INTRODUCTION

Coexistence of sympatric species is facilitated by differences in the use of resources, that is, resource partitioning (Schoener, 1974). Resource partitioning occurs in several dimensions, with regard to resources. Ultimately, the sum of these dimensions constitutes the ecological niche of an organism, that is, the set of biotic and abiotic conditions in which a species can persist (Holt, 2009). This includes both the distribution of a species and its interactions with other species, but also factors relevant to the fine-scale distribution of species (e.g., microhabitats), their biotic interactions as well as their diet (Wiens et al., 2010).

With a notable adaptive radiation in their evolutionary history, and over 1,300 known species worldwide (Fenton & Simmons, 2015), bats have an important role in supporting global ecosystems through their dietary preferences. This is evidenced primarily through the consumption of nocturnal insects and dispersal of nutrients, pollen, and seeds (Patterson, Willig, & Stevens, 2003). Research on the feeding behavior of species is essential to understanding ecosystem function and the impacts of pollution, habitat destruction, and global climate change (Boyles & Storm, 2007; Kunz, Braun de Torrez, Bauer, Lobova, & Fleming, 2011; Vesterinen, 2015; Vesterinen et al., 2016). Furthermore, establishing factors influencing the extinction risk of bats is essential for their conservation, because they help identify endangered species and provide the basis for conservation (Safi & Kerth, 2004). However, these factors may be difficult to discern between species of bats, of which many appear to share portions of their ecological niches, such as habitat and apparently diet.

Table for five, please: Dietary partitioning in boreal bats

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Abstract
Differences in diet can explain resource partitioning in apparently similar, sympatric species. Here, we analyzed 1,252 fecal droppings from five species (Eptesicus nilssonii, Myotis brandtii, M. daubentoni, M. mystacinus, and Plecotus auritus) to reveal their dietary niches using fecal DNA metabarcoding. We identified nearly 550 prey species in 13 arthropod orders. Two main orders (Diptera and Lepidoptera) formed the majority of the diet for all species, constituting roughly 80%–90% of the diet. All five species had different dietary assemblages. We also found significant differences in the size of prey species between the bat species. Our results on diet composition remain mostly unchanged when using either read counts as a proxy for quantitative diet or presence-absence data, indicating a strong biological pattern. We conclude that although bats share major components in their ecology (nocturnal life style, insectivory, and echolocation), species differ in feeding behavior, suggesting bats may have distinctive evolutionary strategies. Diet analysis helps illuminate life history traits of various species, adding to sparse ecological knowledge, which can be utilized in conservation planning.

KEYWORDS
Chiroptera, dietary analysis, metabarcoding, prey size, resource partitioning
Even though some degree of food mixing is required for most species, it is thought that the diets of terrestrial mammals are generally highly specialized (Pineda-Munoz & Alroy, 2014). Indeed, when viewed in its entirety, the dietary diversity in bats is huge, ranging from insectivores, frugivores, and nectarivores to piscivores, carnivores, and even sanguinivores (Kunz, 1998). However, closely related species often occupy similar ecological niches, suggesting that components of the diet overlap to a high degree (Lara, Pérez, Castillo-Guevara, & Serrano-Meneses, 2015; Losos, 2008; Münkemüller, Boucher, Thuiller, & Lavergne, 2015; Razgour et al., 2011; Wilson, 2010). This phylogenetic signal in food webs is associated with the tendency of related species to share habitat and body size (Rezende, Albert, Fortuna, & Bascombe, 2009). For instance, insectivorous bats are generally small, because of the negative correlation between size and echolocation frequency of a bat. High-frequency echolocation calls are needed for the detection of small prey (Brigham, 1991). Nevertheless, species with identical niches rarely exist (Wiens et al., 2010).

Consisting of ca. 430 species sharing similar morphology, the insectivorous family Vespertilionidae [Gray 1821] is a useful group for research on resource partitioning (Aldridge & Rautenbach, 1987; Saunders & Barclay, 1992). Vespertilionidae exhibits only subtle interspecific morphological variation compared to members of the other bat families, even among distantly related species. This has posed a challenge in elucidating their evolutionary history (Jones, Purvis, MacLarnon, Bininda-Emonds, & Simmons, 2002; Van Den Bussche & Lack, 2013). Similarities in morphology are mirrored in diet; the almost cosmopolitan vesper bats are primarily insectivorous (Hoofer & Bussche, 2003; Simmons, 2005; Van Den Bussche & Lack, 2013). However, based on feeding behavior, vesper bat species have been classified to guilds of either aerial-hawking, gleaning, or trawling bats according to their foraging behavior (Norberg & Rayner, 1987). Recent advances in molecular methodology have begun to offer a deeper insight into the cryptic diet of these animals (Roslin, Majaneva, & Clare, 2016; Vesterinen et al., 2016; Vesterinen, Lilley, Laine, & Wahlberg, 2013). Vesper bats within the same feeding guild appear to share a great proportion of their diet (Roswag, Becker, & Encarnação, 2018). Because insectivorous bats opportunistically consume prey that may be periodically abundant (Vesterinen et al., 2013), this leads to significant temporal changes in the diet (Vesterinen et al., 2016), but could additionally result in a large overlap in dietary niches, suggesting resource partitioning occurs in other ecological dimensions.

Here, we unravel the resource partitioning of five resident vespertilionid bats in southwestern Finland through deep dietary analysis, including prey species identification, an estimate for prey body size and temporal changes in diet using fecal DNA barcoding. At high northern latitudes, the distribution of bats is constrained by extreme environmental demands and prey availability is more seasonal than elsewhere in their range (Clare et al., 2014; Shively & Barboza, 2017; Shively, Barboza, Doak, & Jung, 2017). The ranges of these five species (Eptesicus nilssonii [Keyserling & Bläsius, 1839], Myotis daubentonii [Kuhl, 1817], M. mystacinus [Kuhl, 1817], M. brandti [Eversmann, 1845], and Plecotus auritus [Linnaeus, 1758]) show considerable overlap, suggesting that trophic resource partitioning is important in supporting the species in Fennoscandia. We expect to see clear guild-specific segregation in diet between the three different feeding guilds presented by our species, trawling (M. daubentonii), gleaning (P. auritus), and aerial hawking (Figure 1; M. brandti, M. mystacinus, and E. nilssonii), and that we will see at least a partial dietary overlap among the members of the aerial hawker. Because of the opportunistic foraging behavior of insectivorous bats (Vesterinen et al., 2013), we also predict significant temporal changes in diet throughout the sampling season (but see Vesterinen et al., 2016). Finally, we predict a positive correlation between predator and prey size, which could be due to the negative correlation between bat size and echolocation frequency, hindering the ability to detect small prey items (Brigham, 1991). To the best of our knowledge, of the species studied here, molecular data on diet exist only for M. daubentonii (Galan et al., 2018; Krüger, Clare, Greif, et al., 2014; Krüger, Clare, Symondson, Keiis, & Petersen, 2014; Vesterinen et al., 2013, 2016), although the dietary contents of all species have previously been described through morphological analysis of fecal remains (Rydell, 1986; Vaughan, 1997).

**FIGURE 1** One of the study species, Myotis brandti, foraging in its natural environment near the study area in southwestern Finland. M. brandti catches its prey mainly in flight in an open or semi-open environment. The current study is the first ever published molecular analysis of its diet: Geometrid and tortricid moths constituted half of its diet, while mosquitoes, midges, and flies formed another large part of the menu, approximately one-third. Photograph credits: Mr. Risto Lindstedt
2 | MATERIALS AND METHODS

2.1 | Study species

Of the 13 species of bats occurring in Finland, the species sampled here represent the most common and accessible (*Myotis daubentonii, Eptesicus nilssonii, M. brandtii, M. mystacinus, and Plecotus auritus*). Based on both the Finnish Biodiversity Information Facility (www.laji.fi) databases and our own bat sampling, spanning for more than 10 years, these bat species constitute approximately 90%–98% of all bat occurrences in Finland, and have been the focus of most bat research in Finland so far (Jakava-Viljanen, Lilley, Kyheröinen, & Huovilainen, 2010; Laine, Lilley, Norrdahl, & Primmer, 2013; Lilley et al., 2013; Lilley, Stauffer, Kanerva, & Eeva, 2014; Lilley, Veikkolainen, & Pulliainen, 2015; Veikkolainen, Vesterinen, Lilley, & Pulliainen, 2014).

Of the sampled species, only the Northern bat (*Eptesicus nilssonii*) has a range encompassing all of Finland, with records extending far above the Arctic Circle, all the way to Utsjoki at 69°45′27″, 27°1′29″ (Figure 2b; Iso-livari, 1988; IUCN, 2016a). Although records of *M. daubentonii* extend to the Arctic Circle (Figure 2a; IUCN, 2008a; Siivonen & Wermundsen, 2008), the distributions of most of the other focal species, *M. mystacinus, M. brandtii,* and *P. auritus,* are considered to reach their northern limits in central Finland (Figure 2c–e; IUCN, 2008b, 2008c, 2016b). These five species, with the addition of the extremely rare *M. nattererii* and *M. dasycneme,* are most likely the only regularly hibernating species in Finland, whereas the other species

![Figure 2](https://example.com/fig2.png)

**FIGURE 2** The map showing the distribution of each studied bat species in northeastern Eurasia: (a) *Myotis daubentonii,* (b) *Eptesicus nilssonii,* (c) *M. brandtii,* (d) *M. mystacinus,* and (e) *Plecotus auritus* with a star denoting the focal area of the current study. (f) Locations of the roost sites for each bat species in the current study in southwestern Finland: NAU = Nautelankoski (*M. daubentonii*), RUI = Ruissalo (*M. brandtii*), SJÀ = Sahajärvi (*E. nilssonii*), SSA = Särkisalo (*E. nilssonii*), LAI = Laiterla (*P. auritus* and *M. mystacinus*), and ROT = Rotholma (*P. auritus* and *M. brandtii*)
migrate or are infrequent visitors (but see Ijäs, Kahilainen, Vasko, & Lilley, 2017).

2.2 | Field sampling

Fecal pellets were collected between April and July 2014 (Table 1) from day roosts of five species of bats in southwestern Finland, and all these roosts were in buildings within approximately 60 km of each other (Figure 2f). The pellets were collected by placing a clean paper sheet under the roosting bats the day before the collection, and collecting the droppings the next day. The collection was repeated for two or three consecutive days within a period of two weeks. Pellets were stored in RNA later at −20°C until laboratory analysis.

2.3 | Laboratory work

We aimed to pool 25 droppings (from the same roost and same time point) into each sample to maximize the number of droppings without the need to analyze hundreds of fecal pellets individually. Only four samples included less than 25 droppings, and for these, we pooled every available pellet for the given time point per roost. We focused sampling on roosts inhabited by a single species, and likewise, we intended to pool pellets from a single species into a single pooled sample. In total, we initially sampled 1,252 fecal pellets from the five bat species in this study (Table 1). The DNA was extracted using NucleoSpin® DNA Stool Kit (product nr 740472, Macherey-Nagel, Düren, Germany) following the manual (version April 2016/Rev. 01) “Protocol for fresh or frozen stool samples” with following modifications: step 1) we used on average 360 mg (±91 mg) of starting material per sample (samples dried only briefly on paper prior to the weighing), and we increased the amount of lysis buffer ST1 to 1,000 µl to increase the amount of supernatant in the subsequent stages; step 2) we used Tissue Lyser II (Cat No. 85300, Qiagen, Hilden, Germany) 2 × 30 s at full speed; step 3) we centrifuged the samples at 13,000 g for 5 min, after which the supernatant was transferred into a new tube; and in the final step DNA was eluted into 100 µl of SE buffer.

We used a single primer pair (SFF-145f: 5′-GTHACHGCYAYGCHTTYGTAATAAT-3′ and SFF-351r: 5′-CTCCWGRTGDGCWAGRTTTC-3′; primers and PCR setup from Walker, Williamson, Sanchez, Sobek, & Chambers, 2016) to test the DNA extraction success in the pooled samples and confirm the bat species by molecular analysis and another primer pair to amplify the potential prey (ZBJ-ArtF1c: 5′-AGATATTGGAACWTTATATTTTATTTTTGG-3′ and ZBJ-ArtR2c: 5′-WACTAATCAATTWCCAAATCCTC-3′; primers and PCR setup from Zeale, Butlin, Barker, Lees, & Jones, 2011). Despite the proposed bias in Zeale primers toward Diptera and Lepidoptera (Clarke, Soubrier, Weyrich, & Cooper, 2014), we chose these for several reasons: (a) These are the most widely applied markers, (b) many species have been detected using exactly the same primers, even though claimed to be nonamplifiable in the earlier criticism, and (c) we wanted to allow comparison of our results with those of other studies using the same primers (Clare et al., 2014; Kaunisto, Roslin, Sääksjärvi, & Vesterinen, 2017; Koskinen et al., 2018; Krüger, Clare, Greif, et al., 2014; Krüger, Clare, Symondson, et al., 2014; Vesterinen et al., 2013, 2016; Wirta et al., 2015; Eitzinger et al., 2018). The PCR and library construction closely followed Kaunisto et al. (2017).

**Table 1** Information on the sampling details and characteristics of the field and molecular data. *Time/roost sampling points per bat species denote how many times per roost the species was sampled: M. daubentonii was sampled from only a single roost (NAU; see Figure 2 for locations of the roost sites in the current study), E. nilssonii was sampled separately from two roosting sites (SJÄ, SSA), M. mystacinus and P. auritus were sampled from the same roost (LAI), and M. brandtii was sampled at two locations (RUI), one of which was shared by P. auritus (ROT). We found no statistical differences between samples from different bat species in the total reads, total prey species richness, or the average number of prey in each pellet.

|                        | All samples | Myotis daubentonii | Eptesicus nilssonii | M. brandtii | M. mystacinus | Plecotus auritus |
|------------------------|-------------|--------------------|--------------------|------------|---------------|-----------------|
| **Sampling period**    |             |                    |                    |            |               |                 |
| 29th Apr–7th Aug 2014  | 20          | 30                 | 9                  | 10         | 1             | 11              |
| **Pooled samples**     | 51          | 20                 | 9                  | 10         | 1             | 11              |
| **Pellets in total**   | 1,215       | 453                | 225                | 250        | 25            | 262             |
| **Avg. prey species per pellet** | 3.1 ± 1.4 | 3.0 ± 1.7 | 2.9 ± 1.1 | 3.3 ± 0.9 | 4.2 | 3.1 ± 1.6 |
| **Total prey reads**   | 5,449,755   | 1,768,337          | 1,030,783          | 1,128,927  | 119,416       | 1,402,292       |
| **Avg. reads per sample** | 106,858 ± 52,134 | 88,417 ± 42,780 | 114,531 ± 69,513  | 112,893 ± 50,648 | 119,416       | 127,481 ± 51,818 |
| **Prey species**       | 547         | 340                | 301                | 329        | 105           | 277             |
| **Avg. prey species per sample** | 69.7 ± 23.8 | 60.6 ± 22.6 | 71.8 ± 26.9 | 83.3 ± 23.2 | 105.0 | 69.2 ± 17.7 |
**TABLE 2** Prey species observed in the current study. For simplicity, prey species are reported as presence or absence for each bat species. First column stands for the prey number used in the plotweb analysis (Figures 3 and 4). If species name was not available in the molecular species assignment, the BIN cluster number is reported, as listed in Barcode of Life Database (https://v4.boldsystems.org). The bat species are abbreviated as follows: Md = *Myotis daubentonii*, En = *Eptesicus nilssonii*, Mb = *M. brandti*, Mm = *M. mystacinus*, and Pa = *Plecotus auritus*.

| No | Prey taxa            | Md | En | Mb | Mm | Pa |
|----|----------------------|----|----|----|----|----|
| 1  | Araneae              |    |    |    |    |    |
| 2  | Anyphaenidae         |    |    |    |    |    |
| 3  | Anyphaena accentuata | 1  | 1  | 1  | 1  | 1  |
| 4  | Araneidae            |    |    |    |    |    |
| 5  | Larinioides patagiatus | 0  | 1  | 0  | 0  | 0  |
| 6  | Linyphiidae          |    |    |    |    |    |
| 7  | Diplostyla concolor  | 0  | 1  | 0  | 0  | 0  |
| 8  | Ergone sp.           | 0  | 0  | 0  | 0  | 1  |
| 9  | Philodromidae        |    |    |    |    |    |
| 10 | Philodromus cespitum | 0  | 1  | 1  | 0  | 0  |
| 11 | Theridiidae          |    |    |    |    |    |
| 12 | Cryptochaeta riparia | 1  | 0  | 0  | 0  | 0  |
| 13 | Thomisidae           |    |    |    |    |    |
| 14 | Xysticus sp. 1       | 0  | 0  | 1  | 0  | 0  |
| 15 | Xysticus sp. 2       | 1  | 0  | 1  | 0  | 0  |
| 16 | INSECTA              |    |    |    |    |    |
| 17 | Blattodea            |    |    |    |    |    |
| 18 | Coleoptera           |    |    |    |    |    |
| 19 | Cantharidae          |    |    |    |    |    |
| 20 | Podabrus alpinus     | 0  | 1  | 0  | 0  | 0  |
| 21 | Carabidae            |    |    |    |    |    |
| 22 | Acupalpus parvulus   | 0  | 1  | 0  | 0  | 1  |
| 23 | Badister dilatatus   | 0  | 1  | 0  | 0  | 0  |
| 24 | Pterostichus adstrictus | 1  | 1  | 1  | 0  | 1  |
| 25 | Pterostichus melanarius | 1  | 1  | 1  | 1  | 1  |
| 26 | Pterostichus nigrita | 1  | 0  | 0  | 0  | 0  |
| 27 | Cerambycidae         |    |    |    |    |    |
| 28 | Acanthoecus aedilis  | 0  | 1  | 0  | 0  | 1  |
| 29 | Coleoptera sp.       | 0  | 1  | 0  | 0  | 0  |
| 30 | Curculionidae        |    |    |    |    |    |
| 31 | Brachyderes incanus  | 0  | 0  | 0  | 0  | 1  |
| 32 | Strophosoma capitatum| 0  | 0  | 0  | 0  | 1  |
| 33 | Dytiscidae           |    |    |    |    |    |
| 34 | Laccophilus comes    | 0  | 1  | 0  | 0  | 0  |
| 35 | Orectochilus villosus | 1  | 1  | 1  | 0  | 1  |

(Continues)
| No | Prey taxa                        | Md | En | Mb | Mm | Pa |
|----|---------------------------------|----|----|----|----|----|
| 50 | Chironomus sp.1                  | 1  | 1  | 1  | 0  | 1  |
| 51 | Chironomus sp.2                  | 1  | 1  | 1  | 0  | 1  |
| 52 | Cladopelma sp.                   | 1  | 1  | 0  | 0  |
| 53 | Cladopelma sp. 1TE               | 1  | 1  | 0  | 1  |
| 54 | Conchapelopia melanops           | 1  | 1  | 1  | 0  |
| 55 | Conchapelopia sp. 1              | 1  | 0  | 0  | 0  |
| 56 | Chironomus sp.2                  | 1  | 1  | 1  | 0  | 1  |
| 57 | Cladopelma sp.                   | 1  | 1  | 1  | 0  | 1  |
| 58 | Cladopelma sp. 1TE               | 1  | 1  | 0  | 1  |
| 59 | Conchapelopia melanops           | 1  | 1  | 1  | 0  |
| 60 | Conchapelopia sp. 1              | 1  | 0  | 0  | 0  |
| 61 | Demicryptochironomus sp.         | 0  | 1  | 0  | 0  |
| 62 | Dicrotendipes longicollis        | 0  | 1  | 1  | 0  |
| 63 | Dicrotendipes nervosus           | 1  | 1  | 1  | 0  |
| 64 | Dicrotendipes triomus            | 1  | 0  | 1  | 0  |
| 65 | Endochironomus tendens           | 1  | 1  | 0  | 0  |
| 66 | Glyptotendipes bimaculatus       | 0  | 1  | 0  | 0  |
| 67 | Glyptotendipes carchinellus      | 1  | 1  | 0  | 1  |
| 68 | Glyptotendipes lobiferus         | 1  | 1  | 1  | 0  |
| 69 | Glyptotendipes sp.               | 1  | 0  | 0  | 0  |
| 70 | Glyptotendipes sp. 1             | 1  | 1  | 0  | 0  |
| 71 | Heterotrichoscladius marcius     | 0  | 1  | 0  | 0  |
| 72 | Kiefferulus sp.                  | 1  | 0  | 1  | 0  |
| 73 | Metriocnemus sp. 3ES             | 0  | 0  | 1  | 0  |
| 74 | Microchironomus tener            | 0  | 0  | 1  | 0  |
| 75 | Microtendipes chloris            | 1  | 1  | 0  | 0  |
| 76 | Microtendipes pedellus           | 1  | 1  | 0  | 0  |
| 77 | Microtendipes sp.                | 1  | 1  | 0  | 0  |
| 78 | Orthocladiinae sp.               | 1  | 1  | 1  | 0  |
| 79 | Parachironomus digitalis         | 1  | 0  | 1  | 0  |
| 80 | Parachironomus monochromus       | 1  | 0  | 0  | 0  |
| 81 | Paracladopelma sp. 1             | 1  | 0  | 0  | 0  |
| 82 | Paracladopelma sp. 2             | 1  | 0  | 0  | 0  |
| 83 | Paranytarsus dissimilis          | 0  | 0  | 1  | 0  |
| 84 | Polypedilum convictum            | 1  | 0  | 0  | 0  |
| 85 | Polypedilum nubeculosum          | 1  | 1  | 1  | 0  |
| 86 | Polypedilum pedestre             | 0  | 0  | 1  | 0  |
| 87 | Polypedilum sordens              | 1  | 1  | 0  | 0  |
| 88 | Polypedilum sp.                  | 1  | 0  | 1  | 0  |

(Continues)
| No  | Prey taxa                          | Md | En | Mb | Mm | Pa |
|-----|-----------------------------------|----|----|----|----|----|
| 125 | Rhamphomyia caesia                | 0  | 0  | 0  | 1  | 0  |
| 126 | Rhamphomyia nigripennis           | 1  | 1  | 0  | 0  | 1  |
| 127 | Rhamphomyia nr. anaxo             | 1  | 0  | 0  | 0  | 0  |
| 128 | Rhamphomyia sp.                   | 0  | 1  | 0  | 0  | 0  |
| 129 | Rhamphomyia umbripennis           | 0  | 0  | 1  | 0  | 0  |
| 130 | Rhamphomyia valga                 | 0  | 1  | 1  | 0  | 0  |
| 131 | Fanniidae                         |    |    |    |    |    |
| 132 | Fannia minutipalpis              | 0  | 0  | 1  | 0  | 0  |
| 133 | Fannia sociella                  | 1  | 1  | 0  | 0  | 0  |
| 134 | Nycteribia kolenati               | 1  | 1  | 0  | 1  | 0  |
| 135 | Bicellaria simplicipes            | 0  | 1  | 0  | 0  | 1  |
| 136 | Macrocera stigma                  | 0  | 0  | 1  | 0  | 0  |
| 137 | Austrolimnophila unica            | 0  | 1  | 0  | 0  | 1  |
| 138 | Dicranomyia didyma                | 1  | 0  | 0  | 0  | 0  |
| 139 | Dicranomyia frontalis             | 0  | 0  | 0  | 1  | 0  |
| 140 | Dicranomyia modesta               | 1  | 1  | 0  | 0  | 0  |
| 141 | Dicranomyia sp.                   | 1  | 1  | 0  | 1  | 0  |
| 142 | Elophila maculata                 | 1  | 0  | 1  | 1  | 0  |
| 143 | Erioptera divisa                  | 1  | 0  | 1  | 0  | 0  |
| 144 | Erioptera sp.                     | 1  | 1  | 1  | 0  | 0  |
| 145 | Gonomyia tenella                  | 0  | 1  | 0  | 0  | 0  |
| 146 | Helius longirostris               | 1  | 1  | 0  | 0  | 0  |
| 147 | Limonia rubeculosa                | 1  | 0  | 0  | 0  | 0  |
| 148 | Limonia trivittata                | 1  | 0  | 1  | 1  | 0  |
| 149 | Metalimnobia bifasciata           | 1  | 0  | 0  | 1  | 1  |
| 150 | Metalimnobia quadrimotata         | 1  | 1  | 1  | 1  | 1  |
| 151 | Molophilus sp.                    | 0  | 0  | 0  | 0  | 1  |
| 152 | Phylidorea squalens               | 0  | 1  | 0  | 0  | 0  |
| 153 | Rhipidia maculata                 | 1  | 1  | 1  | 0  | 1  |
| 154 | Symplecta stictica                | 1  | 0  | 1  | 0  | 0  |
| 155 | Helina evecta                     | 1  | 1  | 0  | 0  | 0  |
| 156 | Hydrotaea armipes                 | 0  | 0  | 0  | 1  | 0  |
| 157 | Hydrotaea irritans                | 0  | 0  | 0  | 1  | 1  |
| 158 | Muscina levida                    | 0  | 0  | 0  | 0  | 1  |
| 159 | Mydaea new sp. nr urbana          | 0  | 1  | 0  | 0  | 0  |
| 160 | Polietes lardarius                | 1  | 0  | 1  | 0  | 1  |

(Continues)
| No | Prey taxa              | Md | En | Mb | Mm | Pa |
|----|-----------------------|----|----|----|----|----|
| 195| Phorocera obscura     | 1  | 1  | 1  | 0  | 0  |
| 196| Siphona geniculata    | 0  | 1  | 0  | 0  | 0  |
| 197| Tipulidae             |    |    |    |    |    |
| 198| Nephotoma aculeata    | 1  | 1  | 1  | 0  | 1  |
| 199| Nephotoma lunulicornis| 1  | 1  | 0  | 0  | 0  |
| 200| Tipula fascipennis    | 1  | 1  | 1  | 1  | 1  |
| 201| Tipula fulvipennis    | 0  | 1  | 0  | 0  | 0  |
| 202| Tipula lateralis      | 1  | 0  | 0  | 0  | 0  |
| 203| Tipula lunata         | 0  | 1  | 1  | 1  | 1  |
| 204| Tipula maxima         | 1  | 1  | 0  | 0  | 0  |
| 205| Tipula nubeculosa     | 0  | 1  | 1  | 0  | 1  |
| 206| Tipula paludosa       | 1  | 1  | 0  | 0  | 0  |
| 207| Tipula pterei         | 1  | 1  | 0  | 1  | 1  |
| 208| Tipula scripta        | 1  | 1  | 0  | 0  | 0  |
| 209| Tipula truncorum      | 1  | 1  | 0  | 0  | 0  |
| 210| Tipulidae sp.         | 0  | 1  | 0  | 0  | 0  |
| 211| Trichocera regulationis| 1  | 1  | 0  | 0  | 0  |
| 212| Trichocera sp.        | 1  | 0  | 0  | 0  | 0  |

**Ephemeroptera**

| No | Prey taxa              | Md | En | Mb | Mm | Pa |
|----|-----------------------|----|----|----|----|----|
| 213| Procloeon bifidum     | 1  | 0  | 0  | 0  | 0  |
| 214| Caenis horaria        | 1  | 1  | 0  | 1  | 1  |
| 215| Ephemerella vulgata   | 1  | 1  | 0  | 0  | 0  |
| 216| Heptageniida          |    |    |    |    |    |
| 217| Heptagenia sulphurea  | 1  | 1  | 0  | 0  | 0  |
| 218| Siphlonurida          |    |    |    |    |    |
| 219| Siphlonurus alternatus| 1  | 0  | 0  | 0  | 0  |

**Hemiptera**

| No | Prey taxa              | Md | En | Mb | Mm | Pa |
|----|-----------------------|----|----|----|----|----|
| 220| Eucerophis betulae    | 0  | 1  | 1  | 0  | 1  |
| 221| Eucerophis punctipennis| 0  | 1  | 0  | 0  | 1  |
| 222| Cicadellidae          |    |    |    |    |    |
| 223| Lygus pratensis       | 0  | 1  | 1  | 0  | 1  |
| 224| Neolycus contaminatius| 1  | 0  | 1  | 1  | 0  |

**Hymenoptera**

| No | Prey taxa              | Md | En | Mb | Mm | Pa |
|----|-----------------------|----|----|----|----|----|
| 225| Astrophora splenium    | 0  | 0  | 1  | 0  | 1  |
| 226| Diadegma majale        | 0  | 0  | 1  | 0  | 0  |
| 227| Hyposoter PRO-3        | 0  | 0  | 1  | 0  | 0  |
| 228| Mesoschorus sp.        | 1  | 0  | 0  | 0  | 0  |
| 229| Mesoschorus vitticollis| 0  | 1  | 0  | 0  | 0  |
| 230| Pleolphus sp.          | 0  | 0  | 0  | 0  | 0  |

**Trichoceridae**

| No | Prey taxa              | Md | En | Mb | Mm | Pa |
|----|-----------------------|----|----|----|----|----|
| 231| Dolerus vestigialis   | 1  | 0  | 0  | 0  | 0  |
| 232| Pachyprotasis rapae   | 1  | 0  | 0  | 0  | 0  |

**Lepidoptera**

| No | Prey taxa              | Md | En | Mb | Mm | Pa |
|----|-----------------------|----|----|----|----|----|
| 233| Nematopogon swammerdamellus| 1  | 1  | 0  | 0  | 0  |
| 234| Atolmis rubricollis    | 1  | 0  | 0  | 0  | 0  |
| 235| Eilema depressum       | 0  | 0  | 0  | 0  | 0  |
| 236| Argyresthiida          |    |    |    |    |    |
| 237| Argyresthia valida     | 1  | 1  | 1  | 1  | 1  |
| 238| Argyresthia goadartella| 1  | 1  | 1  | 1  | 1  |
| 239| Argyresthia retinella  | 0  | 1  | 0  | 0  | 0  |

**Batrachedrididae**

| No | Prey taxa              | Md | En | Mb | Mm | Pa |
|----|-----------------------|----|----|----|----|----|
| 240| Batrachedra pinicolella| 1  | 0  | 0  | 0  | 0  |

**Bucculatricidae**

| No | Prey taxa              | Md | En | Mb | Mm | Pa |
|----|-----------------------|----|----|----|----|----|
| 241| Bucculatrix cidarella | 0  | 0  | 0  | 0  | 0  |
| 242| Bucculatrix thoracella| 1  | 0  | 0  | 0  | 0  |
| 243| Bucculatrix ulmella   | 1  | 1  | 0  | 0  | 0  |
| 244| Coleophora betulella  | 1  | 1  | 1  | 0  | 0  |
| 245| Coleophora kuehnella  | 0  | 0  | 1  | 0  | 0  |
| 246| Coleophora spinella   | 1  | 0  | 1  | 1  | 1  |
| 247| Coleophora versurella | 1  | 1  | 0  | 0  | 0  |

**Crambidae**

| No | Prey taxa              | Md | En | Mb | Mm | Pa |
|----|-----------------------|----|----|----|----|----|
| 250| Acentria ephemerella  | 1  | 0  | 0  | 0  | 0  |
| 251| Agriphila inquinatella| 1  | 0  | 0  | 0  | 0  |
| 252| Agriphila selasella   | 1  | 0  | 0  | 0  | 0  |
| 253| Agriphila straminella | 0  | 0  | 0  | 0  | 0  |
| 254| Calamotropha pauperella| 1  | 0  | 1  | 0  | 0  |
| 255| Chrysoteuchia culmella| 0  | 1  | 0  | 0  | 0  |
| 256| Crambus lathoniellus  | 1  | 0  | 0  | 0  | 0  |
| 257| Crambus pascuellus    | 0  | 0  | 0  | 1  | 1  |

(Continues)
### TABLE 2 (Continued)

| No  | Prey taxa               | Md | En | Mb | Mm | Pa |
|-----|-------------------------|----|----|----|----|----|
| 258 | Donacaula mucronella    | 1  | 1  | 0  | 0  | 1  |
| 259 | Eolophila nymphaeata    | 1  | 0  | 0  | 1  | 1  |
| 260 | Evergestis extimalis    | 1  | 0  | 1  | 0  | 1  |
| 261 | Nymphula rutiludata     | 1  | 0  | 1  | 0  | 0  |
| 262 | Ostrinia nubilalis      | 1  | 0  | 1  | 0  | 0  |
| 263 | Scoparia ancipitella    | 1  | 0  | 1  | 1  | 1  |
| 264 | Scoparia subfusca       | 1  | 0  | 0  | 0  | 0  |
| 265 | Udea lutealis          | 1  | 0  | 0  | 1  | 0  |

Depressariidae

| No  | Prey taxa               | Md | En | Mb | Mm | Pa |
|-----|-------------------------|----|----|----|----|----|
| 266 | Agonopterix angelicella | 1  | 1  | 1  | 0  | 1  |
| 267 | Agonopterix arenella    | 1  | 0  | 1  | 0  | 1  |
| 268 | Agonopterix ciliella    | 1  | 0  | 1  | 1  | 1  |
| 269 | Agonopterix heracliana  | 1  | 1  | 1  | 0  | 1  |
| 270 | Agonopterix propinquella| 1  | 0  | 1  | 0  | 0  |
| 271 | Depressaria daucella    | 1  | 1  | 1  | 0  | 1  |
| 272 | Depressaria emeritella  | 1  | 1  | 1  | 0  | 1  |
| 273 | Depressaria lobanotidella| 1  | 1  | 1  | 0  | 1  |
| 274 | Depressaria olerella    | 1  | 1  | 1  | 0  | 1  |
| 275 | Depressaria radiella    | 1  | 0  | 0  | 0  | 0  |
| 276 | Depressaria sordidatella| 1  | 1  | 0  | 0  | 1  |

Drepanidae

| No  | Prey taxa               | Md | En | Mb | Mm | Pa |
|-----|-------------------------|----|----|----|----|----|
| 277 | Drepana falcatoria      | 1  | 0  | 0  | 0  | 0  |
| 278 | Falcaria lacertinaria   | 1  | 0  | 1  | 0  | 0  |
| 279 | Tethea or               | 0  | 0  | 0  | 0  | 1  |
| 280 | Tetheella fluctuosa    | 1  | 1  | 1  | 0  | 1  |

Elachistidae

| No  | Prey taxa               | Md | En | Mb | Mm | Pa |
|-----|-------------------------|----|----|----|----|----|
| 281 | Elachista adsicetella   | 0  | 0  | 1  | 1  | 1  |

Endromidae

| No  | Prey taxa               | Md | En | Mb | Mm | Pa |
|-----|-------------------------|----|----|----|----|----|
| 282 | Endromis versicolora    | 0  | 1  | 1  | 0  | 1  |
| 283 | Epermenia illigerella   | 1  | 0  | 0  | 0  | 0  |

Erebidae

| No  | Prey taxa               | Md | En | Mb | Mm | Pa |
|-----|-------------------------|----|----|----|----|----|
| 284 | Calliteara pudibunda    | 0  | 1  | 1  | 0  | 1  |
| 285 | Diaeris sannio          | 1  | 1  | 0  | 0  | 1  |
| 286 | Herminia tarsipennalis | 0  | 0  | 1  | 0  | 1  |
| 287 | Hypena crassalis       | 0  | 1  | 0  | 0  | 1  |
| 288 | Macrochilo cribrumalis | 1  | 1  | 1  | 1  | 1  |
| 289 | Rivula sericealis      | 0  | 0  | 1  | 1  | 0  |
| 290 | Scoliopteryx libatrix  | 0  | 0  | 0  | 0  | 1  |
| 291 | Spilarctia luteum      | 1  | 0  | 0  | 0  | 1  |

Gelechiidae

| No  | Prey taxa               | Md | En | Mb | Mm | Pa |
|-----|-------------------------|----|----|----|----|----|
| 292 | Carpatolechia fugitellae| 0  | 0  | 1  | 0  | 0  |
| 293 | Carpatolechia proximella| 1  | 1  | 1  | 0  | 1  |
| 294 | Caryocolum vicinella    | 1  | 1  | 1  | 1  | 1  |
| 295 | Chionodes electella    | 1  | 1  | 1  | 1  | 1  |

(Continues)
| No | Prey taxa                      | Md | En | Mb | Mm | Pa |
|----|--------------------------------|----|----|----|----|----|
| 339| Scopula floslactata            | 1  | 0  | 0  | 0  | 1  |
| 340| Scopula immutata               | 1  | 0  | 0  | 0  | 0  |
| 341| Selenia dentaria               | 1  | 0  | 1  | 0  | 1  |
| 342| Xanthorhoe montanata           | 1  | 1  | 1  | 0  | 0  |
| 343| Xanthorhoe quadrifasciata      | 1  | 0  | 1  | 1  | 1  |
| 344| Xanthorhoe spadicearia         | 0  | 1  | 0  | 0  | 0  |
| 345| Orthotelia sparganella         | 1  | 0  | 1  | 0  | 0  |
| 346| Caloptilia alchimiella         | 0  | 0  | 1  | 0  | 0  |
| 347| Caloptilia betulicola          | 0  | 1  | 1  | 0  | 0  |
| 348| Caloptilia elongella           | 0  | 1  | 1  | 0  | 0  |
| 349| Caloptilia hemidactylella      | 1  | 0  | 1  | 0  | 0  |
| 350| Caloptilia populetorum         | 0  | 1  | 1  | 0  | 0  |
| 351| Parornix betulae               | 1  | 1  | 1  | 0  | 0  |
| 352| Parornix devoniella            | 1  | 1  | 1  | 0  | 1  |
| 353| Phyllonorycter harrissella     | 0  | 0  | 1  | 0  | 0  |
| 354| Pharmacis fusconebulosa        | 0  | 0  | 1  | 1  | 1  |
| 355| Dendrolimus pini               | 1  | 1  | 1  | 0  | 1  |
| 356| Lasiocampa quercus             | 1  | 1  | 1  | 0  | 1  |
| 357| Macrothylacia rubi             | 1  | 1  | 1  | 0  | 1  |
| 358| Lyocuris clerkella             | 0  | 1  | 1  | 0  | 0  |
| 359| Pseudatemelia elsa             | 0  | 1  | 0  | 0  | 0  |
| 360| Pseudatemelia josephiniae      | 1  | 1  | 0  | 1  | 1  |
| 361| Mompha sturnipennella          | 1  | 0  | 1  | 0  | 0  |
| 362| Mompha subbistrigella          | 1  | 1  | 1  | 0  | 0  |
| 363| Acronicta auricoma             | 1  | 0  | 0  | 0  | 0  |
| 364| Acronicta rumicis              | 1  | 0  | 0  | 0  | 1  |
| 365| Agrochola helvola              | 0  | 0  | 0  | 0  | 1  |
| 366| Agrotis clavis                 | 1  | 1  | 1  | 1  | 1  |
| 367| Agrotis exclamationis          | 1  | 1  | 1  | 0  | 1  |
| 368| Allophyes oxyacanthae          | 0  | 0  | 1  | 0  | 1  |
| 369| Apamea crenata                 | 0  | 0  | 1  | 0  | 1  |
| 370| Apamea remissa                 | 1  | 1  | 1  | 1  | 1  |
| 371| Apamea sclopcina               | 0  | 0  | 0  | 0  | 1  |
| 372| Apamea sordens                 | 1  | 1  | 1  | 0  | 1  |
| 373| Autographa gamma               | 1  | 1  | 1  | 0  | 1  |
| 374| Autographa pulchrina           | 0  | 0  | 0  | 0  | 1  |

(Continues)
| No | Prey taxa                  | Md | En | Mb | Mm | Pa |
|----|----------------------------|----|----|----|----|----|
| 415| Colias palaeno             | 0  | 1  | 0  | 0  | 0  |
| 416| Plutella xyllostella       | 1  | 1  | 1  | 0  | 1  |
| 417| Prays fraxinellla         | 0  | 0  | 1  | 0  | 0  |
| 418| Taleporia tubulosa        | 0  | 1  | 0  | 0  | 0  |
| 419| Gillmeria pallidactyla    | 1  | 0  | 1  | 1  | 0  |
| 420| Dioryctria abietella      | 0  | 0  | 0  | 1  | 1  |
| 421| Agilia tau                | 0  | 1  | 0  | 0  | 1  |
| 422| Saturnia pavonia          | 0  | 0  | 1  | 0  | 1  |
| 423| Deilephila elpenor        | 0  | 0  | 0  | 0  | 1  |
| 424| Morophaga choragella      | 0  | 0  | 1  | 0  | 0  |
| 425| Nemapogon nigralbella     | 0  | 0  | 1  | 0  | 0  |
| 426| Nemapora betulinella      | 0  | 0  | 1  | 0  | 0  |
| 427| Niditinea striolia        | 0  | 0  | 1  | 0  | 0  |
| 428| Triaxomera fulvimitrella  | 1  | 0  | 1  | 0  | 0  |
| 429| Tischeria ekebladella     | 0  | 1  | 1  | 0  | 0  |
| 430| Acleris forsskaleana      | 1  | 0  | 1  | 1  | 1  |
| 431| Acleris lipsiana          | 1  | 0  | 1  | 1  | 1  |
| 432| Acleris logiana           | 1  | 1  | 1  | 0  | 1  |
| 433| Acleris notana            | 1  | 0  | 1  | 0  | 1  |
| 434| Adoxophyes orana          | 1  | 1  | 1  | 1  | 1  |
| 435| Aethes smeathmanniana     | 1  | 1  | 1  | 0  | 1  |
| 436| Agapeta hamana            | 0  | 1  | 0  | 0  | 0  |
| 437| Aleimma loefflingiana     | 0  | 1  | 1  | 1  | 1  |
| 438| Ancyliis badiana          | 1  | 0  | 0  | 0  | 0  |
| 439| Ancyliis laetana          | 0  | 0  | 1  | 0  | 0  |
| 440| Ancyliis mitterbacheriana| 1  | 0  | 1  | 0  | 0  |
| 441| Ancyliis myrtillana       | 1  | 1  | 0  | 0  | 1  |
| 442| Aphelia paleana           | 0  | 1  | 0  | 0  | 0  |
| 443| Apotomis fraterculana     | 1  | 1  | 0  | 1  | 0  |
| 444| Apotomis infida           | 1  | 0  | 0  | 0  | 0  |
| 445| Archips podanus           | 1  | 0  | 1  | 0  | 1  |
| 446| Bactra lancealana         | 1  | 0  | 0  | 0  | 0  |
| 447| Celypha rivulana          | 1  | 0  | 0  | 0  | 0  |
| 448| Clepsis spectrana        | 1  | 0  | 0  | 0  | 0  |

(Continues)
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except we used MyTaq HS Red Mix (product nr BIO-25048, Bioline, UK) polymerase throughout the protocol. In short, the first-step PCR reactions included tagged locus-specific primers targeting either predator or prey COI gene, and the second-step PCR followed directly after this including Illumina-specific adapters with a unique dual-index combination for each single reaction. After this, the individual libraries were pooled (SFF and ZBJ in separate pools at this stage) by equal volume (2 µl each library) and each pool was purified using dual-SPRI (solid-phase reversible immobilization) beads as in Vesterinen et al. (2016). To summarize the SPRI method, 80 µl SPRI was added on top of 100 µl library pool, vortexed thoroughly and incubated at room temperature for 5 min. The mix was then briefly centrifuged and placed on a strong magnet until clear, after

| No | Prey taxa                          | Md | En | Mb | Mm | Pa |
|----|------------------------------------|----|----|----|----|----|
| 491| Paraswammerdamia conspersella      | 1  | 0  | 1  | 0  | 1  |
| 492| Paraswammerdamia nebulella         | 1  | 0  | 1  | 0  | 1  |

**TABLE 2** (Continued)

| No | Prey taxa                          | Md | En | Mb | Mm | Pa |
|----|------------------------------------|----|----|----|----|----|
| 493| Ypsolopa asperella                 | 0  | 1  | 0  | 0  | 0  |
| 494| Ypsolopa falcella                  | 1  | 1  | 1  | 0  | 0  |
| 495| Ypsolopa parenthesella             | 1  | 1  | 1  | 0  | 1  |
| 496| Ypsolopa scabrella                 | 1  | 0  | 1  | 0  | 1  |
| 497| Ypsolopa sylvella                  | 1  | 0  | 1  | 0  | 0  |
| 498| Ypsolopa ustella                   | 1  | 0  | 1  | 0  | 1  |

**Megaloptera**

| SIALIDAE |
|---|
| 499| Sialis lutaria                     | 1  | 0  | 0  | 0  | 0  |

**Neuroptera**

| CHRSYSIDAE |
|---|
| 500| Chrysopa pallens                   | 1  | 1  | 1  | 0  | 1  |
| 501| Chrysoperla carnea                 | 1  | 1  | 0  | 0  | 1  |
| 502| Cunctochrysa albolineata           | 0  | 0  | 0  | 1  | 0  |

**Hemerobididae**

| 503| Hemerobius contumax                | 1  | 1  | 1  | 1  | 1  |
| 504| Hemerobius fenestratus             | 0  | 1  | 1  | 1  | 1  |
| 505| Hemerobius humulinus               | 1  | 1  | 1  | 1  | 1  |
| 506| Hemerobius pini                    | 0  | 1  | 0  | 1  | 1  |
| 507| Hemerobius stigma                  | 1  | 1  | 1  | 1  | 1  |
| 508| Wesmaelius concinnus               | 1  | 1  | 1  | 1  | 1  |

**Neuroptera sp.**

| 509| 0  | 1  | 1  | 0  | 0  |

**ORTHOPTERA**

| PSOCODEA |
|---|
| 510| Sisyra nigra                       | 1  | 0  | 0  | 0  | 1  |

**Trichoptera**

| GOERIDAE |
|---|
| 513| Goera pilosa                        | 1  | 1  | 1  | 0  | 1  |

**Lepidostomatidae**

| 514| Lepidostoma hirtum                 | 1  | 1  | 1  | 0  | 0  |

**Leptoceridae**

| 515| Athripsodes cinereus               | 1  | 1  | 0  | 0  | 0  |
| 516| Ceraclea albimacula                | 1  | 0  | 1  | 0  | 0  |
| 517| Ceraclea annulicornis              | 1  | 0  | 0  | 0  | 0  |
| 518| Ceraclea dissimilis                | 1  | 0  | 0  | 0  | 0  |
| 519| Ceraclea excisa                    | 1  | 0  | 0  | 0  | 0  |
| 520| Ceraclea fulva                     | 1  | 1  | 0  | 1  | 0  |

(Continues)
TABLE 3  Permutational multivariate analysis of variance (adonis) for prey communities for the studied bat species using Bray–Curtis dissimilarity matrix (for RRA) or Jaccard similarity (for presence–absence data) of presence or absence of prey species in each sample. Terms added sequentially (first to last) to the model. The only significant Bonferroni-corrected 𝑝-value (𝑝b) is denoted with an asterisk, indicating that as a whole, the diet changes during the sampling season, although this effect was only observed with the PA data, but not in the RRA data.

| Predictor          | df | 𝑆 | 𝑅2 | 𝑝b |
|--------------------|----|----|-----|-----|
| Relative read abundance data |    |    |     |     |
| Predator           | 4  | 1.46 | 0.12 | 0.0001* |
| Week              | 10 | 0.92 | 0.18 | 0.9544 |
| Predator × Week    | 7  | 0.96 | 0.13 | 0.7598 |
| Residuals          | 29 | 0.57 |       |        |
| Total              | 50 |     | 1.00 |       |
| Presence/absence data |    |    |     |     |
| Predator           | 4  | 1.77 | 0.13 | 0.0001* |
| Week              | 10 | 1.06 | 0.20 | 0.1372 |
| Predator × Week    | 7  | 0.99 | 0.13 | 0.5561 |
| Residuals          | 29 | 0.54 |       |        |
| Total              | 50 |     | 1.00 |       |

Predictor of the sequences were conducted according to Kaunisto et al. (2017). Consequently, paired-end reads were merged (SFF: ∼90% reads successfully merged; ZBJ: ∼85%) and trimmed for quality using program USEARCH with “fastq_maxee_rate” algorithm with threshold 1 (Edgar, 2010). Primers were removed using python program cutadapt (SFF: ∼99% reads passed; ZBJ: ∼96%) (Martin, 2011). We then dereplicated reads using USEARCH “fastx_uniques” algorithm with option “minuniquesize 2”, and then, we applied USEARCH UNOISE3 algorithm to cluster these unique reads into ZOTUs (zero-radius operational taxonomical units; Edgar, 2016). In short, UNOISE algorithm allows the simultaneous a) detection and removal of chimeras (PCR artifacts where two fragments of different origin bind together), point errors (substitutions due to incorrect base calls and gaps due to omitted or spurious base calls), and b) results in ZOTUs (zero-radius OTUs) that are superior to conventional 97% OTUs for most purposes, because they provide the maximum possible biological resolution given the data available (Edgar, 2016). Finally, reads were mapped back to the original trimmed reads to establish the total number of reads in each sample using USEARCH “otutab” algorithm. After processing, our datasets for this study consisted of 5,449,755 prey reads (produced with primers ZBJ-ArtF1c and ZBJ-ArtR2c) and 1,452,602 bat reads (produced with primers SFF-145f and SFF-351r).

The remaining reads (roughly 30% of total output of the sequencing run; ZBJ: 2,618,342 + SFF: 721,684) were used in another study.

We used the following strict criteria for including prey species in the data: (a) Sequence similarity with the reference sequence had to be at least 98% for the ZOTU to be given any (even higher taxa) assignment, and (b) at least ten reads of the final assigned prey species were required to be present in the final data. We assigned the ZOTUs to species as accurately as possible, utilizing a large reference sequence collection orchestrated by the Finnish Barcode of Life campaign (FinBOL: www.finbol.org) and BOLD database (Ratnasingham & Hebert, 2007), and confirmed that all the prey species were actually recorded from (southern) Finland. After the above trimming, we were able to identify and retain 93% of all the prey reads. To account for the even distribution of reads into separate samples, we used ANOVA to test samples from different bat species for differences in the total reads per sample, total prey species richness per sample, and the average number of prey in each pellet (prey richness divided by the number of pooled pellets). The reads originating from bats in the second dataset were used to confirm the bat species identity. The molecular confirmation of bat species revealed a switch in roost occupancy (M. mystacinus to E. nilssonii) in the middle of the sampling season, which resulted in only one pooled sample of M. mystacinus. Also, we removed two mixed samples, containing DNA from two distinct bat species. Labeled raw reads and ZOTUs are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.6880rf1.

A number of metric measurements strongly correlate with the biomass in insects (Garcia-Barros, 2015; Gruner, 2003). Thus, for data on taxon-specific prey size (wingspan for Lepidoptera and thorax length for all the other prey taxa) we referred to earlier dietary studies from Finland (Kaunisto et al., 2017; Vesterinen et al., 2016), or to literature or pictures from reference databases. Wingspan for lepidopteran prey was chosen as it was highly available, accessible, and reliable. The prey taxa where the size could not be determined (e.g., due to a compound taxon that was too large to be reliable or
TABLE 4  Pairwise permutational multivariate analysis of variance (pairwise.adonis) for prey communities for each of the studied bat species using Bray–Curtis dissimilarity matrix (for RRA) or Jaccard similarity (for presence–absence data) of presence or absence of prey species in each sample. Significant Bonferroni-corrected p-values ($p_b$) are denoted with an asterisk. All the bat species pairs significantly differ in their prey species composition, except comparisons with M. mystacinus, which was represented with only one sample.

| Pairs                              | df  | F    | $R^2$ | $p_b$ |
|------------------------------------|-----|------|-------|-------|
| Relative read abundance data       |     |      |       |       |
| Plecotus auritus versus Myotis mystacinus | 11  | 1.29 | 0.11  | 1.00  |
| P. auritus versus M. daubentonii  | 30  | 3.07 | 0.10  | 0.01* |
| P. auritus versus M. brandtii     | 20  | 2.35 | 0.11  | 0.01* |
| P. auritus versus Eptesicus nilssonii | 19  | 2.34 | 0.12  | 0.01* |
| M. mystacinus versus M. daubentonii | 20  | 1.19 | 0.06  | 0.49  |
| M. mystacinus versus M. brandtii  | 10  | 1.03 | 0.10  | 1.00  |
| M. mystacinus versus E. nilssonii | 9   | 1.10 | 0.12  | 1.00  |
| M. daubentonii versus M. brandtii | 29  | 2.24 | 0.07  | 0.01* |
| M. daubentonii versus E. nilssonii | 28  | 1.60 | 0.06  | 0.05* |
| M. brandtii versus E. nilssonii   | 18  | 1.59 | 0.09  | 0.04* |
| Presence/absence data              |     |      |       |       |
| P. auritus versus M. mystacinus    | 11  | 1.16 | 0.10  | 1.00  |
| P. auritus versus M. daubentonii  | 30  | 3.83 | 0.12  | 0.01* |
| P. auritus versus M. brandtii     | 20  | 2.81 | 0.13  | 0.01* |
| P. auritus versus E. nilssonii    | 19  | 2.52 | 0.12  | 0.01* |
| M. mystacinus versus M. daubentonii | 20  | 1.44 | 0.07  | 1.00  |
| M. mystacinus versus M. brandtii  | 10  | 1.21 | 0.12  | 0.88  |
| M. mystacinus versus E. nilssonii | 9   | 1.22 | 0.13  | 1.00  |
| M. daubentonii versus M. brandtii | 29  | 2.55 | 0.08  | 0.01* |
| M. daubentonii versus E. nilssonii | 28  | 2.63 | 0.09  | 0.01* |
| M. brandtii versus E. nilssonii   | 18  | 1.65 | 0.09  | 0.01* |

Informative, such as "Orthoptera sp." were omitted from the prey size analysis. For the predator size analysis, we extracted forearm (FA) length measurements from bat banding data collected from the study area. Forearm length is a standard measurement for bats, and it has been shown to highly correlate with the full body length ($R^2 = 0.933$; Meng, Zhu, Huang, Irwin, & Zhang, 2016). After discarding repeatedly encountered bat individuals, as well as those with unclear identification or no data on size, we ended up with 1,553 distinct individuals from the bat banding data.

2.5 Data analysis

Traditionally, the read count (or read abundance) data produced in metabarcoding studies are directly transformed into presence/absence data, considered to be more cautious and less biased than using read counts. However, the latest opinion on the field seems to suggest that using normalized read abundance data could be even less biased than mere converting to p/a data (Deagle et al., 2018; see also Vesterinen, 2015; Vesterinen et al., 2016). For this reason, we chose to use relative read abundance (RRA: calculated as the proportion of reads per each prey item in each sample). To make the comparison to earlier studies possible, we also prepared the secondary set of analysis using p/a data or more precisely the modified frequency of occurrence (MFO) data throughout the analysis. MFO was calculated as the proportion of occurrences of each prey taxa in each sample scaled to 100% across all prey items (see Deagle et al. (2018) for the terminology and further discussion on the topic).

To begin our data analysis, we calculated prey species accumulation curves to account for sampling adequacy (Colwell & Coddington, 1994). We used R package “iNEXT” to resample the prey reads and frequencies for each bat species and plotted these against accumulated prey species richness (Hsieh, Ma, & Chao, 2016; R Core Team, 2013).

In order to unfold the trophic interactions resolved by the DNA analysis, we used package bipartite (Dormann, Gruber, & Fründ, 2008) implemented in program R to draw interaction webs for each bat predator species using both RRA and MFO data. For those two cases, where two different bat species were observed in the same roost, we constructed additional webs to analyze the diet between separate samples in each location using RRA data. To further estimate patterns among the dietary assemblages of the five species, we used principal coordinates analysis (PCoA) based on Bray–Curtis dissimilarity (Jaccard similarity for presence/absence data) between samples (Davis, 2002; Podani & Miklós, 2002).

Then, to study the effects of predator species and temporal variation (as week number) on variation in prey species composition in each sample, we conducted a permutational multivariate analysis of variance (with Bray–Curtis for RRA and Jaccard for presence/absence data), using 9,999 random permutations to evaluate statistical significance (Anderson, 2001)(PERMANOVA; Anderson, 2001). Analysis of variance was carried out using “adonis” in software R with package “vegan” (Oksanen et al., 2013). Variation was further dissembled using pairwise analysis of variance with package “pairwise.adonis” between all bat species using Bonferroni correction for p-values (Martinez Arbizu, 2017).

Finally, we used information on predator and prey sizes to add dimensions to our attempt to segregate the ecological guilds and predator species. The bat banding data ($n = 1,553$) consisted of unequal sample sizes for the five bat species with unequal variances (Levene’s test for homogeneity of variance: $p = 0.0012$), and thus, to compare the forearm lengths (size) of the five bat species, we used a
Kruskal–Wallis analysis of variance (nonparametric ANOVA) procedure to compare body size (FA length) as a function of predator size using command “kruskal.test” in R (Kruskal & Wallis, 1952). To further study the difference between bat species pairs, we applied the Tukey and Kramer (Nemenyi) test with Tukey-Dist approximation for independent samples with R package “PMCMR” (Pohlert, 2014; Sach, 1997, pp. 395–397, 662–664). The same tests were applied to test prey size (wingspan or thorax length as explained above) differences between the bat species.

### RESULTS

#### 3.1 General aspects of the diet and the study

Altogether, we identified 547 distinct prey species in 13 arthropod orders (Table 1). The main prey order for *M. daubentonii* and *E. nilssonii* was Diptera (56% and 77% of all reads, respectively). For *M. brandii*, *M. mystacinus*, and *P. auritus*, Lepidoptera was the largest prey order (65%, 74%, and 72%, respectively). The only other very abundant prey orders included Trichoptera (15% of reads in *M. daubentonii* diet) and Coleoptera (19% in *P. auritus*). The observed summed prey species richness per bat species varied from 105 prey species to 340 prey species (Tables 1 and 2). From technical point of view, our data show even average distribution of reads across samples (although with high variation), and the average number of prey species per pellet calculated across samples did not differ between bat species (Table 1). The species accumulation curves showed that for *M. mystacinus* the sampling was rather inadequate, but for others more comparable to each other in terms of reads per bat species (Figure 3a), although when using presence/absence data, the curves did not seem to reach the plateau yet (Figure 3b). Nevertheless, we kept *M. mystacinus* in all the analysis, but interpret the results with relevant caution.

### Table 6

| Compared pairs | Bats  
|----------------|------|----------------|----------------|
|                | n = 1,553 |  | n = 1,807 |  | n = 1,642 |  |
| *Plecotus auritus* versus *Myotis mystacinus* | <0.0001 | 0.0008 | 0.9980  |
| *P. auritus* versus *M. daubentonii* | <0.0001  | <0.0001  | <0.0001  |
| *P. auritus* versus *M. brandii* | <0.0001  | <0.0001  | <0.0001  |
| *P. auritus* versus *E. nilssonii* | 0.5700  | 0.0003  | 0.0040  |
| *M. mystacinus* versus *M. daubentonii* | <0.0001  | 0.6635  | 0.2240  |
| *M. mystacinus* versus *M. brandii* | 0.4800  | 0.8516  | 0.1590  |
| *M. mystacinus* versus *Eptesicus nilssonii* | <0.0001  | 0.7223  | 0.3680  |
| *M. daubentonii* versus *M. brandii* | <0.0001  | <0.0001  | 0.9810  |
| *M. daubentonii* versus *E. nilssonii* | <0.0001  | 1.0000  | 0.9580  |
| *M. brandii* versus *E. nilssonii* | <0.0001  | 0.0010  | <0.0001  |

#### Table 5

Tukey and Kramer (Nemenyi) test with Tukey-Dist approximation for independent samples with R package “PMCMR” between all the bat species for bat forearm length, Lepidoptera prey wing span, or other prey body length. The number of records is listed for each group. The significant p-values are bolded (chi-square was corrected for ties)

#### 3.2 Dietary patterns of the studied bats

The quantitative prey assemblages (RRA) seem to be very different for all the bat species, as revealed by the bipartite analysis (Figure 4a). However, when using frequencies (MFO), these patterns are not that clear (Figure 4b). In the current study, different bat species were mainly sampled in different roosts, but luckily prey use does not seem to be vastly related to the roost site, as can be seen from the bipartite analysis from the two sites where two different bat species were sampled from the same roost (Figure 5a,b). The prey use patterns were further illustrated in the PCoA ordinations: Both RRA and presence/absence data ordinations grouped the bat species according to their respective feeding guilds based on differences in the prey species assemblages (Figure 6a,b). In the RRA plotting, first coordinate explained 10.5% and the second coordinate 7.5% of the variation in the data (Figure 6a), and in the plot using presence/absence data, the first and the second coordinates explained 15% and 9.9% of the variation (Figure 6b), respectively, so for
3.3 | Dietary patterns in the feeding guilds

The feeding guilds are also easily separated by looking at the diet at the prey family level (here using percentages from relative read abundance data, but approximately the same ratios can be drawn from the presence-absence data; Table 2): The trawling species (*M. daubentonii*) predominantly consumes a single prey family, Chironomidae (45.8% of all the reads), which is a highly abundant and species-rich family in southwestern Finland (Lilley, Ruokolainen, Vesterinen, Paasivirta, & Norrdahl, 2012; Paasivirta, 2012, 2014), but constrained to the vicinity of aquatic environment, where the bat collects its prey from the water surface (Nilsson, 1997). The gleaner (*P. auritus*) relies on the plentiful moth family Noctuidae (57.2%), which is either caught in flight or from surfaces on vegetation, as some of the prey species are mainly diurnal (Silvonen et al., 2014). The other largely consumed prey family for *P. auritus* was the coleopteran family Carabidae (18.7%), which is most probably foraged from the ground. The third guild, hawks, consists of three bat species (*E. nilssonii*, *M. brandtii*, and *M. mystacinus*), which all have distinct prey family spectrum. *E. nilssonii* is known to be Nematocera specialist (Rydell, 1986), and we can confirm this observation: *E. nilssonii* preyed upon Pediocidae (21.3%), Trichoceridae (18.4%), Tipulidae (13.0%), and also on chironomids (10.7%). The other two hawks relied solely on moths: *M. brandtii’s* menu included Tortricidae (26.5%) and Geometridae (24.3%). Interestingly, at least one very abundant prey species *Agriopis aurantiaria* (Geometridae) only flies during October and after that, so this moth must have been caught by *M. brandtii* as larvae on leaves or while hanging from the tree (Silvonen et al.,...
2014). On the other hand, *M. mystacinus* foraged on the moth families Argyresthiidae (21.0%), Geometridae (16.5%), and Lypusidae (11.3%), which all have distinct life strategies and behavioral ecologies (Silvonen et al., 2014).

3.4 Temporal aspects and predator-prey size analysis

The strong assorting patterns of different bat species seen in plotwebs and PCoA were confirmed when comparing all bat species’ diet’s together in the analysis of variance (Table 3: Predator: RRA data, $df = 4$, $R^2 = 0.12$, $p = 0.0001$; PA data, $df = 4$, $R^2 = 0.05$, $p = 0.0033$). Despite the limited temporal span of the sampling for each bat (Table 1: 8 weeks for *M. daubentonii* and *P. auritus*; 5 weeks for *M. brandtii* and *E. nilssonii*), we tested the dietary variation in time, but found no significant variation between weeks (Table 3: Week). Temporal pattern was same for all bat species (Table 3: Predator $\times$ Week).

When the prey assemblages were analyzed separately in pairwise PERMANOVA between species, the diet was significantly different in all compared pairs, except those with *M. mystacinus*, which was present in the sample with only one sample (Table 4). The same
pattern occurred in both RRA and PA data (Table 4). The diet explained only 6%–13% of the total variance (Table 4).

The bat species differed significantly in size according to the banding data (Figure 7a, Kruskal-Wallis $H = 867.29, df = 4, p < 0.0001$), further confirmed by the pairwise analysis, where all the bats differed from each other significantly (Table 5). Similarly, the prey size differed significantly between bat species (Lepidoptera prey: $H = 118.58, df = 4, p < 0.0001$; other prey $H = 34.5, df = 4,$

**FIGURE 7** (a) Size of adult bats (measured by the length of forearm), (b) size of lepidopteran prey taxa (measured by the wingspan), and (c) size of other than lepidopteran prey taxa (measured by the body length) for each of bat species in the current study. The number of records is denoted for each group.
Diet comparisons between sympatric bat species using molecular methods are still relatively scarce, but often show considerable
overlap in diet, even at the lower taxon level (Krüger, Clare, Greif, et al., 2014; Krüger, Clare, Symondson, et al., 2014; Salinas-Ramos et al., 2015; Ware, 2016). Most studies focus on either closely related species, or species that share a feeding guild, such as the two trawling bats (M. daubentonii and M. dasycneme) in a study by Krüger, Clare, Greif, et al., 2014; Krüger, Clare, Symondson, et al., 2014. In the current study, we compared the diet of five vesperpilionsid bats, representing three different guilds. According to our analysis, all three guilds are clearly evident, with little overlap between the aerial hawks (M. brandtii and E. nilssonii) and the trawling bat (M. daubentonii). These dietary overlaps are likely to be explained by the opportunistic and sporadic consumption of a very few prey items, such as mass-emerging chironomids, moths, mayflies, and caddisflies. Plecotus auritus, the species considered a gleaner and moth specialist, showed a marked difference in PCoA ordination compared to the other two groups. We also discovered a significant difference in the size of prey consumed, with the larger P. auritus consuming larger prey species, whereas the smaller bat, M. brandtii, consumed smaller prey items. This is not surprising as it is generally accepted that the echolocation used by aerial insectivorous bats renders smaller prey items unavailable to larger bats (Brigham, 1991; Waters, Rydell, & Jones, 1995). Additionally, P. auritus, among other members of the genus, possesses a suite of morphological characters (low wing-loading, large pinna, low-frequency hearing), which allow them to use both acoustic gleaning and aerial-hawking foraging strategies to capture prey (Coles, Guppy, Anderson, & Schlegel, 1989; Norberg & Rayner, 1987). It is possible that some noctuid prey individuals have been foraged as larvae, as the flight peak of most noctuid prey in the current study is later than the sampling period. Also, the importance of a comprehensive reference library (Mutanen et al., 2012; Pentinsaari, Hebert, & Mutanen, 2014; Pilipenko, Salmela, & Vesterinen, 2012), which allows the correct and reliable identification of most prey items, needs to be pointed out once more. This offers the possibility of deeper ecological dietary studies, such as prey size analysis (Pentinsaari et al., 2014). While some prey items had not been described with a scientific species-level name in this study, a reliable estimate of their size could be inferred using the so-called barcode index numbers (BIN; Ratnasingham & Hebert, 2013) to trace the images for measurements. This emphasizes the significance of public and easy-accessible reference library systems, such as BOLD (Ratnasingham & Hebert, 2007). Although some studies still rely on OTUs (operational taxonomical units) instead of biological species, we highlight the importance of actual prey species determination, which allows a deeper and more robust insight into dietary ecology.

The molecular work carried out in this analysis not only highlights the deep insight offered by metabarcoding, but also underlines the dynamic and complementary nature of DNA-based analysis. Based on our earlier field work, we had chosen species-specific roosting sites for the diet analysis of five bat species, to obtain an equal sampling effort. However, when confirming the fecal “donor” by the means of metabarcoding, we noticed some discrepancies between the field data and confirmed data, that is, our M. mystacinus roost was confirmed as an E. nilssonii roost. In future, the molecular confirmation of noninvasively collected samples should be a standard approach, either by traditional Sanger sequencing or cost-effective next-generation sequencing (NGS), depending on the number of samples and the predator and prey species. Also, the importance of a comprehensive reference library (Mutanen et al., 2012; Pentinsaari, Hebert, & Mutanen, 2014; Pilipenko, Salmela, & Vesterinen, 2012), which allows the correct and reliable identification of most prey items, needs to be pointed out once more. This offers the possibility of deeper ecological dietary studies, such as prey size analysis (Pentinsaari et al., 2014). While some prey items had not been described with a scientific species-level name in this study, a reliable estimate of their size could be inferred using the so-called barcode index numbers (BIN; Ratnasingham & Hebert, 2013) to trace the images for measurements. This emphasizes the significance of public and easy-accessible reference library systems, such as BOLD (Ratnasingham & Hebert, 2007). Although some studies still rely on OTUs (operational taxonomical units) instead of biological species, we highlight the importance of actual prey species determination, which allows a deeper and more robust insight into dietary ecology.

The main drawbacks of the molecular methods are the highly challenging interpretations of the quantitative aspects of the diet, that is, are the most frequently consumed prey items also the most important in terms of biomass and energy gain? While the current practice in many molecular ecological dietary studies using metabarcoding appears to mostly rely on frequency of occurrence (but see Vesterinen et al., 2016), the read counts may actually hold some important quantitative information (Deagle et al., 2018). Here, we tested our data using both frequency of occurrence and read count data and found no major differences in the outcome of the analysis, or more importantly, in the interpretation of the results. This suggests our data have strong ecological message that holds despite the methodological approach used.

Our study supports the existence of dietary flexibility in generalist bats and dietary niche overlapping, especially in bats of the same feeding guild in a highly seasonal ecosystem (Roswag et al., 2018). In fact, it could be the flexibility in feeding strategies which allows species
to sustain populations in arctic and subarctic regions (Shively et al., 2017). Additionally, a great proportion of niche differentiation most likely also occurs outside the diet dimension where an almost infinite number of possible axes exist for competing species in the $n$-dimensional niche hyper-volume (Hutchinson, 1957). Even minor differences in a number of different axes can result in a substantial overall difference (Privitera et al., 2008). Clearly, the next logical step is to utilize deep dietary analysis, alongside other ecological (LIDAR: light detection and ranging method, etc.) and behavioral (GPS-tracking) datasets to begin to understand niche realization and resource partitioning in species to a far higher accuracy than has been available to date.

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AUTHOR CONTRIBUTIONS

EJV and TML designed the study, collected the data, and wrote the first version of manuscript. ASB collected samples in the field and gathered prey species measurements and the map data. AIEP and EJV conducted the molecular work and data analysis. All authors contributed to the final version of the manuscript.

DATA ACCESSIBILITY

Labeled raw reads and OTUs are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.6880rf1.

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