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ARTICLE

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Arthropod dark taxa provide new insights into diversity responses to bark beetle infestations

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Abstract

Natural disturbances are increasing around the globe, also impacting protected areas. Although previous studies have indicated that natural disturbances result in mainly positive effects on biodiversity, these analyses mostly focused on a few well established taxonomic groups, and thus uncertainty remains regarding the comprehensive impact of natural disturbances on biodiversity. Using Malaise traps and meta-barcoding, we studied a broad range of arthropod taxa, including dark and cryptic taxa, along a gradient of bark beetle disturbance severities in five European national parks. We identified order-level community thresholds of disturbance severity and classified barcode index numbers (BINs; a cluster system for DNA sequences, where each cluster corresponds to a species) as negative or positive disturbance indicators. Negative indicator BINs decreased above thresholds of low to medium disturbance severity (20%–30% of trees killed), whereas positive indicator BINs benefited from high disturbance severity (76%–98%). BINs allocated to a species name contained nearly as many positive as negative disturbance indicators, but dark and cryptic taxa, particularly Diptera and Hymenoptera in our data, contained higher numbers of negative disturbance indicator BINs. Analyses of changes

in the richness of BINs showed variable responses of arthropods to disturbance severity at lower taxonomic levels, whereas no significant signal was detected at the order level due to the compensatory responses of the underlying taxa. We conclude that the analyses of dark taxa can offer new insights into biodiversity responses to disturbances. Our results suggest considerable potential for forest management to foster arthropod diversity, for example by maintaining both closed-canopy forests (>70% cover) and open forests (<30% cover) on the landscape.

KEYWORDS
arthropods, biodiversity, conservation, metabarcoding, national park, natural disturbance, threshold indicator taxa analysis

INTRODUCTION

Increasing human impacts on the biosphere have led to a biodiversity crisis in the form of severe species loss and increasing extinctions (Ceballos et al., 2017; Pimm et al., 2014). To counteract these losses, strategies to preserve species and their habitats are increasingly important (Johnson et al., 2017). Protected areas and wilderness are vital to nature conservation (Di Marco et al., 2019; Hoffmann et al., 2018). For example, a recent meta-analysis showed that species richness and abundance are positively correlated with the increasing naturalness of ecosystems (Pilotto et al., 2020).

While protected areas are subject to a reduced number of human disturbances, they remain subject to natural disturbances. Over one-third of the protected areas in Europe host at least some Norway spruce (*Picea abies* [L.] Karst) (Hagge et al., 2019), a tree species particularly prone to natural disturbances (i.e., disturbances triggered by a natural cause such as windthrows and bark beetle outbreaks). Natural disturbances have increased in Europe's forests over the past few decades (Seidl et al., 2014). With temperatures rising as a result of climate change, it is expected that Norway spruce forests will increasingly come under pressure from bark beetles (Bentz et al., 2019; Jakoby et al., 2019; Sommerfeld et al., 2020). *Ips typographus* (L.) is the most impactful biotic disturbance agent in European forests (Gregoire & Evans, 2007, Bentz et al., 2019,). With warmer temperatures, Norway spruce suffer more drought stress and are less resilient to infestations of *I. typographus* (Honkaniemi et al., 2020), which can spread to higher elevations and latitudes and affect larger areas than a few decades ago (Jakoby et al., 2019).

Natural disturbances can be beneficial for nature conservation because they create dynamic and diverse landscapes (Pulsford et al., 2016). The new structures that result from natural disturbances, like an increased amount of dead wood, open canopy, a diverse understory, vertical diversification. and spatial heterogeneity (Meigs et al., 2017; Senf et al., 2020; Swanson et al., 2011), can provide habitat for rare or endangered species (Bässler & Müller, 2010; Mikolás et al., 2017). For example, Aculeata, Syrphidae and Formicidae can benefit from open forests with warmer microhabitats (Beudert et al., 2015; Lehnert et al., 2013). However, landscapes change drastically after severe disturbance events, which can also have negative consequences for the abundance and diversity of taxonomic groups linked to closed-canopy forests (Lehnert et al., 2013).

Depending on disturbance severity, post-disturbance landscapes can vary in canopy openness, as well as in quantities of dead wood and remnant life trees (Raffa et al., 2008). Disturbance severity is thus a crucial element determining the biodiversity impact of disturbances. Taxa dependent on closed forests, like geometrid moths (Lepidoptera: Geometridae), might suffer in abundance or species numbers from even a low severity disturbance (Kitching et al., 2000). Other taxa, like bees and wasps, might benefit from the semi-open or open forests created after high severity disturbances (Beudert et al., 2015). Acknowledging the fact that species are often associated with different forest structures (Lehnert et al., 2013) suggests that a simple dichotomy of disturbed vs. undisturbed is not sufficient to accurately determine arthropod responses to disturbance.

Comprehensive studies of the impact bark beetle disturbances have on arthropods remain scarce. Lehnert et al. (2013) and Beudert et al. (2015) analyzed a wide range of arthropod species in forests attacked by bark beetles, however, different taxa were sampled on different study plots or at different points in time and only in a single landscape. Furthermore, most previous studies focused on relatively easily identifiable taxonomic or functional

groups within orders of arthropods, for example, saproxylic beetles and ground beetles (Carabidae), Heteroptera (Hemiptera), Aculeata (Hymenoptera), or Syrphidae (Diptera) (Müller et al., 2008, Lehnert et al., 2013, Beudert et al., 2015, Winter et al., 2015, Thom et al., 2017; Table 1). A knowledge gap exists regarding the response of harder-to-detect and often-neglected arthropod taxa, like many families of Diptera. In this study, we analyzed dark and cryptic taxa, which cannot be identified or distinguished by traditional visual observations, in addition to easily identifiable taxa. Dark taxa are organisms that lack a taxonomic identity in the form of a species name (Page, 2016). Among other definitions, cryptic species are considered to be organisms that are morphologically hard to differentiate and have traditionally been considered one species (Hebert et al., 2004) although genetic analyses can further separate them into different species. In this study, we use the term dark taxa for all species that could not be identified with its species name during next-generation sequencing due to missing information in the current metabarcoding data bases and/or because they are not yet described (see also Morinière et al., 2019). Since a huge amount of terrestrial biodiversity belongs to such undescribed species (Mora et al., 2011), considering them in ecological studies can provide new insights into biodiversity responses to natural disturbances. Next-generation sequencing (an enhanced method of DNA sequencing) is a promising tool for analyzing neglected, dark and cryptic taxa (Morinière et al., 2019). It allows scientists to study a broader range of species in less time compared to traditional visual observation and identification of species (Hardulak et al., 2020).

To achieve a more comprehensive understanding of the impact natural disturbances have on arthropod communities, we analyzed the response of arthropods along a gradient of disturbance severity in five European national parks. We used Malaise traps and metabarcoding to investigate responses across a broad range of arthropod taxa. We analyzed changes in the richness of barcode index numbers (BINs; Ratnasingham & Hebert, 2007, 2013) of two different data sets. One data set contained all BINs, including dark and cryptic taxa; and one data set included only BINs that could be associated with a species name. Analyses included different orders of arthropods and specific functional and taxonomic groups at finer resolution (families and genera). Furthermore, we calculated changes in BIN composition with changing disturbance severity and derived community thresholds for each order along the disturbance severity gradient. We aimed at determining disturbance severity thresholds in the responses of different arthropod groups to contribute to the identification of high-conservation-value forests.

METHODS

Study areas and experimental design

The study was conducted in 2018 in five protected areas: Black Forest National Park (Germany), Berchtesgaden National Park (Germany), Bavarian Forest National Park (Germany), Kalkalpen National Park (Austria), and Białowieża Forest (Poland) (Figure 1). Sites in Białowieża Forest were located outside the national park but in an area without management. To exclude impacts of post-disturbance forest management, we selected forest stands

TABLE 1 Observed taxonomic or functional groups within orders of arthropods analyzed in previous studies of bark beetle disturbance impacts on biodiversity

Order	Winter et al. (2015)	Müller et al. (2008)	Beudert et al. (2015)	Lehnert et al. (2013)	Thom et al. (2017)
Coleoptera	Epigeic, Phytophagous, Saproxylic, Pollinating	Saproxylic	Saproxylic, Carabidae	All	Saproxylic, Carabidae
Arachnida	All	-	Araneae, Opiliones	Araneae, Opiliones	Araneae
Collembola	All	-	All	All	-
Hymenoptera	Aculeata	Bees and social wasps	Aculeata, Symphyta	Aculeata, Symphyta	Apocrita
Hemiptera	Phytophagous, Heteroptera	Heteroptera	Cicada, Heteroptera	Auchenorrhyncha, Heteroptera	Heteroptera
Lepidoptera	-	-	Macro-Lepidoptera	Macro-Lepidoptera	-
Neuroptera	-	-	All	All	-
Diptera	-	-	Syrphidae	Syrphidae	Syrphidae

Note: Dashes indicate that the corresponding order was not examined in the relevant study.

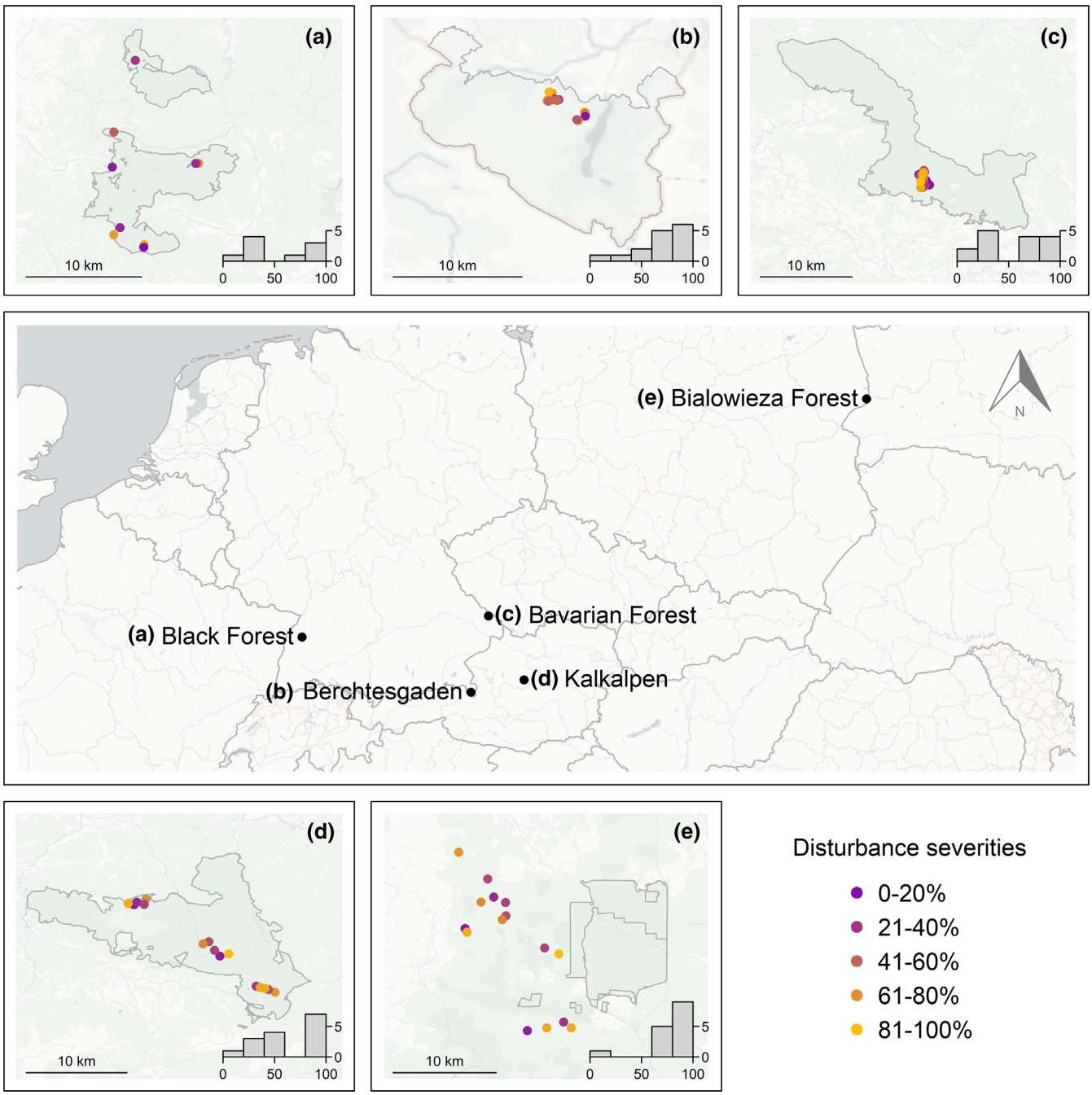

FIGURE 1 Location of the five investigated study areas in Europe and distribution of plots and disturbance severities within study areas. Histograms show the frequency of disturbance severities of the respective study area

that were not salvage logged after disturbance. All study areas had a Norway spruce share on the total tree population of at least 70% prior to disturbance and were affected by outbreaks of *I. typographus*. In each area, we selected 15 circular study plots ($r = 50$ m) along a gradient of disturbance severities from 0% to 100%. Since the Black Forest National Park was only established recently and had rather low levels of bark beetle infestation, we only studied nine plots in this particular study area. In total, this resulted in 69 study plots investigated. The minimum distance between study plots was 110 m. Disturbance severity was measured as the percentage of spruce trees killed by bark beetles. The respective disturbance severities were calculated within a 100-m buffer surrounding each plot to robustly describe the habitat conditions for arthropods.

Processes of dead-wood decomposition and tree regeneration after outbreaks of *I. typographus* occur gradually over the course of years and decades (Senf et al., 2019). We only considered stands between 2 and 20 years after disturbance, such that all affected spruces had lost their needles already (gray attack stage), but the

collapse of snags and decomposition of dead wood was not yet far advanced (Storaunet & Rolstad, 2002). The time since bark beetle outbreaks differed between study areas but was approximately the same within each study area. Consequently, the years since disturbance were included in the area-random effect in our model (see section Statistical analyses). We note that dead wood was present also in undisturbed stands, as the forests under study have not been managed for a considerable period of time. Study plots in Białowieża had higher disturbance severities than originally assumed, because the bark beetle activity of 2017 only became apparent after plot selection. The disturbance gradient is therefore slightly imbalanced in Białowieża (see Figure 1). The study design was also described in Kortmann et al. (2021)).

Dead-wood inventory

We measured all dead Norway spruce trees within a 17.84-m radius (0.1 ha) of the center of each study plot. The heights of standing dead trees were measured with a Vertex IV (Haglöf Sweden AB, Långsele, Sweden). Diameter of standing dead trees was measured at breast height (DBH; at 1.3 m height) using a caliper. Diameter of downed trees was measured at the middle of the stem (D). The length of downed dead wood was measured with a measuring tape. The volume of standing dead trees was calculated as: $\mathrm{DBH}^2 \times \pi \times \mathrm{length}/4 \times 0.43$, with 0.43 being the form factor for Norway spruce, describing the relation of the true volume of the stem to the volume of a cylinder calculated with the DBH (Kramer & Akca, 2008). The volume of downed dead wood was calculated as: $\mathrm{DBH}^2 \times \pi \times \mathrm{length}/4$. The volume of standing broken trees was calculated as: $D2^2 \times \pi \times \mathrm{length}/4$, with the calculation for $D2$ as: $DBH - (\mathrm{DBH}^2 \times 0.04 \times \mathrm{length}/2)$, assuming that the diameter decreases by 4% per meter (Kramer & Akca, 2008). The amount of dead wood was calculated as cubic meters of dead spruce per hectare. Live spruce trees were not included in the analysis.

Arthropod sampling

In the center of each plot, a Malaise trap (i.e., a mesh-tent to catch predominantly flying insects; Matthews & Matthews, 2017) was deployed from April until September 2018. Traps were equipped with collecting bottles filled with 70% ethanol that were emptied once a month to ensure high DNA quality for sequencing (see section Metabarcoding). To evaluate if Malaise traps are suitable for closed forests, we compared changes in arthropod biomass between traps along the disturbance gradient (as a proxy for open and closed forests) and between sampling months (Appendix S1: Figures S1 and S2). The sampled arthropods were separated into two size classes using a sieve (7 mm mesh size) to improve sequencing results by reducing the risk that smaller specimens with underrepresented DNA remain undetected during sequencing (Hardulak et al., 2020). Binning also reduces the differences in the number of hits caused by differences in the size of individuals. We separated arthropods into taxonomic groups based on the five main orders represented in our data (Coleoptera, Hymenoptera, Diptera, Hemiptera, and Lepidoptera). In addition, we clustered the orders into 23 subgroups. We separated families that were well-represented in our data and grouped the remaining ones based on functional information of families or species based on standard literature and expert opinions. Coleoptera were separated into saproxylic and phytophagous taxa, and a group containing the remaining taxa. Hymenoptera were separated into Formicidae, phytophagous and pollinating Hymenoptera, and hymenopteran parasitoids. Diptera were separated into Phoridae, Mycetophilidae, Syrphidae, Sciaridae, dipteran decomposers (feeding on carcasses and dung), parasites, and phytophagous and predatory Diptera. From the Hemiptera group, we selected only phytophagous Hemiptera. Lepidoptera were separated into Noctuidae, Geometridae, and the remaining taxa. Aquatic insects (Trichoptera, Plecoptera, Ephemeroptera), Neuropteroida (Neuroptera, Raphidioptera), Mecoptera (Panorpidae), Araneae, and Opiliones were also included in the analyses.

Metabarcoding

Species identification of arthropods was performed using DNA metabarcoding following the laboratory and bioinformatic pipelines as reported in Hausmann et al. (2020). The entire arthropod samples were dried in a 60°–70°C oven overnight. Dried arthropods were homogenized with stainless steel beads within a FastPrep 96 (MP Biomedicals, Santa Ana, CA, USA). DNA extraction for all samples was carried out in a 90:10 solution of animal lysis buffer (buffer ATL, Qiagen DNEasy Tissue Kit; Qiagen, Hilden, Germany) and Proteinase K. Lysis was performed overnight in a 56°C oven. Samples were then allowed to cool down to room temperature. DNA was extracted from 200 μL aliquots of the lysate with the DNEasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

From each sample, 5 μL of extracted genomic DNA was applied for PCR with Mango TAQ (Bioline, Luckenwalde, Germany), and high throughput sequencing (HTS) adapted mini-barcode primers (Leray et al., 2013; see also Morinière et al., 2016, Morinière et al., 2019). Leray et al. (2013) primer sequences were adapted with Illumina Index sequences to perform a second PCR for

ligation of unique i5 and i7 Illumina Index sequences. Amplification success and fragment length were examined with gel electrophoresis. Amplified DNA of each sample was cleaned up and resuspended in 50 μL molecular water before proceeding. Illumina Nextera XT (Illumina, San Diego, California, USA) indices were ligated to the samples in a second PCR reaction for only seven cycles at the same annealing temperature as in the first PCR reaction. Ligation success was confirmed by gel electrophoresis. DNA concentrations were measured using a Qubit fluorometer (Life Technologies, Carlsbad, California, USA). Samples were combined into 40 μL pools containing equimolar concentrations of 100 ng each. Pools were loaded into a 1% agarose gel and run at 90 V for 45 minutes. Bands of the target amplicon size of 520 bp were excised with sterilized razor blades and purified with a GeneJet Gel Extraction kit (Life Technologies), following the manufacturer's instructions. A final elution volume of 20 μL was used for high-throughput sequencing (HTS) on an Illumina MiSeq using v2 (2 × 250 bp, 500 cycles, maximum of 20 million paired-end reads) chemistry.

Bioinformatics

FASTQ files were combined and sequence processing was performed with the VSEARCH v2.4.3 suite (Rognes et al., 2016) and cutadapt v1.14 (Martin, 2011). Not all of the sequenced samples yielded reverse reads of a sufficient quality to enable paired-end merging. Hence, only forward reads were utilized. Forward primers were removed with *cutadapt*. Quality filtering was conducted with the *fastq_filter* program of VSEARCH (*fastq_maxee* 2, minimum length of 100 bp). Chimeric sequences were filtered out from the large fasta file using *uchime_denovo*. Remaining sequences were clustered into operational taxonomic units (OTUs) at 97% identity. OTU tables were created with *usearch_global*. To reduce likely false positives, a cleaning step was employed that excluded read counts in the OTU table of less than 0.01% of the total. OTUs were blasted against a custom Animalia database downloaded from BOLD in early 2019, including taxonomy and BIN information, by means of Geneious (v.10.2.5; Biomatters, Auckland, New Zealand) and following methods described in Morinière et al. (2016, 2019). The combined results table was then filtered by Hit-%-ID value and total read numbers per OTU. OTUs were then assigned to the respective BIN. Additionally, the API provided by BOLD was used to retrieve BIN species and BIN countries for every OTU, and the Hit-%-IDs were aggregated over OTUs that found a hit in the same BIN and shown in the corresponding column as percent range. To validate the BOLD BLAST results, a separate BLAST search was carried out in Geneious (using the same parameters) against a local copy of the NCBI nucleotide database downloaded in early 2019 (ftp://ftp.ncbi.nlm.nih.gov/blast/db/).

BOLD groups similar CO1 barcode sequences into clusters, which are assigned to a globally unique identifier, termed a barcode index number or BIN (Ratnasingham & Hebert, 2013). This system can be used to verify species identifications when taxonomic information is lacking. The BIN System involves a three-step online pipeline, which clusters similar barcode sequences algorithmically into operational taxonomic units (OTUs). Members of a BIN often belong to a single species as delineated by traditional taxonomy (Hausmann et al., 2013).

Results of the BLAST search are presented as a target sequence, equipped with a sampleID, processID, a BIN, and a percentage value incorporating coverage and identity of a query sequence to the best fitting target (hit percentage). For further analyses, we used all BINs with a hit percentage >75% allocated to an order to include also dark and cryptic taxa, as taxonomic resolution allows for the identification of higher rank taxonomy such as order and family. This data set is hereafter named aBINs. To compare these analyses with a more conservative approach, we created a subset including only BINs with a hit percentage >97% and a species name (see, e.g., Morinière et al., 2019). This data set is hereafter named sBINs.

Statistical analyses

All statistical analyses were conducted with R 3.6.1 (R Core Team, 2019). We fitted generalized linear mixed models (GLMMs) with a negative binomial error term using the glmmTMB function from the glmmTMB package (Brooks et al., 2017) to analyze the impact of disturbance severity and dead-wood amount on alpha diversity of the different groups of arthropods at lower taxonomic levels. Since dead-wood amount and disturbance severity are correlated (Figure 2), we did not use raw dead-wood amounts but rather included the dead-wood residuals (i.e., the residuals of the linear model of disturbance severity and dead-wood amount) in our model to capture the additional information in the data. Dead-wood residuals thus indicate the deviation from the dead-wood amount expected for a given level of disturbance severity. We fitted two separate models, one for the five orders of arthropods and another for the more refined species groups (see end of *Arthropod sampling* for descriptions of the smaller taxonomic levels). Both models had the quantity of BINs as response variable and interactions of

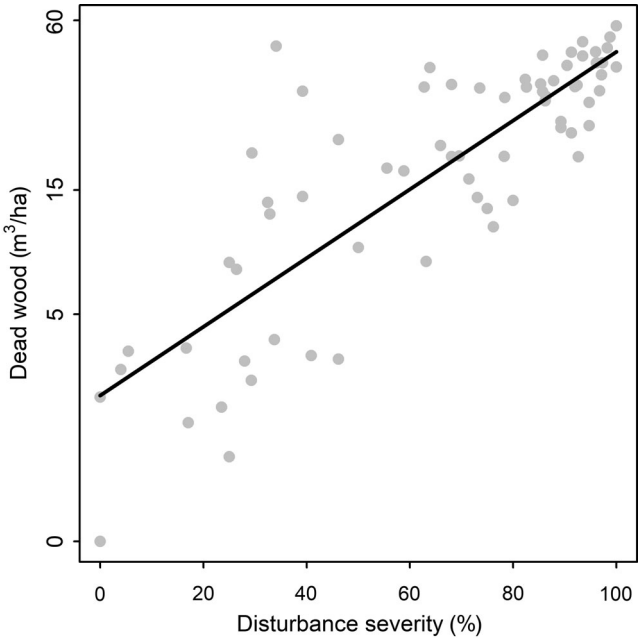

FIGURE 2 Disturbance severity and dead-wood amount for each study plot. Residuals of the dead-wood amount as a function of disturbance severity were used for further analyses to disentangle disturbance severity and dead-wood amounts

the taxonomic groups with disturbance severity and dead-wood residuals as predictors. In both models, we controlled for elevation and included study area (i.e., each national park) as random effect to account for general differences between areas and repeated measurements within these (see Table 2 for an overview of all the used statistical models).

To analyze the effect of the disturbance and dead-wood gradient on β-diversity, we calculated pairwise dissimilarity matrices for each environmental variable and species data. Dissimilarity indices in disturbance severity, dead-wood residuals, and elevation were calculated between all study plots using the vegdist function from the vegan package with Euclidean distances. We used principal coordinates of neighbor matrices (PCNM) to differentiate between large spatial distances (among regions) and small spatial distances (within regions) between the study plots (Borcard & Legendre, 2002). This approach controls for differences between study areas by means of spatial distances among regions. We calculated dissimilarities in species communities between study plots with the vegdist function and Jaccard distances for all plot-pair combinations. Calculations were done on species level for the five arthropod orders (Coleoptera, Lepidoptera, Hemiptera, Diptera, and Hymenoptera), separately (Table 2). We performed multiple regressions on the calculated distance matrices (MRM; Lichstein, 2007). MRMs can be used to test the influence of the environmental dissimilarity matrices (disturbance severity, dead-wood residuals and elevation) and the spatial distance calculated with the PCNM on the calculated community dissimilarities.

TABLE 2 Overview of the analyses and models

Analysis and taxonomic group	Model
GLMM	
Order	richness (aBINs) ~ order + order: disturbance + order: dead-wood residuals + order: elevation + (1|area)
Order	richness (sBINs) ~ order + order: disturbance + order: dead-wood residuals + order: elevation + (1|area)
Smaller taxonomic and functional groups	richness (aBINs) ~ taxon + taxon: disturbance + taxon: dead-wood residuals + taxon: elevation + (1|area)
Smaller taxonomic and functional groups	richness (sBINs) ~ taxon + taxon: disturbance + taxon: dead-wood residuals + taxon: elevation + (1|area)
MRM	
Order	distance (aBINs) ~ distance (disturbance) + distance (dead-wood residuals) + distance (elevation) + distance (among regions) + distance (within regions))
Order	distance (sBINs) ~ distance (disturbance) + distance (dead-wood residuals) + distance (elevation) + distance (among regions) + distance (within regions))
TITAN	
Order	aBINs ~ disturbance
Order	sBINs ~ disturbance

Notes: Smaller taxonomic and functional groups are based on families that were well-represented in our data. The remaining barcode index numbers (BINs) were grouped based on functional information of families or species based on standard literature and expert opinions.

To identify species (BIN) threshold responses across the disturbance severity gradient, we used the threshold indicator taxa analysis (TITAN). TITAN combines a method to detect changes in probability distributions and indicator species analysis (IndVal scores; (Dufrêne & Legendre, 1997) to identify changes in occurrence, frequency, and relative abundance along an environmental gradient. TITAN also assesses congruence among species change-points as an indication of assemblage thresholds

(Baker & King, 2010). The analysis calculates negative ($z-$) and positive ($z+$) responses of single taxa as well as cumulative responses at the community level. We performed TITAN analysis for all arthropod orders using the titan function from the TITAN2 package (Table 2). We considered indicators as pure and reliable when 90% of the replicates matched the observed assignment to negative or positive indicators and 80% had IndVal *O* values <0.05 based on 500 bootstrap replicates.

All analyses were performed for the aBINs (the entire data set including dark and cryptic taxa) as well as the sBINs (the species subset including only BINs that could be allocated to a species name; see description in *Metabarcoding*).

RESULTS

We analyzed 3,864 arthropod BINs in total. These included 248 coleopteran, 1,795 dipteran, 520 lepidopteran, 922 hymenopteran, 106 hemipteran, and 273 remaining BINs. The sBIN subset (excluding all BINs that could not be allocated to a species name) contained 1,711 species. These included 220 coleopteran, 581 dipteran, 451 lepidopteran, 275 hymenopteran, 58 hemipteran, and 126 remaining species. Results of the GLMMs including all BINs showed no clear response of any arthropod order to disturbance severity or dead-wood residuals (Figure 3 and Appendix S1: Table S1). Analyses of the sBINs showed that only species richness of Hymenoptera increased significantly with increasing disturbance severity (Figure 3 and Appendix S1: Table S2).

MRMs of the aBINs showed that differences in disturbance severity between plots had a significant positive effect on beta diversity of Lepidoptera and Hymenoptera (Appendix S1: Table S3). Differences in dead-wood residuals between plots correlated positively with beta diversity of Hemiptera (Appendix S1: Table S3). Distances among regions correlated significantly with dissimilarities of all observed orders (Appendix S1: Table S3). MRMs of the species subset showed similar responses to dissimilarities in disturbance severity and distances among regions but no clear response to differences in dead-wood residuals (Appendix S1: Table S4).

Results of the GLMMs including arthropods at lower taxonomic levels within the aBINs, showed that BIN richness of phytophagous Hymenoptera and Syrphidae increased with increasing disturbance severity. BIN richness of Phoridae and Mycetophilidae decreased with increasing disturbance severity. Increasing dead-wood residuals correlated negatively with BIN richness of pollinating Hymenoptera, Syrphidae, and dipteran decomposers (Table Appendix S1: S5 and Figure 4). Results of the sBINs showed that richness of Noctuidae, phytophagous Hymenoptera, pollinating Hymenoptera, and Syrphidae increased significantly with increasing disturbance severity. Increasing dead-wood residuals correlated negatively with richness of aquatic insects, phytophagous Hymenoptera, pollinating Hymenoptera, phytophagous Diptera, and dipteran decomposers (Appendix S1: Table S6 and Figure 4).

Threshold analysis of all BINs classified 309 BINs as negative and 181 as positive indicators of changing disturbance severities. Within the species subset, 105 species were classified as negative and 92 as positive indicators (Table 3).

Community thresholds of Lepidoptera, Hymenoptera, and Hemiptera were quite similar. Negative disturbance indicators of Lepidoptera, Hymenoptera, and Hemiptera had community thresholds at low disturbance

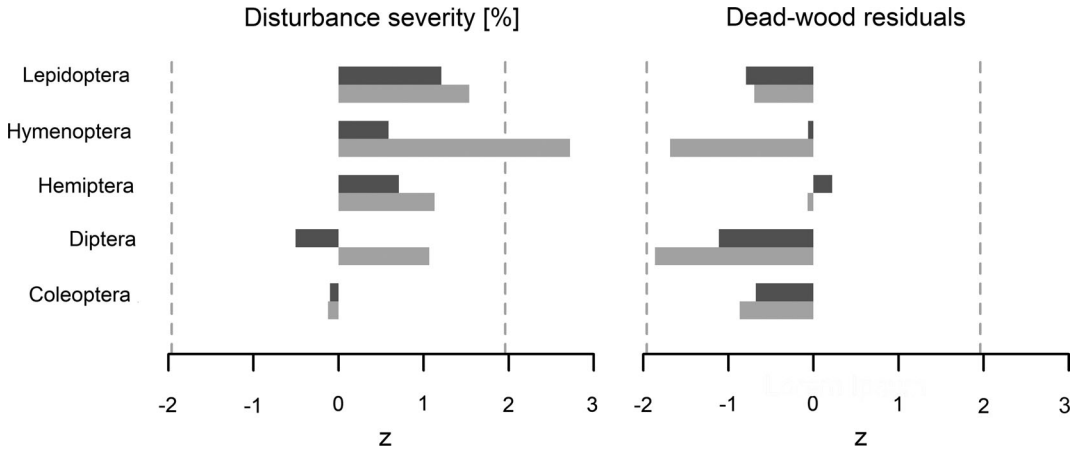

FIGURE 3 *Z* values of the GLMMs with a negative binomial error term calculating the impacts of disturbance severity and dead-wood residuals on alpha diversity of different orders of arthropods. Models were controlled for elevation and study area. Dark gray bars show results of models including all barcode index numbers (BINs), light gray bars show results of models only including BINs allocated to a species name. Thresholds for significant *z* values are symbolized with vertical gray dashed lines

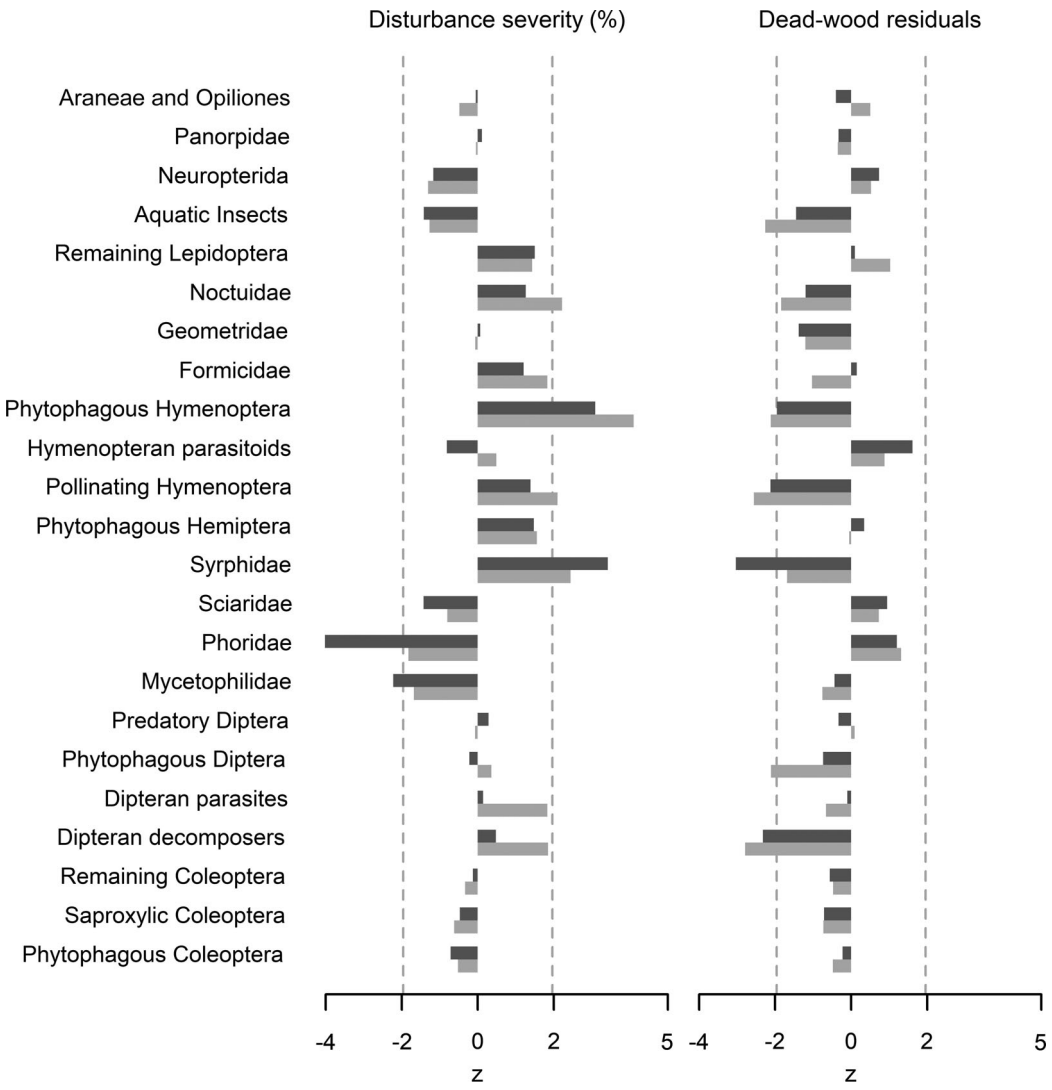

FIGURE 4 *Z* values of the GLMMs with a negative binomial error term calculating the impacts of disturbance severity and dead-wood residuals on alpha diversity of different arthropods at lower taxonomic levels. Models controlled for elevation and study area. Dark gray bars show results of models including all BINs, light gray bars show results of models only including BINs allocated to a species name. Thresholds for significant *z* values are symbolized with vertical gray dashed lines

severities (20.3%, 29.3%, and 29.3%, respectively). Positive disturbance indicators had community thresholds at high severities (75.6%, 97.2%, and 85.7%, respectively; Figure 5a–c,f). By contrast, both negative and positive disturbance indicators of Diptera showed a community threshold at high disturbance severities (73.3% and 75.6%, respectively; Figure 5d,f). Disturbance indicators of Coleoptera showed a negative threshold at 63% and a positive threshold at 96.5% (Figure 5e,f).

DISCUSSION

Impacts on arthropod orders

Analyzing alpha diversity of all BINs, including dark taxa at order level, we found that neither disturbance severity nor dead-wood residuals showed clear effects on any of the orders studied here. Different families and species within the observed orders might respond differently to disturbance with responses canceling out at the order level. This notion is supported by our analyses of community distance matrices (using MRMs), which showed that dissimilarities in lepidopteran and hymenopteran communities were driven by variation in disturbance severity (Appendix S1: Table S3). Our results suggest that for a broad range of arthropods beta diversity between open and closed forests is high, that is, higher dissimilarities in disturbance severity lead to higher beta diversity in arthropod communities. This is in line with the widely accepted concept that environmental heterogeneity is related to high species richness (Stein et al., 2014). This concept was recently tested in detail in a study of temperate forests in Europe, which showed that genetic diversity of Heteroptera, phytophagous and necrophagous Coleoptera,

TABLE 3 Negative and positive indicator taxa for all barcode index numbers (aBINs) and the species subset (sBINs), calculated with threshold indicator taxa analysis (TITAN)

	Positive indicators		Negative indicators	
Taxa	**sBINs**	**aBINs**	**sBINs**	**aBINs**
Coleoptera	**3**	**4**	**21**	**22**
Remaining Coleoptera	0	0	3	4
Saproxylic Coleoptera	3	4	18	18
Diptera	**33**	**64**	**52**	**179**
Dipteran decomposers	3	3	7	15
Dipteran parasites	17	15	12	16
Phytophagous Diptera	5	19	9	50
Predatory Diptera	4	10	5	15
Mycetophilidae	0	1	8	32
Phoridae	0	4	5	43
Sciaridae	1	4	5	7
Syrphidae	3	8	1	1
Hemiptera	**5**	**11**	**0**	**2**
Phytophagous Hemiptera	5	11	0	2
Hymenoptera	**19**	**56**	**8**	**56**
Pollinating Hymenoptera	2	3	1	0
Hymenopteran parasitoids	7	37	4	52
Phytophagous Hymenoptera	9	14	3	4
Formicidae	1	2	0	0
Lepidoptera	**29**	**35**	**21**	**20**
Geometridae	2	4	4	4
Noctuidae	6	6	3	2
Remaining Lepidoptera	21	25	14	14
Unspecified	3	11	3	30
Sum	**92**	**181**	**105**	**309**

Note: Numbers of indicator species for the entire orders are written in boldface.

and Araneae increased especially with increasing horizontal habitat heterogeneity (Heidrich et al., 2020). A previous study has also shown that between-stand heterogeneity is more important for biodiversity than within-stand heterogeneity (Schall et al., 2018).

Impacts on arthropods at lower taxonomic levels

Our analysis of lower taxonomic levels revealed positive effects of disturbance severity on phytophagous Hymenoptera and Syrphidae. This finding is in line with Thom et al. (2017) who also determined Hymenoptera and Syrphidae to be positively affected by natural disturbances. These positive responses might be caused by an increase in productivity of the lower forest layers and a higher plant species diversity in severely disturbed forests. For example, pollinators can benefit from higher amounts of flowering plants in forest gaps (Proctor et al., 2012). Some species might also benefit from increasing insolation and warmer temperatures resulting from canopy opening (Thom et al., 2020). In contrast, BIN richness of Phoridae and Mycetophilidae decreased significantly with disturbance severity. A possible explanation of the decline of Phoridae lies in their association with moist decaying litter (Gorham et al., 1996 as cited in Bouget & Duelli, 2004). For example, Durska (2013) found nearly twice as many species of Phoridae in closed mature forests compared to disturbed and salvage-logged sites. Mycetophilidae are also considered to be linked to moist, damp and dark habitats (Oliveira & Amorim, 2016) and might suffer from canopy opening and the desiccation of

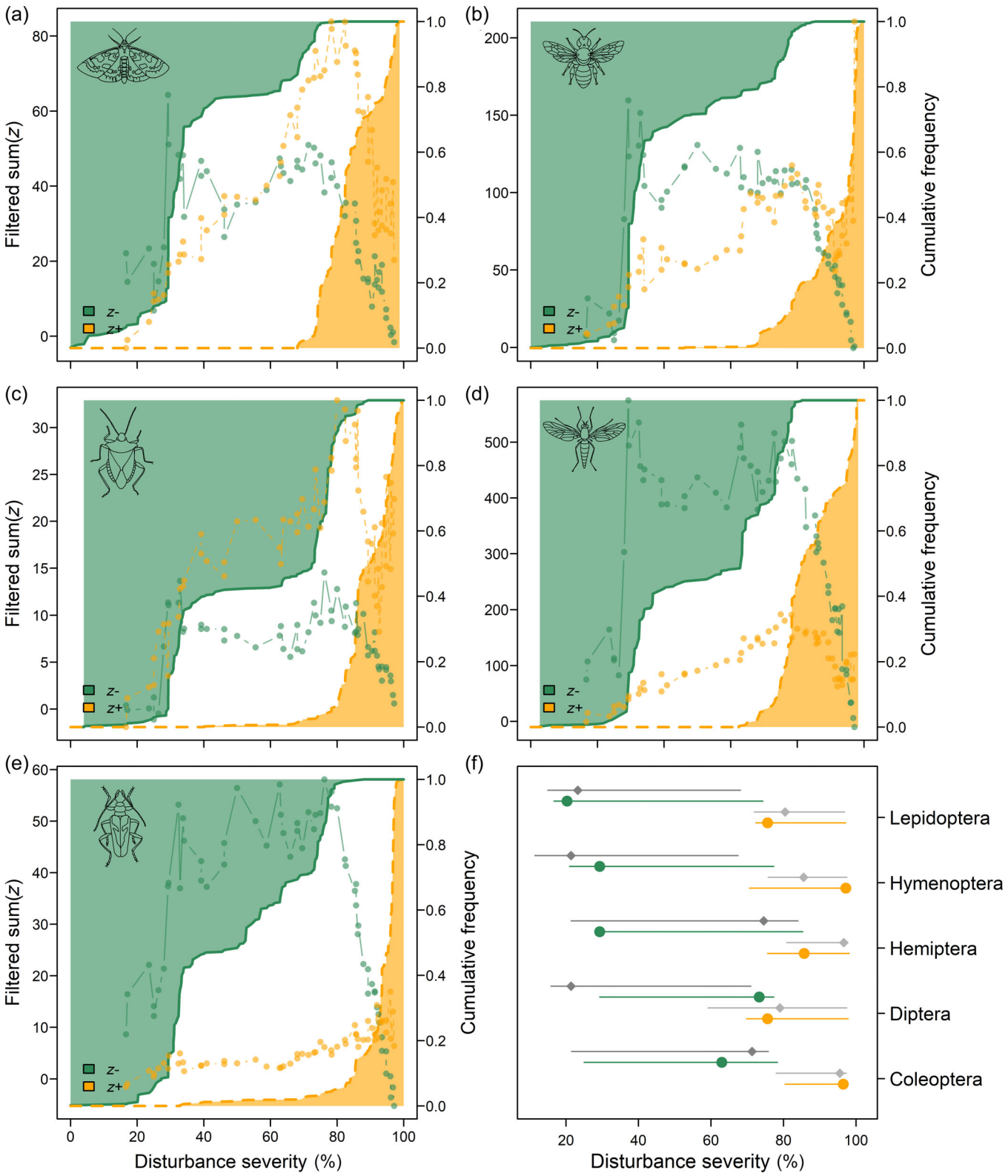

FIGURE 5 Threshold indicator taxa analysis and community change points for BINs of (a) Lepidoptera, (b) Hymenoptera, (c) Hemiptera, (d) Diptera, and (e) Coleoptera communities along a gradient of disturbance severity. Green dots and lines indicate negative indicator taxa ($z-$; species that decrease in occurrence and/or abundance with increasing disturbance severity). Orange dots and dashed lines indicate positive indicator taxa ($z+$; species that increase in occurrence and/or abundance with increasing disturbance severity). Dots show the number of indicator species, with a change point at the according disturbance severity. Green and orange areas in panels a–e show the cumulative frequency distributions of the sum(z) maxima. Dots in (f) show the observed sum($z-$) and sum($z+$) maxima. Horizontal lines in (f) show the 5%–95% quantiles from the bootstrapped change point distribution. Gray lines and points in (f) show results of calculations on species level with the species subset (sBINs) data set

dead wood after a disturbance event. Several Mycetophilidae develop in fungi fruiting bodies and are dependent on this specific substrate (Jakovlev & Siitonen, 2014), which might get lost after desiccation in open forest stands. Still, possible ecological reasons for the response of Phoridae remain speculative, as we have no valid information on feeding habits and general habitat requirements for this family to date. Nevertheless, the different responses of arthropods at lower taxonomic levels analyzed here in combination with the missing signal at order level supports the suggestion that it is necessary to analyze arthropods at family or genus level to be able to detect disturbance responses in species richness.

Contrasting responses of dead-wood residuals and disturbance severity

Our results showed a decrease in BIN richness of pollinating Hymenoptera, Syrphidae, and dipteran decomposers with increasing dead-wood residuals. Dead-wood residuals, in our study, indicate the deviation from the dead-wood amount expected for a given level of disturbance severity. Taxonomic groups with contrasting responses to disturbance severity and dead-wood residuals, such as pollinating Hymenoptera and Syrphidae, might benefit particularly from disturbances in stands with low stocking densities and/or small trees (i.e., a negative deviation from the expected deadwood amount for a given level of severity). Such forests would have relatively low dead-wood amounts even after high severity disturbance, and are therefore likely to maintain a diverse herb and shrub layer (Ares et al., 2010; Roberts, 2004). Diverse and productive lower canopy layers can serve as food resources for phytophagous and pollinating arthropods (Kitching et al., 2000; Proctor et al., 2012). Overall, our findings suggest that canopy openness might be more important for arthropod diversity than the amount of dead wood. Similar effects were shown in a study on saproxylic beetles, where canopy opening had a stronger impact on the abundance of saproxylic beetles than dead-wood amount (Müller et al., 2010). We note, however that saproxylic species and destruents might be underrepresented in our analysis. Malaise traps catch mostly flying insects, which move upwards after hitting the trap and hence get caught in the ethanol bottle (Matthews & Matthews, 2017). In contrast, beetles tend to drop after hitting an obstacle and are generally underrepresented in Malaise traps but can be expected to benefit from increasing amounts of dead wood (Gimmel & Ferro, 2018; Ulyshen & Šobotník, 2018). Hence, a combination of different traps (e.g., pitfall traps, flight-interception traps, and Malaise traps) would cover an even broader range of arthropods.

Community thresholds along the disturbance gradient

Contrasting disturbance responses within orders of arthropods are also visible in our threshold indicator taxa analysis. Each observed order contained both indicators reacting positively and negatively to disturbance severity. Negative indicators of disturbance severity of Lepidoptera, Hymenoptera, and Hemiptera had community thresholds at low to medium severities (20%–30% trees killed). Hence, most of the species that react negatively to disturbance seem to depend on closed forest canopies and disappear already at low disturbance severities. In contrast, Lepidoptera, Hymenoptera, Hemiptera, Diptera, and Coleoptera reacting positively to disturbance had thresholds at high severities (76%–97% infested trees), and generally benefit from open canopies. In this context, we note that the average disturbance severity in Europe between 1986 and 2016 and across all types of disturbance was estimated to 77% of the canopy removed (Senf & Seidl, 2021).

Overall, we find that the majority of arthropod species prefers the extremes within the gradient of disturbance severities. This is in line with the findings of Lehnert et al. (2013), who showed that most species are either indicators for closed or open forests, with only a few species specialized in semi-open conditions. Notably, however, Coleoptera and Diptera responded differently than the other orders in our study. Negative indicators of both orders showed thresholds at rather high severities (63% and 73% of trees killed). This suggests that forests with low to medium disturbance severities remain suitable for some species of Coleoptera and Diptera. Our results underline that further experimental studies are needed to better understand the biological causes of why species favor low or medium disturbance severity.

Analyzing dark and cryptic taxa

To date, the impacts of natural disturbance events on arthropods were reported to be generally positive. For example, Lehnert et al. (2013) found more positive indicators of increased canopy openness than negative ones. However, these previous studies focused mainly on well-established species groups (Table 1). Metabarcoding allowed us to analyze a more comprehensive range of arthropod species, including well-known and often-neglected taxa. For example, the number of indicators of Syrphidae and phytophagous Hymenoptera, frequently assessed taxa in previous studies, were considerably lower than the numbers of the whole order (Diptera and Hymenoptera, respectively) derived from metabarcoding

(Table 3). Diptera and Hymenoptera, in particular, include huge numbers of dark taxa (Hebert et al., 2016; Morinière et al., 2019). In our study, analyses of those two orders showed the highest differences in alpha diversity responses to disturbance severity (Figures 3 and 5). Thus, for Diptera and Hymenoptera, metabarcoding can cover a much broader spectrum of diversity and can help to detect diversity responses that are not otherwise visible.

In the past few years, scientific discourse has suggested that traditional taxonomy is limited in its ability to determine large numbers of species, especially in hyperdiverse groups (Hebert et al., 2016), for example, Ichneumonidae, Cecidomyiidae, and Chironomidae. Although metabarcoding can help to overcome these shortcomings, it also has limitations that need to be considered. The analyzed DNA segments (or amplicons; CO1-5P minibarcode amplicon sequences) can only be allocated to a species with a certain hit probability, which makes distinguishing between closely related species difficult. To date, several hyperdiverse insect orders such as Diptera, Hymenoptera, and Hemiptera lack a complete Linnean taxonomy within the DNA barcoding reference libraries of BOLD (The Barcode of Life Data System, an online database for barcode data; Ratnasingham & Hebert, 2007), which can lead to biases in further analyses of the barcoding data. However, large numbers of species delimitation systems (such as BINs) with referenced DNA barcoding data have already been implemented within the reference libraries. Although most of these "dark taxa" species lack complete Linnean species names, they are still valuable for biodiversity assessment studies. Clearly, metabarcoding is developing fast, and with additional future research it may be a suitable method for efficient large-scale biodiversity assessment and monitoring.

CONCLUSIONS

Malaise traps and metabarcoding grant us more detailed insights into the responses of arthropods to bark beetle disturbances. Our results also suggest that it is helpful to analyze arthropods at lower taxonomic levels to detect variable responses in arthropod communities to canopy opening. Analyses at higher levels run the risk of suggesting stable richness levels, while being the result of diverging responses of the underlying taxa. By analyzing a broad range of arthropods in different taxonomic units, we found highly variable responses to increasing disturbance severity. Most of the observed species are bound to either closed forests or open forests, with few species specializing in intermediate disturbance severities. Our results also highlight that there are many dark taxa responding negatively to disturbance severity. Therefore, traditional observations focusing on a few well-established taxa might miss negative biodiversity responses to natural disturbances. Future studies should consider including classical approaches in combination with barcoding and metabarcoding techniques to allow further insights into the differences between dark taxa and traditional species concepts. We conclude that strong variation in canopy cover as created by variable natural disturbances over large forest areas can generate diverse landscapes with high species richness and beta diversity. To meet the requirements of both species groups, forest management should create landscapes that contain both closed-canopy forests and high severity disturbance patches. To foster arthropod diversity, thinning intensities should remain <30% of trees removed, while high severity disturbances with >70% of canopy trees killed are also of high conservation value.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Metabarcoding data (Kortmann, 2021a) are available via Figshare at https://doi.org/10.6084/m9.figshare.13689772.v1. Environmental data (Kortmann, 2021b) are available via Figshare at https://doi.org/10.6084/m9.figshare.13689883.v1.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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