4		
Pseudomonadota;f_Enterobacteriaceae	3.3		9.4	
Bacillota;*Lactobacillus*	3.3		8.4	
Pseudomonadota;*Acinetobacter*	2.4	7.2		
Bacillota;*Weissella*	2.0	2.7	2.3	
Pseudomonadota;o_Enterobacterales	1.5	4.1[2]		
Pseudomonadota;*Gilliamella*	1.5		2.4[1]	2.1
Pseudomonadota;f_Yersiniaceae			4.0	
Bacillota;*Exiguobacterium*		3.5		
Bacillota;*Spiroplasma*		1.7[2]	[1]	
Bacteroidota;*Dysgonomonas*				2.1
Bacillota;c_Bacilli		1.8		
Pseudomonadota;*Stenotrophomonas*		2.7		
Bacteroidota;*Bacteroides*		2.6		
Pseudomonadota;f_Rhizobiaceae;_		2.4		
Pseudomonadota;*Escherichia-Shigella*		2.1		
Deinococcota;*Deinococcus*		2.2		
Bacillota;f_Ruminococcaceae		2.0		
Pseudomonadota;*Paracoccus*		1.7		
Pseudomonadota;*Rickettsia*			1.6	
Actinomycetota;f_Micrococcaceae		1.6		
Total	73.5	87.2	80.2	80.4

[1] significantly high differential abundance (DA) compared to other beetle groupings

[2] significantly low DA

1.2.3 ***Harpalus***

Alpha diversity

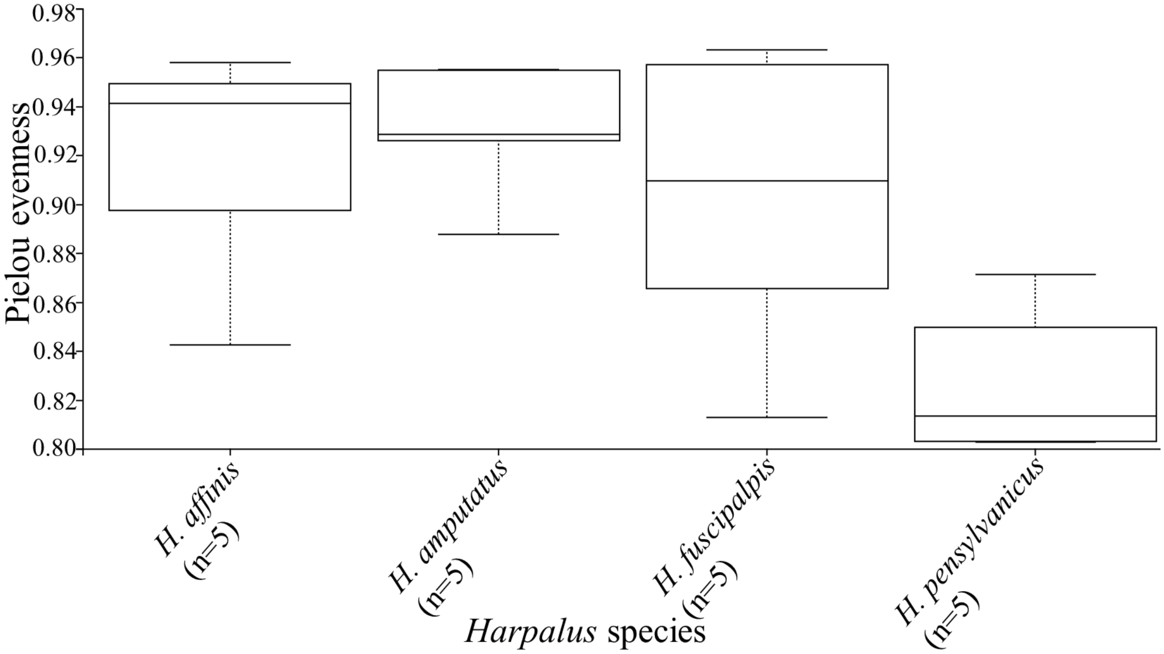


Figure S4: Alpha diversity measure of bacterial species evenness in four species of the *Harpalus* genus. Borderline significant differences (p = 0.052) in alpha diversity in *Harpalus* species groupings were found in a Kruskal-Wallis test. Dots indicate outliers and error bars indicate highest and lowest values. The bottom and top edges of the boxes indicate quartile 1 and quartile 3 respectively and the middle line in the box indicates the median value.

Beta diversity

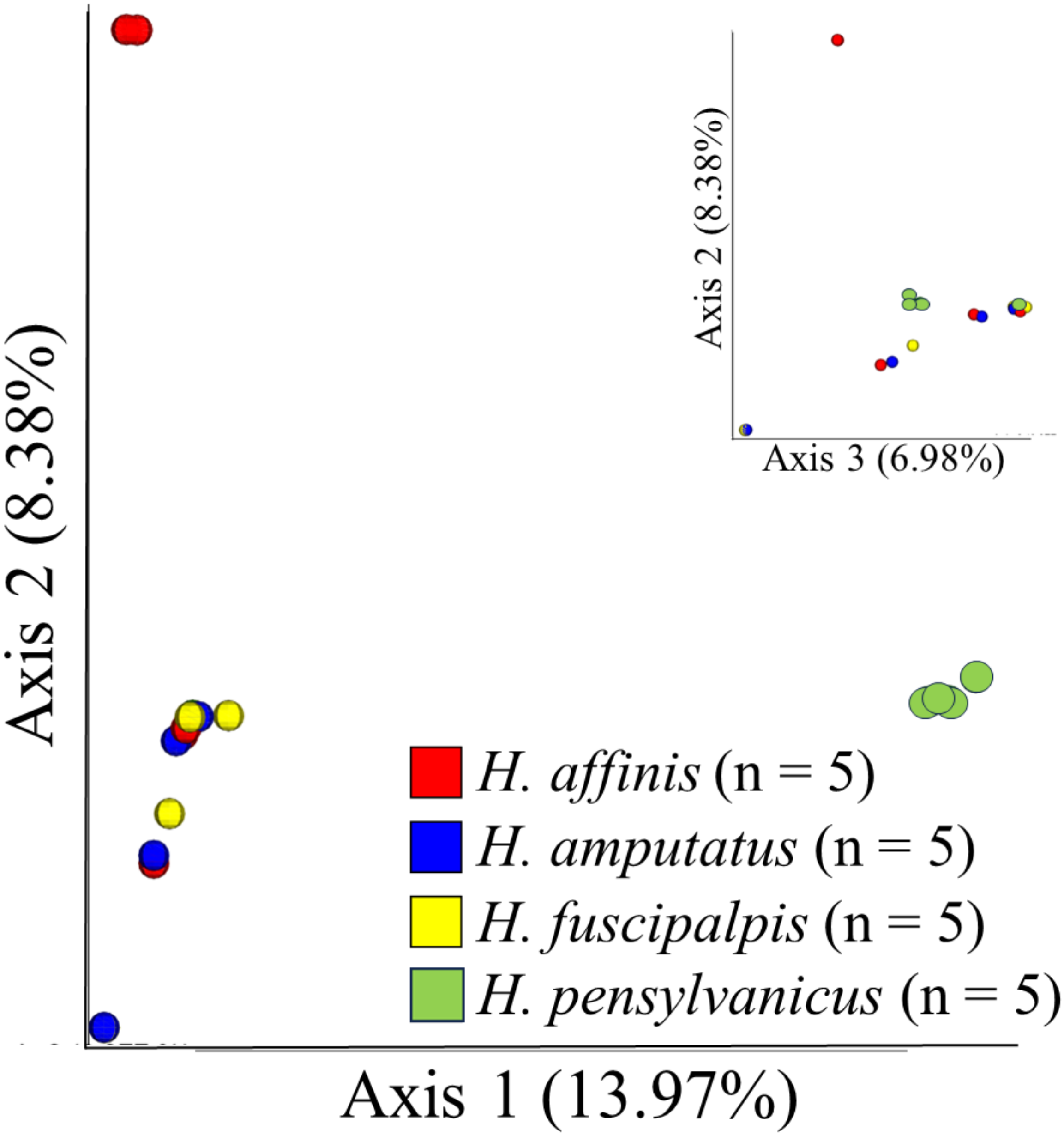


Figure S5: Bray-Curtis principal coordinate analysis (PCoA) for species of *Harpalus*. Inset shows PCoA with axes 2 and 3. The three axes combine to show 29.33% of the beta diversity. Results of the permutational analysis of variance (PERMANOVA) show significant separation (pseudo-F = 1.647, p = 0.001) among samples by species.

Table S5: Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in the gut bacterial community (GBC) of four species of *Harpalus*. Numbers in bold are the highest RA in each column.

	Total (n = 20)	*H. affinis* (n = 5)	*H. amputatus* (n = 5)	*H. fuscipalpis* (n = 5)	*H. pensylvanicus* (n = 5)
Pseudomonadota	**58.2**	35.0[2]	**67.8[1]**	**40.9[1]**	**89.3[1]**
Bacillota	20.6	**42.4[1]**	10.0[2]	24.8[1]	5.4[2]
Actinomycetota	10.7	6.3[1]	12.6[1]	22.3[1]	1.8
Planctomycetota	3.2	1.2	2.0	9.4[1]	
Chloroflexota	2.3	4.9	3.2[1]	0.9	
Bacteroidota	1.2	1.5	1.8[1]	0.5	0.9
Cyanobacteria	0.8	3.2			
Acidobacteriota	0.7	1.2	1.5		
Unidentified phyla	0.6	0.7			1.7
Verrucomicrobiota	0.6	1.7	0.5		
Fusobacteriota		1.0			
Gemmatinonadota		0.7			
Myxococcota				0.7	
Total RA	98.9	99.8	99.4	99.5	99.1

[1] significantly high differential abundance (DA) compared to other beetle groupings

[2] significantly low DA

Table S6: Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera in the gut bacterial community (GBC) of four species of *Harpalus*. Numbers in bold are the highest RA in each column.

	Total (n = 20)	*H. affinis* (n = 5)	*H. amputatus* (n = 5)	*H. fuscipalpis* (n = 5)	*H. pensylvanicus* (n = 5)
Pseudomonadota;c_Alphaproteobacteria	**21.9**				**87.4**[1]
Bacillota;*Lactobacillus*	8.0	**31.9**			
Pseudomonadota;o_Enterobacterales	7.2	2.5	9.4	16.9	
Bacillota;*Spiroplasma*	5.8			**20.3**	1.9[1]
Pseudomonadota;*Methylobacterium-Methylorubrum*	5.6		**16.2**	6.2	
Pseudomonadota;f_Enterobacteriaceae	3.3	4.6	7.0	1.6	
Pseudomonadota;f_Yersiniaceae	2.7		10.1		
Bacillota;*Lactococcus*	2.5	6.5	1.5		2.1
Pseudomonadota;*Escherichia-Shigella*	2.4	3.2	2.5	4.1	
Pseudomonadota;*Acinetobacter*	1.6		6.6		
Bacillota;*Enterococcus*	1.6	3.1	3.0		
Pseudomonadota;*Serratia*		5.6			
Actinomycetota;*Pseudonocardia*				4.8	
Chloroflexota;*JG30-KF-CM45*		2.3			
Pseudomonadota;*Microvirga*		3.8			
Pseudomonadota;*Sphingomonas*				2.4	
Actinomycetota;f_Microbacteriaceae		2.0			
Cyanobacteria;*Tychonema_CCAP 1459-11B*		3.2			
Pseudomonadota;*Stenotrophomonas*			2.1		
Planctomycetota;f_Isosphaeraceae				3.0	
Planctomycetota;f_Gemmataceae				3.0	
Pseudomonadota;f_Comamonadaceae		2.0			
Pseudomonadota;*Pseudomonas*			2.5		
Actinomycetota;*Actinomycetospora*				2.5	
Actinomycetota;f_Intrasporangiaceae				1.7	
Pseudomonadota;*Proteus*		2.2			
unknown phylum					1.7
Bacillota;*Clostridia_vadinBB60_group*				2.4	
Actinomycetota;f_Micrococcaceae		1.6			
Actinomycetota;*Kineosporia*				2.2	
Pseudomonadota;*Hafnia-Obesumbacterium*			1.8		
Planctomycetota;*Tundrisphaera*				2.0	
Bacillota;*Weissella*			1.9[1]		

Bacillota;*[Eubacterium]_coprostanoligenes_group*				1.9	
Total	62.6	74.5	64.6	75.1	93.1

[1] significantly high differential abundance (DA) compared to other beetle groupings

1.2.4 *Nebria*

Beta diversity

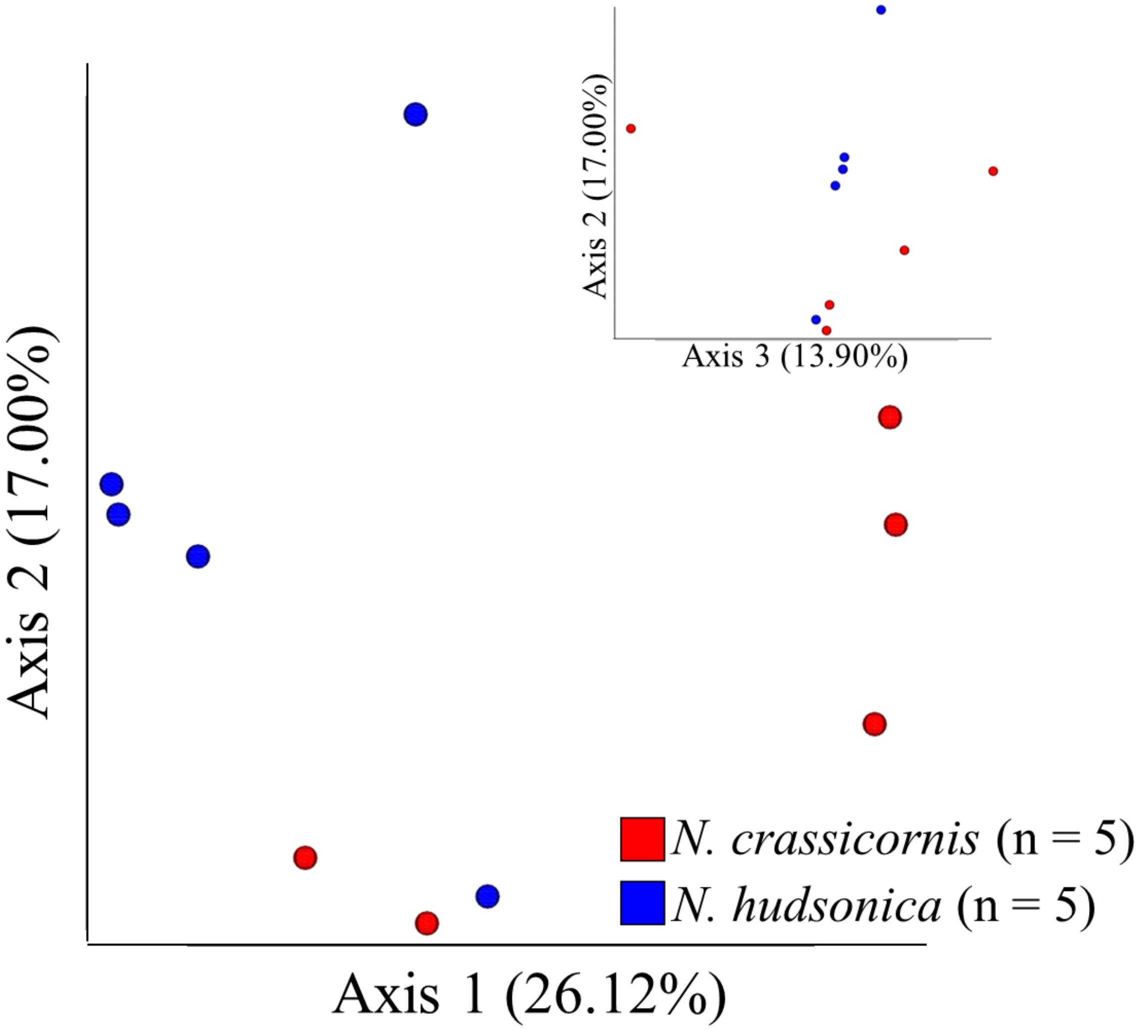


Figure S6: Bray-Curtis principal coordinate analysis (PCoA) for species of *Nebria*. Inset shows PCoA with axes 2 and 3. The three axes combine to show 57.02% of the beta diversity. Results of the permutational analysis of variance (PERMANOVA) show significant separation (pseudo-F = 1.820, p = 0.042) among samples by species.

Table S7: Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in the gut bacterial community (GBC) of two species of *Nebria*. Numbers in bold are the highest RA in each column.

	Total (n = 10)	*N. crassicornis* (n = 5)	*N. hudsonica* (n = 5)
Pseudomonadota	**63.1**	**60.3**[2]	**66.0**[1]
Bacillota	20.6	27.5[2]	13.7[1]
Actinomycetota	6.2	7.4[1]	5.0
Bacteroidota	5.4	1.7	9.1[1]
Planctomycetota	3.3	1.3[2]	5.3[1]
Verrucomicrobiota	0.5	0.5	0.5
Cyanobacteria		0.5	
Total	99.1	99.1	99.6

[1] significantly high differential abundance (DA) compared to other beetle groupings

[2] significantly low DA

Table S8: Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera in the gut bacterial community (GBC) of two species of *Nebria*. Numbers in bold are the highest RA in each column.

	Total (n = 10)	*N. crassicornis* (n = 5)	*N. hudsonica* (n = 5)
Pseudomonadota;*Gilliamella*	**21.0**	**18.1**	23.9[1]
Pseudomonadota;*Pseudomonas*	20.1	8.9[2]	**31.2**
Bacillota;*Enterococcus*	9.2	17.8[1]	
Pseudomonadota;f_Budviciaceae	8.2	16.0	
Bacteroidota;*Dysgonomonas*	4.6		9.1
Bacillota;*Spiroplasma*	3.4	2.6[2]	4.2[1]
Bacillota;o_Lactobacillales	2.8	1.6	4.0[1]
Bacillota;*Carnobacterium*	2.5	5.0	
Pseudomonadota;f_Rhodobacteraceae	2.1	2.7	
Pseudomonadota;*Budvicia*	1.6	3.2	
Bacillota;f_Erysipelotrichaceae	1.5		3.1[1]
Pseudomonadota;*Klebsiella*			2.3
Actinomycetota;*Corynebacterium*			1.9
Pseudomonadota;o_Enterobacterales		1.6	
Total	77.0	77.6	79.8

[1] significantly high differential abundance (DA) compared to other beetle groupings

[2] significantly low DA

1.2.5 *Omophron*

Beta diversity

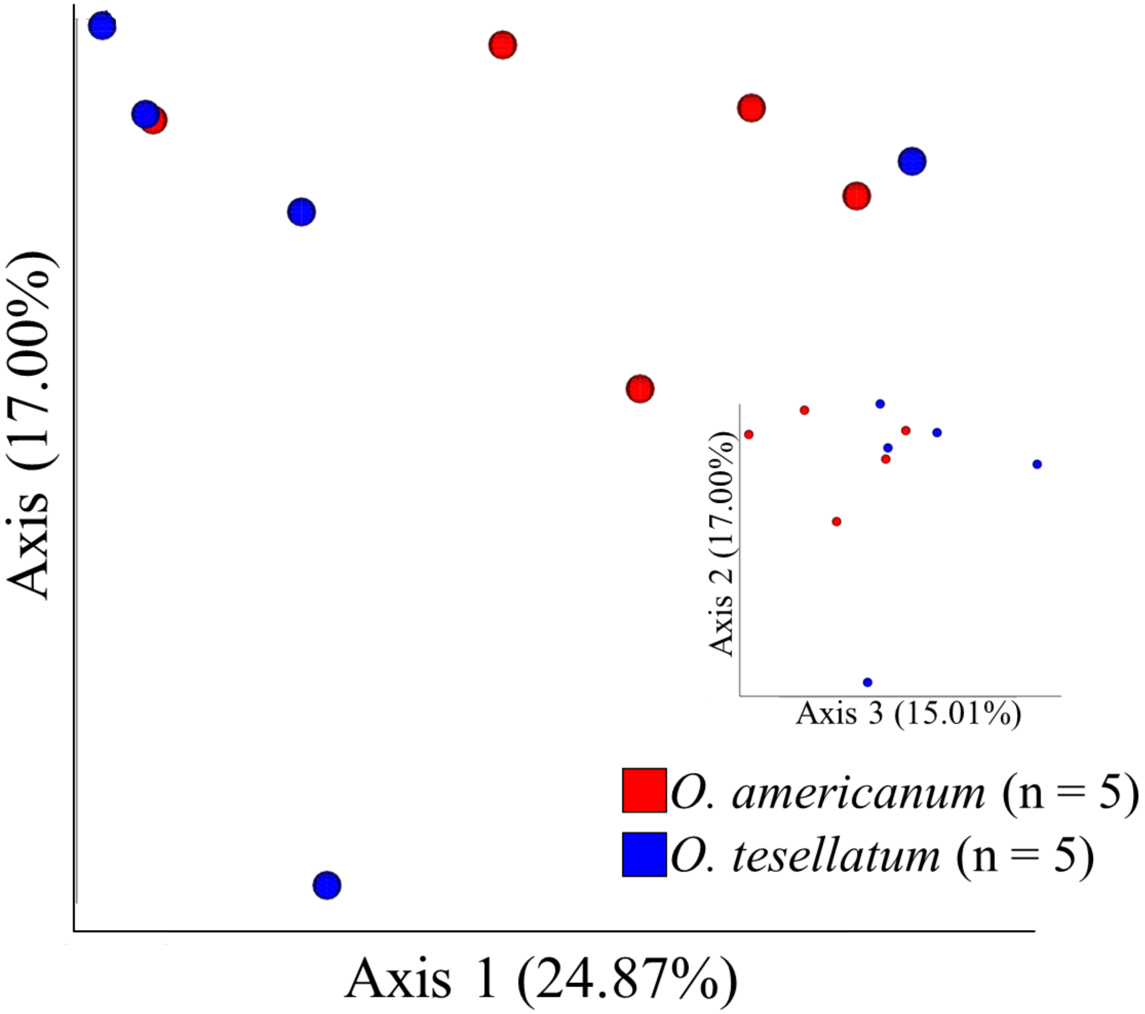


Figure S7: Bray-Curtis principal coordinate analysis (PCoA) for species of *Omophron.* Inset shows PCoA with axes 2 and 3. The three axes combine to show 56.88% of the beta diversity. Results of the permutational analysis of variance (PERMANOVA) did not show significant separation (pseudo-F = 1.071, p = 0.317) among samples by species.

Table S9: Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in the gut bacterial community (GBC) of two species of *Omophron*. Numbers in bold are the highest RA in each column.

	Total (n = 10)	*O. americanum* (n = 5)	*O. tessellatum* (n = 5)
Bacillota	**78.5**	**83.7**[1]	**73.4**[2]
Pseudomonadota	10.1	12.0[1]	8.3[2]
Bacteroidota	7.9	2.1	13.7[1]
Actinomycetota	1.8	2.1[1]	1.4
Fusobacteriota	1.6		2.9
Total	99.9	99.8	99.7

[1] significantly high differential abundance (DA) compared to other beetle groupings

[2] significantly low DA

Table S10: Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera in the gut bacterial community (GBC) of two species of *Omophron*. Numbers in bold are the highest RA in each column.

	Total (n = 10)	*O. americanum* (n = 5)	*O. tessellatum* (n = 5)
Bacillota;*Enterococcus*	**34.1**	**44.5[1]**	23.8[2]
Bacillota;*Lactobacillus*	27.3	26.2[1]	**28.4[2]**
Bacillota;f_Lachnospiraceae	8.2		16.3
Bacteroidota;*Dysgonomonas*	7.2	2.1	12.4[1]
Pseudomonadota;*Gilliamella*	5.4	8.4[1]	2.4[2]
Bacillota;*Spiroplasma*	5.3	10.7	
Actinomycetota;f_Micrococcaceae	1.8	2.1[1]	[2]
Pseudomonadota;*Pseudomonas*	1.7		2.0
Fusobacteriota;*Sebaldella*			2.3
Bacillota;*Breznakia*		1.5[1]	
Bacillota;f_Ruminococcaceae			1.5
Total	91.0	95.5	89.0

[1] significantly high differential abundance (DA) compared to other beetle groupings

[2] significantly low DA

1.2.6 *Pterostichus*

Beta diversity

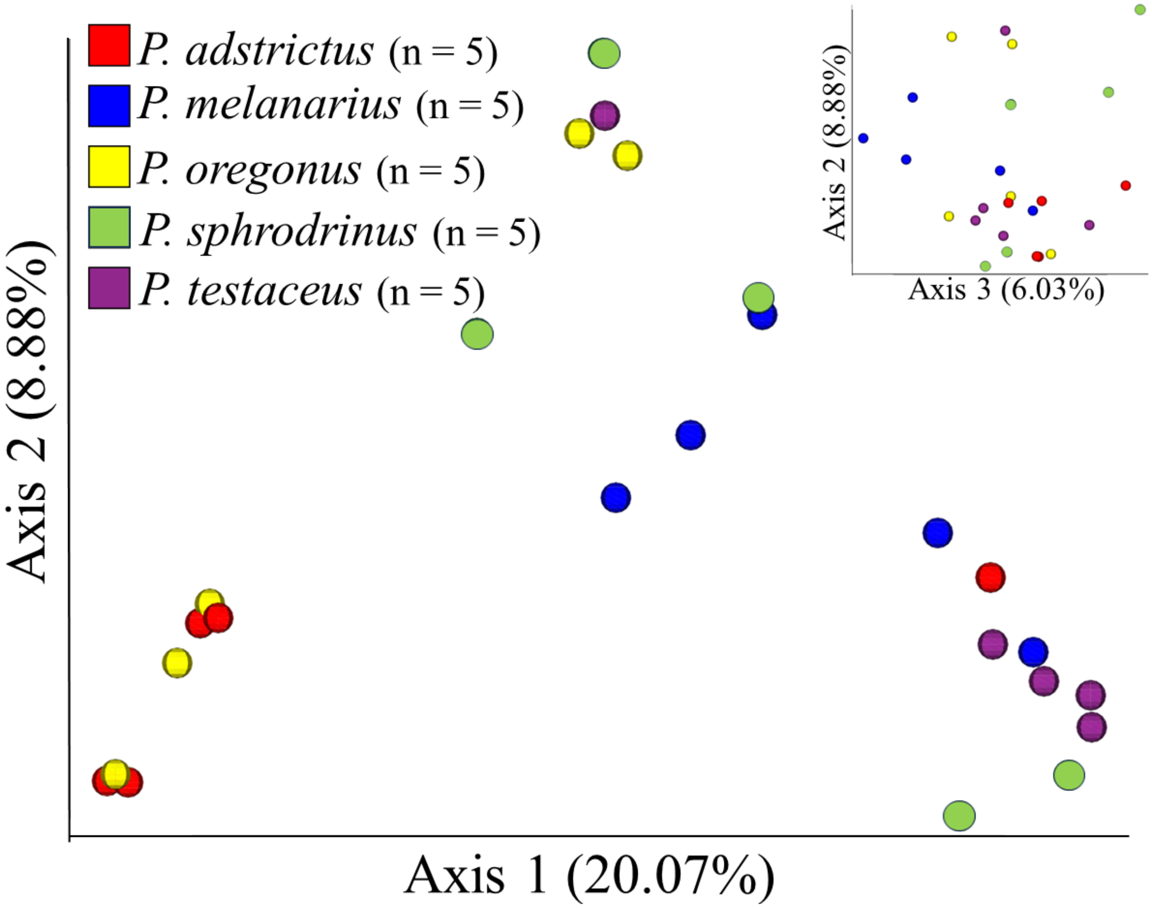


Figure S8: Bray-Curtis principal coordinate analysis (PCoA) for species of *Pterostichus*. Inset shows PCoA with axes 2 and 3. The three axes combine to show 34.98% of the beta diversity. Results of the permutational analysis of variance (PERMANOVA) show significant separation (pseudo-F = 1.634, p = 0.004) among samples by species.

Table S11: Relative abundance % (RA) and significant differential abundance (DA) of bacterial phyla in the gut bacterial community (GBC) of five species of *Pterostichus*. Numbers in bold are the highest RA in each column.

	Total (n = 25)	*P. adstrictus* (n = 5)	*P. melanarius* (n = 5)	*P. oregonus* (n = 5)	*P. sphodrinus* (n = 5)	*P. testaceus* (n = 5)
Pseudomonadota	**57.5**	40.5[1]	41.6[1]	**56.2**[2]	**63.3**[2]	**85.9**[2]
Bacillota	33.5	**57.2**[1]	**57.0**[1]	37.1[1]	12.7	3.5
Fusobacteriota	3.0		[2]	[2]	13.8	1.2[2]
Actinomycetota	2.7	1.8	[2]	4.4[1]	2.7	4.6[1]
Bacteroidota	2.4		1.5[1]	0.6	7.0[1]	2.8
Planctomycetota			[2]	1.0	[2]	0.8
unknown phylum						0.5
Verrucomicrobiota			[2]	0.5	[2]	[1]
Total	99.1	99.5	100.0	99.7	99.6	99.3

[1] significantly high differential abundance (DA) compared to other beetle groupings

[2] significantly low DA

Table S12: Relative abundance % (RA) and significant differential abundance (DA) of bacterial genera in the gut bacterial community (GBC) of five species of *Pterostichus*. Numbers in bold are the highest RA in each column.

	Total (n = 25)	*P. adstrictus* (n = 5)	*P. melanarius* (n = 5)	*P. oregonus* (n = 5)	*P. sphodrinus* (n = 5)	*P. testaceus* (n = 5)
Pseudomonadota;f_Yersiniaceae	**27.6**	13.6	23.4[1]	2.8	**44.0[1]**	**54.5[1]**
Bacillota;*Enterococcus*	19.8	**51.1[1]**	4.6	**35.8[1]**	7.6[1]	
Pseudomonadota;*Gilliamella*	8.3	23.4	3.4	11.8	2.8[1]	
Bacillota;*Lactobacillus*	5.5		**26.0[1]**			
Pseudomonadota;*Pseudomonas*	3.9			6.9		10.4[1]
Pseudomonadota;o_Enterobacterales	3.6			15.6		
Bacillota;o_Lactobacillales	3.4		13.5[1]			2.4
Pseudomonadota;*Pragia*	2.8				14.1	
Fusobacteriota;*Sebaldella*	2.7				13.6	
Pseudomonadota;f_Enterobacteriaceae	2.4			10.7		
Pseudomonadota;*Hafnia-Obesumbacterium*	2.2		9.2	1.9		
Bacillota;*Weissella*			4.1[1]			
Bacteroidota;*Flavobacterium*					5.2[1]	
Bacillota;f_Lachnospiraceae			4.2			
Bacillota;*Spiroplasma*		2.8				
Pseudomonadota;*Acinetobacter*				2.9		
Actinomycetota;f_Microbacteriaceae						1.8[1]
Pseudomonadota;*Buchnera*						3.2[1]
Pseudomonadota;*Burkholderia-Caballeronia-Paraburkholderia*						2.8[1]
Bacteroidota;*Dysgonomonas*			1.5[1]			
Pseudomonadota;f_Acetobacteraceae						2.7
Pseudomonadota;f_Oxalobacteraceae						1.5
Total	82.2	90.9	90.0	88.4	87.3	79.3

[1] significantly high differential abundance (DA) compared to other beetle groupings

Appendix 1.3 Rarefaction of *Cicindela* samples

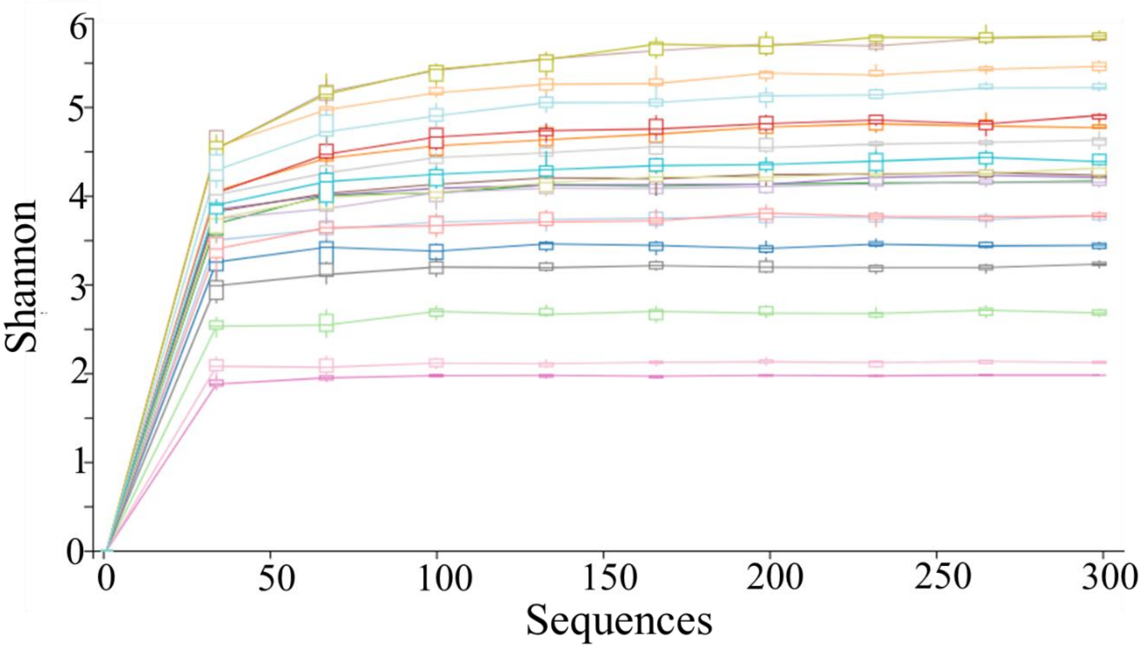


Figure S9: Rarefaction chart for analysis of *Cicindela* species. Samples were subsampled to 299 sequences each.

Appendix 2 Carabid Sample Index

Table S13: Locations and collection dates for all GBC samples available for this study (280 specimens)

Genus/species	**n**	**Collection location**	**Habitat**	**Collection date**	**Lat. and Long.**
Agonum piceolum	5	Castle Mountain, AB	pine forest	Jun. 16-18, 2023	49.349°; -114.411°
Agonum placidum	1	Fairfield Canola, Lethbridge, AB	canola	Aug. 11, 2022	49.703°; -112.693°
Agonum placidum	5	LRDC Canola, Lethbridge, AB	canola	Aug. 6-14, 2022	49.699°; -112.757°
Agonum muelleri	5	Burnt Fork, Stevensville, MT	pine forest	May 18-Jun. 17, 2022	46.448°; -113.899°
Amara farcta	5	Fairfield Canola, Lethbridge, AB	canola	Aug. 5-15, 2022	49.703°; -112.693°
Amara littoralis	2	Oldman River, Lethbridge, AB	riparian	Jun. 7-13, 2022	49.706°; -112.867°
Amara littoralis	1	Oldman River, Lethbridge, AB	riparian	May 9-Jun. 1, 2023	49.706°; -112.867°
Amara littoralis	5	Lethbridge Backyard, AB	urban yard	Apr. 29, 2023	49.731°; -112.832°
Amara littoralis	5	Fairfield Canola, Lethbridge, AB	canola	Aug. 5-15, 2022	49.703°; -112.693°
Amara quenseli	5	Fairfield Canola, Lethbridge, AB	canola	Aug. 5-15, 2022	49.703°; -112.693°
Amara quenseli	5	LRDC Canola, Lethbridge, AB	canola	Aug. 6-16, 2022	49.699°; -112.757°
Amara lacustris	2	Fairfield Canola, Lethbridge, AB	canola	Aug. 5-15, 2022	49.703°; -112.693°
Amara carinata	2	Fairfield Canola, Lethbridge, AB	canola	Aug. 5-15, 2022	49.703°; -112.693°
Amara carinata	5	LRDC Canola, Lethbridge, AB	canola	Aug. 6-16, 2022	49.699°; -112.757°
Amara tenax	1	Oldman River, Lethbridge, AB	riparian	May 9-Jun. 1, 2023	49.706°; -112.867°
Badister neopulchellus	3	Oldman River, Lethbridge, AB	riparian	May14,2023	49.706°; -112.867°
Badister neopulchellus	1	ULeth campus, Lethbridge, AB	urban yard	Jun. 13, 2021	49.677°; -112.868°
Bembidion carinula	5	St. Mary's Reservoir, AB	riparian	Jul. 12, 2022	49.336°; -113.143°
Bembidion quadrimaculatum	4	Fairfield Canola, Lethbridge, AB	canola	Aug. 5-15, 2022	49.703°; -112.693°
Bembidion quadrimaculatum	4	LRDC Canola, Lethbridge, AB	canola	Aug. 6-16, 2022	49.699°; -112.757°
Calathus ingratus	5	Elkwater, AB	pine forest	Aug. 27, 2022	49.678°; -110.184°

Genus/species	**n**	**Collection location**	**Habitat**	**Collection date**	**Lat. and Long.**
Carabus granulatus	2	Oldman River, Lethbridge, AB	riparian	Sep. 05, 2021	49.706°; -112.867°
Carabus granulatus	3	Oldman River, Lethbridge, AB	riparian	Sep. 3-5, 2022	49.706°; -112.867°
Carabus granulatus	2	Oldman River, Lethbridge, AB	riparian	Jun. 13-Sep.5, 2022	49.706°; -112.867°
Carabus granulatus	3	Oldman River, Lethbridge, AB	riparian	Sep. 05, 2021	49.706°; -112.867°
Carabus nemoralis	4	Kootenai Creek, Stevensville, MT	pine forest	Jun. 3-12, 2022	46.537°; -114.155°
Carabus nemoralis	5	Yard, Stevensville, MT	urban yard	May 25-Jun.27, 2022	46.503°; -114.096°
Carabus nemoralis	1	Bitterroot River, Stevensville, MT	riparian	Jun. 5, 2022	46.522°; -114.106°
Carabus taedatus	2	Wilderness Park, Lethbridge, AB	prairie	May 15-jun.1, 2023	49.737°; -112.853°
Carabus taedatus	3	Wilderness Park, Lethbridge, AB	prairie	Jul. 24-Aug. 2, 2022	49.737°; -112.853°
Carabus taedatus	4	Elkwater, AB	pine forest	Aug. 30-31, 2022	49.633°; -110.308°
Cicindela oregona	6	Clark Fork River, Missoula, MT	riparian	Jun. 1-Aug. 28, 2022	46.880°; -113.933°
Cicindela purpurea	5	Wilderness Park, Lethbridge, AB	prairie	May 14-Jun. 12, 2023	49.737°; -112.853°
Cicindela repanda	5	Oldman River, Lethbridge, AB	riparian	Jul. 21, 2022	49.686°; -112.858°
Cicindela repanda	1	St. Mary's Reservoir, AB	riparian	Jun. 8, 2023	49.336°; -113.143°
Cicindela repanda	4	St. Mary's Reservoir, AB	riparian	Jul. 12, 2022	49.336°; -113.143°
Cicindela lengi	2	St. Mary's Reservoir, AB	riparian	Jun. 8, 2023	49.336°; -113.143°
Cicindela lengi	4	St. Mary's Reservoir, AB	riparian	Jul. 12, 2022	49.336°; -113.143°
Chlaenius sericeus	1	Waterton Rsvr., AB	riparian	May 29,2023	49.332°; -113.669°
Chlaenius sericeus	1	Logan Lane, Stevensville, MT	cow pasture	Jun. 15-21, 2022	46.494°; -114.066°
Chlaenius sericeus	1	Oldman River, Lethbridge, AB	riparian	Jul. 17, 2022	49.754°; -112.852°
Chlaenius sericeus	1	Oldman River, Lethbridge, AB	riparian	Jun. 12, 2023	49.738°; -112.864°
Chlaenius sericeus	1	Oldman River, Lethbridge, AB	riparian	Jul. 5, 2022	49.706°; -112.867°
Clivina fossor	5	Fairfield Peas, Lethbridge, AB	peas	Aug. 8-14, 2022	49.701°; -112.693°
Diplous californicus	5	Clark Fork River, Missoula, MT	riparian	Jun. 24, 2022	46.880°; -113.933°
Dyschirius sphaericollis	3	Oldman River, Lethbridge, AB	riparian	Jun. 12-29, 2023	49.738°; -112.864°

Genus/species	**n**	**Collection location**	**Habitat**	**Collection date**	**Lat. and Long.**
Dyschirius truncatus	2	Oldman River, Lethbridge, AB	riparian	Jun. 12-29, 2023	49.738°; -112.864°
Elaphrus americanus	4	Oldman river, Fort Macleod, AB	riparian	Jun. 12, 2023	49.737°; -113.390°
Elaphrus americanus	1	Oldman river, Fort Macleod, AB	riparian	Jul. 26, 2022	49.737°; -113.390°
Harpalus affinis	5	LRDC Canola, Lethbridge, AB	canola	Aug. 11-13, 2022	49.699°; -112.757°
Harpalus amputatus	5	Fairfield Canola, Lethbridge, AB	canola	Aug. 5-15, 2022	49.703°; -112.693°
Harpalus fuscipalpis	5	Grazing Reserve, AB	dry prairie	Jul. 21, 2022	49.845°; -111.896°
Harpalus pensylvannicus	1	LRDC canola, Lethbridge, AB	canola	Aug. 5-15, 2022	49.699°; -112.757°
Harpalus pensylvannicus	4	Fairfield Canola, Lethbridge, AB	canola	Aug. 5-15, 2022	49.703°; -112.693°
Apristus constrictus	2	Oldman River, Lethbridge, AB	riparian	Jul. 16, 2022	49.686°; -112.858°
Apristus constrictus	1	Burnt Fork, Stevensville, MT	pine forest	Jun. 8-23, 2022	46.448°; -113.899°
Syntomus americanus	2	Burnt Fork, Stevensville, MT	pine forest	Jun. 8-23, 2022	46.448°; -113.899°
Microlestes linearis	5	LRDC Alfalfa, Lethbridge, AB	alfalfa	Jun. 2, 2023	49.703°; -112.761°
Nebria crassicornis	5	Beaver Mines Lake, AB	pine forest	Jun. 17, 2023	49.372°; -114.292°
Nebria hudsonica	5	Bitterroot River, Stevensville, MT	riparian	Aug. 29, 2022	46.522°; -114.106°
Omophron americanum	3	Milk River, Aden, AB	riparian	Jul. 23, 2022	49.077°; -111.651°
Omophron americanum	5	Oldman River, Lethbridge, AB	riparian	Jul. 16-17, 2022	49.754°; -112.852°
Omophron tesselatum	5	Milk River, Aden, AB	riparian	Jul. 23, 2022	49.077°; -111.651°
Opisthius richardsoni	5	St. Mary's Reservoir, AB	riparian	Sep. 3, 2022	49.336°; -113.143°
Opisthius richardsoni	5	Clark Fork River, Missoula, MT	riparian	May 26-Aug. 28, 2022	46.880°; -113.933°
Pasimachus elongatus	5	Grazing Reserve, AB	dry prairie	Jul. 21, 2022	49.845°; -111.896°
Pterostichus adstrictus	1	Elkwater, AB	pine forest	Aug. 30-31, 2022	49.633°; -110.308°
Pterostichus adstrictus	2	Elkwater, AB	pine forest	Aug. 29, 2021	49.678°; -110.184°
Pterostichus adstrictus	2	Elkwater, AB	pine forest	Aug. 27, 2022	49.678°; -110.184°

Genus/species	**n**	**Collection location**	**Habitat**	**Collection date**	**Lat. and Long.**
Pterostichus melanarius	1	Logan Lane, Stevensville, MT	cow pasture	Jun. 15-21, 2022	46.494°; -114.066°
Pterostichus melanarius	1	Yard, Stevensville, MT	urban yard	Jun. 16, 2022	46.503°; -114.096°
Pterostichus melanarius	5	Elkwater, AB	pine forest	Aug. 29, 2021	49.678°; -110.184°
Pterostichus melanarius	5	Elkwater, AB	pine forest	Aug. 27, 2022	49.678°; -110.184°
Pterostichus melanarius	5	Fairfield Canola, Lethbridge, AB	canola	Aug. 2022	49.703°; -112.693°
Pterostichus melanarius	5	Oldman River, Lethbridge, AB	riparian	Sep. 3-5, 2022	49.706°; -112.867°
Pterostichus melanarius	3	Willoughby Lane, Stevensville, MT	timothy hay	Jun. 24-28, 2022	46.443°; -114.048°
Pterostichus oregonus	5	Burnt Fork, Stevensville, MT	pine forest	May 22-Jun. 27, 2022	46.448°; -113.899°
Pterostichus sphodrinus	5	Burnt Fork, Stevensville, MT	pine forest	May 22-Jun. 26, 2022	46.448°; -113.899°
Pterostichus testaceus	1	Burnt Fork, Stevensville, MT	pine forest	Jun. 13, 2022	46.448°; -113.899°
Pterostichus testaceus	5	Kootenai Creek, Stevensville, MT	pine forest	Jun. 14-25, 2022	46.537°; -114.155°
Scaphinotus marginatus	1	Burnt Fork, Stevensville, MT	pine forest	Jun. 2, 2022	46.448°; -113.899°
Scaphinotus marginatus	1	Castle Mountain, AB	pine forest	Jun. 16-18, 2023	49.349°; -114.411°
Scaphinotus relictus	1	Burnt Fork, Stevensville, MT	pine forest	Jun. 2, 2022	46.448°; -113.899°
Trechus oregonensis	6	Castle Mountain, AB	pine forest	Jun. 16-18, 2023	49.349°; -114.411°