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Diet analysis of bats killed at wind turbines suggests large-scale losses of trophic interactions

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Abstract

Agricultural practice has led to landscape simplification and biodiversity decline, yet recently, energy-producing infrastructures, such as wind turbines, have been added to these simplified agroecosystems, turning them into multi-functional energy-agroecosystems. Here, we studied the trophic interactions of bats killed at wind turbines using a DNA metabarcoding approach to shed light on how turbine-related bat fatalities may possibly affect local habitats. Specifically, we identified insect DNA in the stomachs of common noctule bats (*Nyctalus noctula*) killed by wind turbines in Germany to infer in which habitats these bats hunted. Common noctule bats consumed a wide variety of insects from different habitats, ranging from aquatic to terrestrial ecosystems (e.g., wetlands, farmland, forests, and grasslands). Agricultural and silvicultural pest insects made up about 20% of insect species consumed by the studied bats. Our study suggests that the potential damage of wind energy production goes beyond the loss of bats and the decline of bat populations. Bat fatalities at wind turbines may lead to the loss of trophic interactions and ecosystem services provided by bats, which may add to the functional simplification and impaired crop production, respectively, in multi-functional ecosystems.

KEYWORDS

bat fatalities, biodiversity decline, food web, green-green dilemma, renewable energy, wind energy production, wind energy-biodiversity conflict

1 | INTRODUCTION

Globally, land use intensification has driven large-scale losses of biodiversity and changes in species interactions across a variety of ecosystems (Foley et al., 2005; Gámez-Virués et al., 2015). Specifically, intensification of agricultural practice via the promotion of monocultures and chemical crop treatment has led to landscape simplifications, impaired producer–consumer interactions, and the loss of diversity and ecosystem functions (Dainese et al., 2017). While past land use changes have mostly been practiced for improving food production, current land use changes are mostly related to the conversion of farmland into places for energy production from renewable sources. Green energy production involves, for example, planting non-food crop for generating bioenergy via fermentation processes and establishing infrastructures such as solar parks and wind turbines for producing electricity from solar and wind power, respectively (Lu et al., 2009). This current wave of land use intensification turns agroecosystems into multi-functional energy-agroecosystems—a term used in the remainder of the text to describe arable fields used for both crop and energy production. The rationale for establishing multi-functional energy-agroecosystems lies in the need to reduce global carbon dioxide emission rates, and thus many countries have committed to this important goal. Yet, the consequences of multi-functional energy-agroecosystems for biodiversity and ecosystem resilience remains by and large unknown.

Currently, it is poorly understood how energy-producing infrastructures feed back on the diversity and functioning of farmland, including adjacent habitats not used for crop production. For example, wind turbines may alter the microclimate due to increased evaporation caused by changes in the airflow (Armstrong et al., 2014), and because surface temperatures may increase downwind of wind turbines (Walsh-Thomas et al., 2012). Besides, vibrational noise of wind turbines may reduce earthworm abundance with likely cascading effects on soil quality and vegetation (Velilla et al., 2021). Wind turbine platforms and verges may host a higher diversity of pollinators and wildflowers than adjacent field sites, which could help introducing pollinator services to farmland areas (Pustkowiak et al., 2018). Yet, wind turbines also kill large numbers of insects, which could potentially reduce insect-mediated ecosystem services and insect biomass for predators (Voigt, 2021). Additionally, wind turbine facilities lead to displacement of nesting and wintering birds (Leddy et al., 1999; Stevens et al., 2013). Avoidance of wind parks is also known for some bats and raptors (Barré et al., 2018; Garvin et al., 2011; Millon et al., 2018; Reusch et al., 2022), which may lower predation rates on insects or rodents, respectively. Most prominently, trophic interactions can be largely altered when predators die at wind turbines after colliding with the spinning blades (Baerwald et al., 2008; Thaxter et al., 2017; Voigt et al., 2015), yet the spatial scale and specificity of this effect is by and large unknown. As a first step toward an improved understanding of how trophic interactions are altered by turbine-related fatalities, we investigated the trophic interactions of bats shortly before they were killed at wind turbines.

In the temperate zone, bats belonging to the ecological guilds of edge- and open-space foraging species are most vulnerable at wind turbines (Rydell et al., 2010; Voigt et al., 2015). The ecological guild of edge-space foraging bats consists of aerial-hawking species that forage next to structural elements, such as forest edges, whereas open-space foraging bats hunt insects in the unobstructed aerial column far away from natural or man-made structures (Denzinger & Schnitzler, 2013; Heim et al., 2017). Estimated annual losses of bats at single wind turbines may range from 3 to 12, or even 70 fatalities per year and turbine, depending on the geographic region, on landscape features adjacent to turbines, and on the type of wind turbine (Brinkmann et al., 2011; Măntoiu et al., 2020; Rydell et al., 2010; Voigt et al., 2022). These seemingly small numbers add up to hundreds of thousands of fatalities per year for a country like Germany that hosts 30.000 onshore wind turbines (Voigt et al., 2015). In Europe, the legal protection of all bat species requires the implementation of mitigation schemes such as curtailing the operation of turbines at periods of high bat activity (Brinkmann et al., 2011; Voigt et al., 2015). In North America, such mitigation schemes are also efficient, yet their practice is restricted to those regions where protected bats occur, e.g., species listed on the endangered species act in the U.S.A. (Arnett et al., 2011, 2016). In most countries, the efficiency of these mitigation schemes are rarely controlled and old wind turbines operate without such curtailments (Voigt et al., 2022). More importantly, in the vast majority of countries worldwide curtailments are not practised at all to protect bats from colliding with spinning blades. As a consequence, the current practice of wind turbine operation may put populations of vulnerable species at stake (Frick et al., 2017; Friedenberg & Frick, 2021; Zahn et al., 2014). In our study, we looked beyond the population level effect of bat fatalities at turbines by asking what trophic interactions will disappear when bats get killed at wind turbines? This is a relevant question because worldwide open and edge-space foraging bats are known for their ecosystem services. For example, open-space foraging bats such as *Tadarida brasiliensis* and *Chaerephon plicatus* are known to hunt economically relevant pest insects, contributing with billions of U.S. dollars of savings to national economies and the income of local farmers (Boyles et al., 2011; Ghanem & Voigt, 2012; Wanger et al., 2014). In Europe, common noctule bats (*Nyctalus noctula*) are open-space foraging bats, and they are the species with the highest collision risk in central Europe (Rydell et al., 2010). Thus, we used common noctule bats as a model to shed light on what trophic interactions might get lost by fatalities at wind turbines. Specifically, we asked: (1) What species of insects common noctule bats consumed at the time of death, and (2) to which habitats common noctule bats were connected via their insect diet. Further, we asked (3) whether common noctule bats fed on pest insects shortly before dying. Answering these questions may shed light on the trophic consequences of bat fatalities at wind turbines, including the loss of ecosystem services provided by insect-feeding bats.

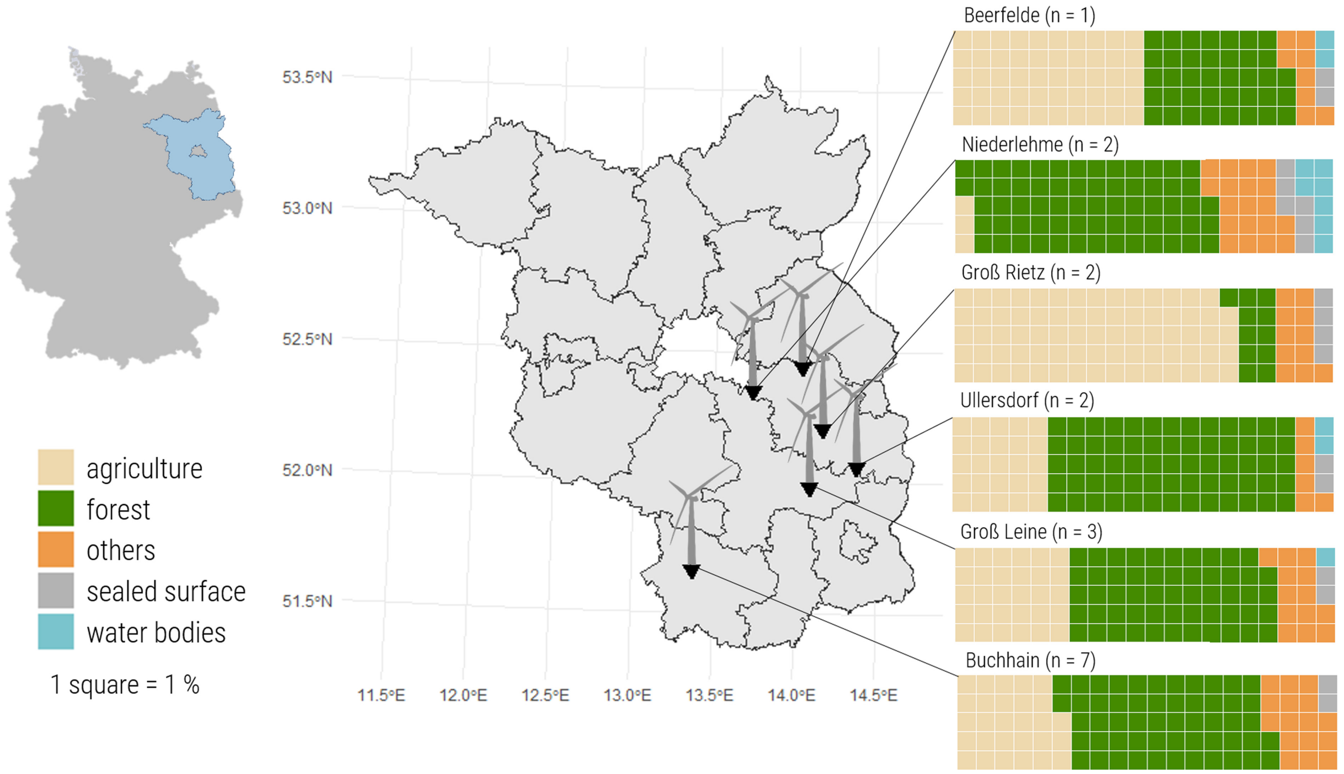

FIGURE 1 Sampling region in the federal state of Brandenburg in the Northeast of Germany. Black triangles on the Brandenburg map (middle) show the locations of those wind turbines where carcasses were collected. Charts on the right illustrate landscape composition of sampling areas within a 5.2 km radius (mean maximum flight distance of common noctule bats from roosts [Roeleke et al., 2020]) and number of bat carcasses found at each site (1 square = 1%)

2 | METHODS

2.1 | Study area and sampling

We obtained 17 fresh carcasses of common noctule bats (*Nyctalus noctula*) from the repository of the Landesumweltamt Brandenburg, Germany (Dürr, 2021). All carcasses originated from search campaigns performed during the summer/autumn migration period at wind turbines in the Northeast of Germany between 2015 and 2019 (Figure 1, Table 1). Carcasses were kept frozen at −20°C from the time of retrieval at a wind turbine until they were defrosted at the Leibniz Institute for Zoo and Wildlife in Berlin, Germany. To ensure a high quality of genetic data, we only used those carcasses that lacked signs of decay, such as holes caused by scavengers, detaching fur, maggots, and smell. After this visual and olfactory check, we verified the species and dissected the animals. We transferred the stomach including its content into a sterile vial, which was put on cool packs. All samples were then preserved in a −80°C freezer.

TABLE 1 Sampling details of carcasses of common noctule bats (*Nyctalus noctula*) included in this study

Sample ID	Sex	Sampling date	Location
1	Male	23.7.2015	Buchhain
2	Female	8.8.2015	Buchhain
3	Female	16.8.2015	Buchhain
4	Male	30.8.2015	Buchhain
5	Female	15.8.2015	Buchhain
6	Female	7.8.2015	Buchhain
7	Male	12.8.2015	Ullersdorf
8	Male	19.8.2015	Ullersdorf
9	Female	30.8.2018	Buchhain
10	Male	7.8.2019	Groß Rietz
11	Female	13.8.2019	Beerfelde
12	Male	7.8.2020	Groß Leine
13	Female	3.8.2020	Groß Leine
14	Female	3.8.2020	Groß Leine
15	Unknown	7.8.2020	Niederlehme
16	Unknown	28.8.2019	Niederlehme
17	Unknown	19.8.2020	Groß-Rietz

2.2 | DNA extraction from stomach content

Throughout the laboratory work, we strictly applied protocols to prevent contaminations by alien DNA or PCR products. The presence of contaminations was checked through all laboratory steps using different negative controls. After thawing samples, DNA was extracted from the stomachs by applying NucleoSpin© Food Kit (Macherey-Nagel GmbH & KG, Düren, Germany) as outlined in the manufacturer's instructions. We performed two DNA extractions for each stomach content sample. The concentration of the extracts was determined by fluorometric quantification in a Qubit Fluorometer (Qubit fluorometric quantification dsDNA High Sensitivity Kit, ThermoFisher Scientific, Walham, USA). Some of the DNAs had to be cleaned and concentrated using a DNA Clean and Concentrator Kit (Zymo Research, 17062 Murphy Ave, Irvine, CA 92614, USA) to get rid of PCR-inhibitors.

2.3 | CO1 mini barcode, PCR and library construction

We performed a double-PCR strategy with dual indexing. The first PCR amplified the target region CO1 (Cytochrome oxidase subunit 1) region (Galan et al., 2018, for PCR conditions, see Supplements). PCR products were checked with agarose gels and stronger products were purified with magnetic beads (CleanNGS, GC biotech, Waddinxveen, Niederlande). The concentration of each product was measured using fluorometric quantification (Quant-iT™ dsDNA Assay Kit, high sensitivity, ThermoFisher Scientific, Walham, USA) in a Tecan plate reader (infinite M200, Tecan, Switzerland). The second PCR added the indices to the target region. Between 1 and 1.5 ng from the first PCR product were used as a template for the indexing PCR. Every product had its unique index combination. Again, products were checked with an agarose gel and cleaned twice with magnetic beads (CleanNGS, GC biotech, Waddinxveen, Niederlande). All products were quantified by fluorometric quantification in the plate reader (Quant-iT™ dsDNA Assay Kit, high sensitivity, ThermoFisher Scientific, Walham, USA) and pooled in equimolar concentration. If necessary, the final library was purified and concentrated by using CleanNGS beads. The quality and integrity of the library were confirmed using the Agilent 2200 TapeStation with D1000 ScreenTapes (Agilent Technologies, Santa Clara, California, USA).

2.4 | MISEQ sequencing

Sequences were generated at the Berlin Centre for Genomics in Biodiversity Research (BeGenDiv) in three runs on the Illumina MiSeq platform (Illumina, San Diego, California, USA) using v3 chemistry with 600 cycles. The quality of the generated reads was evaluated using FastQC v.0.11.9 and multiqc. The remaining adapter sequences were removed using cutadapt (version 2.8).

2.5 | Data filtering, taxonomic assignment and removal of contaminant reads

Data filtering and analyses were done in R version 4.1.0 (R Core Team, 2021) and R Studio 1.4.1717. Sequencing reads processing from quality control to taxonomic assignment was performed using the R package "dada2" (Callahan et al., 2016). In brief, sequencing forward and reverse reads were trimmed to a length of 160 base pairs (bp) after a quality check. Furthermore, forward and reverse primers were trimmed as well. Forward and reverse sequencing reads were dereplicated, and a core sequence-variant inference algorithm, using an error model developed before, was applied to calculate abundance p-values for each unique sequence to identify and drop reads caused by errors. Afterwards, denoised forward and reverse paired reads were merged if they overlap exactly by 20 nucleotides minimum. Finally, chimaeras were removed.

We assigned taxonomy to the inferred Amplified Sequence Variants (ASVs) up to species level based on the reference database provided by Heller and colleagues for CO1 provided by the BeGenDiv (Heller et al., 2018). Taxonomy was assigned based on the single best hit or a last common ancestor (in case of multiple best hits) with 50 out of 100 bootstrap replicates as minimum bootstrap confidence for assigning a taxonomic level. Assigned ASVs were compiled by sample ID and species annotation into a single occurrence matrix for further analysis. For post-sequencing removal of reads caused by contamination, we used the R package "microDecon" (McKnight et al., 2019) which uses the proportions of ASVs in blank samples (negative controls) to systematically identify and remove contaminant reads from the metabarcoding data set.

2.6 | Further data preparation

We summed up reads for pseudo-biological replicates and removed reads which were only present in one of two technical replicates to further increase the power and quality of our data set. We then limited the dataset to the data where we could make a clear identification at the species level.

2.7 | Insect species classification

The final metabarcoding data set including taxonomic assignment was complimented by categorization of detected insect species based on literature information through a google search. In particular, habitat assignment was based on regional online databases. Categories include main ecosystem (aquatic, terrestrial), imago (forest, grassland, ruderal, agriculture, wetlands, multiple, unknown) and larval habitat (soil, forest, water, grassland, agriculture, ruderal, wetlands, multiple, other, unknown) and pest status (0 = no, 1 = yes). Insects were only categorized as pests if they were known to cause economic or ecological damage or to be classified as nuisance pests, thereby potentially also transmitting diseases or pathogens to humans.

2.8 | Species accumulation curve

We analyzed diversity of the common noctule bat diet, hereafter called insect diversity, by estimators of species richness (Hill number $q = 0$, Chao et al., 2014). To estimate how comprehensive our dietary analysis was, we used a rarefaction analysis ("iNEXT"; Hsieh et al., 2020). We extrapolated the data based on double the sample size.

3 | RESULTS

The metabarcoding sequencing of the stomach content of 17 common noctule bats yielded 549,650 reads. After data filtering, chimaera and contamination removal, 206,126 total reads remained in the final data set distributed over 89 different ASVs. Of these, 58 (52%) were identified to the species level. In total, we documented 46 insect species in the stomach contents of common noctule bats belonging to nine insect orders (Table 2; Supplementary) and one Arachnida. Coleoptera and Lepidoptera were the most species-rich groups and were also represented in most samples.

The number of insect orders found within a sample varied from 1 to 6 (mean = 2.5 ± 1.7; mean ± standard deviation) with the number of species detected in a given stomach sample ranging from 1 to 15 (Figure 2). On average, 4.7 ± 4.3 species were found in stomachs of common noctule bats. The most dominating prey species were *Harpalus griseus* and *Spondylis buprestoides* (41% of stomachs analyzed), followed by *Dendrolimus pini* (24%), *Amara bifrons*, *Calathus cinctus*, *Curculio elephas*, and *Harpalus froelichii* (18% each).

TABLE 2 Overview of detected insect orders, number of corresponding species within the stomachs of 17 common noctule bats, distribution over samples and how many reads belonging to insect order in total

Insect order	Number of detected species	Number of samples in which insect order was detected	Total number of reads belonging to insect order
Coleoptera	17	13	128,775
Diptera	6	4	1542
Ephemeroptera	1	2	132
Hemiptera	5	5	364
Hymenoptera	1	2	444
Lepidoptera	12	11	41,857
Neuroptera	1	1	18
Orthoptera	2	2	602
Trichoptera	1	2	287

The majority of insect species (89%) identified in stomachs of common noctule bats originated from terrestrial ecosystems. A small portion of species were from the orders *Diptera*, *Ephemeroptera*, and *Trichoptera*, with larval stages developing in aquatic habitats. All in all, common noctule bats fed on insects from a wide variety of habitats, including farmland, forests, grasslands, and wetlands (Figure 3).

In total, 20% of documented insect species were pest insects, including (sorted by descending prevalence): *Spondylis buprestoides* (Coleoptera), *Curculio elephas* (Coleoptera), *Cydia splendana* (Lepidoptera), *Tipula oleracea* (Diptera), *Culiseta annulata* (Diptera), *Pentatoma rufipes* (Hemiptera), *Plutella xylostella* (Lepidoptera), *Pollenia rudis* (Diptera), *Serropalpus barbatus* (Coleoptera) (Figure 4). Two of the observed pest species are known to be silvicultural pests. The proportion of silvicultural pests in the 17 samples accounted for about 42% of the total number of pest species detections. We observed two nuisance species, representing ~11% of all insect species. Five insect species were agricultural pests, representing 47% of the total number of pest species detections (Figure 4). Overall, we observed pest insects in 12 out of 17 analyzed stomach contents of common noctule bats. Rarefaction analysis shows that our analysis underestimated the true taxonomic diversity of the diet of common noctule bats, i.e., a species

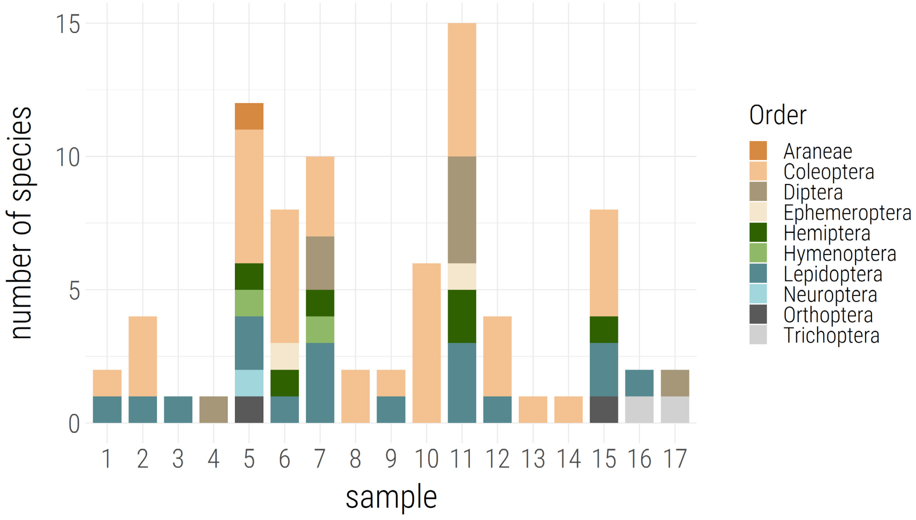

FIGURE 2 Number of detected species per stomach sample from a total of 17 common noctule bats and corresponding order. In total, we detected 46 different insect species from 9 insect orders and a single *Araneae* species

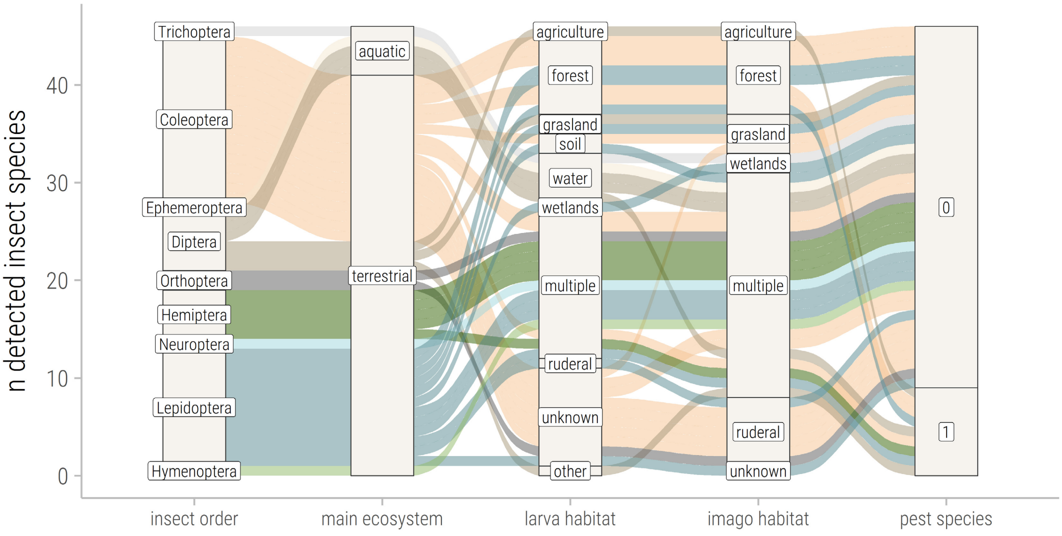

FIGURE 3 Alluvial plot indicating the species diversity of consumed insects (sorted according to order), their affiliation with either aquatic or terrestrial ecosystems, their association to various habitats as larvae and imago, and their categorization as non-pest insects (0) or pest insects (1). Colors of connecting lines indicate inspect species of the same order

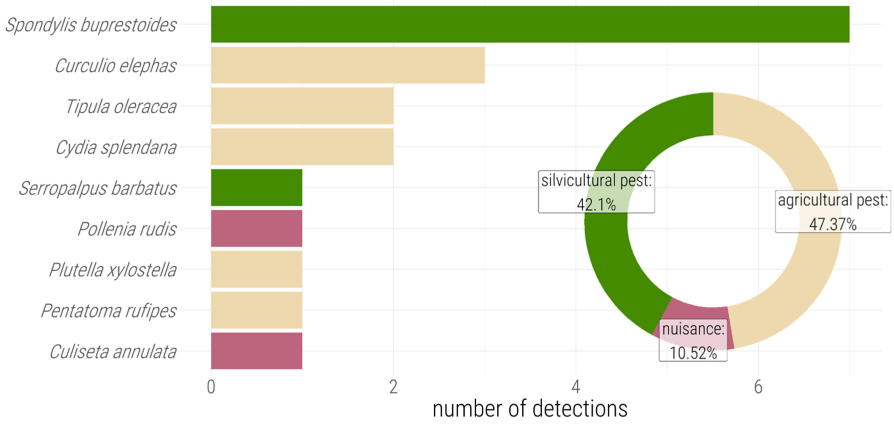

FIGURE 4 Number of stomachs in which pest species were detected and overall percentage of the pest type based on number of detections

accumulation curve did not flatten after extrapolation the sampling effort to twice the sample size (supplementary).

4 | DISCUSSION

We conducted a dietary study based on DNA metabarcoding of the stomach contents of bat carcasses found below wind turbines to assess the loss of trophic interactions in energy agroecosystems. We chose the European common noctule bat as the focus species, as this bat is most frequently killed at turbines in Central Europe (Rydell et al., 2010). Based on the stomach contents of 17 carcasses, we inferred that common noctule bats consumed mainly insects from terrestrial food webs shortly before dying at wind turbines. This is consistent with GPS tracking studies from lowland regions in Germany, which showed that common noctule bats foraged over a wide variety of terrestrial habitats such as grasslands, farmland, urban areas, and forests (Reusch et al., 2022; Roeleke et al., 2016, 2018, 2020; Voigt et al., 2020). The low number of species consumed by individual bats is consistent with a metabarcoding study of the stomach content of Northamerican bats found dead below wind turbines (Foo et al., 2017). The low proportion of insect species with aquatic larval stages found in the diet of common noctule bats is surprising considering that this species forages also around waterbodies (Reusch et al., 2022; Roeleke et al., 2016, 2018, 2020; Voigt et al., 2020). Possibly, small-bodied insects with aquatic larval stages, such as Ephemeroptera, Diptera (Chironomonidae and Culicidae) and Trichoptera, were rare in our study region in late summer when carcasses were found. Ephemeroptera and Trichoptera were more dominant in the diet of common noctule bats in a previous metabarcoding study from southern Sweden (Rydell et al., 2016). This discrepancy may have been caused by the lower abundance of water bodies at our study site compared with southern Sweden, which might translate into differences in the diet of common noctule bats known to feed opportunistically on the most abundant insect taxa in the aerial column (Eichstädt, 1995).

Based on the number of DNA reads as a proxy for the consumed organic mass for a given insect species, common noctule bats obtained most organic mass from beetles (Coleoptera) and moths (Lepidoptera), and only small amounts of organic mass from insects of other orders. Indeed, *Spondylus buprestoides*, a relatively large-sized cerambycid beetle was one of the dominant beetle species in the diet of the studied bats. Our study suggests that the loss of trophic interactions caused by fatalities at turbines may feed back on a variety of habitats adjacent to turbines, such as grassland, ruderal areas, and forests where bats fed on insects. This is consistent with findings from North America, indicating that high collision risk species hunt insects at wind turbines or in adjacent habitats (Foo et al., 2017; Valdez & Cryan, 2013). So far, no study has looked at the implications for trophic interactions in the affected habitats in which bats foraged before they were killed at wind turbines. However, previous studies highlighted that excluding bats from consuming insects by cage exclosures alters the trophic interactions in habitats. For example, an experimental study confirmed that preventing bats from foraging in a temperate zone forest increases the level of herbivory in the canopy of broad-leaved oaks (Böhm et al., 2011).

We observed that 20% of observed insect taxa were agricultural and silvicultural pest insects or nuisance insects, indicating that some of the studied common noctules provided ecosystem services at the time of death at wind turbines. This is consistent with findings from Northamerica indicating that bats of the genus *Lasiurus* also consumed pest insects shortly before dying at wind turbines (Foo et al., 2017). In North America, the experimental exclusion of insectivorous bats from foraging over maize fields led to increased herbivory by insects, resulting in significant crop damage (Boyles et al., 2011). A literature review confirmed that bats play an important role in controlling pest insects and can even suppress arthropod outbreaks at the regional level (Maas et al., 2016). These field experiments demonstrate that the trophic interactions of bats, including their ecosystem services, are not compensated by other predators such as birds (Maas et al., 2016). Accordingly, it is likely that losses of bats caused by collisions with turbines may lead to permanent changes in the food webs of arable fields. In the case of common noctule bats, the range of affected habitats could be large given the high mobility of this species, which may cover distances of several dozen kilometers during a single foraging trip (Reusch et al., 2022; Roeleke et al., 2016, 2018, 2020; Voigt et al., 2020) and several hundred kilometers during the migration period (Lehnert et al., 2018).

We obtained carcasses only from the late summer and autumn period, because most fatalities of common noctule bats occur during this period of the year. During spring migration, common noctule bats migrate over shorter periods of time and also higher above ground compared with summer/fall migration (O'Mara et al., 2019), which leads to lower fatal interactions of common noctule bats with wind turbines. Currently, we are unaware of the trophic interactions common noctule bats engage in springtime when, for example, agricultural pest insects are more abundant and most relevant for the growing crop. Future studies should therefore focus as well on the diet of common noctule bats and other high collision risk species throughout other parts of the year to capture the seasonal variation in the diet of bats (Tiede et al., 2020). This could be achieved by studying the insect diet based on metabarcoding of fecal matter. It is important to note that bat fatalities at wind turbines lead to a permanent loss of bat-mediated ecosystem services throughout the whole year, i.e., also during periods when pest insects are more abundant than during the times when carcasses are most often found below wind turbines.

The loss of bat-mediated ecosystem services may be pronounced in species that have a strong record of feeding on pest insects. In Southern Europe, for example, bats of the genus *Pipistrellus* and the species *Miniopterus schreibersii* are known to feed on a variety of pest insects (Aizpurua et al., 2018; Puig-Montserrat et al., 2015). On Madagascar, open-space foraging bats of the genus *Mops* and *Miniopterus* feed on paddy swarming armyworms (*Spodoptera mauritia*) and grass webworms (*Herpetogramma licarsisalis*) (Kemp et al., 2019). In the U.S.A., Brazilian free-tailed bats (*Tadarida brasiliensis*) feed on cornworms (*Helicoverpa zea*), which damage corn production (Cleveland et al., 2006), and Wrinkle-faced bats (*Chaerephon plicatus*) feed on planthoppers (*Sogatella furcifera*) in Thailand, which cause damage to rice plants (Wanger et al., 2014). The loss of these bat-mediated ecosystem services may reduce crop production with potentially negative impact on national economies and farmer households. Further, the loss of bat-mediated ecosystem services may have to be compensated for by a larger pesticide treatment of the corresponding crops with potentially cascading negative effects on ecosystems, biodiversity, and human health.

The magnitude of trophic interactions lost by turbine-related bat fatalities is difficult to evaluate because it depends largely on the cumulative number of bat fatalities in a region. For Central Europe, it has been estimated that a single wind turbine may cause on average 14 bat fatalities per year, yet numbers vary largely across Europe (Brinkmann et al., 2011; Măntoiu et al., 2020; Rydell et al., 2010; Voigt et al., 2022). Two independent sources suggest that 75% of the 30.000 onshore wind turbines in Germany are currently operating without any curtailing restrictions for bat conservation (Fritze et al., 2019; KNE, 2020). Based on the conservative estimate of only 10 bat fatalities per year and wind turbine and the aforementioned proportion of turbines operating with curtailment, cumulative fatality numbers would amount to ~240.000 killed bats per year for Germany alone, of which 32% are thought to be common noctule bats (Voigt, 2020). Although these numbers bear a high level of uncertainty, they hint toward the magnitude of the impact that wind turbines may have on bat populations. Recent national reports and papers document a decline in the population size of common noctule bats (BfN, 2018; Printz et al., 2021), which is most likely related to fatalities at wind turbines. Similar patterns are confirmed for North America. For example, it has been estimated that more than 600,000 bats are killed per year at wind energy facilities in the U.S.A. (as of 2012; Hayes, 2013), and more than 47,000 bats per year in Canada (as of 2013; Zimmerling & Francis, 2016). These high numbers of bat casualties may lead to population declines of high collision risk species in North America (Frick et al., 2017; Friedenberg & Frick, 2021), similar to what is observed and predicted for corresponding species in Europe. Overall, large numbers of turbine-related fatalities and associated population declines suggest large-scale permanent losses of trophic interactions with currently unknown impacts on local food webs. Admittedly, a direct link between wind turbine operations and changes in, e.g., insect herbivory or other insect-mediated functions have not been demonstrated so far.

Our study provides insights into the trophic interactions of common noctule bats killed by turbines, yet we acknowledge that our study is limited because of the small sample size, a limited sampling period, and a small geographic coverage. The number of involved carcasses was relatively low, owing to the problem that carcass searches are no longer commissioned on a regular scale in Germany and because we depended on carcasses that were sufficiently fresh to yield insect DNA. Unfortunately, the digestive capacity of bat stomachs is still high shortly after the death of bats, which leads to a continuous and rapid degradation of insect DNA in the carcasses. This may have caused an underestimation of the diversity of insect species consumed by common noctule bats. However, previous metabarcoding studies on bat stomachs detected a similar number of insect species (Foo et al., 2017), which supports our findings. Furthermore, COI is a protein-coding gene and known for primer mismatches when targeting genetically diverse taxonomic groups, such as insects (Clarke et al., 2014). Additionally, DNA-metabarcodes targeting COI induces a taxonomic bias in PCR amplification toward well-matched taxa, i.e., typically leading to a bias toward species from the orders Lepidoptera and Diptera (Piper et al., 2019). To account for this bias, we decided to base our analysis primarily on presence-absence data rather than pure read counts. Also, carcasses originated exclusively from wind turbines operating in northeastern Germany, and therefore, we have no information on which trophic interactions are eliminated in other areas of the species' distribution range. Lastly, we may have underestimated the number of trophic interactions that are lost by turbine-related fatalities of common noctule bats because the species accumulation curve observed for the insect diet of studied animals was rising with the number of carcasses analyzed. As a consequence, the true number of trophic interactions of common noctule bats may be significantly higher than documented for the 17 carcasses of this study.

5 | CONCLUSIONS

Our metabarcoding approach yielded information on trophic interactions of common noctule bats at wind turbines and in adjacent habitats before the time of death. The data suggest that mostly terrestrial ecosystems, such as grassland, ruderal areas, crop fields, and forests, might be affected by the turbine-related losses of bat-mediated insect consumption. Common noctule bats consumed a large variety of insect species, yet with a large portion of beetles and moths. Pest insects contributed with about 20% of all consumed insect species to the diet. Turbine-related fatalities could have long-lasting effects on local food webs. Considering the migratory behavior of bat species like common noctule bats, the loss of trophic interactions may be relevant not only for those habitats adjacent to wind turbines but also for regions from which bats originate from and to which they are migrating. These first insights call for more in-depth studies on the effect of turbine-related bat fatalities on habitats in multi-functional energy-agroecosystems. Accounting for these effects in energy-agroecosystems adds an important perspective on the question how ecologically sustainable wind energy production is when no mitigation scheme for the prevention of bat fatalities is practised.

AUTHOR CONTRIBUTIONS

Christian C. Voigt conceived the idea, both authors designed the study, Carolin Scholz contributed to the genetic analyses, conducted the statistical analyses, and designed the graphs. Christian C. Voigt wrote the original draft and both authors discussed and finalized the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data are available at the following public repository https://doi.org/10.5281/zenodo.6548192

ETHICS STATEMENT

Research for this paper did not involve any experiments with animals.

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SUPPORTING INFORMATION

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