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Unexpected soundscape response to insecticide application in oak forests

Oliver Mitesser[1] | **Sophia Hochrein**[1] | **Zuzana Burivalova**[2] | **Sandra Müller**[3] | **Christian Strätz**[4] | **Andrew M. Liebhold**[5,6] | **Benjamin M. L. Leroy**[7,8] | **Torben Hilmers**[9] | **Kostadin B. Georgiev**[10] | **Soyeon Bae**[1,11] | **Wolfgang Weisser**[7] | **Jörg Müller**[1,12]

[1] Field Station Fabrikschleichach, Chair of Conservation Biology and Forest Ecology, Biocenter, University of Würzburg, Rauhenebrach, Germany

[2] Department of Forest and Wildlife Ecology and The Nelson Institute for Environmental Studies, University of Wisconsin–Madison, Madison, Wisconsin, USA

[3] Department of Geobotany, Faculty of Biology, University of Freiburg, Freiburg, Germany

[4] Bureau for Ecological Studies C. Strätz, Bayreuth, Germany

[5] USDA Forest Service Northern Research Station, Morgantown, West Virginia, USA

[6] Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic

[7] Terrestrial Ecology Research Group, Department of Life Science Systems, TUM School of Life Sciences, Technical University of Munich, Freising, Germany

[8] Hans Eisenmann-Forum for Agricultural Sciences, Technical University of Munich, Freising, Germany

[9] Chair of Forest Growth and Yield Science, Department of Life Science Systems, TUM School of Life Sciences, Technical University of Munich, Freising, Germany

[10] Hessian Agency for Nature Conservation, Environment and Geology, Biodiversity Center, Giessen, Germany

[11] Centre of Biodiversity and Sustainable Land Use, University of Göttingen, Göttingen, Germany

[12] Department of Conservation and Research, Bavarian Forest National Park, Grafenau, Germany

Correspondence
Oliver Mitesser, Chair of Conservation Biology and Forest Ecology, Biocenter, University of Würzburg, Glashüttenstr. 5, 96181 Rauhenebrach, Germany.
Email: oliver.mitesser@uni-wuerzburg.de

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Abstract

Rachel Carson's warning of a silent spring directed attention to unwanted side effects of pesticide application. Though her work led to policies restricting insecticide use, various insecticides currently in use affect nontarget organisms and may contribute to population declines. The insecticide tebufenozide is used to control defoliating Lepidoptera in oak forests harboring rich insect faunas. Over 3 years, we tested the effect of its aerial application on bird populations with autonomous sound recorders in a large, replicated, full factorial field experiment during a spongy moth (*Lymantria dispar*) outbreak. The soundscape analysis combined automated aggregation of recordings into sound indices with species identification by experts. After pesticide application in the year of the outbreak, acoustic complexity in early summer was significantly reduced. The soundscape analysis showed that the reduction was not related to birds, but instead to the large reduction in caterpillar feeding and frass dropping. Effects on the vocal activity of birds were smaller than originally expected from a related study demonstrating tebufenozide's negative effect on bird breeding success. The legacy of the pesticide treatment, in terms of soundscape variation, was not present in the second year when the outbreak had ended. Our results showed a dimension of insecticide-induced acoustic variation not immediately accessible to the human ear. It also illustrated how a multifaceted soundscape analysis can be used as a generic approach to quantify the impact of anthropogenic stressors in novel ways by providing an example of remote and continuous sound monitoring not possible in conventional field surveys.

KEYWORDS
defoliation, *Lymantria dispar*, oak forest, pest control, soundscape, tebufenozide

INTRODUCTION

The world's increasing demand on resources has created multiple anthropogenic stressors on biodiversity (Reid et al., 2019; Wagner et al., 2021). The simultaneous rise in many of these stressors, as well as interactions among them, makes it increasingly difficult to identify causal relationships under real-world conditions (Outhwaite et al., 2022). Prominent examples are recent observations of declines in insect abundance and diversity, raising challenging questions about the relative impact of land use, climatic factors, and agrochemicals (Harvey et al., 2023). The heterogeneity in data and responses across different regions, taxonomic groups, and habitats complicates attempts to make general conclusions (Harvey et al., 2023; Outhwaite et al., 2022; van Klink et al., 2020).

The use of insecticides in agriculture and forestry has vastly diversified over the last 100 years, as have concerns about the secondary impacts on biodiversity. As awareness of such impacts has grown, more specific toxins have been developed to avoid harming nontarget taxa and the broader environment. The insecticide DDT (dichlorodiphenyltrichloroethane), now widely known to be harmful to ecosystems, once replaced arsenicals as one such targeted toxin in the 1940s (Pérez-Criado & Bertomeu Sánchez, 2021). Despite the ban on DDT in the United States and many other countries since the 1970s, new insecticides also have the potential to adversely affect biodiversity in farmland (Geiger et al., 2010). In the 21st century, vertebrates are exposed to fewer toxins because the use of organophosphates decreased (Schulz et al., 2021). Yet, across Europe, insecticides used in agriculture still pose increased risks for aquatic invertebrates (Wolfram et al., 2021). In the United States, there remains concern about the exposure of aquatic invertebrates, pollinators, and terrestrial plants to insecticides despite a decrease in the use of agrochemicals (Schulz et al., 2021).

In forests, the application of insecticides to large areas is less common compared with agriculture due to considerably higher economic thresholds (Thompson, 2011). However, aerial applications are still performed to suppress sporadic outbreaks of defoliating caterpillars (Holmes & MacQuarrie, 2016; Matyjaszczyk et al., 2019; Rindos & Liebhold, 2023; United States Department of Agriculture Forest Service, 2022). One of the most significant defoliator species is the spongy moth (*Lymantria dispar* L.). Across its native range, spanning most of temperate Palearctic, this insect feeds on hundreds of tree species, but in Europe and parts of Asia, it predominantly feeds on oak, *Quercus* spp. Accidentally introduced to North America in the 19th century (Elkinton & Liebhold, 1990; McManus & Csóka, 2007), the species has spread over about one third of its potential range (Morin et al., 2005) and causes extensive economic damages (Aukema et al., 2011; Coleman et al. 2020). Outbreaks typically develop synchronously over large areas every 8–12 years, persisting locally for 1–3 years (McManus & Csóka, 2007). Products currently registered for suppression treatments are almost exclusively Lepidoptera-specific larvicides that are only active upon ingestion by caterpillars.

The entomopathogenic bacterium *Bacillus thuringiensis* var. *kurstaki* (Btk) has long been established as the primary treatment option across Europe and North America (Liebhold et al., 2021; Zúbrik et al., 2021). It is sometimes substituted by tebufenozide, a synthetic agonist of the ecdysone hormone that governs the molting process in Lepidoptera. Tebufenozide shows higher environmental stability, which allows for more consistent effectiveness, albeit at the cost of longer lasting environmental side effects (Leroy et al., 2023). It is deployed in the US spongy moth management strategy (United States Department of Agriculture, 2012) and was applied to 48% of the area treated during outbreak suppression efforts from 2013 to 2022 (United States Department of Agriculture Forest Service, 2022). In Germany, tebufenozide has been the main treatment for spongy moth management since its approval for use on forests in 2018 (Hahn et al., 2021).

Temperate mixed-oak forests are among the most diverse forest habitats in Central Europe. These ecosystems are specifically associated with an abundant and diverse assemblage of Lepidoptera (Brändle & Brandl, 2001; Southwood et al. 2004). Larval populations peak in the spring, when they become a cornerstone of the food web as the primary food source for invertebrate and vertebrate predators (Perrins, 1991; Southwood et al., 2013). The impacts of Btk and tebufenozide on Lepidoptera communities are well documented. Both substances cause substantial community-wide population reductions, although species-specific responses may vary owing to differences in physiological sensitivity and exposure patterns (e.g., Boulton et al., 2007; Butler et al., 1997; Leroy et al., 2023; Peacock et al., 1998). Although non-lepidopteran species are not directly affected, several studies reported indirect effects on fitness and survival of prey communities (e.g., Hochrein et al., 2022; Silva et al., 2021) and raise the question of previously overlooked mechanisms. In addition, it remains challenging to transfer the results of laboratory assays to field level (Schmidt-Jeffris, 2023). The strong reduction in caterpillar availability that quickly follows insecticide application may alter the structure of the food web by disrupting trophic interactions. However, laboratory and observational studies indicate that the effects of spongy moth outbreaks on nontarget Lepidoptera can exceed the impacts of pesticides used to suppress outbreaks (Manderino et al., 2014; Scriber, 2004). Both types of impacts are important factors to consider for balancing conservation and pest control objectives. However, indirect effects and management trade-offs remain poorly understood because replicated experiments complementing observation studies are lacking (Leroy et al., 2021).

Effects of pesticides on birds have been documented for decades (Mitra et al., 2011). Both direct and indirect impacts on reproduction (Fry, 1995), behavior (Walker, 2003), and survival (Loss et al., 2015) or via the food chain (Boatman et al., 2004) demonstrate a broad spectrum of causal relationships. In addition, interactions of invertebrate prey, parasitoids, and hyperparasitoids might be interrupted by insecticides with guild-specific response (Leroy et al., 2023) complicating analyses. Narrow-spectrum insecticides, such as Btk and tebufenozide, are in general not expected to have direct impact on vertebrates. However, altered behavioral patterns have been reported in warblers following aerial spraying of tebufenozide (Holmes, 1998) and Btk (Awkerman et al., 2011). In both studies, these behavioral shifts were associated with alteration in specific reproductive metrics: lower clutch size and hatch rates in *Leiothlypis peregrina* (Parulidae) in tebufenozide-treated habitats (Holmes 1998) and lower nest success and nestling weights in *Helmitheros vermivorum* (Parulidae) in Btk-treated habitats (Awkerman et al., 2011). Hochrein et al. (2022) demonstrated a reduction in numbers of successful songbird broods in the year of tebufenozide application. Thus, both substances have been linked to important but subtle, indirect effects on the behavior and reproduction of several species of insectivorous birds. It remains unclear whether insecticide treatments affect bird communities beyond single species due to a lack of community-level impact assessments.

One way to measure subtle fitness effects in bird communities indirectly induced by insecticides could be monitoring changes in their acoustic activity (e.g., Stansley & Roscoe, 1999). Declines in frequency of singing behavior may in some species reflect an increase of time allocated to foraging at the expense of singing-associated behaviors, such as pair communication and territorial defense. Acoustic communication is energy demanding, and its magnitude therefore might correlate with bird fitness. Since Carson's (1962) emphasis of silent soundscapes as indicators of reduced biodiversity, bioacoustic methods have improved dramatically (Burivalova et al., 2019; Pijanowski et al., 2011). Modern sound recorders allow automatic and highly standardized recording of environmental sounds, including biophony (animal sounds). Various soundscape indices, which aggregate rich data resulting from long-term recordings to an interpretable level, have been developed to detect changes in ecosystems (Alcocer et al., 2022; Bradfer-Lawrence et al., 2019; Ross et al., 2021). Sound diversity has been used to detect community shifts and homogenization after selective logging in tropical forests (e.g., Burivalova et al., 2018; Campos-Cerqueira et al., 2020), response of terrestrial and marine communities to hurricanes (Gottesman et al., 2021), and climate change effects on biodiversity (Krause & Farina, 2016). Soundscape indices correlate with bird diversity, depending on environmental context and study design (Bateman & Uzal, 2022; Gasc et al., 2015; Sueur et al., 2014; Towsey et al., 2014). However, they are typically not restricted to a specific species group as traditional point count methods would be. Morrison et al. (2021) simulated and extrapolated effects on soundscape indices across all of North America and Europe and found “a pervasive loss of acoustic diversity and intensity of soundscapes across both continents over the past 25 years.”

The application of tebufenozide in 2019 was anticipated to alter the amount and composition of food in treated plots. As a consequence, we expected detectable effects on avian vocal activity, used here as a proxy for bird fitness. We established a full-factorial experiment with a spatial range of 70 km and 11 blocks. Each of the blocks was composed of 4 treatments differing in spongy moth egg mass density (high vs. low) and tebufenozide application (sprayed vs. control): high-density sprayed (HS), high-density control (HC), low-density sprayed (LS), and low-density control (LC). To test the hypothesis of reduced songbird activity indirectly caused by tebufenozide, we deployed one autonomous sound recorder per plot and evaluated the temporal pattern of different acoustic indices. In addition, we employed expert knowledge on bird vocalizations for manual species recognition, which constitutes a novel approach of combining technologically advanced and traditional methods to disentangle relevant mechanisms and effects on particular groups of organisms.

METHODS

The experiment was conducted in Franconia, northwestern Bavaria, Germany, in an approximately 2400-km^2 region delimited by the cities of Würzburg (west), Schweinfurt (north), Bamberg (east), and Bad Windsheim (south) (Figure 1). Plots in that region provided sufficient variation in spongy moth densities with tebufenozide application by helicopter legally permitted. Local forest stands are dominated by deciduous oaks (*Quercus robur* L. and *Quercus petraea* Mattuschka) that have experienced recurrent and spatially synchronous *L. dispar* outbreaks since the early 1990s (Lemme et al., 2019). A detailed description of the experimental design has already been provided by Leroy et al. (2021).

Local district foresters surveyed spongy moth populations in the administrative regions of Upper Franconia, Middle Franconia, Lower Franconia, and Swabia during fall 2018 by counting egg masses on tree trunks and branches. Moth egg masses were counted on the lower 2 m of tree trunks along a transect typically comprising 10 trees of the dominant social class. Based on the abundance of egg masses on the underside of lower canopy branches of each tree and additional data on stand structure, vitality, and past outbreak history, we calculated a defoliation risk index (DRI) to identify areas at high or low risk of defoliation characterized by high or low expected levels of population densities for the summer of 2019 (details in Supporting Information S1 of Leroy et al. [2021]). Oak-dominated areas with high (>1) and low (<0.5) DRI were selected, excluding young stands (<70 years) and recently sprayed sites (within the last 5 years). Spatial blocks of comparable forest plots were chosen (see Figure 1), and tebufenozide spraying was randomly

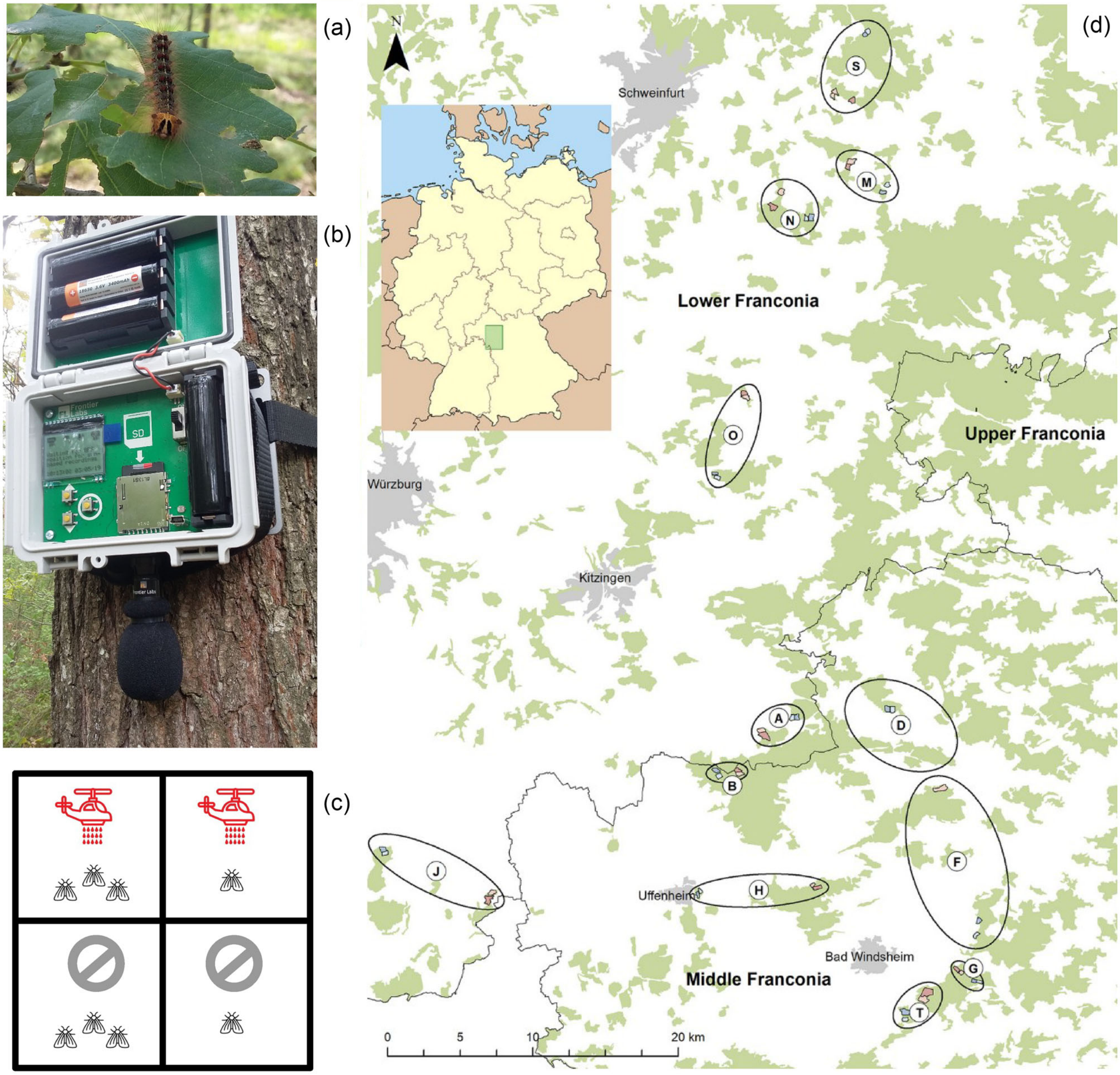

FIGURE 1 (a) *Lymantria dispar* caterpillar, (b) sound recorder attached to a tree trunk, (c) 4 treatment combinations (spongy moth high-density sprayed, spongy moth high-density control, spongy moth low-density sprayed, and spongy moth low-density control), and (d) 11 blocks of the study design in northwestern Bavaria, Germany. Adapted from figure 2 in Leroy et al. (2021). Parts (a) and (b) by Sophia Hochrein.

allocated to one plot per class of expected density (high or low, corresponding to high [>1] or low [<0.5] DRI), resulting in 4 plots per block: high-density (outbreak) sprayed (HS), high-density control (HC), low-density sprayed (LS), and low-density control (LC). We used 11 of these blocks (i.e., 44 plots) for the assessment of songbird community response.

Jacobs et al. (2022) used terrestrial laser scanning in our experimental sites to demonstrate higher oak leaf area in tebufenozide-treated plots and greater crown defoliation in plots initially (2019) characterized by high spongy moth egg mass densities. Defoliation (as well as refoliation) in high-density plots without tebufenozide application was also tracked by satellite-based lidar data (Bae et al., 2021). The impacts of tebufenozide and defoliator outbreaks on secondary tree growth (Hilmers et al., 2023) and caterpillar communities (Leroy et al., 2023) differed among treatments, defoliation intensities, and even trait composition.

The insecticide Mimic (Spiess-Urania Chemicals) (240 g/L active ingredient [a.i.] tebufenozide) is a growth disruptor that specifically targets Lepidoptera larvae, the dominant food source of insectivorous birds. Mortality occurs following ingestion by caterpillars. It was applied at the maximal legal rate of 750 mL diluted in 50 L of water per ha (i.e., 180 g a.i./ha) from 3 to 23 May 2019, targeting the early developmental stages

(L2, L3), when larvae are still small and feeding less than in later stages (Leroy et al., 2021). Mimic was deployed from a Bell 208 helicopter fitted with a Simplex spraying system (Simplex Aerospace) at several meters above the canopy for about an hour per plot. It was applied on entire plots (area ranging from 6.7 to 27.8 ha), and flights were conducted in dry-weather, low-wind conditions (i.e., wind speed below 2.5 m/s) and were organized in blocks whenever applicable.

We recorded soundscapes from April to September in 2019, 2020, and 2021, with Bioacoustic Audio Recorders by Frontier Labs. We deployed one recorder at each plot's center. We attached recorders to tree trunks (<30 cm dbh) at a height of 2.5 m. We chose trees that had no branches or leaves near the recorder that could cause noise. The microphone (omnidirectional sensor element with 80 dB S/N ratio, 14 dBA self-noise, flat frequency response [±2 dB] from 80 to 20 kHz, a fixed gain of 20 dB, and an 80-Hz high-pass filter for filtering out low-frequency wind noise) pointed toward the ground. We programed the timing of recordings with the scheduler software from Frontier Labs (http://www.frontierlabs.com.au/) in relation to the exact time of sunrise and sunset determined by the GPS location of the device. We recorded 2-min segments with a break of 10 min between recordings (10 min/h) around sunrise (from 2 h before to 4 h after sunrise) and sunset (from 3 h before to 3 h after sunset). At each plot, we thus typically recorded 120 min per day, for ∼150 days each year. We chose a sampling rate of 44.1 kHz with a gain of 40 dB because sampling originally targeted birds.

More than 70 bioacoustic indices have been suggested for ecological soundscape analysis (Buxton et al., 2018). Each of them takes a different approach to mathematically summarize the acoustic properties of a recording and performs differently in representing biophony, geophony, or anthrophony (Bradfer-Lawrence et al., 2019). For ecological research, Bradfer-Lawrence et al. (2019) selected and recommended several indices, including the acoustic complexity index (ACI) (Pieretti et al., 2010). The ACI correlates particularly well with bird species richness (Sueur et al., 2014; Towsey et al., 2014); however, reviews by Alcocer et al. (2022) and Bateman and Uzal (2022) challenged the suitability in general and fueled an ongoing discussion. The ACI is nevertheless one of the most widely used indices in studies of biodiversity, and its relationship to birds' vocal activity appears to be strongest in temperate ecosystems, such as ours (Eldridge et al., 2018). We calculated a standard set of acoustic indices with the program AnalysisProgram.exe (Towsey et al., 2018). These indices included ACI; low-, medium-, and high-frequency cover; and events per second. We aggregated the resulting data at different temporal scales (per day [Figure 2] and per hour [Figure 5]) in R 4.0.2 (R Core Team, 2020). Soundscape saturation and acoustic diversity index were calculated according to Burivalova et al. (2018) and Ross et al. (2021), respectively, on a daily basis. Data preprocessing was carried out with the package stringr (Hadley, 2019). We used high amplitude and clipping index to identify recordings with very loud wind and rain noises and excluded all recordings with values >0 from further analysis.

For each year, a professional ornithologist (Kostadin B. Georgiev) listened to several 100 sound files, noted bird songs incidence (i.e., bird counts), and identified corresponding species, blinded to the provenance of the recordings. Separable individuals were counted once in each minute of the 2-min recordings; however, distinction between individuals was not always clear. The expert analyzed recordings from each plot, including 5 or 6 days evenly distributed in April, May, and June, with usually 2 of these days in June. Five sound recordings (i.e., 10 min) were selected each day in an hourly sequence centered at sunrise.

In blind testing, another expert (Christian Strätz) listened to and visually inspected spectrograms of 150 sound files. Files were selected from control and sprayed plots within 5 days around the 171st day of the year (20 of June 2019) with maximum difference in ACI level (see RESULTS) and when larvae typically had reached one of the 2 final stages of development (usually 5 instars for males and 6 for females [Eastern, 1989]). Recording time of the selected files was after sunset from 21:00 to 24:00, when spongy moth larvae typically feed in the canopy of the trees. After listening to each audio file, we assigned a caterpillar sound level to each, ranging from 0 to 4 based on a gradual scale of 5 intensity classes from absent caterpillar sound (level 0) to maximum intensity (level 4). Expert analysis of caterpillar sound recordings was conducted with Audacity 2.1.3 and BCAnalyze 3 pro Standalone 290719 software (example spectrogram in Appendix S4).

To statistically compare the acoustic index time series between treatment and control (Figure 2), we calculated confidence bands for the mean of plot-wise daily mean values for each treatment combination (Schenker & Gentleman, 2001). Generalized additive models (GAMs) with negative binomial error distribution (R package mgcv [Wood, 2023]) allowed identification of differences in bird counts and species richness (response variables) (Figure 3) with respect to tebufenozide treatment and initial egg mass density as fixed factors and geographical plot coordinates constituting an isotropic smoother to account for potential spatial autocorrelation (similar to plot as a random factor in a generalized linear mixed model). The GAMs were calculated separately for 2019, 2020, and 2021. Detailed model structures and outputs are provided in Table 1 and Appendix S8. Relationships between ACI and caterpillar abundance (Appendix S7), caterpillar sound level (Appendix S6), as well as bird counts and species numbers (Figure 4; Appendix S2) were evaluated by Spearman rank correlation analysis. Bird community composition based on the expert bird identification from audio files was tested for effects of the 4 treatments in all 3 years separately, in early (prior to spraying in 2019), mid (May), and late phases during the breeding season. We utilized a nonparametric multivariate statistical permutation analysis of variance (PERMANOVA) to test the null hypothesis that the centroid or spread of distances between bird species matrices differs among treatment groups. Distances between bird species matrices were quantified with Bray–Curtis dissimilarities based on bird counts. To additionally test for

TABLE 1 Results and specification of the generalized linear mixed model linking bird counts and species richness from June of 2019, 2020, and 2021 to predictors initial egg mass density (high vs. low) and insecticide application (present or absent) correcting for spatial autocorrelation via a smoothing term based on geographical plot coordinates[a].

Model[b] and year	Predictor	Estimate	SE	z	Pr(>|z|)
Bird counts ~ density + insecticide + *s*(plot X, plot_Y, bs="tp"), 2019					
	Density low	−0.081	0.047	−1.735	0.083
	Insecticide present	−0.085	0.046	−1.838	0.066
Bird counts ~ density + insecticide + *s*(plot_X, plot_Y, bs="tp"), 2020					
	Density low	−0.015	0.045	−0.332	0.740
	Insecticide present	−0.021	0.045	−0.457	0.647
Bird counts ~ density + insecticide + *s*(plot_X, plot_Y, bs="tp"), 2021					
	Density low	0.103	0.068	1.507	0.132
	Insecticide present	−0.132	0.068	−1.933	0.053
Species ~ density + insecticide + *s*(plot_X, plot_Y, bs="tp"), 2019					
	Density low	−0.097	0.073	−1.341	0.180
	Insecticide present	−0.119	0.073	−1.631	0.103
Species ~ density + insecticide + *s*(plot_X, plot_Y, bs="tp"), 2020					
	Density low	−0.003	0.073	−0.039	0.969
	Insecticide present	−0.091	0.073	−1.244	0.213
Species ~ density + insecticide + *s*(plot_X, plot_Y, bs="tp"), 2021					
	Density low	−0.024	0.080	−0.293	0.770
	Insecticide present	−0.136	0.081	−1.682	0.093

[a] Visualization of underlying data in Figure 3.

[b] Variables: bird counts, number of distinguishable bird individuals; species, species number; density, initial caterpillar density; insecticide, indicator of insecticide absence or presence; plot_X and plot_Y, geographic plot coordinates.

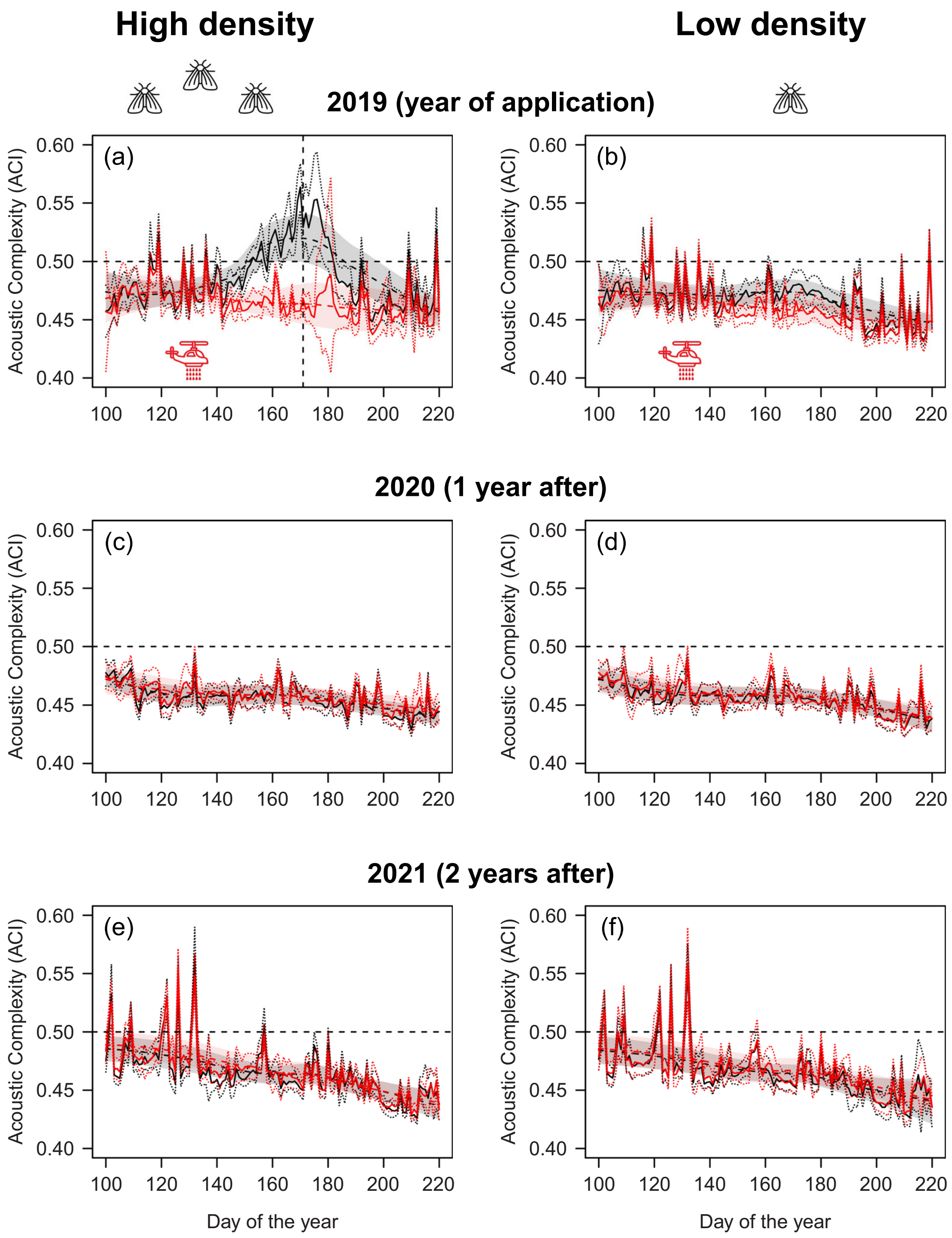

FIGURE 2 Time series of the acoustic complexity index (solid lines, daily mean of daily plot means; dotted lines, 95% confidence interval smoothed by second-order local polynomial regression) in (a, b) 2019, (c, d) 2020, and (e, f) 2021 (rows) in plots with initially high (left column) or low densities (right column) of spongy moth caterpillars and with (red) or without (black) tebufenozide treatment (AIC, acoustic complexity index). Observation intervals begin on the 100th day of the year (10 April 2019 and 2021, 9 April 2020) and end on the 220th day of the year (8 August 2019 and 2021, 7 August 2020) (dashed curves, daily means averaged over 11 plots [see METHODS]; shading, 95% confidence bands; dashed horizontal lines, 0.5 level; dashed vertical line in panel [a], the day [171] of the maximum acoustic complexity). The ACI of the 171st day is presented with higher temporal resolution in Figure 5.

pairwise differences among treatment combinations, we used the R package pairwise Adonis (Martinez Arbizu, 2020), which provides a wrapper function for multilevel comparisons with the adonis() function from the R package vegan (Oksanen et al., 2024).

Data and code are available at https://figshare.com/s/7fa5ac4d0c974f639aaa.

RESULTS

Among a number of soundscape indices, the most sensitive index to the treatments in our experiment was the ACI, an indicator for biological activity (more indices in Appendix S1A–F). In areas that were treated with tebufenozide, acoustic complexity decreased monotonically from April to August for all years

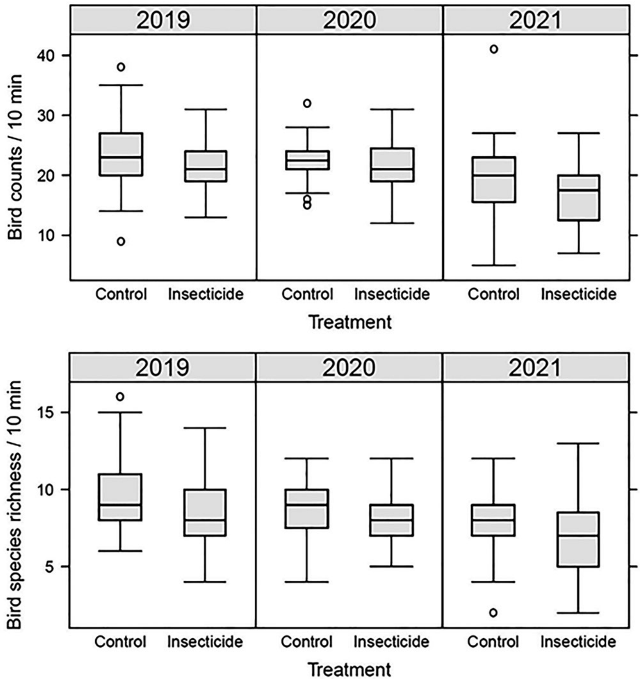

FIGURE 3 Bird counts (upper row) and number of bird species (lower row) detected for control and tebufenozide treatments in June of the 3 years of observations from audio files ($n = 22$ for both treatment levels; whiskers extend to the most extreme data point, which is no more than 1.5 times the interquartile range from the box). High- and low-density plots are pooled. None of the small differences are statistically significant. For statistics, see Table 1. Years 2019, 2020, and 2021 comprise 85, 88, and 83 days, respectively, with 5 evaluated sound files.

(Figure 2) and similarly in sites with both high (Figure 2a,c,e) and low (Figure 2b,d,f) initial densities of spongy moth caterpillars. Daily averages of acoustic complexity in plots and treatments ranged from 0.4 to 0.5. In contrast, in control plots without tebufenozide application, acoustic complexity showed a hump-shaped response with a peak in mid-June (171st day of the year, 20 June), most pronounced in high-density spongy moth control plots (black lines in left column of Figure 2). Acoustic complexity was >0.5 for more than a month. The significant difference in ACI between control and sprayed plots indicated by nonoverlapping confidence intervals in 2019 (Figure 2a) but not in 2020 and 2021 (Figure 2c,e).

In the experimental plots, bird species richness and the number of song and call incidences (bird counts), proxies for abundance, slightly decreased from control to sprayed plots in June for all years (Figure 3). However, changes in median by approximatively 1 unit for both, bird counts and species number, were very small and not significant (summary statistics in Table 1).

The pattern was even more uniform when considered across the entire season in 2020 and 2021 (Appendices S3 & S8). For high-density plots, we found a difference in species composition between sprayed and control plots only late in 2019 ($F = 3.1685$, $p = 0.001$). There was no difference between sprayed and control low-density plots. Yet, these differences hardly explained the observed soundscape patterns (Figure 2) because the ACI levels were not correlated overall with the expert's bird counts and bird species richness. The 2 measures were only correlated in the sprayed plots (Figure 4, right side) but specifically not in the control plots in 2019 (Figure 4, left side), which had the prominent peak in the ACI in summer (Figure 2a). Thus, an additional explanation was required.

The detailed analysis at higher temporal resolution of sound data on day 171 (20 June 2019) showed that the response in acoustic complexity between sprayed and control plots was most pronounced at night (Figure 5a), when the majority of bird acoustic activity had already ceased. Furthermore, the difference was greatest at high frequencies (Figure 5b), compared with mid and low frequencies (Figure 5c,d). High (>11 kHz), mid (1–11 kHz), and low (<1 kHz) frequency indices (Figure 5b–d) indicated the fraction of spectrogram cells above an amplitude threshold.

The seasonal (Figure 2) and daily patterns (Figure 5) of acoustic complexity corresponded with known timing of larval development of spring-feeding folivores in forests. First, larval densities followed the greening of the forest in the spring, with maximum larval biomass in June. Second, spongy moth larvae climb up trees in the evening to feed in the canopy at night, but during the day, they climb down from the canopy to seek cryptic resting sites suggesting that the observed diurnal (Figure 5) as well as the seasonal acoustic patterns (Figure 2) might be directly linked to caterpillar activity. Thus, the reduced sound index level in sprayed plots might simply result from the depression in abundance and activity of the target species due to tebufenozide application.

Our manual evaluation of spectrograms of individual sound files validated this interpretation. It showed low caterpillar sound intensities in sprayed plots and medium to very high sound intensities in control plots (Appendix S5). In high-density plots without tebufenozide treatment, we found a strong correlation between caterpillar sound level evaluated by the expert and the ACI (Spearman $r = 0.84$, $p < 0.0001$) (Appendix S6). Larval abundance, typically recorded in early June after tebufenozide application in May, was correlated with the ACI averaged across 1 week around its maximum on day 171st of the year (20 June) in sprayed and control plots of both density levels (Spearman $r = 0.76$, $p < 0.0001$) (Appendix S7).

DISCUSSION

We found a clear effect of an insecticide treatment on the ACI in a forest habitat. However, we did not find support for the hypothesis that observed soundscape variation results from missing bird songs. Our results showed that an indirect impact of tebufenozide treatment on birds within 3 years after application cannot be positively detected based on acoustic analysis. In the treated plots of the experiment, caterpillar abundance was more than 80% lower than in control plots in the days after spraying (Leroy et al. 2023). A small fraction of the acoustic response in 2019 might be attributed to birds because the rise of acoustic complexity in unsprayed plots (Figure 5a) was also present after sunrise when caterpillars' activity decreased

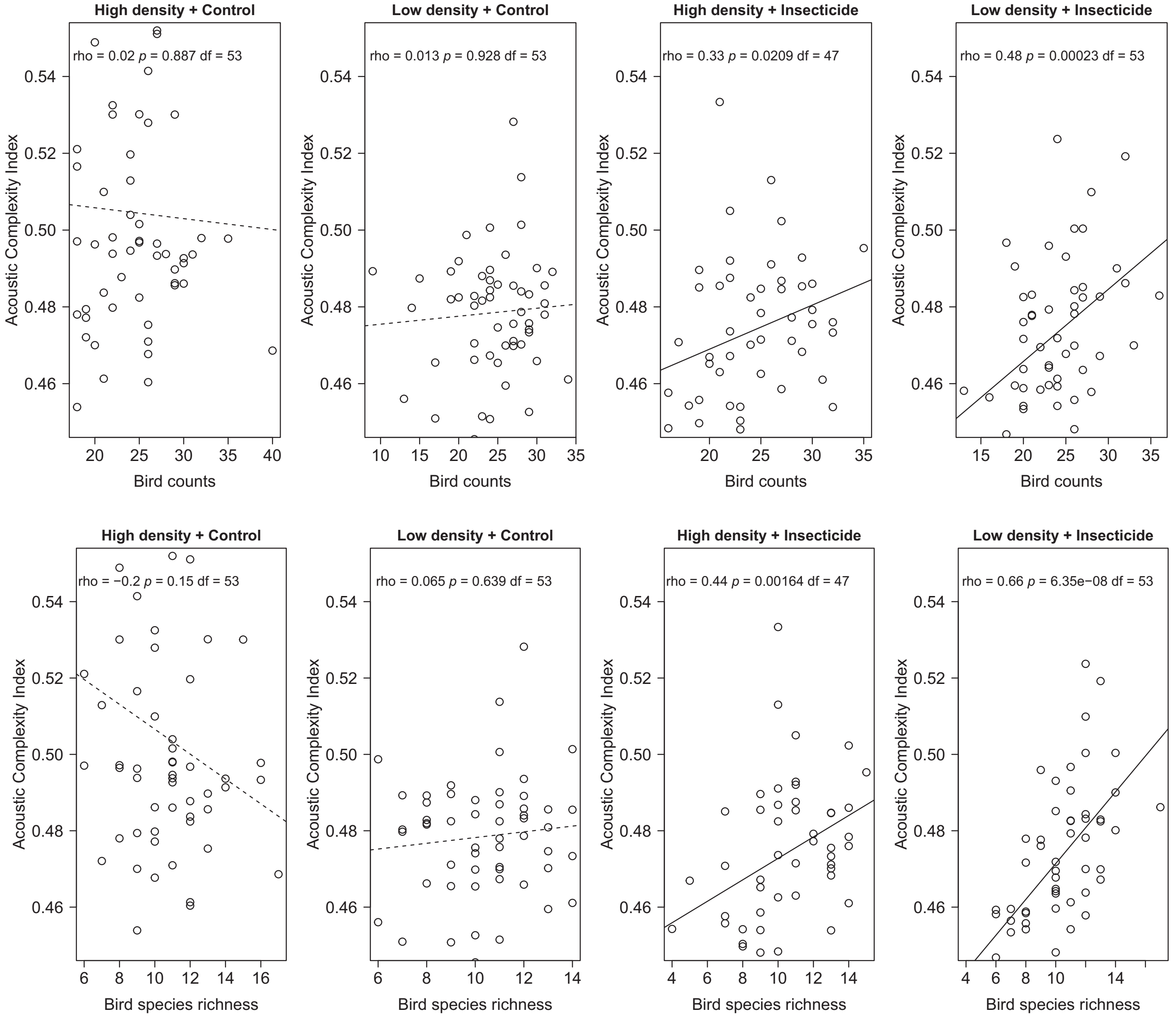

FIGURE 4 Mean acoustic complexity level at 5-day intervals for each annotated audio file relative to bird counts (upper row) and species number (lower row) in 2019 for high or low initial caterpillar density and control or tebufenozide treatments ($n = 11$ for each combination) (solid lines, significant Spearman rank correlations; dashed lines, nonsignificant relationships).

(but see Lance et al. [1987], who documented weaker diurnal rhythms of spongy moth larval feeding during outbreaks). Yet, caterpillars dominated the soundscape response to the treatment. Differences in acoustic indices were most pronounced at night, when spongy moth caterpillars enter the canopy for feeding (Lance et al., 1987), and their dominance in the soundscape was confirmed by expert listening analysis (Appendix S5). Finally, the lack of a correlation between bird community composition identified by the expert and the ACI in untreated control plots throughout the treatment year and partly in the 2 subsequent years (Figure 4; Appendix S2) also supports the conclusion that tebufenozide application did not meaningfully influence bird community composition as measured by the ACI. The nonsignificant effects of tebufenozide on bird counts and species number (Figure 3; Table 1) also matched this pattern. By combining results from both methods (acoustic index analysis and expert analysis of recordings), we were able to identify decreased spongy moth populations as the dominant change following treatment, and this illustrated how a combination of methods often can provide a more comprehensive understanding of changes than can any single approach.

In 2022, another study in the same area and with similar experimental design directly investigated the impact of tebufenozide on breeding success of cavity-nesting birds using standardized nest boxes (Hochrein et al., 2022). They found that successful broods in nest boxes were reduced by 42% in the year of tebufenozide application. In the second year, breeding impairment in the second brood was still visible but no longer significant. The reduction in breeding success did not result in a reduction in bird species and adult individuals present on

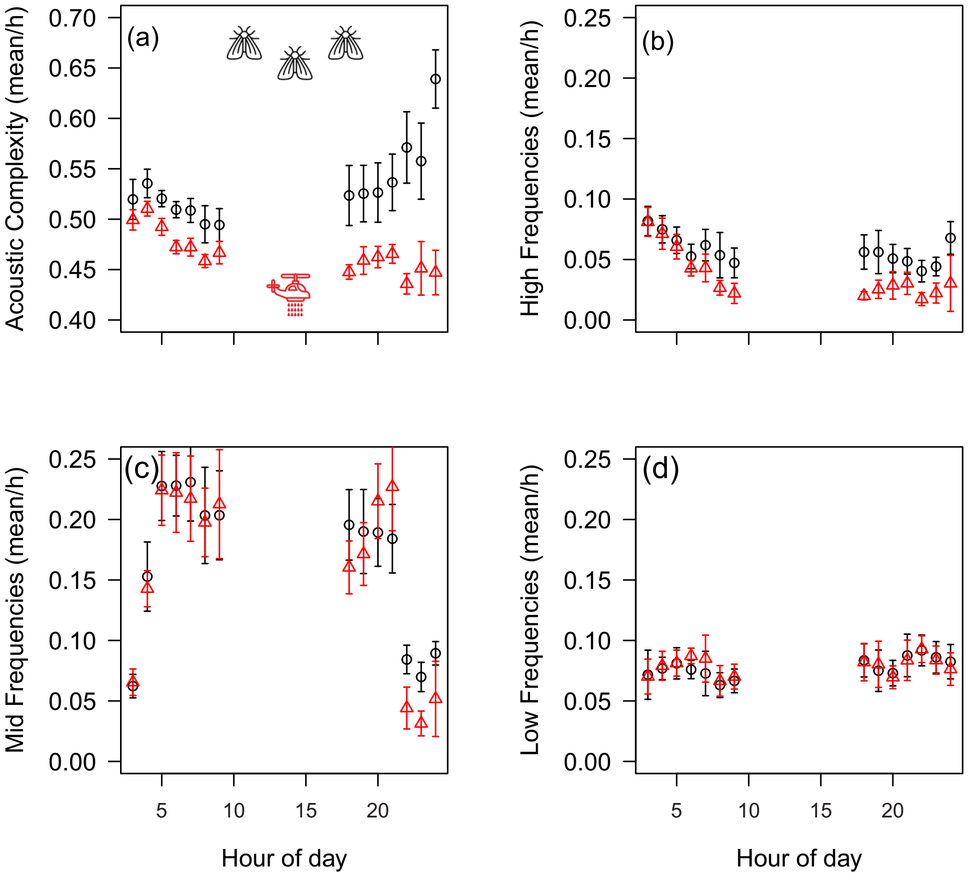

FIGURE 5 Hourly mean of the (a) acoustic complexity index, (b) high frequency index (>11 kHz), (c) midfrequency index (1–11 kHz), and (d) the low frequency index (<1 kHz) for 5 days centered on the 171st day of the year during 2019 (year of tebufenozide application) and high moth density plots (red triangles, average of plots with tebufenozide applications [$n = 11$]; black dots, values in control plots [$n = 11$]; error bars, SE of estimated means averaged over plots).

the experimental plots (Table 1). This could be due to nesting habitats still being occupied by territorial parents while the offspring are moving into new habitats. Taken together, sound index patterns and bird community observations from expert identifications could not show an effect in adult songbird communities, despite the observed reduced reproduction in treated forests for at least 1 year (Hochrein et al., 2022).

Reasons for only minor effect on bird soundscapes

In the early spring, which corresponds to the typical timing of *L. dispar* suppression treatments, leaf-chewing caterpillars comprise a large portion of the insect biomass in European deciduous oak forests (Brändle & Brandl, 2001; Southwood et al., 2004). Insectivorous birds supply their nestlings almost entirely with caterpillars as food sources, and some species of passerines are believed to synchronize the timing of breeding with tree budburst and peak availability of caterpillar biomass in May (Ceia et al., 2016; Perrins, 1991). The second brood observed by Hochrein et al. (2022) coincided well with the maximum of acoustic complexity we detected in early June. A reduction of live caterpillar biomass following an application of the larvicide diflubenzuron, a molting disruptor akin to tebufenozide, had detrimental effects on forest bird breeding success and could be characterized by a reduced fraction of caterpillars in the diet of great (*Parus major*) and blue (*Parus caeruleus*) tit nestlings in sprayed areas (Sample et al., 1993; Schönfeld, 2009). Similar to diflubenzuron, tebufenozide is a larvicide that kills insects by altering the molting process. However, it is highly selective to Lepidoptera (Dhadialla et al., 1998).

Caterpillars compose more than half of the nestling diet in many bird species (Ceia et al., 2016; Nour et al., 1998). Turcek's (1948) list of bird species that feed on *L. dispar* caterpillars included great and other tits as well as nuthatches. However, hairy caterpillars are less preferred food items (Krištín & Patocka, 1997; Whelan et al., 1989) despite the ability of birds to handle caterpillars with urticating setae, such as *L. dispar* (Turcek, 1948; Whelan et al., 1989). Ceia et al. (2016) observed great and blue tits preying on *L. dispar* larvae and adults, but together they comprised only 5% of all prey items. *Lymantria dispar* might be less favored and thus dampen community-level response of birds to tebufenozide application. The weakness of the tendency we saw in bird counts and species number

(Figure 3) might be due to many birds being generalist predators that are adapted to drastic seasonal changes in the composition of their prey community in undisturbed conditions. As shown in Southwood et al. (2004), the biomass of free-living caterpillars collapses toward the end of June, and birds switch to a diet dominated by other prey types, such as spiders in the early summer (Burger et al., 2012; Maziarz & Wesołowski, 2010). Thus, tebufenozide treatments may merely force an earlier dietary shift in birds, whereas *L. dispar* outbreaks likely do not delay the shift due to the low preference for hairy caterpillars.

In addition, the ACI might not be sufficiently sensitive to detect differences in avian species richness or abundance that may have been present in this study. Relationships between abundance and acoustic indices have not been widely explored in soundscape ecology (but see Buxton et al. [2016] and Pieretti et al. [2010]). Thus, it is interesting that we found a significant correlation of abundance with ACI when spongy moth has been removed by spraying (Figure 4; Appendix S2, right half of figures). Moreover, if vocally dominant bird species were less affected by spraying, then an impact on acoustic complexity might not be detectable, even though abundance or even species richness of less vocally dominant species was reduced.

Caterpillar activity

We found advantages and disadvantages of using a broad, generic acoustic index approach in evaluating the consequences and legacy of selective insecticide application and in monitoring pest species incidence in temperate forests. Although aimed at birds, it turned out that here the ACI responded more strongly to differences in caterpillar-generated sound. Several studies successfully disentangled the impact of different species groups on sound indices and pointed to the relevance of non-avian vocalizers, such as amphibians, mammals, and specifically insects, in the acoustic analyses of bird communities (Eldridge et al., 2018; Ferreira et al., 2018). Although Buxton et al. (2018) considered insect-generated sound mainly as noise when evaluating the correlation of sound indices with biodiversity, in our study, it represented the principle observed treatment effect. In other studies, specific sound measurements were taken to detect presence of particular insect species (Desjonquères et al., 2020; van der Lee et al., 2020). The ACI detects temporal changes in frequency and amplitude modulation over frequency bins, within a frequency range where most birds are typically vocalizing (Eldridge et al., 2018; Pieretti et al., 2010). Many studies calculate this index for the frequency range of 500 Hz to 24 kHz, thus effectively eliminating much of potential anthrophonic and geophonic contributions, which are typically characterized by lower frequencies and minor modulation in amplitude and frequency (McGrann et al., 2022). In accordance with Gasc et al. (2015), McGrann et al. (2022) theorized about a negligible effect on ACI from biophony that exhibits minimal frequency modulation, such as is the case for insect sounds. We found that the opposite is true when insect sound is generated by caterpillar activity, mainly from frass dropping in outbreak populations, but also chewing and caterpillar movement on tree trunks. This, in addition, could explain the response of the soundscape index events per seconds (Appendix S1A). Our results also showed limitations and caveats of the acoustic index analysis. For example, the choice of a different acoustic index, restricting soundscapes to daylight hours, certain frequencies, or refraining from using expert knowledge would have limited our study and altered conclusions.

Need of causal analyses

Findings in ecoacoustic studies often stimulate discussion about alternative explanatory processes rather than reliably tracking a specific factor. The lack of experimental selectivity limits understanding of the impacts of various chemical controls against pest species, silvicultural measures, and environmental noise on species communities. Furthermore, specific disturbances might favor one but disfavor another group of vocalizing species. For instance, selective logging can have opposing effects (e.g., detrimental to birds [Burivalova et al., 2021], but beneficial for insects [Campos-Cerqueira et al., 2020]). Here, detailed causal analyses are required to elucidate the relevant mechanisms. Understanding ecological responses might require complex explanatory pathways to explain correlations between acoustic index change and biodiversity loss (Burivalova et al., 2021; Pillay et al., 2019).

Even in our simple case of soundscape variation due to tebufenozide application, neither a sound index analysis nor an expert-based field survey alone would have generated a comprehensive representation of the relevant processes. Naïve employment of sound index analysis could easily have led to a misinterpretation of results, for example, when associating the decrease in acoustic complexity in the sprayed areas with a decrease in bird vocal activity. Thus, acoustic indices should be interpreted with caution and not necessarily as proxies for species diversity because interpreting changes in the acoustic signal is often not straightforward and causal relationships and drivers often cannot be identified with certainty. In contrast, expert-based field surveys are strongly limited by the availability of an expert at single plots or the huge amount of recorded sound files an expert would have to listen to. For an undocumented pattern, such as the seasonal sound profile of caterpillars, with an a priori unknown temporal peak position and range, this can easily result in overlooking the entire extent of the phenomenon. Thus, automated sound index analysis with high temporal resolution and range to identify interesting periods followed by expert evaluation of the relevant time slots resulted in an efficient combination. Our results demonstrated that the dynamics in acoustic space of our ecosystems and landscapes are still poorly understood and that there is much to be learned from observing acoustic patterns and integrating these methods into observational and particularly experimental studies (Ross et al., 2023).

Current developments in sound source identification techniques (Grinfeder et al., 2022) that allow automatic separation of different components of the soundscape (e.g., avian vocalization from insect stridulations or biophony from geophony) will improve the interpretability of detected changes in acoustic dynamics. In addition, advances in species detection algorithms (Kahl et al., 2021; Stowell, 2022), identification of individuals, their abundance, or even call types will allow exploration of acoustic space in more detail and consolidate the emerging field of soundscape ecology in relation to other fields (e.g., chemical ecology).

Difficulty of tracking and quantifying caterpillars in large areas with high spatial and temporal variation

Spongy moth is one of the most deleterious forest defoliating insect pests, has substantial economic and ecological consequences (Aukema et al., 2011; Bradshaw et al., 2016; Rindos & Liebhold, 2023), has greatly expanded its geographical range during the last decades, and exhibits outbreaks over large areas (Coleman et al., 2020; Liebhold et al., 1992). Methods to track its damage are important. In the United States, aerial sketch mapping is used to map the extent of spongy moth defoliation. For example, ∼526,000 ha of spongy moth defoliation was mapped in 2023, and ∼647,500 ha was mapped in 2022 (United States Department of Agriculture Forest Service, 2022). Remote sensing by spaceborne multispectral sensors (e.g., Townsend et al., 2012) has been experimentally applied to map spongy moth outbreaks in the United States; however, image capture can be obscured by cloud cover and adverse weather conditions. Coarse spatial and temporal resolution of imagery also constrains these methods, limiting their operational application. Technological improvements in the temporal and spatial resolution of such data may lead to new operational systems for monitoring highly dynamic defoliation over large spatial extents (Bae et al., 2021). Soundscape analysis is a promising method to overcome previous limitations, too, and could yield further insight when combined with other methods (Dixon et al., 2023). Recorders can be deployed and repaired easily, effective means of interconnection are available, and their cost and energy consumptions are likely to decrease in the future. Bioacoustic surveys could provide added value specifically when utilized to evaluate how well spraying programs control the pest they are targeting. This would require additional and more detailed research on the quantitative correlation between remaining population size of pest species and sound index level, when variation in potential dosage and timing of insecticide application is generated experimentally.

Although the wide-ranging effects of insecticides have decreased in the last decades, it is still vital to continuously monitor the impact, direct and indirect, of new-generation, targeted insecticides. Here, inspired by Carson's (1962) concerns and her methodological stimulus, we used soundscapes to investigate the indirect impacts of a highly effective insecticide. Our experimental study, focused on the consequences of a spongy moth outbreak and the application of tebufenozide, demonstrates that combining 2 ecoacoustic methods, soundscape analysis and expert knowledge of animal sounds, can document and explain an unexpected soundscape response. Although impacts on birds' vocalizations were negligible, we discovered that the soundscape was substantially changed due to caterpillar activity. Thus, this combination of methods is a promising new tool to not only address basic research questions but also to bridge the gap to application and management (Doohan et al., 2019).

AUTHOR CONTRIBUTIONS

Jörg Müller, Wolfgang Weisser, Sophia Hochrein, and Oliver Mitesser designed the research. Sophia Hochrein, Sandra Müller, Benjamin M. L. Leroy, Torben Hilmers, and Kostadin B. Georgiev conducted the research. Oliver Mitesser contributed new reagents or analytic tools. Oliver Mitesser, Jörg Müller, Sophia Hochrein, Zuzana Burivalova, Sandra Müller, Kostadin B. Georgiev, and Christian Strätz analyzed the data. Oliver Mitesser, Jörg Müller, Sophia Hochrein, Zuzana Burivalova, Sandra Müller, Christian Strätz, Andrew M. Liebhold, Benjamin M. L. Leroy, Torben Hilmers, Kostadin B. Georgiev, Soyeon Bae, and Wolfgang Weisser wrote the paper. Kostadin B. Georgiev identified birds by listening to selected recordings. Christian Strätz identified caterpillar sound intensity by listening to selected recordings.

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ORCID

Oliver Mitesser https://orcid.org/0000-0002-3607-877X
Zuzana Burivalova https://orcid.org/0000-0001-5730-7546
Kostadin B. Georgiev https://orcid.org/0000-0003-0475-9439

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SUPPORTING INFORMATION

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

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