

RESEARCH PAPER

# More intraguild prey than pest species in arachnid diets may compromise biological control in apple orchards



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## Abstract

Understanding the full diet of natural enemies is necessary for evaluating their role as biocontrol agents, because many enemy species do not only feed on pests but also on other natural enemies. Such intraguild predation can compromise pest control if the consumed enemies are actually better for pest control than their predators. In this study, we used gut metabarcoding to quantify diets of all common arachnid species in Swedish and Spanish apple orchards. For this purpose, we designed new primers that reduce amplification of arachnid predators while retaining high amplification of all prey groups. Results suggest that most arachnids consume a large range of putative pest species on apple but also a high proportion of other natural enemies, where the latter constitute almost a third of all prey sequences. Intraguild predation also varied between regions, with a larger content of heteropteran bugs in arachnid guts from Spanish orchards, but not between orchard types. There was also a tendency for cursorial spiders to have more intraguild prey in the gut than web spiders. Two groups that may be overlooked as important biocontrol agents in apple orchards seem to be theridiid web spiders and opilionids, where the latter had several small-bodied pest species in the gut. These results thus provide important guidance for what arachnid groups should be targets of management actions, even though additional information is needed to quantify all direct and indirect interactions occurring in the complex arthropod food webs in fruit orchards.

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**Keywords:** Gut metabarcoding; Philodromus; Opiliones; Theridiidae; Araneidae

## Introduction

The consensus among scholars studying ecosystem services in agricultural landscapes is that management should be designed to promote the diversity and abundance of natural enemies, as a means to reduce pest attack and damage to

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crops (Herz et al., 2019; Landis, Wratten, & Gurr, 2000; Michalko, Pekár, & Entling, 2019; Naranjo, Ellsworth, & Frisvold, 2015; Snyder, 2019; Tscharnkte et al., 2016). This focus on non-chemical means to reduce pest damage is well motivated, both because negative side effects from chemical pesticides are common but also because of the high loss rates in many crops caused by pest damage if unchecked (Cross, Fountain, Marko, & Nagy, 2015; Oerke, 2006; Pimentel, 2005; Sharma et al., 2020). In fact, natural enemies may provide more than \$5.5 billion worth of benefits to humans across all crops in the US alone (Losey & Vaughan, 2006; Naranjo et al., 2015).

Despite this consensus, the reality is that we often lack information on the efficiency or even diet of natural enemies in agricultural fields, what species significantly feed on pests and whether negative side effects through indirect interactions in food webs, such as intraguild (IG) predation on other natural enemies, outweigh positive effects from the consumption of pest species (Garcia, Olimpí, Karp, & Gonthier, 2020; Grass, Lehmann, Thies, & Tscharnkte, 2017; Martin, Reineking, Seo, & Steffan-Dewenter, 2013; Rosenheim, Kaya, Ehler, Marois, & Jaffee, 1995; Straub, Finke, & Snyder, 2008). For instance, Grass et al. (2017) showed that tree sparrows disrupt biocontrol by preferentially feeding their chicks with aphid predators. In their study, one important aphid predator, syrphid larvae, actually increased following bird exclusions. One group of iconic biocontrol agents with highly variable and often poorly known diets are spiders and other Arachnida. Spiders and other arachnids are common and voracious predators in most terrestrial ecosystems, and the global consumption by spiders has been estimated to exceed 400 million tons of prey annually, thus exceeding the total meat consumption by humans (Nyffeler & Birkhofer, 2017). From a biocontrol perspective, however, arachnids may be poor friends as their broad diet may include both predatory insects, parasitoids, other spider species and even juveniles of their own kind (Hodge, 1999; Korenko & Pekár, 2010; Mezöfi, Markó, Nagy, Korányi, & Markó, 2020; Saqib, Liang, You, & Gurr, 2021), and such intraguild predation may reduce the top-down control of herbivore populations (Finke & Denno, 2005; Schmidt-Entling & Siegenthaler, 2009).

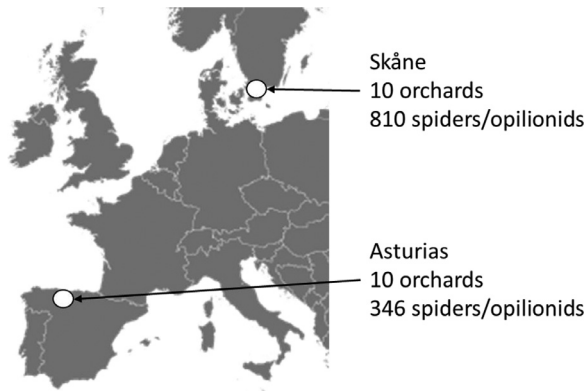
The limited data on IG predation from many crop production systems affect our ability to estimate the biocontrol potential of different natural enemies and therefore to design cropping systems that selectively promote those natural enemies that are most beneficial (Michalko et al., 2019; Straub, Finke, & Snyder, 2008). One reason for the sparsity of quantitative data on IG predation has been the logistical difficulty of collecting accurate diet data. Diet data through direct observations or morphological determination from gut contents are very time consuming to collect (Mezöfi et al., 2020), and are often biased because larger prey items are more easily detected. The development of molecular tools, however, has drastically increased our capacity to collect

and process diet data; where even small amounts of prey DNA in predator guts can be identified and quantified (Liu, Clarke, Baker, Jordan, & Burrige, 2020; Nielsen, Clare, Hayden, Brett, & Kratina, 2018; Pompanon et al., 2012). Studies using diet metabarcoding have previously shown the diversity of prey in spider diets (Cuff et al., 2021; Verschut, Strandmark, Esparza-Salas, & Hambäck, 2019), even though quantitative estimation of diet from metabarcoding data should be treated cautiously (Deagle et al., 2019). Previous studies, however, typically focus on single, or a few related, spider species, with a limited focus on the broader network perspective that would allow us to separate the roles of different enemy species (but see Roubinet et al., 2017).

In this study, we used DNA metabarcoding to estimate the relative importance of IG prey, pest species and other prey in spider and opilionid diets in Swedish and Spanish apple orchards with the aim to identify predators with biocontrol potential and groups that rather counteract biocontrol by consuming other predators. The reason for focusing on spiders and opilionids is that these are among the most abundant natural enemies in fruit orchards (Happe et al., 2019), and have often been implicated as important biocontrol agents (Cross et al., 2015). When studying the function of predators in food webs, we should separate the mechanisms underlying diet choice and the prey's functional role. Predators seldom select prey based on the prey's feeding mode but rather on traits determining catchability and profitability, even though feeding mode may correlate with these other traits (Matsumura et al., 2004). For instance, spiders do not catch heteropteran predators because these are predators but because heteropterans are suitable prey. On the other hand, spiders may avoid attacking other spiders just because they also pose a risk for the attacker (Polis, Myers, & Holt, 1989). For these reasons, we analyzed predator diets from the perspective of prey taxonomy separately from the prey functional role.

## Materials and methods

*Study sites and sample collection:* Spiders and opilionids were collected in 10 apple orchards in south Sweden (4 organic and 6 with integrated pest management) and 10 low input orchards in northwestern Spain (Fig. 1), within the research program EcoFruit (see also Samnegård et al., 2019). The motivation for including these orchards was logistic feasibility, because we had already collected density data on spiders and prey in a separate project (Hambäck, Porcel, Tasin, & Samnegård, 2021; Happe et al., 2019), and to include regional variability. In these previous projects, we estimated arthropod densities (predators and potential prey) by beating one representative apple branch (height 1.5–2 m) on 24 trees per orchard once within 2 weeks after peak flowering. Here, we complement the density data with diet analyses from predator guts. Spiders and



**Fig. 1.** Map showing the location of study regions. In Sweden, sampled orchards included some with organic production ( $N = 4$ ) & others managed according to IPM ( $N = 6$ ). In Spain, sampled orchards ( $N = 10$ ) used chemical control but at very low input. Orchards varied in size (1–20 ha) & apple trees were 5–20 years old.

opilionids were collected individually by hand-picking from leaves (Sweden) or from beat sampling sheets (Spain) in the same part of the orchards as the density data. In both cases, spiders and opilionids were directly transferred to individual tubes with 95% ethanol to reduce contamination risk. Collected individuals were identified to the lowest taxonomic level possible, to species for adults and to genus or family for most juveniles. The intention was to compare prey availability from beat sampling with predator diets to estimate prey selection, but because beat sampling bias against flying species such as Diptera and Hymenoptera (Hambäck et al., 2021), and against small-bodied taxa such as mites, Collembola and Thysanoptera, we present density data separately and do not perform selection analyses. Estimated densities correctly show differences within groups and between sites, and are compared as such, but not between groups and within sites.

**Gut metabarcoding:** Details for primer design, PCR amplification and library building are provided in Appendix A and summarized here. DNA was extracted from dissected abdomens, for larger specimens, and whole abdomens for smaller specimens. To detect and barcode prey DNA, we sequenced three sections, depending on predator taxon, within the Folmer region of COI (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994), using two new primers designed to reduce amplification of spiders, except for Lycosidae, (NoAranR) and opilionids (NoOpiF) respectively, and one previously developed primer designed for Lycosidae (NoSpi2, Lafage et al., 2020), combined with general forward or reverse primers. The theoretical and realized amplification successes of novel primers on relevant prey groups were evaluated in silico using PrimerMiner (Elbrecht & Leese, 2017) compared with downloaded sequences from GenBank ([www.ncbi.nlm.nih.gov/genbank](http://www.ncbi.nlm.nih.gov/genbank)), and *in vitro*, through PCR of reference prey (Appendix A: Table 1 & 2). The primers successfully amplified almost all prey groups,

**Table 1.** Summary of predation events (PE) & sequence counts for recorded prey taxa (Taxa# = number of unique species except for Acari where taxon identity is unclear) in arachnid guts from Spanish & Swedish apple orchards.

Prey taxon group	Taxa #	Spain		Sweden	
		PE	Sequence #	PE	Sequence #
Acari	18	20	5893	185	125,701
Annelida	5			5	1248
Araneae	42	74	42,625	362	631,122
Chilopoda	1			3	7981
Coleoptera	39	50	43,979	80	155,001
Collembola	18	11	10,945	293	555,842
Dermoptera	1			24	71,965
Diptera	187	384	978,153	608	1482,394
Ephemeroptera	1			4	4637
Gastropoda	2			11	4060
Auchenorrhyncha	14	35	56,453	52	92,338
Sternorrhyncha	23	38	5660	204	159,645
Heteroptera	29	115	356,042	94	302,487
Hymenoptera	70	63	34,047	184	189,702
Isopoda	4			12	42,901
Lepidoptera	52	22	26,067	124	290,895
Neuroptera	5			30	27,951
Opiliones	3	1	15	2	18
Orthoptera	1			7	572
Psocoptera	19	85	71,031	47	41,483
Thysanoptera	9	18	12,321	136	286,608

except for Psyllidae and Aphididae, two important pests on apple. We therefore complemented gut metabarcoding with a diagnostic PCR for *Cacopsylla mali* (for spiders) and *Dysaphis plantaginea* (for opilionids) (Appendix A).

In the PCR amplification, NoAranR (reverse primer 5′–3′ TGTTTCATCCDGTNCCWG) combined with the forward primer LCO146 amplified a 317 bp fragment, NoOpiF (forward primer 5′–3′ CCHGAYATAGCWTTYCCHC-GAATA) combined with the reverse primer fwR2n (Vamos, Elbrecht, & Leese, 2017) amplified a 306 bp fragment, and NoSpi2 (forward primer, Lafage et al., 2020) combined with fwR2n amplified a 295 bp fragment. For post-process identification of individual spider guts, we used a dual tagging approach. In the PCR, an eight base pair–tag was included on the 5′–end of the primers (Binladen et al., 2007) and thereafter Illumina–adaptors bearing unique indices were ligated to the phosphorylated amplicons. Sequencing were done in two batches using parallel high throughput sequencing on the Illumina MiSeq3 platform at SciLifeLab in Stockholm, one involving NoAranR (477 specimens) and one involving NoOpiF and NoSpi2 (429 specimens).

We processed sequence data using OBITOOLS 1.2.9 (Boyer et al., 2016) as described in Verschut, Strandmark, Esparza-Salas, & Hambäck, 2019 and summarized here. PE-sequences with a minimum combined quality score of 40 were assembled, trimmed from PCR primers and

**Table 2.** Mean percent sequences of different prey groups in guts of common arachnid families (Sweden/Spain) from metabarcoding, calculated from lme's. If there is only one F- & p-value for a family, this means that the family-by-region interaction was non-significant ( $p > 0.05$ ) & percent sequences are means across both regions. If there are two F- & p-values, the first value shows the mean for Swedish spiders & the second value shows the mean for Spanish spiders. Bold & underlined F- & p-values indicate that predatory families have different percent sequences ( $p < 0.05$ ), & bold & underlined percentages indicated differences compared to other families. Sample size indicates the number of analyzed spider guts.

	F-value (p)	Opiliones	Araneidae	Clubiona	Philodromus	Salticidae	Theridiidae	Thomisidae
Sample size		334/14	24/34	13/10	125/17	-/18	20/94	17/18
Acari	<b><u>6.9 (0.0001)</u></b>	<b><u>5.6</u></b>	0	0.7	2.4	0	1.3	0.8
Auchenorrhyncha	<b><u>3.4 (0.005)</u></b>	2.7	3.7	0	1.4	<b><u>17.8</u></b>	1.0	0
Sternorrhyncha	1.7 (0.1)	3.2	0.4	2.3	5.3	0.9	4.6	0.6
Psocoptera	1.8 (0.1) /	0.7/	3.5/	0/	4.3/	-/	0/	0/
	<b><u>4.6 (0.002)</u></b>	<b><u>23.9</u></b>	1.1	0	0.6	2.9	10.5	7.1
Heteroptera	1.2 (0.3) /	3.8/	1.8/	2/	5.3/	-/	1.8/	0.3/
	<b><u>5.3 (0.0005)</u></b>	18.9	1.9	17.2	<b><u>48.0</u></b>	<b><u>42.1</u></b>	18.3	1.0
Nematocera	<b><u>2.9 (0.01)</u></b>	19.5	30.8	17.2	15.0	<b><u>4.6</u></b>	24.0	<b><u>4.3</u></b>
Brachycera	2.0 (0.1) /	11.4/	23.9/	22.3/	7.6/	-/	24.7/	2.8/
	<b><u>8.0 (0.0008)</u></b>	3.9	<b><u>46.0</u></b>	20.5	10.8	12.2	26.4	<b><u>46.5</u></b>
Formicidae	<b><u>3.9 (0.002)</u></b>	1.8	0.6	0	0.6	0	2.2	<b><u>15.0</u></b>
Collembola	1.1 (0.4)	11.9	4.4	4.7	5.3	4.2	6.0	5.2
Thysanoptera	<b><u>10.0 (0.0001)</u></b> / 1.4 (0.3)	1.2/	13.6/	<b><u>34.7/</u></b>	<b><u>42.6/</u></b>	-/	10.5/	15.5/
		0	1.2	9.0	1.8	0.6	0.2	0
Lepidoptera	<b><u>2.6 (0.04)</u></b> /	6.0/	7.4/	3.7/	1.3/	-/	0.4/	2.3/
	1.6 (0.2)	0	0.4	0	4.1	7.4	5.5	2.2
Web spiders	<b><u>3.9 (0.006)</u></b> / <b><u>6.2 (0.0001)</u></b>	<b><u>8.4/</u></b>	0/	5.5/	2.3/	-/	0.7/	0/
		<b><u>26.7</u></b>	1.1	4.1	0.4	3.9	1.5	2.5
Cursorial spiders	<b><u>9.2 (0.0001)</u></b> / 0.6 (0.8)	4.1/	0.2/	3.0/	0/	-/	6.2/	<b><u>50.7/</u></b>
		2.6	3.8	0	11.4	0	1.7	0

demultiplexed after filtering into individual samples according to their respective tagged primer and index combination using NGSFILTER. We dereplicated the reads with the “obiuniq” command and filtered the data for PCR errors with the “obiclean” command by keeping “head” sequences (sequences with no variants with a count  $> 5\%$  of their own count) and discarding singletons and erroneous sequences (sequences with a count  $< 5\%$  of their own count; Boyer et al., 2016). We used the compacted data set (13,741,954 sequences), containing representative sequences with information on abundance per PCR sample, for taxonomic assignment.

We identified unique sequences (22,152) using a 97% similarity threshold in the Barcode of Life Database ([www.boldsystems.org](http://www.boldsystems.org) (Ratnasingham & Hebert, 2007)), and pooled sequence counts to lowest taxon identity. Some sequences could not be sorted to species, due to low variability in target sequences. A very small number of sequences (1600) were discarded as likely contaminations (human, cow and bird DNA). Sequences identified as plant identities (35,055; mainly *Malus* sp.), fungal or bacterial sequences (195,325; mainly *Wolbachia* sp.) were discarded. Primers also amplified consumer sequences and we discarded sequences (4988,218) belonging to the same genus as the consumer individual. Discarded sequences mainly came from *Philodromus* when using NoAranR and Opiliones when using NoOpIF.

These cleaning procedures removed roughly half of all sequences, leaving 6152,034 sequences for further analysis. As a final cleaning step, we used a probabilistic approach to remove potential tag jumping errors through a combination of fixed and species-specific thresholds. In the first step, we excluded prey with less than 6 sequences detected in the consumer individual. In the second step, we assumed that other errors (such as tag jumping) were related to the number of retrieved sequences for the prey. Thus, we estimated a prey-specific threshold based on the number of sequences for the given prey and the number of predators from which sequences of this prey were retrieved (Cirtwill & Hambäck, 2021). For example, assume that 10,000 sequences were retrieved for prey A from a total of 10 guts. With a 2% error rate, the threshold for prey A would be  $(10,000 \times 0.02 / 10 = 20)$ , and prey A was removed from the predator diet when having less than 20 scored sequences. Notice that these last cleaning steps only removed about 35,000 sequences.

Translating reads to semi-quantitative diet estimates can be based both on read number and prey presence/absence, each with separate problems (Deagle et al., 2019). We calculated both estimates for each consumer individual; the proportion of prey sequences (hereafter sequence number) and the number of predation events on a given prey (hereafter predation events). A predation event is a binary estimate assuming that all sequences from a prey species retrieved



from a predator individual comes from the consumption of one prey individual (see also [Verschut, Strandmark, Esparza-Salas, & Hambäck, 2019](#)). These diet estimates were used to calculate mean dietary proportions at the level of predator family per site, where dietary proportions were based on prey taxonomy or coarse functional groups (IG prey, apple pest, other herbivore, detritivores, fungivores and semi-aquatic species) (see Appendix A: Table 3 for details). Apple pests were defined broadly as species that have been recorded feeding on apple, whether they are considered pests or not.

Some sequences were likely resulting from secondary predation or parasitism. For instance, sequences from a parasitic wasp may have arrived in a spider gut from consumption of the wasp itself, a parasitized host or a generalist that had consumed the wasp species. Such secondary predation cannot be identified, but was likely in some cases where host and wasp DNA were found in the same spider. However, co-occurrence of host and wasp DNA cannot resolve whether the spider has eaten a parasitized host or a wasp with remnant host DNA, but both cases indicate death of the parasitoid. The only parasitoids excluded were a few instances of known spider parasitoids (*Sinarachna pallipes* and *Zatyota percontatoria*).

**Statistical analyses:** To compare prey taxonomic composition within guts and between predator families, we used a PERMANOVA implemented with `adonis2` (with option ‘by=margin’) in `vegan` ([Oksanen et al., 2019](#)) with relative prey occurrences within site as response variable and predator family (Opiliones were only represented by Phalangidae) and study region (Spain and Sweden) as explanatory variables. Because initial tests indicated interaction effects between predator family and region, analyses were run separately for the two regions. When analyzing the Swedish data set, we included orchard type (IPM vs. Organic) as explanatory variable. Differences in diet composition were illustrated using Non-Metric Dimensional Scaling (NMDS) and a heatmap, and further compared statistically in separate linear mixed effects models (lme’s) for major prey groups with predator family, region and family-by-region as fixed effect and orchard as random effect, weighted by the number of analyzed specimens per orchard. When the family-by-region interaction was significant, separate analyses were performed for each region. In the lme’s, we only included prey groups with >5% content in any spider or opilionid family and p-value thresholds were sequentially Bonferroni-corrected based on 11 tested prey groups (subclass for mites, suborder for Diptera and Hemiptera and order for other groups). To compare diet composition between predator families based on prey function, we similarly performed lme’s on IG prey, apple pests, other herbivores, fungivores, detritivores and prey with semi-aquatic larvae with predator family as fixed effect and orchard as random effect. Finally, to compare arthropod abundance between Spanish and Swedish orchards, we performed `adonis2` with region as explanatory variable. For PERMANOVA tests, we used `betadisper` to confirm multivariate homogeneity. For lme’s, we

confirmed residuals normality and homogeneous distribution. All statistics were performed in R 3.6.3 ([R Core Team, 2020](#)).

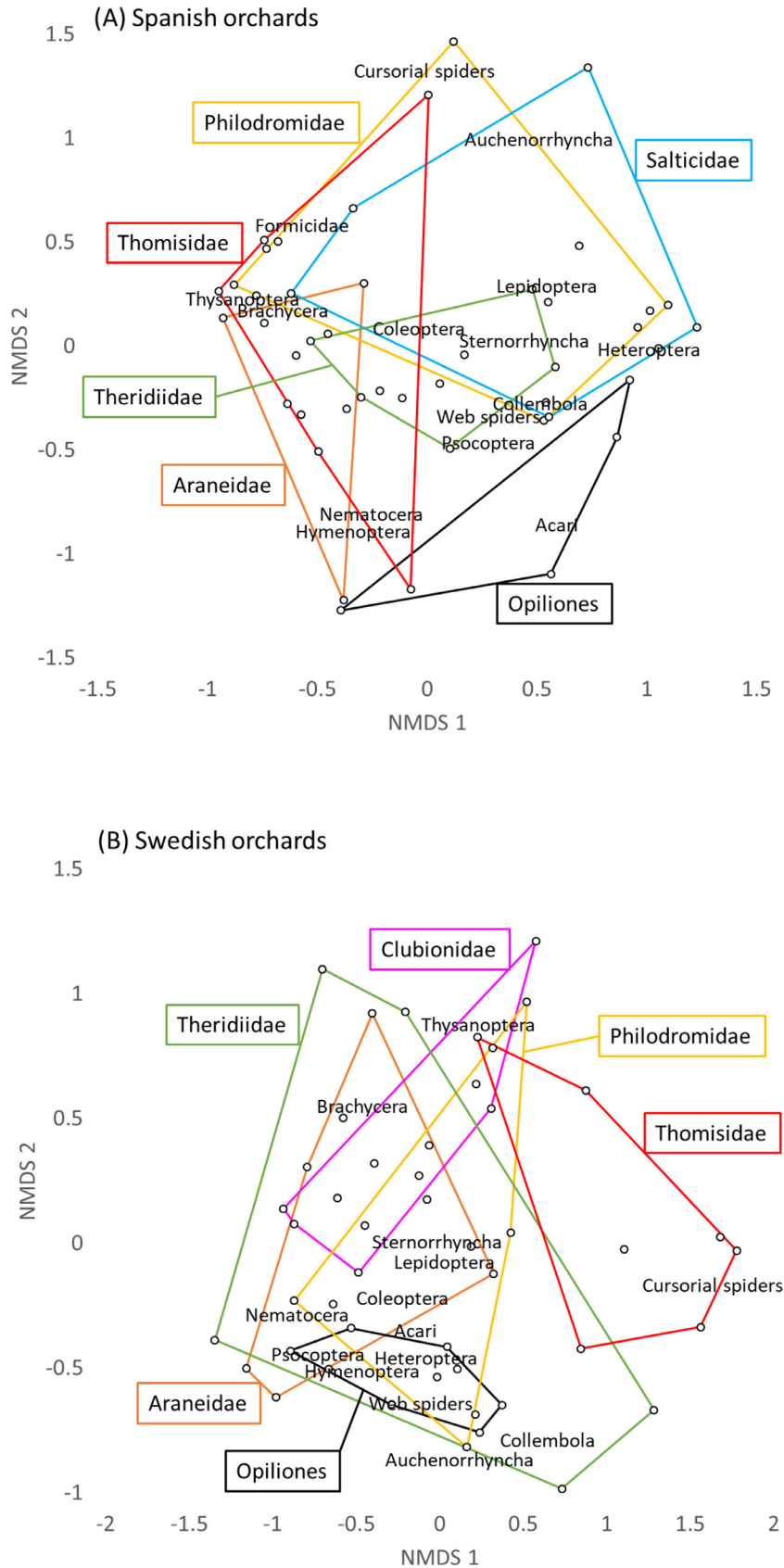
## Results

After cleaning, and excluding predator individuals with no remaining prey sequences, we retrieved 6117,782 prey sequences (or 3383 predation events) from 788 spider and opilionid individuals (about 7760 prey sequences per predator) belonging to >61 species for further analysis (for species lists see Appendix A: Tables 3 & 4). In Spain, sampled predator groups were dominated by web spiders: Theridiidae (tangle-web spiders,  $N = 90$ ) and Araneidae (orb-weaver spiders,  $N = 34$ ), followed by cursorial spiders: Thomisidae (crab spiders,  $N = 18$ ), Salticidae (jumping spiders,  $N = 18$ ), *Philodromus* (running crab spiders,  $N = 17$ ) and Opiliones (harvestmen,  $N = 14$ ). In Sweden, the most sampled groups were instead Opiliones ( $N = 334$ ) and *Philodromus* ( $N = 125$ ), followed by Araneidae ( $N = 24$ ), Theridiidae ( $N = 20$ ), Thomisidae ( $N = 17$ ) and *Clubiona* (sac spiders,  $N = 13$ ). The age structure of Opiliones and *Philodromus* differed between regions, with adult Opiliones (65%) and juvenile *Philodromus* (96%) dominating in Swedish samples and juvenile Opiliones (100%) and adult *Philodromus* (88%) dominating in Spanish samples. Other groups consisted mainly of adult spiders.

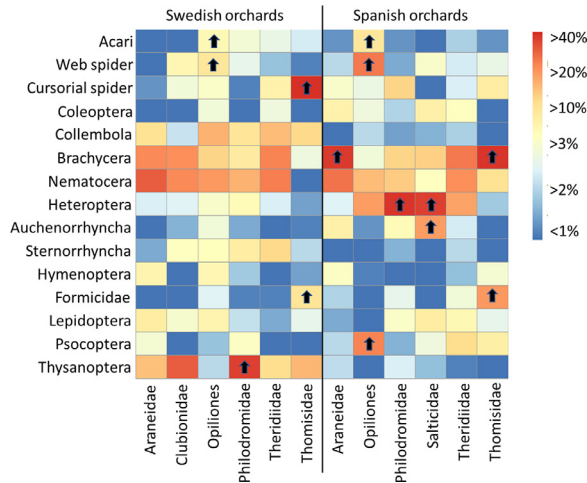
Because sequence number and predation events correlated across prey (Appendix A: [Fig. 1](#)), we only present results for sequence number. The largest number of identified prey sequences in spider and opilionid guts were Diptera (38%), Heteroptera (11.5%) and Araneae (11%), but sequences were also identified to mites, earthworms, slugs, isopods and centipedes ([Table 1](#)). In total, we recorded 543 prey taxa in the guts and the vast majority were identified to species or sets of closely related species, except for mites that rarely could be identified beyond family. In the species count, there was a majority of Diptera (187 species), Hymenoptera (70 species) and Lepidoptera (52 species) ([Table 1 & Appendix A: Table 3](#)).

## Analyzing predator diets based on prey family

Because some spider families were poorly sampled, we limit our analyses to opilionids and the five most sampled spider groups per region. The first PERMANOVA showed that region and predator group interactively affected gut content composition ( $F_{10,108}=2.2$ ,  $p<0.001$ ). When splitting the data set, we found that predator groups differed in gut contents in both regions (Spain:  $F_{4,44}=3.5$ ,  $p<0.001$ ; Sweden:  $F_{5,41}=3.7$ ,  $p<0.001$ ). The NMDS suggested that the gut content compositions were consistently different between spiders and opilionids for Spanish but not Swedish orchards, connected to several prey groups, and between specific spider families for both Spanish and Swedish orchards ([Fig. 2](#)).



**Fig. 2.** NMDS plots based on mean diet proportions within orchards, for (A) Spanish & (B) Swedish apple orchards. Arachnid groups belong to three broader categories: web spiders (Araneidae & Theridiidae), cursorial spiders (Clubionidae, Philodromidae, Salticidae & Thomisidae) & harvestmen (Opiliones). Points indicate family mean per orchard.



**Fig. 3.** Heat map showing diet proportions (means weighted by the square-root of the number of individuals analyzed per site) based on number of sequences for the six most common arachnid groups in Spanish & Swedish apple orchards. Arrows indicate prey groups that were more common ( $P < 0.05$ ) in the gut of this arachnid group compared to other arachnid groups, & black arrows indicate significances retained at the Bonferroni-corrected threshold ( $P < 0.0045$ ).

In Sweden, there was also an effect of management ( $F_{1,41}=2.6$ ,  $p < 0.02$ ). The heatmap suggested different prey composition between regions (Fig. 3); Collembola, Sternorrhyncha and Thysanoptera were more common in guts from Swedish orchards whereas Formicidae, Heteroptera, Coleoptera and Psocoptera were more common in guts from Spanish orchards. These patterns were illustrated in a food web (Figs. 4 & 5), showing that the quantitatively most important prey items were Diptera (particularly Brachycera) and Heteroptera in Spanish orchards, and Diptera and Thysanoptera in Swedish orchards. There were also differences among predators in their use of dipteran groups (Fig. 5).

When comparing arthropod communities between regions, the beat sampling indicated regional differences ( $F_{5,39}=3.5$ ,  $p < 0.001$ ); Auchenorrhyncha, Coleoptera, Formicidae, Heteroptera and Psocoptera were more abundant in Spanish orchards, whereas Diptera and Lepidoptera were more abundant in Swedish orchards (Fig. 6).

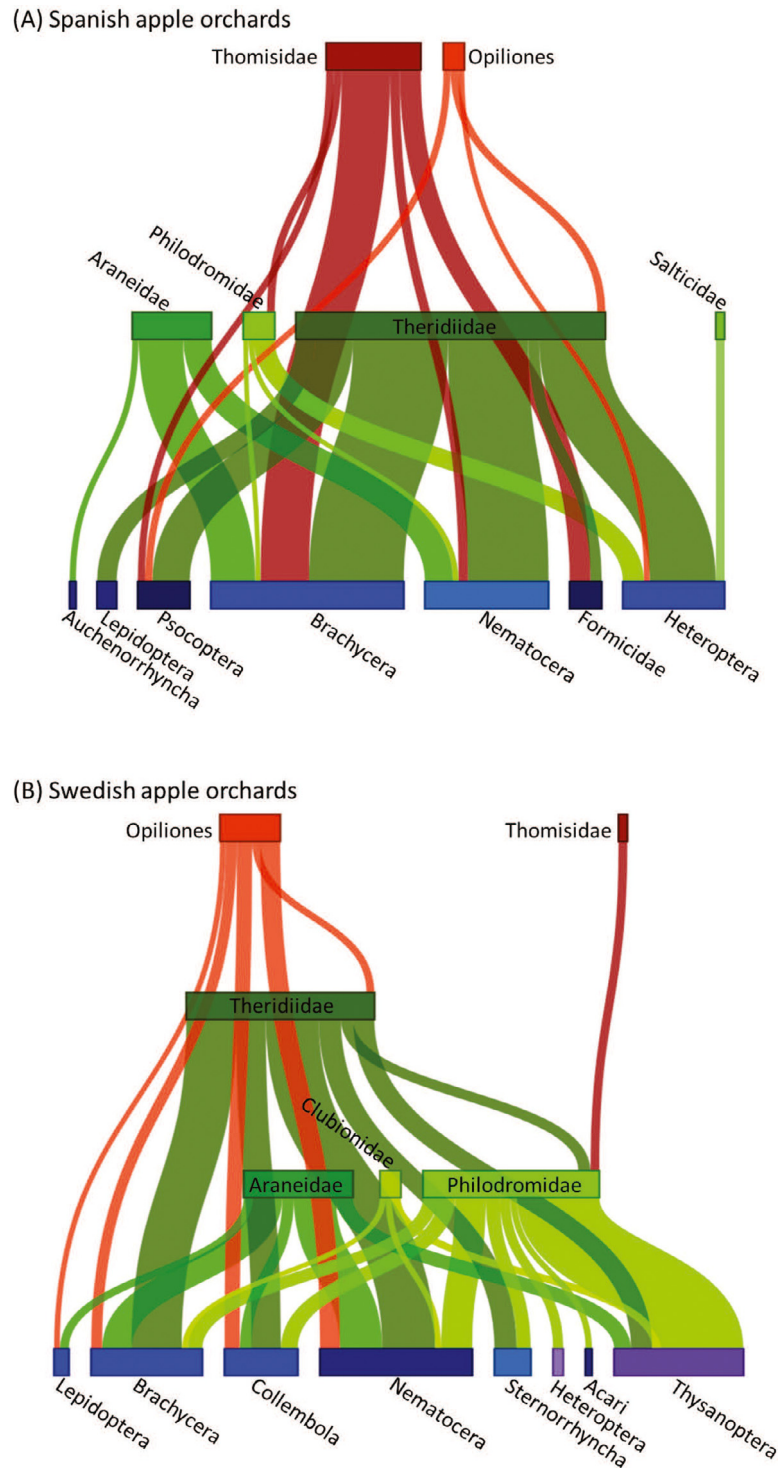
When comparing diets using lme's, patterns observed in the PERMANOVA were repeated with some additional family differences (Fig. 3, Table 2). The family-by-region interaction was significant for Brachycera, Heteroptera, Psocoptera, Thysanoptera, Web spiders and Cursorial spiders, and for these groups we separated the analysis by region. For both regions, opilionid guts contained higher proportions of web spider and Acari sequences compared to spider guts. Other consistent differences between regions were that Salticidae contained higher proportions of Auchenorrhyncha compared with other spiders and opilionids and thomisid spiders contained a higher proportion of Formicidae than other groups. In Spain, opilionid guts also contained higher proportions of Psocoptera sequences than spider guts.

Diet differences for other predator groups were less consistent between regions (Table 2). In Sweden, Thomisidae guts contained higher proportions of cursorial spider sequences (mainly *Philodromus*) than guts from other groups, and guts from *Philodromus* and *Clubiona* contained higher proportions of Thysanoptera sequences than guts from other groups. In Spain, Araneidae and Thomisidae guts contained higher proportions of Brachycera sequences than guts from other groups, and *Philodromus* and Salticidae guts contained higher proportions of Heteroptera sequences than guts from Theridiidae, Araneidae and Thomisidae. Finally, in Sweden we found that guts contained a higher proportion of Heteroptera in organic (6.3%) than IPM (1.6%) orchards ( $p < 0.005$ ), whereas other prey groups were equally common between organic and IPM orchards ( $p > 0.1$ ).

### Analyzing predator diets based on prey functional groups

When analyzing prey data based on prey's functional role, it was apparent that IG predation was very common for both opilionids and spider groups. On average, 33.5% of prey sequences in predator guts belonged to other predator species. About half of the IG prey (16.4%) were predators such as spiders, but sizable fractions were heteropteran bugs (9.4%) and hymenopteran parasitoids (6.0%). Interestingly, IG prey were considerably more common in guts from Spanish (44.2%) than from Swedish (24.4%) specimens ( $F_{1,18}=21.6$ ,  $p < 0.0003$ ), mainly due to a higher proportion of predatory Heteroptera (several anthocorid and mirid species, Appendix A, Table 3) in guts from Spanish (16.7%) than from Swedish (2.9%) specimens ( $F_{1,18}=23.0$ ,  $p < 0.0002$ ). In Sweden, Thomisidae had the highest content of IG prey (51%), almost exclusively *Philodromus* spiders. In Spain, no significant differences in IG prey were observed between predator groups, but proportions were very high in *Philodromus* guts (71.9%).

In total, 14.1% of sequences in gut samples belonged to herbivore species known to feed on apple, with the most common being Thysanoptera (9.5% - in 128 guts), Lepidoptera (1.8% - 63 guts), aphids (1.2% - 20 guts, mainly *Dysaphis plantaginea* and *Aphis pomi*) and *Cacopsylla mali* (0.8% of sequences, 85 guts) (Appendix A: Table 3). The lme indicated an interaction of region and predator family, because the proportion of apple pests was uniformly low (1–6%) in guts from Spanish orchards ( $F_{6,33}=1.0$ ,  $p > 0.4$ ) while being higher (22.8%) and more variable in guts from Swedish orchards ( $F_{6,33}=9.2$ ,  $p < 0.0001$ ). In Sweden, high gut contents of apple pests were found in *Clubiona* (42.5%) and *Philodromus* (48.7%) compared to other groups (8.5% - 18.4%), but these sequences mostly originated from Thysanoptera, that are less serious apple pests. For other pests, there was a tendency of higher proportions of *Cacopsylla* in Opiliones (3.4%) ( $F_{5,33}=2.4$ ,  $p < 0.06$ ), and a higher

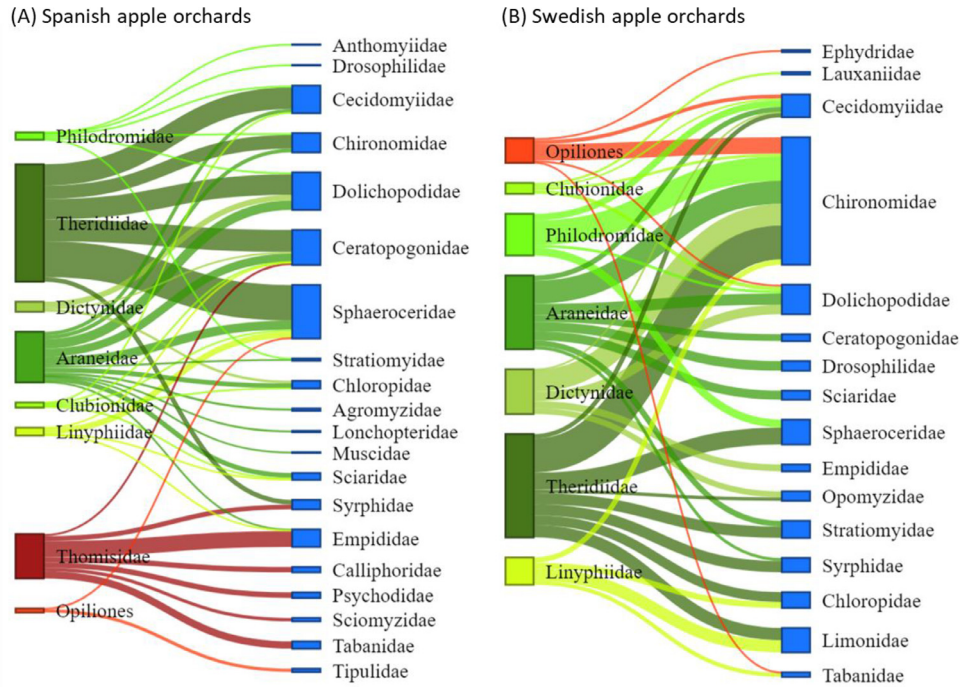


**Fig. 4.** Sankey diagrams of trophic flows in (A) Spanish & (B) Swedish apple orchards, calculated as outputs from lme's (Table 2). Width of spider/opilionid boxes indicates relative abundance, width of flow paths indicates relative importance in consumer diet (diet% x abundance) & width of prey boxes indicate relative importance in consumer diets across all consumer species (sum of diet% x abundance). To increase clarity, only flows > 0.01 of total are shown.

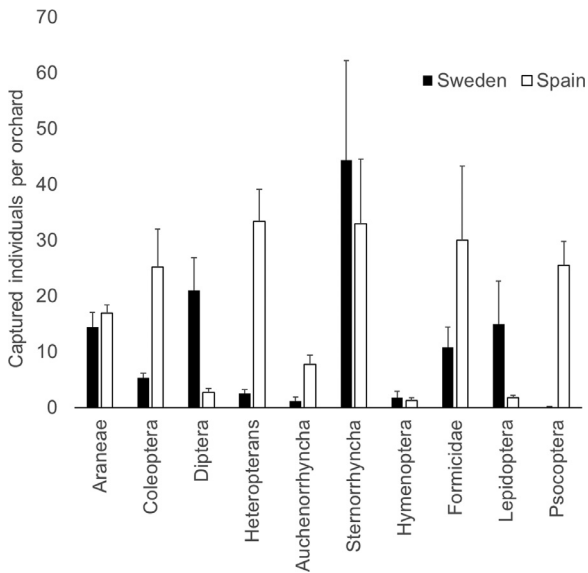
proportion of lepidopteran pests in *Clubiona* (5.5%) and Opiliones (3.3%) ( $F_{5,33}=3.4$ ,  $p<0.02$ ). For other herbivores, we found no effect of region or region-by-predator group interaction, but they were more common in Araneidae

(24.8%), Salticidae (24.6%), Theridiidae (17.9%) and Opiliones (16.6%) compared to other groups (<8.9%) ( $F_{6,75}=3.5$ ,  $p<0.005$ ). Other functional prey groups did not vary between region or predator groups (on average: 11.9%





**Fig. 5.** Sankey diagrams of spider & opilionid use (>1%) of Diptera in (A) Spanish & (B) Swedish apple orchards. Width of spider/opilionid boxes indicates relative abundance, width of flow paths indicates relative importance in consumer diet (diet% x abundance) & width of prey boxes indicate relative importance in consumer diets across all consumer species (sum of diet% x abundance).



**Fig. 6.** Arthropod abundances ( $\pm$ S.E.) in Spanish & Swedish apple orchards from beat sampling. Note that abundances for mites, Collembola & Thysanoptera are missing.

fungivore prey, 8.2% detritivore prey and 9.4% prey with aquatic larvae). As expected, diagnostic PCRs indicated a higher proportion of Opiliones containing aphid DNA than metabarcoding (40.9% vs. 19.5%), and a higher incidence of psyllid DNA in spider guts (Araneidae: 20%, *Philodromus*: 9.1%, Theridiidae: 18.4%, Thomisidae: 11.5%) compared with metabarcoding (<1%).

## Discussion

The most important take home message from this study is that common natural enemies (harvestmen and spiders) in apple orchards frequently feed on other natural enemies, potentially compromising control of apple pests. Although molecular gut content analyses have problems for estimating predation rates, it is notable that a third of sequences belonged to natural enemy species. In fact, this number may be even higher because field quantification of cannibalism is difficult using molecular tools, due to identical DNA of predator and prey, and because used primers in this study reduced amplification of spider DNA. However, our study also suggests diet differences between predator families (spiders and opilionids) (see also Michalko & Pekar, 2016; Nyffeler, 1999), offering the potential for targeted actions that shift natural enemy communities to species less prone to intraguild predation. For instance, our study indicates that cursorial spiders (Thomisidae crab spiders and *Philodromus* spiders) are more prone to intraguild predation than web spiders. Crab spiders in the Swedish orchards had a particular high frequency of *Philodromus* spiders in their guts, but in the Spanish orchards the same group of *Philodromus* spiders were heavy consumers of Heteroptera, many of which are known enemies of apple pests such as aphids and psyllids (Jerinić-Prodanović & Protić, 2013; Sigsgaard, 2010).

The comprehensive metabarcoding of spider and opilionid diets was possible through development of two almost universal primers that reduce amplification of these predator groups. The primer design strategy was a compromise

between more universal primers, which are often dominated by consumer DNA, and primers that completely exclude consumer DNA and thereby affect amplification of other spiders (Cuff et al., 2021; Lafage et al., 2020; Pompanon et al., 2012; Verschut, Strandmark, Esparza-Salas, & Hambäck, 2019). The *in silico* and *in vitro* testing indicated the broad applicability of NoAranR and NoOpiF for diet analyses, but with limitations. Even though the output did not perfectly match the theoretical testing, reduced amplification of aphids and psyllids is likely for these primers. Because these prey groups are important in apple orchards, we complemented the metabarcoding with diagnostic PCR, as a cost-efficient solution to the problem.

The complexity of trophic interactions in the spider part of the orchard food webs (for a recent review on spider predation ecology see Michalko et al., 2019) is well illustrated by *Philodromus* spiders. *Philodromus* spiders are not only involved in intraguild interactions as both prey and predator (Fig. 4), they are also known to feed heavily on aphids and psyllids in orchards (Gajski & Pekár, 2021; Korenko et al., 2010; Lefebvre et al., 2017; Mezöfi et al., 2020; Michalko & Pekár, 2015; Pekár, Michalko, Loverre, Líznavová, & Černečka, 2015; Petrakova et al., 2016), even though predation may vary by orders of magnitude seasonally between sites. We found aphid DNA in 3 of 17 (18%) *Philodromus* individuals in Spain and in 37 of 125 (30%) individuals in Sweden, but only 9% contained psyllid DNA. The lower aphid and psyllid consumption in our study may be due to site differences, differences in spider age structures or to our sampling later in the season, but it is notable that opilionids in our study still had quite high incidences of both aphid (41%) and psyllid (25%) DNA in the gut. *Philodromus* spiders instead seem to have consumed mainly aphids (30%) and Thysanoptera (60%) in Sweden and Heteroptera in Spain, reflecting relative abundance of these two prey groups (Fig. 6). Another arachnid family with higher aphid consumption than *Philodromus* were the Theridiidae (see also Wyss, Niggli, & Nentwig, 1995). These web spiders likely consume mainly winged aphids, whereas cursorial spider also consume sedentary life stages, but their role in aphid control may deserve more attention.

This study also included harvestmen, which are abundant in many agricultural systems, including apple orchards (Happe et al., 2019; Schmaedick & Shelton, 2000; Vink, Teulon, McLachlan, & Stufkens, 2004), but are seldom or ever included in biocontrol studies (but see Papura et al., 2020), perhaps because of their perceived broad diet. However, harvestmen diets vary considerably between species, and some species, including the ones considered here, are mainly arthropod predators (Acosta & Machado, 2007). The harvestmen in this study had mainly consumed small prey species, mites, bark lice (Psocoptera), psyllids, aphids and theridiid web spiders, with smaller amounts of snail and earthworm DNA. Opilionid roles also differed between regions; they consumed more apple pests in Swedish orchards but more natural enemies (mainly

Theridiidae) in Spanish orchards. Compared with spiders, harvestmen seem like poor competitors as they lack venom, but harvestmen instead have a specialized glandular structure on their pedipalps that strongly glues prey to the pedipalp surface (Wolff et al., 2016), enabling harvestmen to efficiently catch both jumping prey such as Collembola and flying prey. This trait may also allow safe capture of spiders, as glued spiders could not turn around and inject venom in the predator.

To conclude, the results from this study question the consensus that management to improve biocontrol in apple orchards should aim to benefit all natural enemies. The arthropod food web in apple orchards, involving spiders, harvestmen and a range of other arthropod predators, has a high complexity where some natural enemies may even reduce biocontrol of pest species through intra-guild predation. Moreover, evaluating the biocontrol potential of natural enemies should also account for ontogenetic diet shifts (Verschut, Strandmark, Esparza-Salas, & Hambäck, 2019), where some life stages feed on pest species whereas other life stages are intraguild predators (see also Polis, Holt, Menge, & Winemiller, 1995). For this purpose, more detailed and longer-term studies are needed to evaluate the net effect of arachnids on pest damage. Exclusion experiments would be ideal, but are seldom feasible for excluding parts of the arachnid community and over sufficiently long time. Other outstanding questions concern the environmental drivers affecting the arachnid community. This and other studies show that the natural enemy community in apple orchards varies regionally and locally, both due to prey availability and to environmental factors such as local or landscape structures. For instance, Happe et al. (2019) found opilionids to be more abundant in IPM than in organic orchards and both spiders and opilionids to be more abundant in less complex landscapes. This study complements previous work showing that diets of spiders and opilionids show only small differences between organic and IPM orchards. Clearly more studies are needed to disentangle the arthropod food web in these orchards that would allow us to provide growers with scientifically sound management guidelines, but understanding the enemy diets is an important step and our study provides the tools needed to further pursue that goal.

## Declaration of Competing Interest

The authors have no competing financial or personal interests that would conflict with the content of this paper.

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## Data accessibility

The data that support the findings of this study are openly available in Dryad at <http://doi.org/10.5061/dryad.gtht76hkz> (Hambäck et al., 2021)

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## Supplementary materials

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.baae.2021.09.006](https://doi.org/10.1016/j.baae.2021.09.006).

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