ORIGINAL ARTICLE

Density-independent prey choice, taxonomy, life history, and web characteristics determine the diet and biocontrol potential of spiders (Linyphiidae and Lycosidae) in cereal crops

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Abstract

Spiders are among the dominant invertebrate predators in agricultural systems and are significant regulators of insect pests. The precise dynamics of biocontrol of pests in the field are, however, poorly understood. This study investigates how density-independent prey choice, taxonomy, life stage, sex, and web characteristics affect spider diet and biocontrol. We collected spiders in four genera of Linyphiidae (i.e., *Bathyphantes*, *Erigone*, *Tenuiphantes*, and *Microlinyphia*), and individuals from the Lycosidae genus *Pardosa*, and their proximate prey communities from barley fields in Wales, UK between April and September 2018. We analyzed the gut contents of 300 individual spiders using DNA metabarcoding. From the 300 spiders screened, 89 prey taxa were identified from 45 families, including a wide range of pests and predators. Thrips were the dominant prey, present in over a third of the spiders sampled, but a type IV functional response appears to reduce their predation at peak abundances. Spider diets significantly differed based on web characteristics, but this depended on the genus and sex of the spider and it was not the principal separating factor in the trophic niches of linyphiids and lycosids. Diets significantly differed between spider genera and life stages, reflected in different propensities for intraguild predation and pest predation. Adult spiders predated a greater diversity of other predators, and juveniles predated a greater diversity of pests. Overall, *Tenuiphantes* spp. and *Bathyphantes* spp. exhibited the greatest individual potential for biocontrol of the greatest diversity of pest genera. The greater trophic niche complementarity of *Pardosa* spp. and *Erigone* spp., however, suggests that their complementary predation of different pests might be of greater overall benefit to biocontrol. Sustainable agriculture should aim to optimize conditions throughout the cropping cycle for effective biocontrol, prioritizing provision for a diversity of spiders which predate a complementary diversity of pest species.

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1 | INTRODUCTION

As insecticides decline in use due to increased resistance, regulation and detrimental environmental effects, alternative and integrated pest management is increasingly pertinent (Fountain et al., 2007; Loetti & Bellocq, 2017; MacFadyen et al., 2009; Pekár, 2013; Whitehorn et al., 2012). Specialist predators and parasitoids offer effective biocontrol, but given their dependence on a narrow niche of host/prey taxa, pest populations can reach large sizes before these biocontrol agents intervene (Ammann et al., 2020; Jordan et al., 2020; Levie et al., 2005; Sunderland et al., 1997). Polyphagous generalist predators such as spiders, which are abundant in arable fields (Nyffeler & Sunderland, 2003), can be effective conservation biocontrol agents of crop pests, even when they first emerge or arrive (Alderweireldt, 1994; Boreau De Roincé et al., 2013; Chapman et al., 2013; Juen et al., 2002; Korenko et al., 2010; Riechert & Lockley, 1984; Symondson et al., 2002). Generalists do, however, increase trophic network complexity by forming many weak links with other species, which can reduce the stability of these networks (McCann et al., 1998; Wootton & Stouffer, 2016). Pest suppression by specialists with complementary trophic niches will have a greater quantitative effect on pest populations than generalists with overlapping niches and resultantly high competition, particularly when those generalists are engaging in intraguild predation of other natural enemies (Finke & Snyder, 2008; Michalko et al., 2019). Many specialists, however, lack niche complementarity and are vulnerable to the loss of their prey, suggesting that a diversity of natural enemies, ranging from specialist to generalist, will produce the most robust natural suppression of pests (Jonsson et al., 2017; Michalko et al., 2019).

The trophic interactions of spiders vary greatly between taxa, with each species' trophic niche determined by its body size, generality, hunting mode, and stratum (e.g., canopy-based webs vs. ground-hunting; Sanders et al., 2015). Cursorial and web-building spiders represent one significant division with implications for biocontrol; for example, ground-hunting spiders can predate more intraguild prey (Hambäck et al., 2021). However, significant differences in diet can even be found within families with relatively consistent foraging behavior. The Linyphiidae subfamily Linyphiinae feed on more aphids than the confamilial Erigoninae spiders (Harwood et al., 2004); however, more frequently itinerant Erigoninae spiders may encounter more aphids when actively searching for prey on the ground (Gavish-Regev et al., 2009; Harwood et al., 2004). The different nutritional requirements of spiders, depending on sex and life stage, are likely to be a determining factor in differences in their foraging ecology too (Toft, 2013). As spiders develop, their trophic niche expands, resulting in differences between adult and juvenile diets with respect to taxonomic composition, diversity, and prey size (Bartos, 2011; Mezőfi et al., 2020). This ontogenetic determination

of diet is thought to be driven by spiders' need for a relatively consistent size ratio of predator and prey, and may be more important than prey density in determining trophic interactions (Murphy et al., 2020). Pest predation by spiders is thought to vary greatly between sexes, with female spiders tending to eat more aphids than males (Harwood et al., 2004). It is difficult, however, to predict the extent of pest predation via functional traits, with taxonomy, life stage, and morphology sometimes serving as poor predictors of predation rates (Bell et al., 2008).

Some variation in spider foraging behavior may relate to differences in web characteristics. Spider webs are extended phenotypes that indicate investment in foraging by individual spiders (Welch et al., 2016). While wolf spiders (Lycosidae) and many other families do not build webs, those of linyphiids are intricate sheet-and-tangle webs comprising dense horizontal sheets with vertical capture lines (Haberkern et al., 2020). Even within Linyphiidae, web form and function can greatly vary. Erigoninae spiders will often build small webs near to the ground which they leave regularly to hunt itinerantly, whilst Linyphiinae spiders produce larger sheet webs several centimeters higher in the vegetation (Sunderland et al., 1986). The spatial separation of the webs of different species may facilitate their complementary biocontrol activity through niche separation (Harwood et al., 2003) and reduced intraguild predation (Opatovsky et al., 2016). These web characteristics can further deviate based on life stage and sex (Harwood et al., 2001, 2003). Many spiders are thought to avoid building webs in the proximity of other active webs, further enhancing niche separation (Opatovsky et al., 2016). Spiders will rapidly colonize web locations with high prey densities, sometimes engaging in territorial contests to hold them (Bollinger et al., 2015; Harwood et al., 2001; Riechert & Lockley, 1984; Samu et al., 1996) and altering web structures for taxon-specific prey capture (Welch et al., 2016).

High predator fitness requires a diverse diet of pest and non-pest prey (Harwood et al., 2009). Spiders often preferentially forage for pests such as aphids, which have been recorded in the diet of up to 63% of spiders under field conditions (Sunderland et al., 1986; Welch et al., 2016). Some pest species can elicit a toxic effect on spiders (Bilde & Toft, 2001), but may be regularly predated regardless given the nutritional benefit they confer upon the spider, especially when diluted by other prey (Michalko et al., 2021; Toft, 1995). Spider foraging is thought to reactively redress nutritional deficiencies (Mayntz et al., 2005) and optimal nutritional intake can be achieved either by ingestion of high-quality prey or nutritionally complementary alternative prey (Michalko et al., 2020; Toft, 2013). Although alternative prey is thought to positively affect spider-mediated biocontrol efficacy by enhancing predator fitness (Ostman, 2004; Roubinet et al., 2017), this is taxon-specific and may increase intraguild predation, reducing the overall benefit of spider-mediated

biocontrol (von Berg et al., 2012; Davey et al., 2013; Moreno-Ripoll et al., 2014; Traugott & Symondson, 2008). To effectively harness their conservation biocontrol potential, the factors affecting the diet of spiders in the field must first be understood (Chapman et al., 2013). The functional response of predators to prey, effectively determining prey choice, is itself determined by encounter rates, the search and handling time required, predator hunger, innate behaviors, current physiology, and past experience with the prey (Holling, 1966; Peterson et al., 2016; Welch et al., 2016). Generalist invertebrate predators are thought to employ prey switching, which is disproportionate feeding on the most common prey to increase foraging efficiency (Cornell, 1976). Spiders have, however, been shown to forgo abundant prey in favor of less locally abundant species, indicating prey choice beyond density-dependent predation (Agustí et al., 2003; Welch et al., 2016).

Here, we analyzed the diets of common cereal crop spiders (Linyphiidae and Lycosidae) using DNA metabarcoding. The principal aim of this study was to identify differences in dietary intake between spiders and the factors responsible for those differences, ultimately to compare the biocontrol potential of different spiders and identify optimal candidates for biocontrol. The following hypotheses were tested: (i) spider diet differs between spider genera, life stages, and sexes, (ii) differences in the size and height of spider webs account for dietary differences between spiders, (iii) the diversity of intraguild and pest prey differs between spiders based on taxonomy and life history, and (iv) spiders exhibit density-independent (i.e., not proportional to relative abundance) prey choice with observable preferences differing based on functional traits.

2 | MATERIALS AND METHODS

2.1 | Fieldwork

Money spiders (Araneae: Linyphiidae) and wolf spiders (Araneae: Lycosidae) were the two most common families present in field surveys, so were prioritized for collection. Spiders were visually located along transects in two adjacent barley fields at Burdons Farm, Wenvoe in South Wales (51°26′24.8′′N, 3°16′17.9′′W) and collected from occupied webs and the ground, between April and September 2018. Surveys and sampling were conducted five days per week across this period. Each transect was adjacent to a randomly selected tramline and they were distributed across the entire field. The areas searched were 4 m^2 quadrats at least 10 m apart and all observed linyphiids and lycosids were collected in approximately 15-minute searches. The spiders included in this study were taken from 64 locations across 24 days (Table S3) along the aforementioned transects. Spiders were individually placed into 1.5-ml microcentrifuge tubes containing 100% ethanol using an aspirator, regularly changing meshing, at least every five spiders, to limit potential cross-contamination between spiders (spiders were also subsequently washed during transferral to fresh ethanol at the identification and, separately, dissection stages). Linyphiids

occupying webs were prioritized for collection, but ground-active linyphiid spiders were also collected. For each spider taken from a web, the height of the web from the ground and its approximate dimensions were recorded, the latter calculated as approximate web area. Spiders were taken to Cardiff University, transferred to fresh ethanol, adults identified to species-level and juveniles to genus, and stored at −80°C in 100% ethanol until subsequent DNA extraction. To obtain data on local prey density, 4 m^2 of ground and crop stems were suction sampled using a 'G-vac' for 30 s at each quadrat from which spiders were collected, with the collected material emptied into a bag. Organisms were immediately killed with ethyl-acetate and material frozen for storage before sorting into 70% ethanol in the laboratory.

All suction-sampled invertebrates were identified to family level due to the restriction of many of the metabarcoding-derived dietary data to this level, and the difficulty associated with finer taxonomic resolution of many taxa. Exceptions included springtails of the superfamily Sminthuroidea (Sminthuridae and Bourletiellidae, which were often indistinguishable following suction sampling and preservation due to the fine features necessary to distinguish them) which were left at super-family, mites (many of which were immature or in poor condition) which were identified to order level and wasps of the superfamily Ichneumonoidea (which were identified no further due to obscurity of wing venation due to damage).

2.2 | Extraction and high-throughput sequencing of spider gut DNA

Given their prevalence in field collections, dietary analysis was carried out for the linyphiid genera *Erigone*, *Tenuiphantes*, *Bathyphantes* and *Microlinyphia* (Araneae: Linyphiidae), and the Lycosidae genus *Pardosa*. Spiders were transferred to and washed in fresh 100% ethanol to reduce external contaminants prior to identification via morphological key (Roberts, 1993). Abdomens were removed from spiders and again washed in and transferred to fresh 100% ethanol. DNA was extracted from the abdomens via Qiagen TissueLyser II and DNeasy Blood & Tissue Kit (Qiagen) as per the manufacturer protocol, but with an extended lysis time of 12 h to account for the complex and branched gut system in spider abdomens (Krehenwinkel et al., 2017). At least one extraction negative (blank tubes treated identically to samples) was included per 12 spiders (each extraction typically contained 24 spiders, thus two extraction negatives), which was included in subsequent PCR and high-throughput sequencing to detect instances of lab/reagent contamination.

For amplification of DNA, two primer pairs were used. BerenF-LuthienR (Cuff et al., 2021) amplified a broad range of invertebrates including spiders, and TelperionF-LaureR, amplified a range of invertebrates but fewer spiders (modified from TelperionF-LaurelinR (Cuff et al., 2021) via one base-pair change from Laurelin; 5′-ggrtawacwgttcawccagt-3′). Primers were labeled with unique 10 bp molecular identifier tags (MID-tags) so that each individual had a unique pairing of forward and reverse tags for identification

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of each spider post-sequencing. PCR reactions of 25 μl contained 12.5 μl Qiagen PCR Multiplex kit, 0.2 μmol (2.5 μl of 2 μM) of each primer and 5 μl template DNA. Reactions were carried out in the same thermocycler, optimized via temperature gradient, with an initial 15 min at 95°C, 35 cycles of 95°C for 30 s, the primer-specific annealing temperature for 90 s and 72°C for 90 s, respectively, followed by a final extension at 72°C for 10 min. BerenF-LuthienR and TelperionF-LaureR used annealing temperatures of 52°C and 42°C, respectively.

Within each PCR 96-well plate, 12 negative controls (extraction and PCR), two blank controls, and two positive controls were included (i.e., 80 samples per plate), based on Taberlet et al. (2018). Positive controls were mixtures of invertebrate DNA comprised of non-native Asiatic species in four different proportions (Table S1) and blanks were empty wells within each plate to identify tag-jumping into unused MID-tag combinations. PCR negative controls were DNase-free water treated identically to DNA samples. A negative control was present for each MID-tag to identify any contamination of primers. All PCR products were visualized in a 2% agarose gel with SYBRSafe (Thermo Fisher Scientific, Paisley, UK) and placed in categories based on their relative brightness. The concentration of these brightness categories was quantified via Qubit dsDNA High-sensitivity Assay Kits (Thermo Fisher Scientific, Waltham, MA, USA) with at least three representatives of each category per plate. The PCR products were then proportionally pooled according to these concentrations. Each pool was cleaned via SPRIselect beads (Beckman Coulter), with a left-side size selection using a 1:1 ratio (retaining ~300–1000 bp fragments). The concentration of the pooled DNA was then determined via Qubit dsDNA High-sensitivity Assay Kits and pooled together into one library per primer pair. Library preparation for Illumina sequencing was carried out on the cleaned libraries via NEXTflex Rapid DNA-Seq Kit (Bioo Scientific, Austin, USA) and samples were sequenced on an Illumina MiSeq via a V3 chip with 300-bp paired-end reads (expected capacity ≤25,000,000 reads). Bioinformatic analysis followed (Drake et al. 2021); (Appendix S1).

2.3 | Statistical analysis

All analyses were conducted in R v4.0.0 (R Core Team, 2020). Initial multivariate analyses used binary data (i.e., presence/absence) given the various problems inherent to quantifying metabarcoding data (Deagle et al., 2013, 2019). Prey species that occurred only once across all of the dietary samples were removed before further analyses to prevent outliers skewing the results, which is particularly problematic for non-metric multidimensional scaling. Spider diets were compared between variables using multivariate generalized linear models (MGLMs) via 'manyglm' in the 'mvabund' package (Wang et al., 2012) with a binomial error family and Monte Carlo resampling. Model-independent variables included spider genus, spider life stage (juvenile or adult, the latter defined by fully developed genitalia), spider sex, and all two-way

interactions between these variables. Pairwise two-way interactions were also included between the aforementioned variables and Julian day to account for how seasonality may affect these relationships.

Coarse dietary differences were visualized by non-metric multidimensional scaling (NMDS) via metaMDS in the 'vegan' package (Oksanen et al., 2016) with Jaccard distance in two dimensions and 999 tries. For NMDS, outliers (usually samples containing rare taxa) were identified by plotting and subsequently removed to facilitate separation of samples and achieve minimum stress. For visualization of the effect of categorical variables against the dietary NMDS, spider plots were created using 'ordispider' with 'ggplot' and 'RColorBrewer' (Neuwirth, 2014) and 'viridis' (Garnier, 2018) color palettes. Spider diet was compared against web characteristics for spiders for which both web and dietary data were available using the MGLM process outlined above, but with models containing web height, web area, an interaction between the two, and pairwise interactions between genus, life stage, and sex with the two web variables. This model used the same binomial error family as above, but with a 'cloglog' link function. For visualization of the effect of continuous variables against the NMDS, surf plots were created with scaled colored contours using the function 'ordisurf' of the 'ggplot' package in R.

All prey taxa were classified as agricultural pests, natural enemies (simplified as 'predators'), or excluded from subsequent analyses of intraguild predation and biocontrol (Table S2). Intraguild predation and biocontrol variables were created by counting the number of natural enemy taxa, and, separately, of agriculturally relevant 'pest' taxa (taxa containing species that commonly adversely affect agricultural productivity; Table S2) in each spider's diet. These resultant count data (effectively the diversity of pests and natural enemies predated by each individual spider) were separately analyzed against spider genus, life stage, and sex via GLM. 'Site' (denoting the 4 m^2 area from which spiders were collected within fields) was initially included as a random effect in generalized linear mixed-models, but no significant effect was observed when comparing this model against a standard GLM via a likelihood ratio test of nested models using the 'lrtest' command in the 'lmtest' package (Zeileis & Hothorn, 2002). Standard GLMs were thus used to avoid issues relating to singularity in the mixed models. The assumptions for the resultant Poisson error family GLMs were tested using the 'testResiduals' function of the 'DHARMa' package (Hartig, 2020). Intraguild predation and biocontrol differences between significant terms were visualized using violin plots with the quartiles, median and 95% upper limit annotated using the 'geom_violin' function in 'ggplot2'.

In situ spider prey choice was analyzed using network-based null models in the 'econullnetr' package (Vaughan et al., 2018) with the 'generate_null_net' command, visually represented with the 'plot_preferences' command. Binary dietary data were used alongside suction sample count data to represent prey availability. These suction sample data, as described above, were collected at the same sites as the spiders three days after spider collection. Prior to the

taxonomic prey choice analysis, an hemipteran identified no further than order level through dietary analysis was removed due to the inability to pair it to any present prey taxa with certainty. Standardized effect sizes (SES) were extracted for all comparisons for each individual spider and compared between genera, life stages, and sexes using permutational multivariate analysis of variance (PerMANOVA) using the 'adonis' function of the 'vegan' package with 9999 permutations and a Euclidean distance matrix to determine overall differences in prey choice.

3 | RESULTS

3.1 | Dietary observations and differences

From the 300 spiders screened, data were recovered from 244 (38 *Bathyphantes*, 35 *Erigone*, 26 *Microlinyphia*, 21 *Pardosa*, 124 *Tenuiphantes*, 105 females, 93 males (46 unsexed due to immaturity), 168 adults, 76 juveniles). Across these 244 spiders, 89 different prey ZOTUs were identified from 45 families. Spiders contained prey from an average of 2.57 ZOTUs and 2.23 families with 81.3% (244) of spiders containing detectable prey DNA. The general amplification primers BerenF-LuthienR recovered a total of 7,351,188 reads post-bioinformatics, comprising an average of 745 (±2,773 SD) prey reads and 23,359 (±11,507 SD) predator reads across all samples. The spider-exclusion primers TelperionF-LaureR recovered a total of 6,574,698 reads post-bioinformatics, comprising an average of 5,046 (±11,279 SD) prey reads and 9,958 (±17,585 SD) predator reads across all samples. The ratio between predator and prey reads did, however, differ between spider genera (Figure S1). Through the application of stringent minimum sequence copy thresholds, we believe that our data present a conservative snapshot of the diet of these spiders. It is, of course, always possible that false positives persist in the data, but best practice measures such as cleaning of spider abdomens prior to DNA extraction, thorough sterilization of all equipment, and implementation of controls at every step of the work (accounting for reagent and ambient contamination) should reduce the prevalence of these instances.

Coarse dietary differences were observed between genera, sexes, and life stages (Table S4-S7). In the full analysis, specific spider diets were significantly related to spider genus (MGLM: Dev = 543.8, d.f. = 232, p = 0.001; Figure S2-S3, Table S8), spider life stage (Dev = 151.4, d.f. =231, p = 0.001; Figure S4-S5, Table S8), spider sex (Dev = 147.8, d.f. = 229, p = 0.001; Figure S6-S7, Table S8), and interactions between genus and life stage (Dev = 43.2, d.f. = 225, p = 0.043), and genus and sex (Dev = 110.9, d.f. = 219, p = 0.001; Table S8), but not life stage and sex (MGLM: Dev = 37.5, d.f. = 217, p = 0.149). Pairwise interactions between Julian day and genus (MGLM: Dev = 223.7, d.f. = 212, p = 0.001) and maturity (MGLM: Dev = 53.9, d.f. = 211, p = 0.002) were also significant, suggesting these associations may change over time; the interaction between Julian day and sex was marginally insignificant (MGLM: Dev = 52.8, d.f. = 210, p = 0.079).

3.2 | Web differences

Active (i.e., occupied) webs were measured in association with 147 spiders within the focal genera of this study during sampling (Figure S8, Table S9). When considered as single response variables, diet was not significantly related to specific web heights (MGLM: Dev = 33.83, d.f. = 79, p = 0.414) and marginally insignificantly to web area (MGLM: Dev = 48.90, d.f. = 78, p = 0.066). When web characteristics interacted with other variables, however, several associations were shown to significantly affect diet: web area with web height (MGLM: Dev = 52.82, d.f. = 77, p = 0.024), web height with genus (MGLM: Dev = 166.39, d.f. = 74, p = 0.001), web height with sex (MGLM: Dev = 101.01, d.f. =67, p = 0.003), and web area with genus (MGLM: Dev =79.74, d.f. =71, p = 0.010; Figure S9-S10; Table S10). Interactions between web height and life stage (MGLM: Dev =49.98, d.f. =70, p = 0.144), web area and life stage (MGLM: Dev =32.80, d.f. =69, p = 0.110), and web area and sex (MGLM: Dev =35.89, d.f. =65, p = 0.655) did not significantly affect diet.

3.3 | Intraguild predation and biocontrol

A significantly greater diversity of pest taxa were regularly predated by juveniles than adults (Adult-Juvenile GLM: mean diff. = 0.289 ± 0.132, z = 2.193, p = 0.028; Figure 1), and pest prey diversity differed significantly between genera (Figure 2, Table S11), but not between sexes (Female-Male GLM: mean diff. = 0.157 ± 0.113, z = 1.387, p = 0.165). Intraguild prey diversity significantly differed between spider genera (Figure 2, Table S12), but not between life stages (Adult-Juvenile GLM: mean diff. = −0.391 ± 0.374, z = −1.046, p = 0.296; Figure 1) nor sexes (Female-Male GLM: mean diff. = −0.396 ± 0.241, z = −1.645, p = 0.100).

3.4 | Prey choice analysis

All groups of spiders exhibited taxonomically biased prey choice beyond density-dependent selection across the 81 taxa identified from community and dietary samples (Figures 3-5 & Figure S11-S13). The overall effect sizes generated by the expected versus observed predation tests were not significantly different between genera, life stages, or sexes, but different spider genera (Figure 3 & Figure S11), life stages (Figure 4 & Figure S12) and sexes (Figure 5 & Figure S13) exhibited distinct taxonomic biases in their prey choice. The prey choice analysis revealed that the network exhibited lower weighted nestedness (standardized effect size (SES) = −2.476, observed = 0.637, expected = 0.720) and
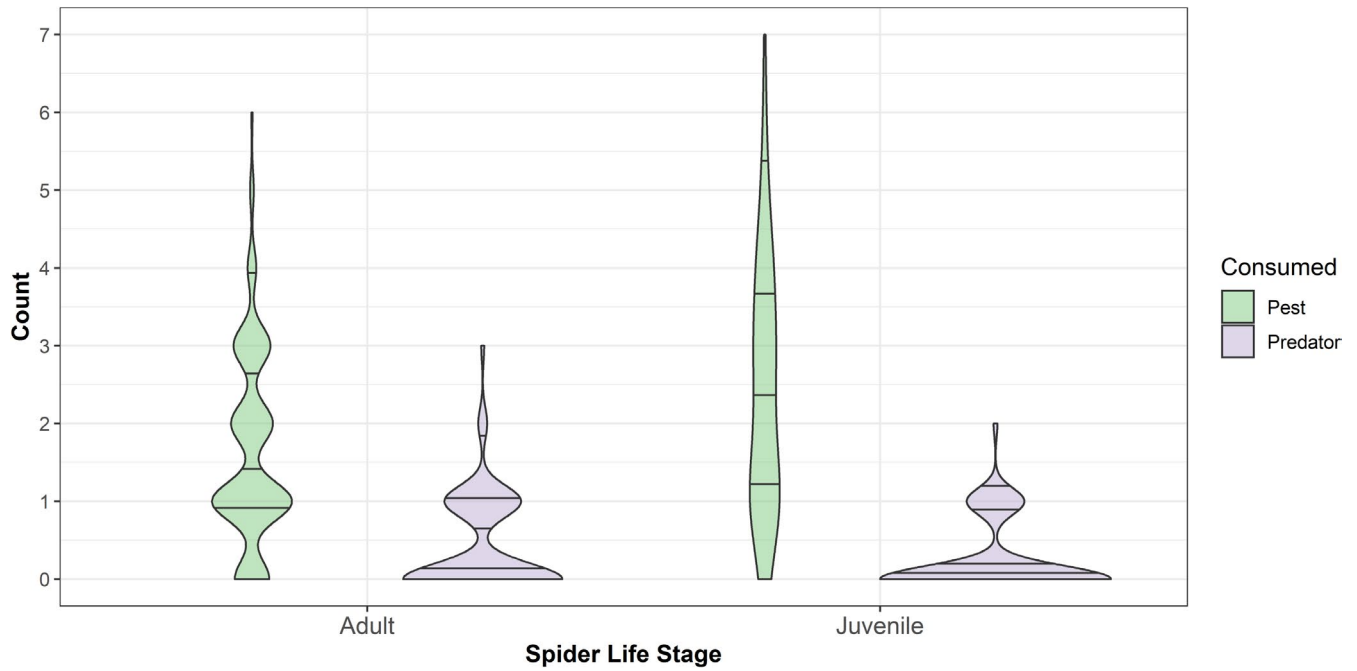
FIGURE 1 Frequency of pest and predator taxa consumed by adult and juvenile spiders. Horizontal lines within the plotted shapes denote, from bottom to top, the lower quartile, median, upper quartile, and upper 95% CI. The width of the plotted shape differs to reflect the relative frequency of predators that predated different numbers of prey taxa
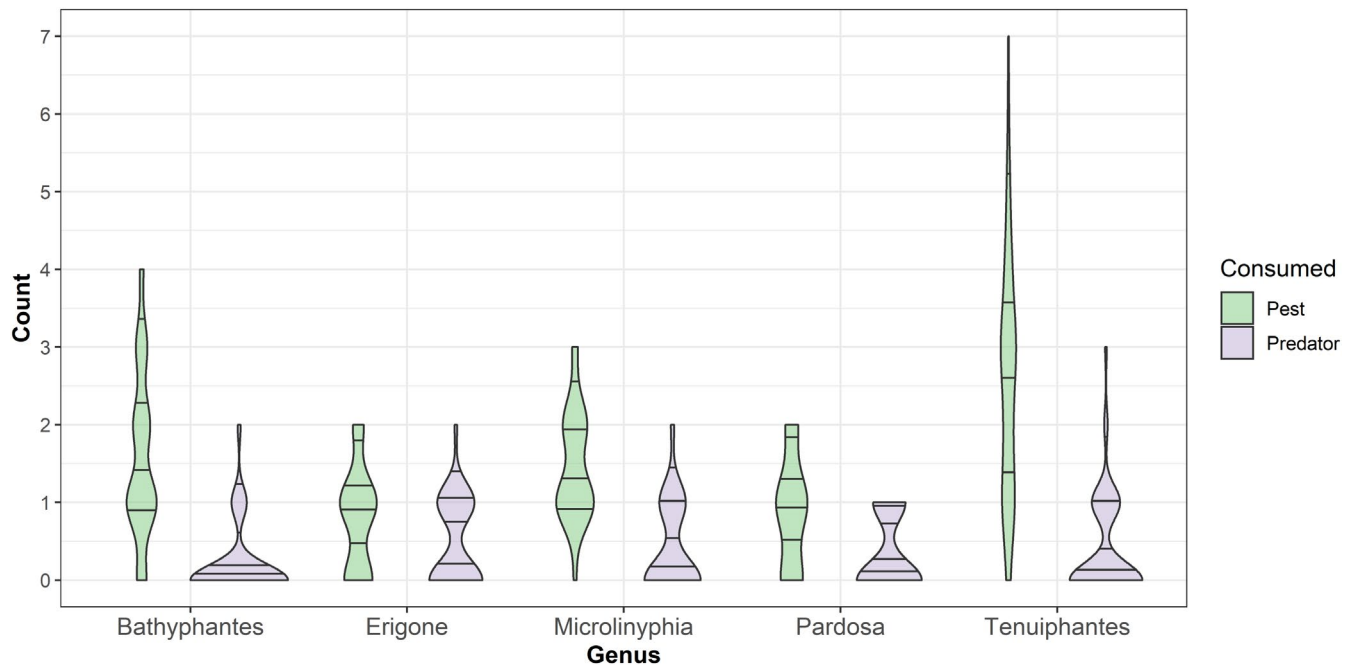
FIGURE 2 Frequency of pest and predator taxa consumed by each spider genus. Horizontal lines within the plotted shapes denote, from bottom to top, the lower quartile, median, upper quartile, and 95% CI. The width of the plotted shape differs to reflect the relative frequency of predators that predated different numbers of prey taxa

linkage density (SES = −5.318, observed = 8.961, expected = 10.190) than expected by the null models, but similar weighted connectance (SES = −1.248, observed = 0.152, expected = 0.160) and interaction evenness (SES = 1.886, observed = 0.912, expected = 0.906) to the null models (Figure 6). Spider partner diversity (SES = −2.712, observed = 3.366, expected = 3.499) and spider generality (SES = −2.528, observed = 11.041, expected = 12.162), and prey partner diversity (SES = −6.420, observed = 2.666, expected = 2.878) and prey vulnerability (SES = −8.199, observed = 6.862, expected = 8.218) were lower than expected (Figure 6).
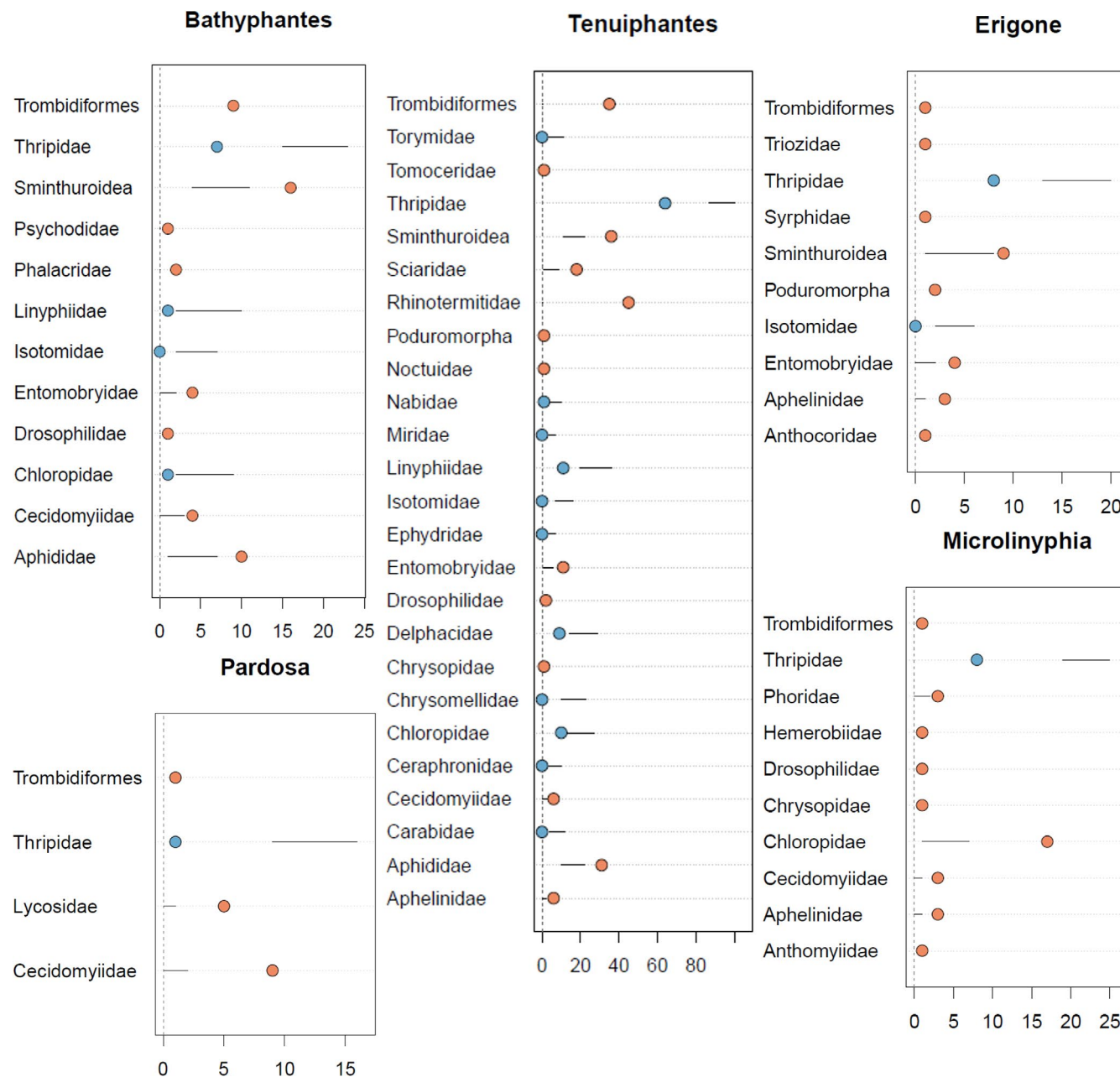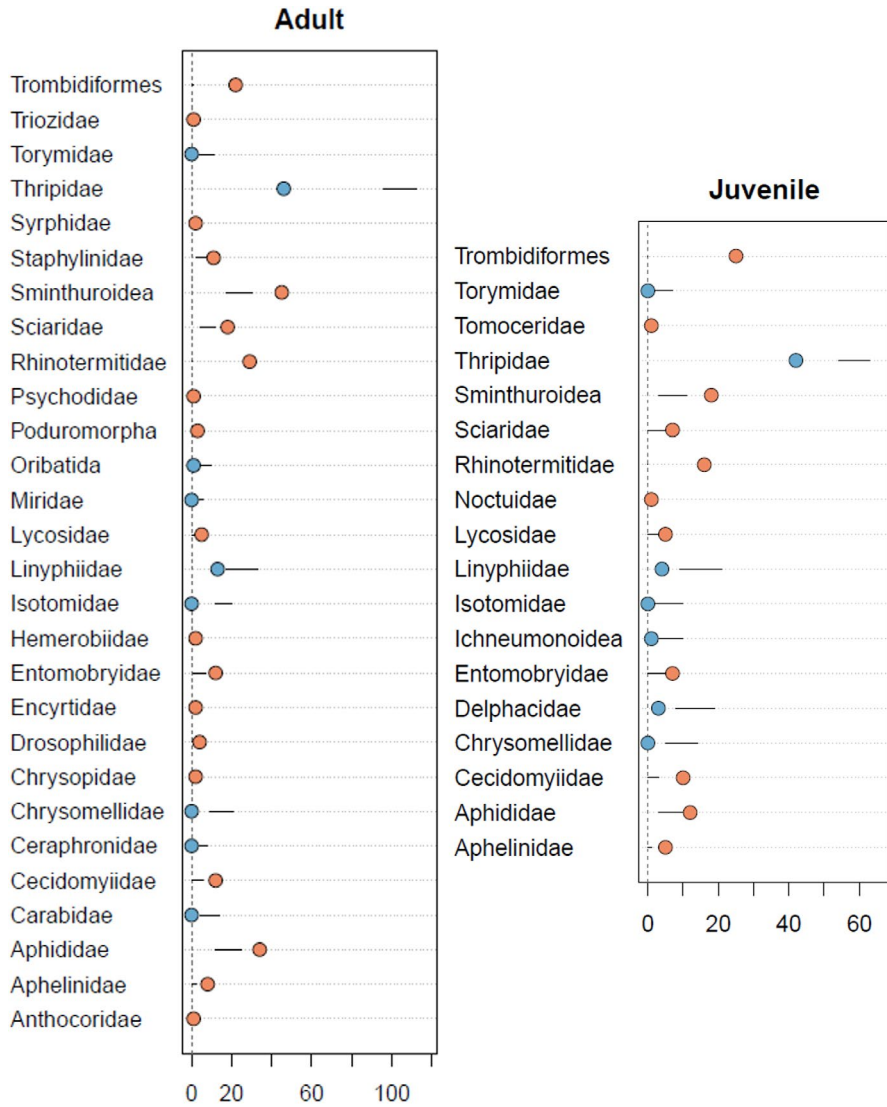
FIGURE 3 Significant deviations from expected frequencies of trophic interactions for spider genera predating prey taxa in the field. Horizontal lines denote 95% confidence limits of the frequency of predation calculated in the null models, whereas circles represent the observed frequency of predation. Blue =lower consumption than expected (avoidance), red =higher than expected (consumed more frequently than predicted from relative abundance). The full results are given in Figure S11

4 | DISCUSSION

Key differences in dietary intake between spiders with different functional traits were identified, elicited through distinct prey preferences, ultimately resulting in their variable benefit to conservation biological control. Specifically, juvenile spiders and those of the genera *Bathyphantes* and *Tenuiphantes*, were especially beneficial to biocontrol individually (i.e., predated a high diversity of pests and relatively few natural enemy species), but the greater niche separation of other genera (e.g., *Pardosa* and *Erigone*) may be of greater overall benefit to biocontrol.

4.1 | Broad dietary observations

Whilst predation of thrips, the most frequently predated prey family, may simply be a function of their disproportionate abundance, the frequency of their predation was significantly lower than expected. Thrips may be nutritionally suboptimal when consumed in high numbers, requiring spiders to consume alternative prey to redress nutritional deficits following an 'overdose' of certain nutrients like carbohydrates (Mayntz et al., 2005; Schmidt et al., 2012; Schmidt et al., 2012). This highlights the importance of alternative prey in

FIGURE 4 Significant deviations from expected frequencies of trophic interactions for spider life stages predating prey taxa in the field. Horizontal lines denote 95% confidence limits of the frequency of predation calculated in the null models, whereas circles represent the observed frequency of predation. Blue =lower consumption than expected (avoidance), red =higher than expected (consumed more frequently than predicted from relative abundance). The full results are given in Figure S12

facilitating predation of pests by generalist predators (Harwood & Obrycki, 2007, 2014). Equally, these insects may be too small to be energetically beneficial (Okuyama, 2007). Similarly, aphids, another prevalent prey group mostly comprising pests in this study, were frequently consumed (19% of spiders), but less than the 38–63% of linyphiids or 20% of lycosids (0 of which contained aphid DNA in this study) reported in previous studies (Kuusk et al., 2008; Sunderland et al., 1986). The greater read depth afforded to each sample in this study, however, should have successfully captured a greater diversity than in some previous metabarcoding studies of spider diet (Cuff et al., 2021), though the uneven exclusion of some spider genera by TelperionF-LaureR may have skewed this between taxa for this primer pair (exemplified in Figure S1).

4.2 | Dietary differences based on spider life history and taxonomy

While dietary differences were identified between spiders based on life history and taxonomy, these relationships changed over time.

The specific prey that leveraged these time-dependent relationships were not the prey principally associated with the other variables (i.e., significant interactions between Julian day and other variables for specific prey species did not occur alongside significant univariate relationships for the variable interacting with Julian day) except in the case of *Sminthurus viridis*, for which the association with different genera changed over time. The diet of juveniles tended to include smaller and less mobile prey, with adults targeting typically larger but 'riskier' intraguild prey (Mezőfi et al., 2020; Murphy et al., 2020; Sanders et al., 2015). Adults appeared to predate more Trombidiidae; however, this could be the detection of ectoparasitism by these mites of recent prey or the spider itself, the latter being more likely for adult spiders given their larger body surface and a greater time in situ (Tomić et al., 2015). Ecological differences between sexes may explain their overall dietary difference, particularly increased itinerance in male spiders (Foelix, 2011). Female spiders have previously been recorded predating pests such as aphids at a greater rate than male spiders (Harwood et al., 2004); the opposite was identified in this study, with more than twice as many male spiders having detectable aphid DNA in their guts.

FIGURE 5 Significant deviations from expected frequencies of trophic interactions for spider sexes predating prey taxa in the field. Horizontal lines denote 95% confidence limits of the frequency of predation calculated in the null models, whereas circles represent the observed frequency of predation. Blue =lower consumption than expected (avoidance), red =higher than expected (consumed more frequently than predicted from relative abundance). The full results are given in Figure S13
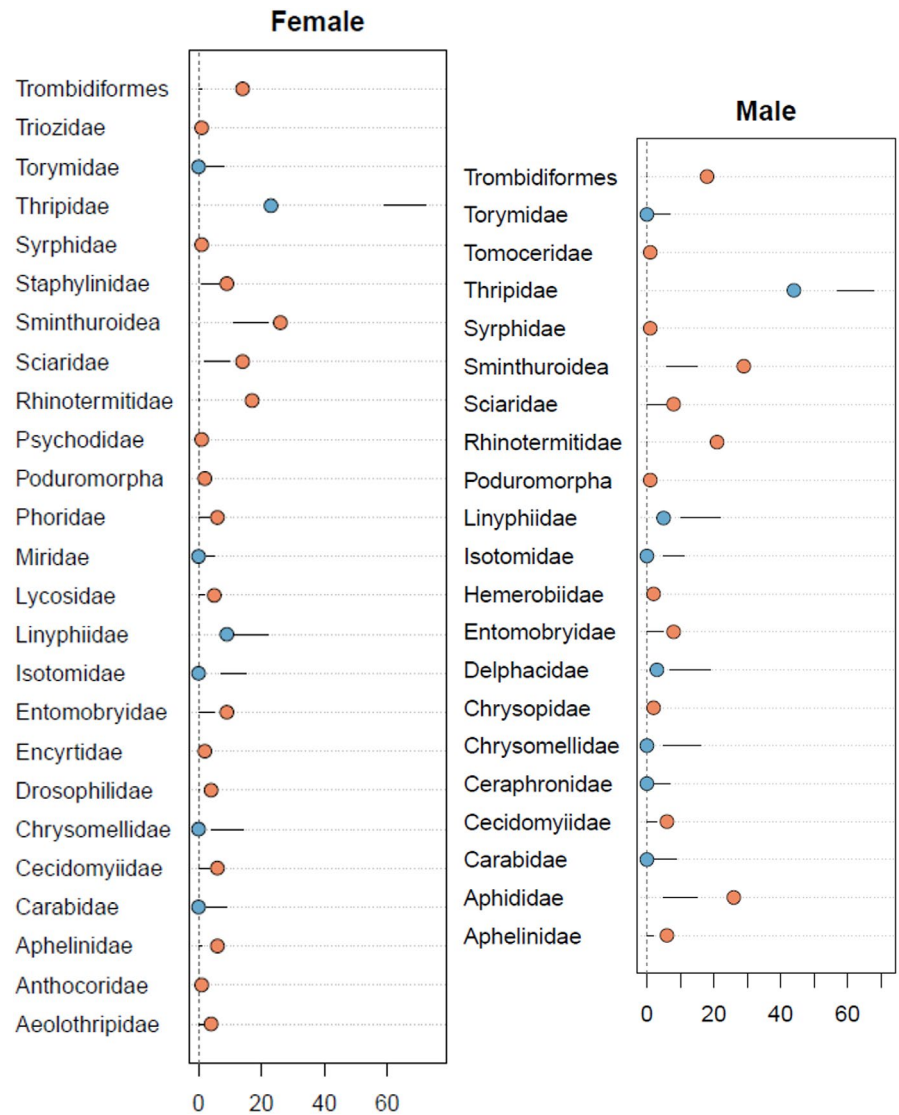
The disparity in diet between spider genera is unsurprising given the different life histories and ecological niches of the focal genera. From the NMDS analysis, the genera can be roughly visually split into two groups: first, *Microlinyphia* and *Pardosa*; and second, *Erigone*, *Bathyphantes*, and *Tenuiphantes*. The latter group all typically employ sit-and-wait foraging at similar positions in the crop canopy (except *Erigone* which is typically more itinerant (Sunderland et al., 1986)), whilst *Pardosa* hunt more actively, despite remaining in relatively small areas (Hallander, 1967). That the diet of *Microlinyphia* is less distinct from that of *Pardosa* highlights that web-building is not necessarily the principal separating factor in the trophic niche differentiation of linyphiids and lycosids. During this study, however, female *Microlinyphia* were almost exclusively found at the apex of the crop canopy, sometimes inhabiting large sheet webs unlike the small close-to-the-ground webs of the males. This separation of the sexes might necessitate a high degree of mobility, possibly resulting in a greater level of itinerancy that might increase the probability of encountering those prey met by highly mobile *Pardosa*, albeit with the inclusion of more canopy-based species. Female *Microlinyphia* were also markedly larger than most of the other linyphiid individuals

studied, possibly allowing them to mechanically subdue and predate larger prey otherwise only attainable by *Pardosa* among the focal genera of this study.

Oscinella spp. were mostly predated by *Microlinyphia*, with a few instances of *Tenuiphantes* and *Bathyphantes* predation. That only *Pardosa* and *Erigone* did not predate these flies could indicate that they are typically found higher in the crop, most accessible by the female *Microlinyphia* in the canopy. Eupodid mites were only predated by *Bathyphantes* and *Tenuiphantes*, suggesting their presence at a specific crop height given the vertical co-occurrence of these two genera. Bourletiellid springtails were, however, almost exclusively predated by *Tenuiphantes* and *Erigone*, which differ in their web positioning. The summer in which many of these spiders were collected (2018) was particularly hot and dry locally and *Tenuiphantes* were regularly collected from the edges of cracks in the ground where the soil had dried and contracted. *Tenuiphantes* may have sought prey from these ground fissures during arid periods, thus increasing predation of subterranean prey such as springtails. *Reticulitermes lucifugus* was detected in the diet of 36% of *Tenuiphantes* individuals, but is not recorded in the United Kingdom; this is discussed in Appendix S2.
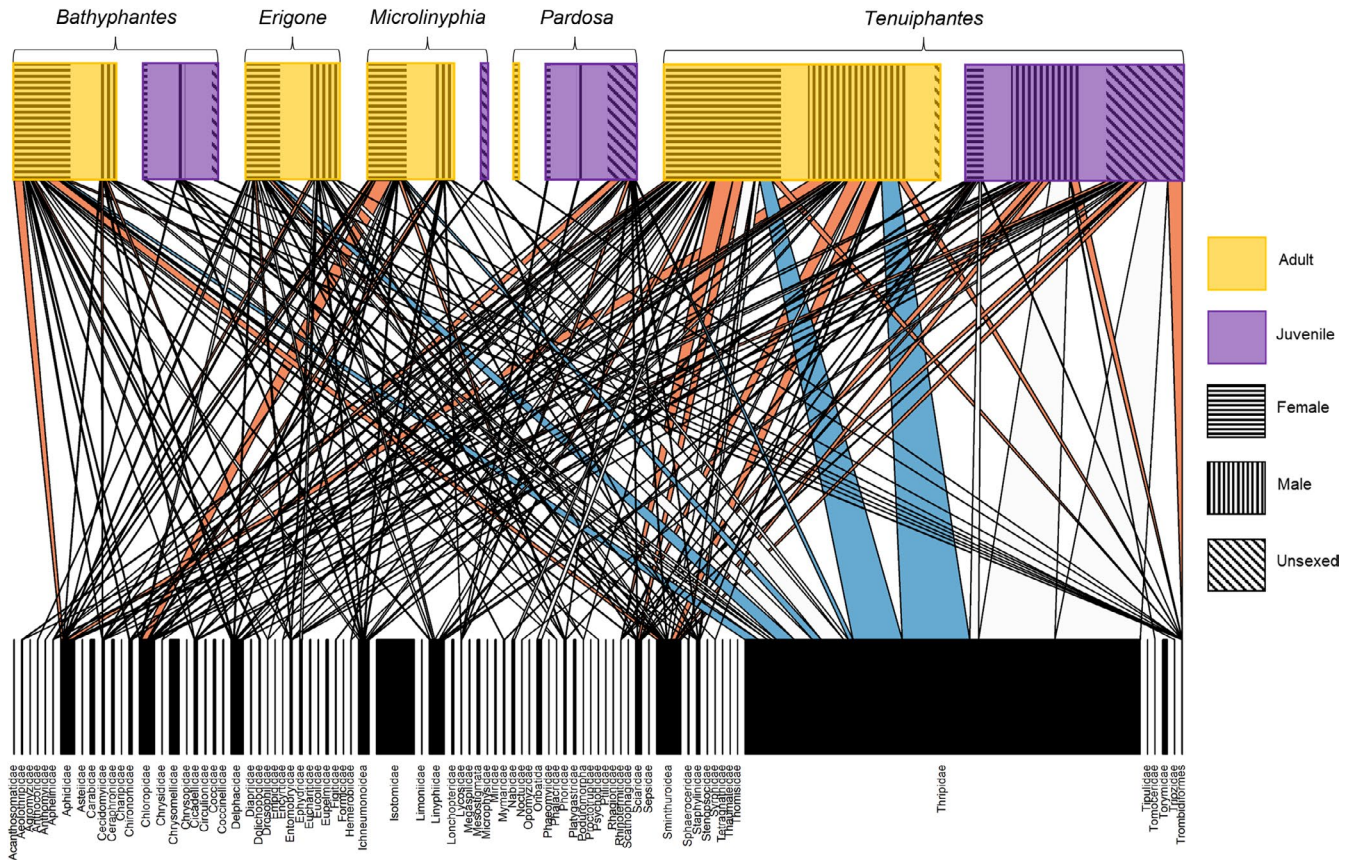
FIGURE 6 Spider-prey network structure based on prey choice null models. Spider groups (upper level) are separated by life stage (orange =adult, purple =juvenile) and sex (horizontal lines =female, vertical lines =male, diagonal lines =unsexed). The width of links at the top denotes the relative exploitation of the lower resource, and at the bottom denotes the relative abundance of that resource in the local community. Red and blue links denote significant preference and avoidance of that resource by the linked consumer group, respectively, based on comparison of observed predation frequency with that expected by prey choice null models

4.3 | Webs as a potential determinant of spider diet

Variable web structure and positioning is a noteworthy consideration regarding the distinction in diet between linyphiid genera. *Tenuiphantes* and *Bathyphantes* build larger and higher webs than those of *Erigone*, and females have been reported to build slightly larger webs than males (Sunderland et al., 1986), all ratified by the results of this study. These web characteristics interactively explain some dietary variation, particularly when considered alongside taxonomy and life history, suggesting that linyphiids adjust their webs for differential prey capture to modulate their foraging efficiency for certain prey in response to spatial variation in prey availability (Welch et al., 2016). Web area and height interactively affect diet, indicating that just the height or area of the web alone is not sufficient to meaningfully modify the dietary options available to the spiders. The only individual prey taxon significantly associated with a particular web area and height was Cecidomyiidae sp., which were separately found to be predated significantly more by those spiders which occupy higher levels in the crop. The genus of the spider is particularly important in determining the effect of web area and height modulation. Given the observed differences in web

characteristics between different genera, this is possibly indicative of restriction of web characteristics to a taxonomically determined range, or variations in species behavior interacting with these differences. Life stage does not, however, appear to affect the relationship between web characteristics and diet, suggesting that life stage is a greater constraint on trophic interactions possibly due to the reduced capacity of juveniles to successfully predate larger, 'riskier' prey that enter their webs.

Importantly, spiders will invade web sites of other spiders to monopolize prey-rich sites (Bollinger et al., 2015; Harwood et al., 2001; Riechert & Lockley, 1984; Samu et al., 1996), which may have occurred in this study. It is difficult to confirm such instances, but the web characteristics could deviate from those typically representative of the occupying spider's species, sex and life stage if it were to colonize the web of a spider differing in one of these traits. Noteworthy too is the degree of itinerance exhibited by the spiders, particularly in *Erigone* spp., which would alter foraging behavior and prey availability beyond the restriction of web-based foraging (Alderweireldt, 1994). The plasticity of web characteristics observed and the impact that this can have on diet indicates that the capacity of spiders to adaptively alter web characteristics is of fundamental

importance to their foraging ecology. Factors other than spider taxon, sex and life stage, or prey availability may influence web morphology, such as meteorological conditions; for example, sheet-and-tangle web spiders are more likely to build their webs under cover to avoid rainfall (Haberkern et al., 2020). Equally, spatial separation of webs from those of other spiders is an important driver of web form and function (Opatovsky et al., 2016), and consideration of the spatial dynamics of these webs could rationalize suboptimal web placements. Further study is required to elucidate the many mechanisms likely to be affecting web structure and function and how this impacts diet.

4.4 | Differences in spider biocontrol based on functional traits

The diversity of pests and intraguild predators predated by each spider group differed substantially, but it is important to note that this does not necessarily equate to differences in overall biocontrol. The diversity of pests consumed is important in assessing the biocontrol generalism of a predator, but the presence–absence data inherent to metabarcoding are preclusive to any quantitative analysis of predation pressure on pests. This analysis may thus neglect the difference between focused predation of few pest individuals and weak predation of many. The results do, however, provide valuable insight into niche complementarity between these generalist biocontrol agents. The ratio of interguild to intraguild prey diversity appears to be consistent with other studies using similar methods (Saqib et al., 2021). That juveniles predated a greater diversity of pests indicates the importance of maintaining spider population turnover *in situ* to suppress a broad range of pests. Pest predation by juveniles may comprise a greater number of their trophic links as a result of these taxa being largely herbivorous and thus less 'risky' prey, whereas 'bolder' adults will have greater success in attacking other predators. The toxins sometimes present in these herbivores may, however, be detrimental to development (Bilde & Toft, 2001), highlighting the importance of non-toxic herbivorous prey for the provision of developing spiderlings. *Tenuiphantes* predated the greatest diversity of pests among the focal genera, while *Bathyphantes* similarly predated many pest taxa, but also far fewer predators. This indicates a possible benefit to ensuring sufficient crop height for these genera early in the cropping season since their webs exist slightly higher in the crop (Sunderland et al., 1986). This could indicate a benefit to winter over spring cropping for spider-mediated biocontrol. Many spiders present early in the cropping season are, however, thought to be juvenile immigrant spiders (Opatovsky et al., 2012). That juveniles had the greatest breadth of biocontrol increases the hypothetical benefit of juvenile immigrant spiders, particularly given the ecologically turbulent process of crop cycling (Cuff et al., 2021; Opatovsky & Lubin, 2012).

When assessing the biocontrol efficacy of a predator, the natural enemies with which it co-occurs are of paramount importance. As highlighted in this study, intraguild predation is a critical problem

in the provision of biocontrol services by generalist predators, but equally important is the niche complementarity of the full spectrum of natural enemies present (Michalko et al., 2019; Schmitz, 2007). Generalist predators establish many weak trophic links, rather than a few strong links as is the case for specialists (McCann et al., 1998; Wootton & Stouffer, 2016). This suggests that generalist predators provide two key functions in the natural enemy community: suppression of early pest populations, and suppression of pests for which there are no or few specialist natural enemies. The complementarity of these generalist spiders can, however, be determined spatially (e.g., separation of their webs, as shown in this study), temporally (e.g., diel activity or phenological differences) or by different prey specialization or preferences (Michalko et al., 2019). While this study showed that some genera (particularly *Bathyphantes* and *Tenuiphantes*) predated a greater diversity of pests, the dietary distinction between those genera was low. The greatest distinction in diet existed between those genera that predated the fewest pest taxa (i.e., *Erigone*, *Microlinyphia* and *Pardosa*), possibly suggesting that these genera together may have an overall greater impact on pests through their niche complementarity and thus reduced competition. Differences in consumed pest diversity between life stages and sexes were smaller but could sustain this niche complementarity even in natural enemy populations lacking taxonomic diversity. This niche complementarity can ultimately be encouraged by the provision of suitable conditions in cereal crops, such as sufficient early crop height and adequate alternative prey provision. This could be achieved by habitat manipulation through additional compost applications (Agustí et al., 2003) or by increasing habitat heterogeneity, for example, by the introduction of intercropping, field margins, or beetle banks (Butts et al., 2003; Mansion-Vaquie et al., 2017; Michalko et al., 2017; Sunderland & Samu, 2000), which can profoundly affect trophic links (Staudacher et al., 2018). By managing the generalist predator populations of cereal crops to maximize the activity of those spiders with the greatest impact on biocontrol, particularly when they have complementary trophic niches, conservation biocontrol can be increasingly employed for the sustainable control of crop pests in integrated pest management schemes.

4.5 | Spider prey choice analyses

Spider prey choice deviated from density-dependent selection, with preferences differing between genera, sexes, and life stages. That spider generality and prey vulnerability were lower than expected supports the notion that spiders are selectively predating a smaller number of prey than expected, possibly to seek specific nutrients or as a result of their specific ecologies. That this network was less nested than predicted by null models suggests that the distinction between diets of these groups is not necessarily a result of niche width changing with development, evolution or between sexes. Several consistent prey preferences arose, notably the significantly lower-than-expected predation of Thripidae, likely due to the overwhelming abundance of thrips considering

that they still comprised the most frequently predated family. The predisposition of G-vac suction samplers to thrips (Zentane et al., 2016), may have resulted in their over-representation but this finding nevertheless suggests that spiders exploit alternative prey to diversify their diet. That some linyphiids predated other linyphiids significantly less than expected may be an artefact of the lack of detection of cannibalism via metabarcoding given the removal of conspecific reads as presumed host detection; all remaining con-familial detections in the diet are instances of inter-specific predation. Cannibalism in linyphiids and lycosids is well-documented, particularly in juveniles (Hallander, 1970; Lesne et al., 2016; Turnbull, 1973) and as an outcome of courtship (Wilder & Rypstra, 2010); if it was detectable in this study, linyphiid cannibalism could elevate con-familial predation to a level proportional with abundance or higher, especially given the benefit to competing for optimal web sites despite the risk that poses.

Coarse differences in prey choice can be observed between spider genera. Most immediately, *Pardosa* exhibits few deviations from expected dietary proportions. Given the ground-active habit of *Pardosa* (Kuusk et al., 2008) and their restricted access to flying and climbing prey, suction sampling could simply align more closely with their prey interception behavior. It is, however, likely that the larger metabolic requirement of the larger-bodied and more active *Pardosa* (Andersson, 1970; Greenstone & Bennett, 1980) may reduce their selectiveness. Even when *Pardosa* encounters toxic prey, it is thought that they must consume at least one individual in order to subsequently identify that pest as toxic, thereby reducing a presumed major driver of choice in initial encounters (Toft & Wise, 1999). The preference of *Erigone* and *Tenuiphantes* for often hypogeal springtails is likely a consequence of the lower webs or *Erigone* (Sunderland et al., 1986), while for *Tenuiphantes* it could ratify their aforementioned retreat into ground fissures during arid periods to access prey (Blamires et al., 2016). The greater-than-expected predation of aphids by both *Bathyphantes* and *Tenuiphantes* indicates that their heightened potential for biocontrol is disproportionate to their expected contribution.

Life stages and sexes exhibited overlapping preferences with some key distinctions. Adult spiders exhibited preference for predators such as staphylinids, hemerobiids, and chrysopids, whereas juveniles preferred herbivorous and detritivorous taxa like tomocerids and noctuids. This further ratifies the increased propensity for intraguild predation in adult spiders, but predation of relatively innocuous prey, often pests, by smaller and less experienced juveniles. The slightly greater number of preferences for ground-hunting predators (e.g., staphylinids, lycosids) in female spiders may reflect their larger mean body sizes (Walker & Rypstra, 2003) and thus a greater mechanical advantage in subduing such prey. Overall differences between the sexes could, at least in cases such as *Erigone*, relate to itinerancy in males modulating the taxonomic range of their encounters (Foelix, 2011). The preference of only males for aphids is, however, contrary to previous literature which has indicated a female-biased predation of aphids (Harwood et al., 2004).

Importantly, the foraging behavior of spiders depends on their functional response to prey density (i.e., the relationship between prey density and predation rate; Holling, 1965; Solomon, 1949). Spiders are known to exhibit all three basic functional responses to prey density, and some rarer responses such as a dome-shaped response (i.e., predation increases with density up to a point, after which predation gradually declines with increasing prey density; Bressendorff & Toft, 2011; Denno et al., 2003; Holling, 1965; Michalko & Košulič, 2016; Michalko et al., 2019; Schmidt et al., 2012; Vucic-Pestic et al., 2010). While the data in this study do not facilitate inspection of these relationships at an individual level, that we can observe the most abundant prey group, thrips, being the most widely predated but still relatively avoided suggests that these spiders conform to the dome-shaped type IV functional response reported elsewhere in spiders (Bressendorff & Toft, 2011). This response can be indicative of nutritional imbalance as a result of over-consumption of particular prey (Bressendorff & Toft, 2011; Michalko et al., 2019; Schmidt et al., 2012), as was suggested above in the case of thrips. Most spiders are thought to exhibit a type II functional response, in which predation rate increases logarithmically with prey density, eliciting the greatest predation pressure at low prey densities, for example, when pests are first establishing populations (Michalko et al., 2019; Riechert & Lockley, 1984). Importantly, these functional responses are dynamic and prey-specific, with the type of response to each prey depending on its nutritional quality, spatio-temporal co-occurrence with the predator, and its ease of capture.

4.6 | Summary

This study successfully expands our understanding of spider foraging ecology by assessing not only differences in diet, but also in the density dependence of their trophic interactions. We assessed spiders as candidates for biocontrol by comparing the diet of spiders against various functional traits. Dietary differences were highlighted between spiders of different genera, sexes, and life stages, seemingly driven by density-independent prey choice. That *Bathyphantes*, *Tenuiphantes*, and juvenile spiders individually exhibited greater biocontrol efficacy does not necessarily equate to their heightened benefit to agriculture. Additional research is required to investigate the relationship between generalism and biocontrol, specifically to identify whether niche complementarity of generalists is of greater importance than suppression of a greater number of the same pest taxa. Regardless, that the predation of some pests by most of these spiders was greater than expected based on their abundance suggests an inherent importance of spiders as biocontrol agents.

CONFLICT OF INTEREST

AUTHOR CONTRIBUTIONS

J.P.C., J.R.B., I.P.V, C.T.M., P.O.-t.W., and W.O.C.S. conceived the ideas and oversaw the project; J.P.C. carried out the field and laboratory work; J.P.C., L.E.D., and I.P.V. analyzed the data; J.P.C. and M.P.T.G.T. identified and processed the invertebrate communities; J.P.C. and L.E.D. carried out the bioinformatic analysis; J.P.C. led writing of the manuscript. All authors commented upon and contributed to the drafts and approved the final manuscript for publication.

DATA AVAILABILITY STATEMENT

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