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RESEARCH ARTICLE

Response of parasitoid communities to insecticide application during a *Lymantria dispar* outbreak in mixed oak forests

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Abstract

1. In the temperate mixed oak forests of Central Europe, outbreaks of insects such as the spongy moth, *Lymantria dispar*, can cause severe defoliation and insecticide is sometimes applied for their control. Parasitoids, mainly Hymenoptera and Diptera, are among the most diverse and important natural enemies of caterpillars in these forests. However, due to their cryptic lifestyle and taxonomic difficulties, we lack knowledge on the impact of insecticide applications on complex host-parasitoid networks.
2. In a large-scale field experiment, we tested the effect of spraying the lepidopteran-specific insecticide Mimic (tebufenozide) on the abundance and community composition of both adult and larval parasitoids. We combined morphological identification, DNA barcoding and metabarcoding to identify parasitoids adult or inside caterpillars, both sampled by canopy fogging during an outbreak and two subsequent years. We analysed the abundance of parasitoids, community composition and network specialisation using statistical methods that account for sample incompleteness in host-parasitoid data.
3. For adult parasitoid assemblages, we found strong annual effects on abundance, with highest numbers of adult parasitoids occurring in the outbreak year, as well as on annual changes in community composition, but no effect linked to insecticide application. However, the abundance and species number of immature parasitoids revealed negative effects of insecticide application, while community composition was only affected by annual variation.

4. Coverage-based network analyses showed a reduction of taxonomic network diversity and network specialisation associated with insecticide application in the first 2 years.
5. *Synthesis and applications*: This real-world experiment shows that parasitoid populations respond immediately to large-scale outbreaks but only limited to local disturbances. Results indicate that this group of natural enemies exhibits high mobility, enabling them to track host populations across large spatial scales. However, our observation of reduced network specialisation after insecticide application is a warning signal that ecosystem function, and consequently natural pest control services, may be impaired by human interference at the local stand scale.

KEYWORDS
canopy parasitoids, DNA metabarcoding, forest management, host–parasitoid network, incomplete sampling data, integrative taxonomy, network specialisation, temporal dynamics

1 | INTRODUCTION

Forests host much of the world's terrestrial biodiversity. In temperate regions of Europe, canopies of mixed oak forest are particularly ecologically valuable as they possess unique arthropod communities with high species diversity (Kennedy & Southwood, 1984; Southwood et al., 2004; Valencia-Cuevas & Tovar-Sánchez, 2015). These communities can be affected by natural disturbances, such as cyclical outbreaks of lepidopteran defoliators. One of the most damaging of these phytophagous insects is the spongy moth, *Lymantria dispar* L. (Lepidoptera: Erebidae), which causes widespread defoliation (Johnson et al., 2005) sometimes leading to extensive host tree mortality (Davidson et al., 1999) and disruption of associated arthropod communities (Redman & Scriber, 2000). Predators, entomopathogens, and parasitoids, mainly Diptera and Hymenoptera, are the principal natural agents that control spongy moth outbreaks and form complex interaction networks (Alalouni et al., 2013; Elkinton & Liebhold, 1990). However, these agents do not always maintain spongy moth populations at sub-outbreak levels, leading to high population densities in many regions. In order to mitigate unwanted effects of outbreaks, lepidopteran-specific insecticides are sometimes applied to supress populations of the spongy moth, evoking debate about adequacy efficacy as well as collateral damage on these hyper-diverse forest ecosystems (Butler et al., 1997; Leroy et al., 2019).

Spongy moth outbreaks are characteristically synchronised over large areas and occur periodically at intervals of 5 to 13 years (Hlásny et al., 2016; Johnson et al., 2005). They can cause widespread damage with serious economic and ecological impacts on mixed oak-broadleaf forests (Aukema et al., 2011; Morin & Liebhold, 2016). Canopy arthropods can potentially be affected by these outbreaks through changes in the quality and quantity of leaves (Nykänen & Koricheva, 2004; Redman & Scriber, 2000) and increased apparent competition resulting from a proliferation of spongy moth antagonists (Redman & Scriber, 2000; Timms & Smith, 2011). Arthropod community structure may be altered as these intensified bottom-up and top-down pressures asymmetrically impact different species, including parasitoids (Redman & Scriber, 2000; Timms & Smith, 2011). Although parasitoids are natural regulators of the spongy moth (Hoch et al., 2001), the response of parasitoid communities to insecticides and outbreaks is poorly understood particularly at a regional scale.

Despite their ecosystem function and ubiquity in terrestrial ecosystems, most parasitoid communities remain poorly documented, mainly due to their cryptic lifestyle and incompletely resolved taxonomy (Jones et al., 2009; Miller et al., 2021). It is known that they are sensitive to small-scale environmental disturbances as manipulation of habitat structure has been shown to shift species distribution patterns, abundances (Gibb & Hochuli, 2002; Komonen et al., 2000) and even parasitism rates (Roland & Taylor, 1997). Parasitoid communities may respond numerically to host densities (Armstrong et al., 2016; Kondoh, 2003) but parasitoid populations may also respond spatially via convergence in areas of high host availability (Eveleigh et al., 2007; Greyson-Gaito et al., 2021). It is likely that species with narrow host ranges, such as parasitoids of the spongy moth (Alalouni et al., 2013; Zankl et al., 2023), would be particularly sensitive to rapid changes in host availability following outbreaks and their management with insecticides (Shaw & Hochberg, 2001).

Even more unknown than abundance and diversity responses of parasitoid communities to outbreak-associated disturbances are their effects on interactions among caterpillars of Lepidoptera and Symphyta (Alalouni et al., 2013; Žikić et al., 2017). Networks of species interactions provide insights into ecological communities, and it is important to distinguish between the response of species diversity and the interaction network, as interaction networks may change despite only small changes in species diversity (Tylianakis et al., 2007). The spongy moth supports a complex trophic network with high diversity and host specificity

which reflects high niche partitioning (Cordeiro et al., 2020). This leads to a highly modular network, in which subsets of species interact more with each other than with other members of the community, which is generally expected to be stable (Stouffer & Bascompte, 2011; Teng & McCann, 2004). However, the loss of species in host-parasitoid networks may lead to network destabilisation (Thébault & Fontaine, 2010), and similar results are expected from the creation of host scarcity through the use of insecticides.

In this study, we assess the impact of a Lepidoptera-specific insecticide (Mimic) application, under outbreak and non-outbreak conditions, on parasitoid abundance, community composition, and network architecture in mixed oak forests. We use traditional taxonomy, DNA barcoding, and metabarcoding to identify caterpillars and parasitoids sampled by canopy fogging over a 3-year interval. Specifically, we hypothesise that (1) the decline of caterpillar densities following insecticide treatment is reflected in both reduced abundance and altered community composition of parasitoids, (2) that Mimic application reduces the abundance of parasitoids and has strong effects on their species composition and (3) that complex host-parasitoid networks are disrupted by insecticides reducing network specialisation.

2 | MATERIALS AND METHODS

2.1 | Experimental design and study area

A detailed description of the study design and study area can be found in Leroy et al. (2021). Briefly, the research area covered a 2400 km^2 region in northwestern Bavaria, Germany. The forests in this region are characterised by mixed stands, including species such as deciduous oak (*Quercus robur* L. and *Quercus petraea* Matt.). They experienced a severe outbreak of the spongy moth *L. dispar* between 2018 and 2020. Twelve experimental blocks were established, each consisting of four structurally comparable oak-dominated forest stands with an area of at least 7 ha.

Based on egg mass surveys in fall 2018, two plots in each block were classified as high and low spongy moth density plots. Each stand with high or low spongy moth density was randomly assigned to either suppression of spongy moth populations by insecticide application or to no treatment, serving as a control. The legal basis for the use of forest insecticides in Bavaria is provided by the Bavarian State Institute of Forestry (LWF). Approval has been applied for from the authority for all areas with Mimic treatments. Further details of the approval process can be found in Leroy et al. (2021). The insecticide Mimic® with the active ingredient (a.i.) tebufenozide (Spiess-Urania Chemicals, Hamburg, Germany) was aerially applied at a concentration of 750 $mL ha^{-1}$ (180 g a.i. ha^{-1}) between 3 and 23 May 2019. This time window coincided with the peak activity of spongy moth larvae. As tebufenozide selectively targets lepidopteran larvae (not affecting Symphyta larvae), it is recommended for the suppression of spongy moth outbreaks. This study resulted in a final design of 48 plots arranged in 12 blocks, each with the four treatments: high spongy moth density + Mimic, high spongy moth density + control treatment, low spongy moth density + Mimic and low spongy moth density + control treatment.

2.2 | Arthropod sampling

We sampled caterpillar and adult parasitoid communities by fogging 60-m^2-canopy areas, covering one major tree, in each plot between 2019 and 2021. Single tree canopy fogging is an effective method for sampling canopy-dwelling arthropods (Basset et al., 1997; Floren et al., 2022) with only limited disruption to ecosystems, as the insecticide used (natural pyrethrum) breaks down very quickly into non-toxic compounds (Oguh et al., 2019). Timing of sampling sessions was set to coincide with the peak caterpillar abundance (April–July) and absence of precipitation and wind. This resulted in different sampling periods among years, with most individuals sampled in May for all 3 years (details can be found in Leroy et al., 2023). The sampling areas were swapped each year to prevent potential carry-over effects from fogging in the year before. At each location, four 3 × 5 m tarpaulins were placed on the forest floor around a single mature oak tree to collect insects falling from the fogged tree crowns. Insects were knocked down with a 2.5% oil-pyrethrum solution fogged into the canopy with SwingFog SN50 machines (Swingtec, Isny, Germany). Fogging machines were operated until the canopy area above the sheets was fully coated by the fog cloud. All arthropods on the sheets were collected after 30 min and stored at −18°C until further processing. No ethical approval was required for this study.

2.3 | Species determination

Adult parasitoids and caterpillars were sorted from the canopy arthropod samples. Adult parasitoids of three taxa (Microgastrinae, Sarcophagidae and Tachinidae) were usually morphologically identified by expert taxonomists; if morphological determination was not possible, specimens were assigned to a molecular operational taxonomic unit (mOTU) as species proxies based on DNA barcoding using the cytochrome *c* oxidase subunit I (COI) gene. All larvae were counted and separated into three groups: (1) spongy moth caterpillars, (2) sawfly larvae and (3) other species. Non-spongy moth larvae were initially classified into morphospecies based on visual characteristics and second via metabarcoding.

DNA barcoding and metabarcoding for the species-level identification of the caterpillars and the immature parasitoids inside the caterpillars was carried out at the University of Guelph's Centre for Biodiversity Genomics. The detailed DNA barcoding protocol is described in Leroy et al. (2023). DNA extraction and amplification followed standard protocols (https://ccdb.ca/resources/) and employed SMRT sequencing of asymmetrically labelled COI amplicons (Hebert et al., 2018). To minimise the inhibition in the PCR from secondary plant compounds in the gut of the caterpillars, a DNA extraction

protocol typically used for plants was employed for the caterpillar samples (Ivanova et al., 2008). Each caterpillar was homogenised and 50 μL of the resultant lysate from each caterpillar homogenate were transferred to a well in a 96-well microtiter plate and subjected to bind-wash-elute DNA extraction procedure. The initial step involved combining and mixing lysates with binding buffer (contains 5M GuSCN). After mixing, the samples were transferred to 96-well glass fibre filter plates (Pall Corporation) and then positioned on a vacuum manifold to bind DNA to the silica membrane. Following four DNA wash steps, and removal of residual wash buffers and evaporation of remaining ethanol, the DNA was eluted into 80 μL of elution buffer for subsequent use as a template for the PCR. The full-length (658 bp) COI barcode region was amplified with asymmetrically labelled fusion primers supplied by Integrated DNA Technologies. Each PCR contained a 1:1 mixture of two forward oligos (LepF1 and LCO1490) tailed with a unique forward Unique Molecular Identifier (UMI), and a 1:1 mix of two reverse oligos (LepR1 and HCO2198) tailed with a unique reverse UMI. All oligos contained a five-nucleotide pad at the 5′ end of the primer. After PCR, 9216 unique amplicons (including controls) were combined from 24,348-well microplates into a single amplicon pool. The pool was purified with AMPure beads and quantified on a Qubit Fluorimeter. SMRTbell libraries were prepared using a standard PacBio protocol for short inserts with the Express Template kit 2.0. The SMRTbell library for each amplicon pool was sequenced on its own 8M SMRT cell on Sequel II with a 10 h movie time and 150 pM on plate loading concentration. Post-sequencing analysis of circular consensus reads, including contig assembly and taxonomy assignments, were completed on the mBRAVE platform (Ratnasingham, 2019).

DNA barcodes were obtained from 86% of the sequenced caterpillars. All barcodes were aligned to voucher sequences in the BOLD database via BLAST searches with a minimum sequence similarity threshold of 97%. To improve taxonomic resolution, the resulting taxonomic assignments were compared to the preliminary morphological identifications. Preference was given to species identities determined by DNA barcoding when there was a conflict between the methods and when individuals lacked a morphological identification. Morphological assignments were retained for caterpillars without barcodes if both methods agreed for most specimens assigned to a particular morphotype. However, if this validation was not possible (i.e. for singletons), only morphological assignments with high confidence were retained. All sequence data are available through BOLD dataset DS-LDMPOAK1D.

2.4 | Statistical analyses

All statistical analyses were performed in R 4.1.1 (R Core Team, 2020). Based on the experimental study design we evaluated the effects of spongy moth density and Mimic among years (Leroy et al., 2021) on parasitoid abundance, species number and community composition. We applied generalised linear mixed models using the glmm. TMB package (Brooks et al., 2017) and used abundance (number of individuals found on the 3 × 5 m tarpaulin) and species number of parasitoids as response variables. Abundance and diversity data from different sampling sessions were pooled by year. However, reducing the dataset to only the main peak (May) showed generally similar patterns (respective R script is provided). Predictor variables included sampling years (2019, 2020 and 2021) and, to analyse the parasitoid response to the different treatments over time, insecticide treatment (control/Mimic) and spongy moth (high/low) in interaction with year. Block was included as random effects to account for the experimental design. To identify the main drivers of the parasitoid assemblage at a block level, we used multiple regression models on distance matrices (MRM; Lichstein, 2007) using the *MRM* function from the ecodist package (Goslee & Urban, 2007). After assigning numerical dummy variables for the categorial predictors insecticide treatment (0 = control, 1 = Mimic) and spongy moth density (0 = low density, 1 = high density), distance matrices were calculated as Bray-Curtis dissimilarities using the function *vegdist* from the vegan package (Oksanen et al., 2020). The distance matrices for sampling years were created as Euclidian distances using the function *dist* provided by the vegan package. To make the estimates comparable, all distances were scaled to a zero mean and unit variance.

For the analysis of the caterpillar-parasitoid network, we pooled data from the high and low spongy moth densities for each year to increase statistical power and tested only the effect of Mimic. Quantification of taxonomic network diversity and network specialisation was done using the package iNEXT.link, which accounts for incomplete sampling data (Chiu et al., 2023). The taxonomic network diversity incorporates interaction richness and evenness among the strength of interactions in an ecological network, with a high taxonomic network diversity indicating even interaction frequencies among networks. Using the *iNEXT.link* function, sample-size (number of caterpillar-parasitoid links) based rarefaction and extrapolation curves and coverage-based (standardised level of sample completeness) rarefaction and extrapolation curves were computed to assess taxonomic network diversity while taking sampling effort into account. Calculations were based on the Hill numbers $q = 0$ (interaction richness; rare interactions), $q = 1$ (the exponential of Shannon entropy; common interactions) and $q = 2$ (the inverse of the one-complement of the Gini–Simpson index; dominant interactions) (Hill, 1973). Network specialisation was computed using the function *Spec.link* as a function along with the Hill numbers and with a standardised sample coverage of 90% (other standardised coverage values yielded the same ordering and significance). Calculations of network specialisation were based on two unevenness measures, with unevenness measured by the normalised slope of the Tsallis-entropy profile (1-E1) and the normalised slope of the Hill-number profile (1-E3) (Chao & Ricotta, 2019). Both measures are based on the evenness of link frequencies in networks and quantify whether interactions are specialised (i.e. the distribution of link frequencies among the interactions is very uneven) or generalised (i.e. the distribution of link frequencies among the interactions is even; Chiu et al., 2023). Significant differences are indicated by non-overlapping confidence intervals.

3 | RESULTS

Over the 3 years, 978 adult parasitoids of the taxa Microgastrinae, Sarcophagidae and Tachinidae were sampled and assigned to 99 species/mOTUs. The metabarcoding of caterpillars revealed 63 lepidopteran species, 16 Symphyta species and 1074 records representing 122 putative species (BINs) from four parasitoid families (Braconidae—500 BINs, Ichneumonidae—495 BINs, Tachinidae—73 BINs and Eulophidae—6 BINs). We found seven parasitoid species specific to the spongy moth (Figure S1).

3.1 | Parasitoid abundance, species number and community composition

The abundance and species diversity of adult parasitoids from three families varied significantly among years, with the lowest number in 2021 (Figure 1a, Table S1). Community composition of adult parasitoids was best explained by year (Figure 2a; Figure S2A). Mimic treatment and spongy moth density did not affect abundance, species diversity nor community composition of adult parasitoids.

The abundance and species number of immature parasitoids from four families varied among years, with the lowest numbers in 2021. Abundance was significantly reduced by Mimic in 2019, and species number decreased in Mimic-treated areas in 2019 and 2020 (Figure 1b, Table S1). Community composition of immature parasitoids differed among years (Figure 2b), however, based on the 48-plot design, community variance was best explained by year and Mimic application (Figure S2B). Neither abundance nor species number nor community variance of immature parasitoids were affected by spongy moth density.

3.2 | Taxonomic network diversity

Sample-size-based rarefaction and extrapolation showed that taxonomic network diversity rose consistently with increasing sample size for all Hill numbers for both treatments (control, Mimic) and both years (2019, 2020), although for dominant interactions ($q=2$) network diversity of control treatments plateaued within the extrapolation curve (Figure 3a). For common ($q=1$) and dominant ($q=2$) interactions, no differences in taxonomic network diversity between treatments were detected. However, for rare interactions ($q=0$), the interaction diversity was greater for networks in areas without Mimic treatment than in those treated with Mimic in 2019 and 2020.

The coverage-based taxonomy network diversity curve showed an increase of interactions with increasing sample coverage for all treatments in both years. However, sample coverage was incomplete in both years for both treatments (Figure 3b). For rare interactions ($q=0$), the control area had higher network diversity (more interactions) than Mimic-treated areas when sample coverage exceeded 75% in 2019 and 80% in 2020. For common and dominant interactions ($q=1$ and $q=2$), interaction diversity did not differ between treatments.

3.3 | Network specialisation

Network specialisation was lower for rare, common, and dominant interactions in the Mimic-treated areas than in the control areas for 2019 and 2020 (Figure 4). Measuring network specialisation by 1-E3 (normalised slope of Hill-number profile) yielded a similar pattern, with a higher specialisation in control treated areas.

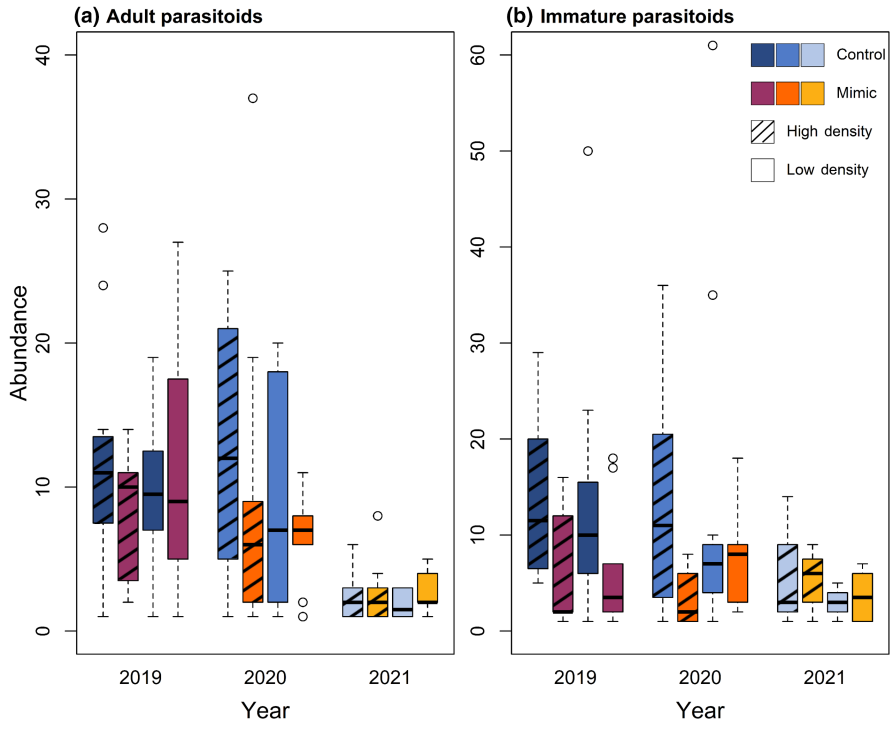

FIGURE 1 Effects of insecticide treatment (Mimic) and spongy moth density on the abundance of (a) adult parasitoids from three families and (b) immature parasitoids from four families over 3 years.

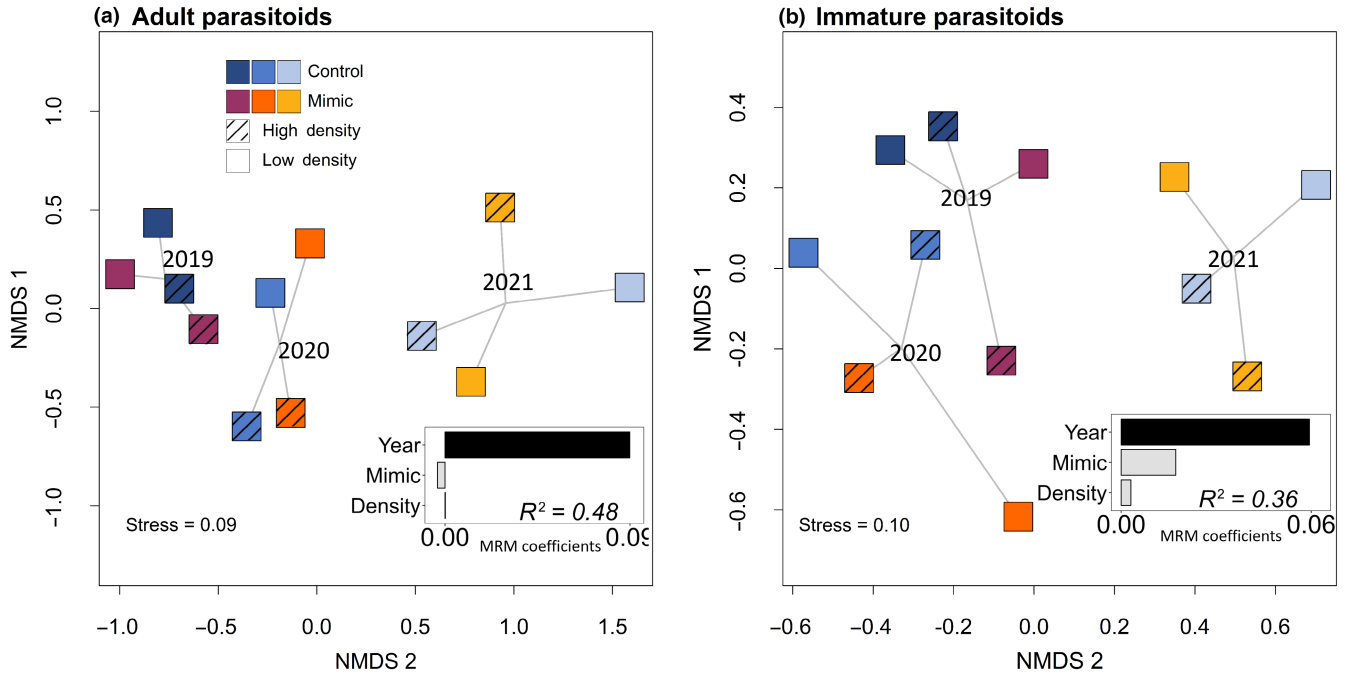

FIGURE 2 Nonmetric multidimensional scaling (NMDS) ordination spider-plot of (a) adult parasitoids from three families and (b) immature parasitoids from four families over three years. Filled symbols show the four treatments: High spongy moth density + Mimic, high spongy moth density + control, low spongy moth density + Mimic and low spongy moth density + control over three years. Multiple regression on distance matrices (MRM) coefficients of the predictors (year, Mimic treatment, spongy moth density) are added to the corresponding NMDS plots. Black bars indicate significance in MRM. NMDS and MRM results of both parasitoid datasets based on the 48-plot design are presented in the Supporting Information.

4 | DISCUSSION

This real-world experiment and use of DNA-based identification provides important insights into the effects of insecticide spraying with Mimic (tebufenozide) on forest canopy parasitoid communities in the presence of both outbreak and non-outbreak spongy moth populations. Both adult parasitoids and immature parasitoids in caterpillars showed strong annual variation with immature parasitoids responding to Mimic applications with a decrease in abundance and species number, producing a shift in community structure. Spongy moth density had no effect on parasitoids, neither on adults nor on immatures. Although metabarcoding provided detailed insights into host-parasitoid interactions, not all possible interactions were detected. Here, the rarefaction-extrapolation curves indicate that many more caterpillars would have to be collected to reach an asymptote, particularly to characterise rare host-parasitoid links (Figure 3), even for dominant host-parasitoid links in Mimic-treated areas. However, it was clear that the caterpillar parasitoid network controlled for sample coverage responded strongly to Mimic application, with reduced taxonomic network diversity for rare interactions and reduced specialisation. As sample coverage systematically varies with Mimic treatment due to reduced number of caterpillars, neglecting sampling effects would have impeded valid conclusions.

We hypothesised that parasitoid diversity and community will strongly respond to spongy moth densities and Mimic application. Contrary to expectations, the abundance, species number and community structure of adult parasitoids were not reduced or affected by Mimic application or by spongy moth abundance. Similarly, spongy moth densities had only limited effects on herbivore assemblages (Leroy et al., 2023). However, adult parasitoids showed a steady decline in abundance and species number over the 3 years. Although spongy moth densities were not identified as a main driver for adult parasitoid abundance, their gradual decline may be linked to the outbreak conditions which peaked in 2018 and 2019. Parasitoid populations studies suggest an oscillating host-parasitoid pattern, with parasitoids tracking host populations with a time lag (Williams et al., 1992) and perceiving cues about host abundance and distribution (Gould et al., 1990). The spatial scale at which higher trophic levels operate is generally larger than that of lower trophic levels and adult parasitoids are highly mobile (Cronin & Reeve, 2005). Hence, the population dynamics of adult parasitoids are likely driven by large-scale rather than local events. However, our data on adult parasitoid species consisted of three families and represents a subset of the total adult parasitoid community in the canopy, only some of which attack caterpillars affected by the Mimic treatment. This might be an alternative explanation for why adult parasitoids did not respond to Mimic application.

In contrast to adult parasitoids, the abundance and species number of immature parasitoids were very sensitive to Mimic applications and also showed annual variation. As shown by Leroy et al. (2023), plots treated with Mimic massively reduced the number of *L. dispar* caterpillars and other lepidopteran larvae,

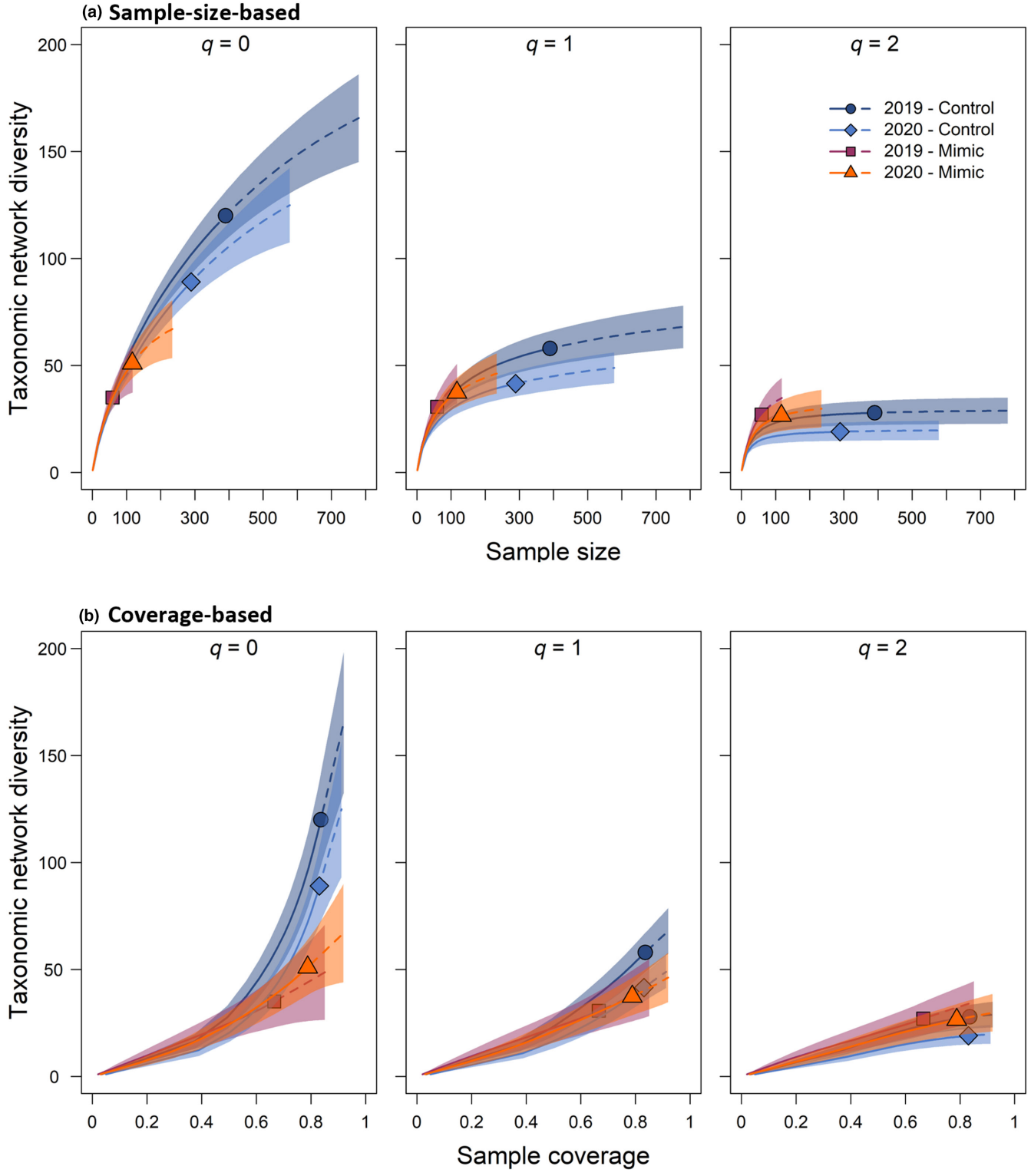

FIGURE 3 (a) Sample-size-based rarefaction (solid lines) and extrapolation (dashed lines; up to twice the reference size) curves and (b) coverage-based rarefaction (solid lines) and extrapolation curves (dashed lines) comparing taxonomic network diversity as a function of Hill numbers for control and Mimic-treated areas for 2019 and 2020. Symbols denote observed data points. All shaded bands denote 95% confidence intervals obtained from a bootstrap method with 100 replications. Data for high and low spongy moth densities was pooled and taxonomic network diversity for all 3 years is shown in the Supporting Information.

creating a scarcity of hosts for parasitoids. In the immature life-stage, endoparasitoids are non-mobile, directly dependent on their host and directly affected by host abundance (Stireman & Singer, 2003). Therefore, abundance and species number reduction of immature parasitoids by Mimic application can be viewed as a direct feedback loop resulting from host scarcity. However,

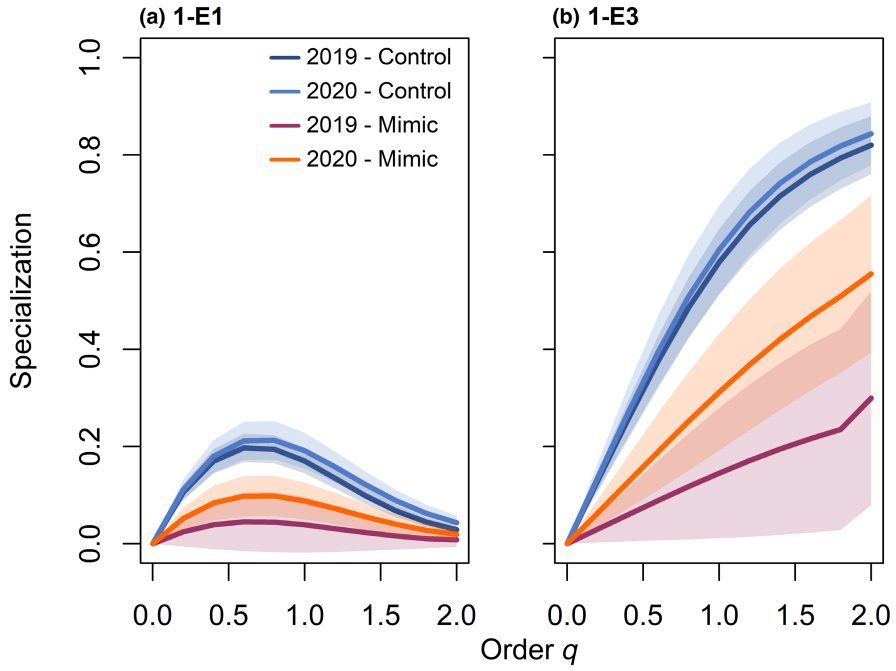

FIGURE 4 Specialisation profiles as a function of Hill numbers based on two unevenness measures (a) 1-E1 (normalised slope of Tsallis-entropy profile) and (b) 1-E3 (normalised slope of Hill-number profile), computed at a coverage level of 90% for control and Mimic-treated areas for 2019 and 2020. All shaded areas denote 95% confidence bands obtained from bootstrap analysis with 100 replications. Data for high and low spongy moth densities was pooled and specialisation profiles for all 3 years are shown in the Supporting Information.

comparisons between the life stages of parasitoids should be made with caution, as the adult and immature parasitoid families studied were distinct, with little overlap.

For both adult and larval parasitoids, we found that communities were very distinct across the 3 years because of marked species turnover over time. Similar patterns have already been documented for *L. dispar* parasitoids (Williams et al., 1992), but have also been found for parasitoid communities of the spruce budworm, with differences in community composition before, during, and after outbreaks (Greyson-Gaito et al., 2021; Royama et al., 2017). Such community differences may reflect distinct preferences of the parasitoids to their host and other caterpillar densities (Greyson-Gaito et al., 2021), but host spectra of parasitoids might also provide an explanation. Polyphagous parasitoid species should remain abundant during non-outbreak conditions, while populations of specialists will be numerically elevated during outbreaks. Their presence will change the composition of the community or even displace other species through interspecific competition (Eichhorn, 1996). Both explanations for temporal variation of the parasitoid community suggest that the species composition is strongly dependent on the phase of the spongy moth outbreak cycle. It is therefore likely that changes in parasitoids occur periodically just as spongy moth populations go through their outbreak cycles. However, sampling at a single point in the season provides only a limited snapshot of the parasitoid community, as there are likely to be parasitoid species that have completed development before the sample was taken (early season) as well as species that occur later (late season). Variation in the timing of sampling from year-to-year may also result in differences in parasitoid communities. Although canopy fogging is an effective method of sampling arthropods (Basset et al., 1997; Floren et al., 2022), it is important to note that the application of the knockdown insecticide causes a short-term disturbance of the ecosystem. However, given the variation in fogging areas from year to year, the quick degradation of the insecticide, and the high mobility of parasitoids, it seems that we can exclude carry-over effects from year to year due to the sampling method.

By analysing links between caterpillars and immature parasitoids, we reveal that Mimic interferes with the complex network, which is consistent with our hypothesis. Importantly, our analyses of the caterpillar-parasitoid network indicate that some interactions await detection and thus, some natural interactions are not reflected in our data. However, after standardising sampling coverage, we found that the diversity of taxonomic networks for rare interactions was lower in areas with Mimic application. This finding is particularly important, as reduced taxonomic diversity indicates that the number of links declined so that fewer interactions between caterpillars and parasitoids can occur. A lower frequency of interactions may result in reduced ecosystem services, that is lower top-down pressure on herbivorous insects and ultimately not mitigating the consequences of outbreaks, the reduced network diversity over two consecutive years indicating medium-term disruption of network architecture. We could not analyse the network's temporal dynamics over a longer period, but long-lasting disruption of network dynamics could even promote future spongy moth outbreaks if the minimum level of interaction of natural controls is disrupted. With the spatial extent of spongy moth outbreaks predicted to increase under climate change (Régnière et al., 2009), followed by potential reorganisation of the network structure (Thompson & Gonzalez, 2017), the effect of altered network structure may be amplified by insecticide usage in future scenarios.

An important feature of the present study is that specialisation networks were consistently more specialised in controls compared to Mimic-treated areas. Higher specialisation in control areas suggests that there are both very frequent and very rare interactions

between caterpillars and parasitoids. This finding aligns with studies pointing out that under high spongy moth densities, specialised species become more active and, together with generalist species, contribute to natural control of spongy moth populations (Alalouni et al., 2013). Therefore, the reduction of network specialisation under Mimic application is likely caused by a scarcity of spongy moths, resulting in a community of parasitoids consisting of generalists and with only evenly distributed interactions. However, it should be noted that the Mimic treatment was applied over several days and it cannot be guaranteed that all caterpillars included in the analyses were equally affected by the insecticide or equally sensitive to it. Given that the disruption of the networks was constant over both years, we interpret these results as direct Mimic effects. Certainly, it would be interesting to study whether changes in network architecture and specialisation have impacts on parasitism rate as specialist parasitoids are more effective at locating their hosts and thus more likely to provide efficient control (Gunton & Pöyry, 2016; Stiling, 1987).

Network studies often rely on sampled data, but capturing all species and interactions in natural environments is challenging, leading to incomplete observations (Chacoff et al., 2012; Jordano, 2016). These limitations can skew calculations of ecological networks and their associated indices, affecting conclusions drawn from them (Dorado et al., 2011; Vizentin-Bugoni et al., 2016). The evaluation of networks based on the concept of sampling coverage as proposed by Chiu et al. (2023) takes into account sampling effects that are not per se considered in the commonly used specialisation index H2' (Blüthgen et al., 2006). However, detecting all interaction links, especially for rare and specialised species, is difficult or even impossible, as evidenced by high proportions of undetected interactions in our results (see increasing rarefaction curves, Figure 3), highlighting the importance of analyses that consider bias introduced by under-sampling, specifically when sample coverage level correlates with treatment.

Despite their obvious importance, our understanding of the diversity patterns of parasitoids is significantly constrained by their small body size, the difficulty of morphological identification, their complex life cycles and their high species diversity. We addressed these challenges through a large-scale field experiment that involved collecting 20,000 caterpillars by canopy fogging and then identifying 1000 interaction links, using DNA barcoding, metabarcoding and morphology. Despite the intensity of this work and the application of new statistical methods, many questions remain about how insecticide treatments affect the diversity of parasitoids and their interactions in mixed oak forests. This highlights that we are only now beginning to identify and understand natural parasitoid communities and their response to environmental change.

5 | CONCLUSIONS

Our study, based on parasitoid data from a real-world experiment obtained through DNA sequencing provides new insights into a very diverse parasitoid community that interacts with host caterpillars in a highly fine-tuned way. The synchronous dynamics of parasitoids over 3 years in oak forests indicate that they are structured at a larger landscape scale and respond immediately to caterpillar outbreaks, highlighting their high mobility. In our study, the diversity and composition of the parasitoid community was dominated by the course of the outbreak and the subsequent dynamics of the caterpillar population. However, our network approach suggests that insecticide application reduces network specialisation during the treatment and the following year. Disruption of finely tuned caterpillar-parasitoid interactions has potentially far-reaching consequences for ecosystem services and must be considered in sustainable forest management. Despite our great effort to measure host-parasitoid interactions, with more than 20,000 individual caterpillars analysed, our analyses show high sample incompleteness, highlighting the need for statistical methods that can account for unobserved links.

AUTHOR CONTRIBUTIONS

Benjamin M. L. Leroy, Jörg Müller and Wolfgang W. Weisser conceived the ideas for the project and designed methodology; Benjamin M. L. Leroy, Evgeny V. Zakharov, Paul D. N. Hebert, Amelie Höcherl, Hans-Peter Tschorsnig, Daniel Whitmore and Jeremy Hübner collected the data; Marina Wolz, Oliver Mitesser and Jörg Müller analysed the data; Marina Wolz, Andrew M. Liebhold and Jörg Müller led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interests to declare.

DATA AVAILABILITY STATEMENT

Data available from the figshare repository https://doi.org/10.6084/m9.figshare.25664013.v1 (Wolz et al., 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Venn diagram illustrating the number of unique and shared parasitoid species from three different caterpillar hosts: Spongy moth (*Lymantria dispar*), Symphyta larvae and Lepidoptera larvae (excluding *L. dispar*).

Table S1. Results of generalized linear mixed effect models for effects of year (2019, 2020, 2021) and the interaction of sampling year with insecticide treatment (control/Mimic) and spongy moth density (high/low) on the abundance and species number of adult parasitoids from three taxa (Microgastrinae, Sarcophagidae and Tachinidae) and immature parasitoids from four families (Braconidae, Ichneumonidae, Tachinidae, Eulophidae).

Figure S2. Nonmetric multidimensional scaling (NMDS) ordination spider-plot of (A) adult parasitoids from three taxa (Microgastrinae, Sarcophagidae and Tachinidae) and (B) immature parasitoids from four families (Braconidae, Ichneumonidae, Tachinidae, Eulophidae) over three years.

Figure S3. (A) Sample-size-based rarefaction (solid lines) and extrapolation (dashed lines; up to twice the reference size) curves and (B) coverage-based rarefaction (solid lines) and extrapolation curves (dashed lines) comparing taxonomic network diversity as a function of Hill numbers for control and Mimic-treated areas for 2019, 2020 and 2021.

Figure S4. Specialization profiles as a function of Hill numbers based on two unevenness measures (A) 1–E1 (normalised slope of Tsallis-entropy profile) and (B) 1–E3 (normalised slope of Hill-number profile), computed at a coverage level of 90% for the years 2019, 2020 and 2021.

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