Climate-induced forest dieback drives compositional changes in insect communities that are more pronounced for rare species

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Species richness, abundance and biomass of insects have recently undergone marked declines in Europe. We metabarcoded 211 Malaise-trap samples to investigate whether drought-induced forest dieback and subsequent salvage logging had an impact on ca. 3000 species of flying insects in silver fir Pyrenean forests. While forest dieback had no measurable impact on species richness, there were significant changes in community composition that were consistent with those observed during natural forest succession. Importantly, most observed changes were driven by rare species. Variation was explained primarily by canopy openness at the local scale, and the tree-related microhabitat diversity and deadwood amount at landscape scales. The levels of salvage logging in our study did not explain compositional changes. We conclude that forest dieback drives changes in species assemblages that mimic natural forest succession, and markedly increases the risk of catastrophic loss of rare species through homogenization of environmental conditions.

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Insects are vital components of biodiversity, providing important ecosystem services such as pollination and pest regulation, while also performing disservices as disease vectors and plant pests. Global changes, including those of climate, land use and land cover, can lead to degradation and habitat loss, chemical and light pollution or invasive species. These changes have caused major decreases in biomass, abundance and species richness of insects, and this accelerating decline has become a major cause for concern. Yet, understanding the relative impacts of these drivers underlying insect decline and its propensity is a complex task.

Forests are thought to act as buffer zones from rapid anthropogenic changes in adjacent areas, and contribute to biodiversity conservation by providing refuge for rich insect communities. However, forests are increasingly suffering from climate-induced tree dieback, regardless of protection measures, which could have long-term consequences for forest biodiversity. Tree diebacks largely result from more frequent, intense and longer droughts, especially in Europe. Indeed, severe summer droughts can induce tree mortality in particular if they trigger insect pests and pathogen outbreaks. While heatwaves can negatively impact some insect pest species or pathogens by imposing heat stress, others can benefit from it, favouring distribution range expansion and population outbreaks, which can cause massive forest diebacks. Bioclimatic models predict that the extent, severity and duration of droughts will increase as a result of climate change, notably in temperate and Mediterranean areas.

Drought-induced forest diebacks can cause major structural changes such as an increase in canopy openness and reduction in foliage density, which in turn increases light availability, potentially changing the community structure of understory plants and their associated herbivorous insects. While insect decline is independent of forest protection status, it has been presented as lower in terms of species richness for plots undergoing dieback. Indeed, tree dieback can also increase the availability of resources stored in vital trees (i.e. deadwood), sunlight and microhabitats, increasing species richness for multiple insect groups including bees, wasps, hoverflies, saproxylic beetles, as well as multiple red-listed insect taxa following insect pest outbreaks, or canopy-dwelling beetles in declining oak forests. However, no significant change in species richness of ground-dwelling carabids and spiders has been associated with climate-induced dieback in a beech-dominated forest. Meanwhile, forest management practices, like salvage logging (i.e. cutting down dying trees to salvage their timber value), can also have contrasting effects on biodiversity.

For instance, species richness of saproxylic insects increased with bark beetle outbreaks, but decreased with deadwood harvesting. Even though specific to each of these well-studied taxa previously stated, species richness in general appears to be boosted by tree diebacks, while salvage logging seems to have no effect overall.

The identification of samples down to species level is important in ecological studies because adaptive response to disturbances can be highly species-specific. Unfortunately, studies on the response of insects to forest disturbance often focus on a few well-known taxa and exclude important hyper-diverse groups such as Diptera and Hymenoptera that are difficult to identify to species level and as a result leave changes undetected. In addition, studies on the response of insect biodiversity to forest disturbances are based on species richness, which has been used as a surrogate for ecosystem functionality. However, richness alone is often a poor indicator of biodiversity change compared to ecological guilds within the community. Quantifying these changes requires high taxonomic resolution that is often not available. The morphological identification that richness measures are typically derived from are often limited to well-known insect biodicators that are overrepresented in the literature, hence biasing species richness per se and impeding further holistic community-based and ecological studies. Unfortunately, accurate species identification of hyper-diverse insect groups such as dipterans and hymenopterans is difficult because of the taxonomic impediment, often hampering—or limiting to few remarkable groups—the study of these species-rich taxa despite their ecological importance. The use of DNA barcoding as a tool for species identification now allows bypassing this taxonomic impediment. As exemplified by Wang et al., targeting poorly described Chinese entomofauna, the use of DNA metabarcoding allows researchers to tackle more comprehensive insect biodiversity studies and facilitates the documenting of all trophic guilds and their responses to forest disturbances. The authors suggested that forest diebacks induced by bark beetle outbreaks could drive a transition from homogenous plantations to more biodiversity-friendly heterogenous forests.

To measure the response of insects to forest dieback and subsequent salvage logging, there is a need to sample as many taxa as possible to detect any possible variation among different taxa and functional groups. For instance, floricolous species are expected to increase in diversity and abundance following tree dieback due to greater canopy openness. Similarly, parasitoid wasps, which are rarely included in this kind of ecological study, may benefit from temperature rises and associated tree diebacks, because the more complex a forest is the more diverse the parasitoid communities are. Parasitoids may also suffer from poorer host quality, especially of sap-feeders directly affected by tree health under drought conditions. Forests undergoing diebacks are thus expected to induce guild-specific responses, which may have contrasting effects on species richness of each insect group depending on their respective ecology. A comprehensive approach to sampling and identification of taxa is therefore needed to improve our understanding of the effects of forest disturbances on total insect biodiversity.

Here, we study insect diversity in montane Pyrenean forests dominated by silver fir (Abies alba Mill), a conifer species sensitive to drought that suffered severe climate-induced diebacks due to several heatwaves that have occurred since 2003. Current forest management practices often implement salvage logging at the first signs of dieback. Despite the high conservation value of Pyrenean silver fir forests, the effects of dieback and salvage logging on associated insect fauna remain unknown. To address this gap, we studied the communities of aerial insects over 56 natural (i.e. non-experimentally modified) silver fir forest plots (Fig. 1) that varied in dieback level and management practices. From late spring to early autumn 2017, insects were mass-trapped monthly using Malaise traps, resulting in 222 samples that we analysed using DNA metabarcoding of a fragment of the mitochondrial COI gene region. Based on BOLD DNA reference libraries, the recovered Molecular Operational Taxonomic Units (MOTUs) were taxonomically identified. Ecological functions (i.e. floricolous and parasitoid insects) were attributed using family-level information from published literature whenever possible. We used generalized linear models to assess whether dieback levels and salvage logging influence the structure and diversity of forest insect communities and functional guilds.

We hypothesized that species richness would remain stable based on similar amounts of species gain and loss throughout the levels of forest dieback and salvage logging, but that changes in species composition of local insect communities would occur across sites with different disturbances. We also hypothesized that functional guilds would respond differently to forest dieback intensity; in particular the diversity of parasitoid and floricolous insects in areas of high forest dieback with greater canopy openness. As expected, we found no change in species richness...
but variations in community compositions across dieback levels and management practices. We performed zeta order analyses\textsuperscript{28,29} to further investigate the dataset structure and identify the nature of these community changes, as well as the species compositional and functional turnover. We also used zeta analyses in multi-site generalized dissimilarity modelling (zeta.msgdm)\textsuperscript{30} to study the impact of environmental features (i.e. geographic distance to the nearest plot, altitude, canopy openness, total amount of deadwood, basal area, tree diversity, density of very large trees and both Tree-related Microhabitats (TreM) diversity and density) in driving these community structural changes and further discuss their associated consequences for forest management and conservation. Finally, we used the fourth-corner model\textsuperscript{31} to highlight hypothesized winning and losing insects to forest disturbances as well as idiosyncratic responses of insect orders and functional groups.

**Results**

Diverse insect community with high temporal and spatial turnover. A total of 59,321,436 raw sequencing reads (individual forward and reverse reads) were obtained from the sequencing, and 11,802,769 sequences were recovered after complete demultiplexing and data processing including applying our criteria of at least 3 reads present in each of the 3 PCR replicates for each retained MOTU. Overall, the mean number of reads per sample was 55,937 ± 22,120 (SD) (max. 157,012; min. 12,669). Of the 222 Malaise-trap samples studied, 211 samples yielded results after demultiplexing.

Metabarcoding recovered thousands of locally rare species and unnamed “dark taxa” (i.e. species-level ‘arthropod’ taxa in DNA reference libraries without a species name assigned and/or species-rich taxa, often small in body size and undescribed)\textsuperscript{32}. From the 211 Malaise-trap samples, we recovered 2972 MOTUs (see Supplementary Data 1 for the complete MOTU list), and we estimated a total richness of ~4000 MOTUs with iNEXT extrapolation set on incidence frequency datatype and Hill number \( q = 0 \) parameter to account for observed MOTU diversity, with species accumulation curves approaching saturation (Fig. 2a, Supplementary Fig. 1). Our 4-month sampling effort (from late spring to early autumn) thus captured ca. 75% of the total Malaise-trappable insect diversity in the sampled areas allowing us to reach ~90% sample coverage (Fig. 2a–c). Applying a threshold of 97% sequence similarity for species-level discrimination and keeping unambiguous taxonomic matches for higher taxonomic ranks, 100%, 84.8%, 75.4% and 52.4% of the total 2972 insect MOTUs were assigned to a total of 15 orders, 258 families, 1193 genera and 1558 species names, respectively (Supplementary Data 1 and 2). Two orders, Diptera and Hymenoptera, together represented 73% of all MOTUs, as expected with Malaise-trapped samples (Fig. 3a), with 641 (49.7%) and 371 MOTUs (41.4%) identified to species, respectively (Supplementary Fig. 3). As with the whole dataset (Fig. 2a), the accumulation curves of the five most represented insect orders approached asymptotes (Fig. 3b).

We observed a high rate of temporal species turnover across months, as evidenced by only 360 MOTUs (12% of the total MOTUs diversity) being detected in all 4 months (i.e. from mid-May to mid-September) (Fig. 2d). Thus, even though our sampling was efficient at recovering high biodiversity, sampling throughout the complete growing season is likely to increase the total diversity occurring in the region (Fig. 2d). After grouping monthly temporal replicates into sample sites, geographic turnover was also observed, with only 45% of the total insect species sampled occurring in both of the sampled districts Aure valley (Central French Pyrenees) and Sault plateau (Eastern French Pyrenees) (Fig. 2a), the remaining 31% and 24% being specific to each, respectively. In addition, these district-specific taxa were the most rarely caught throughout all the different plots of their respective districts (Supplementary Fig. 2a).

Climate-induced dieback influences the composition but not the species richness of insect communities. We grouped individual sample sites into three dieback categories (low, medium, high) based on the proportion of drought-affected and dying trees...
for each plot. Insect species richness was compared across the three dieback categories but also across three defined stand types including management practice (healthy, stands expressing dieback but not salvaged—hereafter ‘disturbed’—, stands expressing dieback and being salvage logged—hereafter ‘salvaged’). No response could be detected in terms of changes in species richness from all insect or functional groups tested, neither for the three levels of forest dieback nor the stand types (Table 1). However, a significant difference in species richness was found between the two sampled districts for Coleoptera only, with more species of beetles detected in Sault plateau (Table 1).

As the dataset was geographically structured (i.e. Aure valley and Sault plateau districts), we analysed the dataset using the nearest-neighbour (NN) sample-selection scheme (Fig. 4). The NN model fitted better to a power-law function than to an exponential one (Fig. 4c, d; $\text{AIC}_{\text{NN, Exp}} = 0.89$, $\text{AIC}_{\text{NN, PL}} = -122.78$), which was consistent with community assembly being driven by niche differentiation over stochastic assembly. We also
ran a zeta-diversity analysis with the non-geographically structured all-combinations (ALL) sample-selection scheme (Supplementary Fig. 4), and the results were similar but showed a much weaker fit to the power-law function (Supplementary Fig. 4c, d; AIC$_{\text{ALL, exp}} = 6.3$, AIC$_{\text{ALL, PL}} = -20.97$). The rapid decline in zeta diversity between zeta orders 2 and 10 indicated that compositional turnover was mainly driven by rare species; few species were shared in 10 or more sites (Fig. 4a; Supplementary Fig. 2b). However, re-visualising the decline curve in Fig. 4a as a zeta retention-rate curve (Fig. 4b) showed that the few species that did occur in ≥10 sites ($n = 42$ species) were highly prevalent but only two species were shared by 55 out of 56 total sites. Interestingly, this decline curve also demonstrated the drop of species retention rate at 28 sites (equivalent to the number of sampled plots in each district), accounting for the strong geographic effect that induced very few shared species between Aure valley and Sault plateau. Re-analysis using the ALL sample-selection scheme produced similar results in shared species but smoothed geographic effects (Supplementary Fig. 4).

The impacts of drought-induced forest dieback intensities and stand types on community compositional changes were evaluated for the total sampling (Fig. 5a). Overall, forest dieback was found to induce significant changes in insect community assemblages for all tested groups but Coleoptera (Supplementary Table 1). However, no significant variation in community composition was found across stand types, hence no effect of salvage logging could be detected (Supplementary Table 1). Regardless of dieback intensity and stand type, each insect community assemblage tested differed significantly between districts (Fig. 5b, Supplementary Table 1). Each dieback category hosted particular sets of species, yet insect communities of low dieback level plots were more similar to each other and more distinct than those of medium and high dieback level plots (Fig. 5a; Supplementary Fig. 2b). Furthermore, taxa specific to a particular dieback level were mostly rare taxa (i.e. taxa with low prevalence) (Supplementary Fig. 2b). Finally, community composition variations found across districts were reflected by Sault plateau plots sharing more species than those of Aure valley plots (Fig. 5b).

**Winners and losers of forest disturbances.** Zeta decline analyses allowed us to assess community assemblages and species...
Retention rates across plots for the five main insect orders represented and for functional groups (floricolous/non-floricolous and parasitoid/non-parasitoid species) within each dieback category and stand type. We detected that species were retained differently within both dieback intensity gradient and stand types, regardless of the model scheme used (Figs. 6 and 7; Supplementary Figs. 5 and 6). Both Lepidoptera and Hymenoptera showed a rapid drop and a lack of structure in zeta ratio along the dieback gradient (Fig. 6a–c) and between the different stand types (Fig. 7a–c), indicating there were no species shared across zeta order ranges of both environmental gradients. Similar zeta declines were observed at higher zeta orders for Coleoptera at low dieback level and in healthy stands (Figs. 6a and 7a), as well as for Diptera and Hymenoptera at high dieback level and in disturbed stands (Figs. 6c and 7b). These results highlighted that rare species shaped Coleoptera communities mostly at low dieback level, whereas common Coleoptera species were found across plots of higher dieback gradients. Conversely, both Diptera and Hymenoptera species assemblages were less diverse at low dieback level and healthy stands while high dieback level and disturbed stands favoured complete species turnover with no common species retained (Figs. 6a–c and 7a, b). Regarding functional assemblages, all had common species likely to be found across low and medium dieback gradient, as well as healthy and salvaged forest stands (Figs. 6d–e and 7d–f). Nevertheless, while a similar pattern was observed for non-floricolous and non-parasitoid species assemblages at high dieback level or within disturbed stands, zeta ratio of decline for both parasitoid and floricolous species cohorts fully dropped, indicating species compositional turnover within the two functional groups and no core species shared across 18 to 23 zeta order range (Figs. 6f and 7e). This lack of structure was likely driven by effects of high dieback level and disturbed stands observed on both Diptera and Hymenoptera, which included many taxa of pollinators and/or parasitoids (Figs. 6c and 7b). Overall, while few drops in species retention rates due to geographic effect were noticeable throughout the different environmental conditions (Figs. 6 and 7), main effects of both dieback level and stand types on species retention rates remained visible in ALL model scheme (Supplementary Fig. 5–6).

Table 1 Impact of forest dieback and salvage logging on insect species richness.

| Studied group | Condition          | Degree of freedom | Chi-square | P-value (95% confidence) | Significance |
|---------------|--------------------|-------------------|------------|--------------------------|--------------|
| Total insects | Dieback level effect | 2                 | 2.110      | 0.3483                   | N.S.         |
|               | Stand type effect   | 2                 | 0.945      | 0.6230                   | N.S.         |
|               | District effect     | 1                 | 0.520      | 0.4710                   | N.S.         |
| Coleoptera    | Dieback level effect | 2                 | 0.357      | 0.8366                   | N.S.         |
|               | Stand type effect   | 2                 | 0.906      | 0.6357                   | N.S.         |
|               | District effect     | 1                 | 13.957     | 0.0002                   | ***          |
| Diptera       | Dieback level effect | 2                 | 1.978      | 0.3719                   | N.S.         |
|               | Stand type effect   | 2                 | 1.806      | 0.4053                   | N.S.         |
|               | District effect     | 1                 | 1.815      | 0.7779                   | N.S.         |
| Hemiptera     | Dieback level effect | 2                 | 0.142      | 0.9315                   | N.S.         |
|               | Stand type effect   | 2                 | 1.640      | 0.4404                   | N.S.         |
|               | District effect     | 1                 | 1.587      | 0.2078                   | N.S.         |
| Hymenoptera   | Dieback level effect | 2                 | 2.724      | 0.2562                   | N.S.         |
|               | Stand type effect   | 2                 | 1.239      | 0.5384                   | N.S.         |
|               | District effect     | 1                 | 1.042      | 0.3074                   | N.S.         |
| Lepidoptera   | Dieback level effect | 2                 | 0.742      | 0.6899                   | N.S.         |
|               | Stand type effect   | 2                 | 0.150      | 0.9279                   | N.S.         |
|               | District effect     | 1                 | 0.925      | 0.3361                   | N.S.         |
| Floricolous   | Dieback level effect | 2                 | 2.330      | 0.3119                   | N.S.         |
|               | Stand type effect   | 2                 | 0.372      | 0.8301                   | N.S.         |
|               | District effect     | 1                 | 0.572      | 0.4495                   | N.S.         |
| Non-floricolous | Dieback level effect | 2                 | 2.100      | 0.3499                   | N.S.         |
|               | Stand type effect   | 2                 | 1.341      | 0.5114                   | N.S.         |
|               | District effect     | 1                 | 0.109      | 0.7415                   | N.S.         |
| Parasitoids   | Dieback level effect | 2                 | 2.422      | 0.2979                   | N.S.         |
|               | Stand type effect   | 2                 | 0.798      | 0.6709                   | N.S.         |
|               | District effect     | 1                 | 3.223      | 0.0073                   | N.S.         |
| Non-parasitoids | Dieback level effect | 2                 | 2.018      | 0.3646                   | N.S.         |
|               | Stand type effect   | 2                 | 1.136      | 0.5666                   | N.S.         |
|               | District effect     | 1                 | <0.001     | 0.9860                   | N.S.         |

Generalized linear models for species richness variations of different study groups (i.e. total insects, five individual insect orders, and four functional groups) compared across respective environmental conditions of diebacks (i.e. low, medium and high forest dieback levels) and stand types (i.e. healthy, disturbed and salvaged logged). Functional groups (i.e. Parasitoids/non-parasitoids, floricolous/non-floricolous insects) were assigned using each MOTU’s taxonomic family. Significance is given by *** while N.S. stands for non-significant.
Winning and losing insect orders in terms of prevalence over dieback gradient and stand types were assessed using a “fourth corner” modelling. We found a higher prevalence in low dieback level stands and conversely, a detrimental effect of salvage logging over Coleoptera (Fig. 8). Furthermore, the lack of a decrease in species retention rate for both Hymenoptera and Diptera at low dieback level or in healthy stands (Figs. 6a and 7a) could be explained by a lower prevalence of these two orders in these environments (Fig. 8). Interestingly, Hymenoptera but not Diptera were winning from a particular level of dieback, especially with a higher prevalence at medium dieback level (Fig. 8a), while both Lepidoptera and Hemiptera diversity were favoured by stand disturbances in general (Fig. 8b).

Finally, by analysing congruences between MOTUs and dieback gradients using IndVal analyses, we highlighted species-specific responses to forest dieback. We significantly associated MOTUs to low and medium but not to high dieback levels (Supplementary Table 2). These MOTUs could be linked to specific forest dieback conditions with particular environmental niches and therefore be considered losing over the general dieback gradient. The remaining species may either be too poorly sampled across plots to assess significant linkage or likely to be spread across multiple levels of dieback.

Environmental drivers of species turnover. When assessing the contribution of our eight variables as drivers of the compositional turnover across zeta orders, we found that distance between plots played an important role in explaining the observed variance, especially in a two-plots to 10-plots comparison—hence dominated by rare species—and even greater at zeta orders above 28, thus on common species (Fig. 9a). This increase in geographic effect was in accordance with the relative number of plots in each respective district, for which communities were significantly different (Fig. 5b; Supplementary Table 1). Besides distance, both altitude and canopy openness, and to a lesser extent density of large trees played a major role in driving community composition of rare species (Fig. 9a, zeta order 2). Similar results were observed at zeta order 10, but TreM diversity, TreM density and volume of deadwood became more impactful, while canopy openness had less of an effect. Curve slopes indicated a high but discontinuous and decreasing impact of canopy openness on community changes above ~0.2 (rescaled value), while community composition had a relatively constant sensitivity to altitude at low zeta orders overall (Fig. 9a, zeta order 2–20). Interestingly and according to the slope, community changes were sensitive to the density of large trees, quickly showing a slight unhook but with an important and continuous impact of this environmental driver at up to 0.2–0.4 (rescaled value) overall (Fig. 9a, zeta order 2, 10). Finally, the bigger the zeta order was, the more impact TreM diversity, TreM density and deadwood volume on-site had, as only these three environmental factors (aside from distance) were found to drive compositional turnover (Fig. 9a, zeta order 50). TreM at high zeta orders showed a similar impact trend as community openness at low zeta orders on community composition, plateauing at ~0.9 at zeta order 20, 1.5 at zeta order 28, 3 at zeta order 40 and 5 at zeta order 50, while deadwood amount followed patterns of the density of large trees (Fig. 9a, zeta order 20–50). Interestingly, TreMs density also affected overall compositional turnover but had less impact than TreM diversity in driving community composition. The nine tested variables explained 20–40% of the species composition turnover variance across all zeta orders. Distance excluded, the eight remaining variables only accounted for 15–20% of the variation, hence most of the variance remained unexplained (Fig. 9b). This suggests a complex multifactorial effect of environmental factors, but not random assemblies (Fig. 4c–d; Supplementary Fig. 4c–d), with many variables yet to be explored, in driving the insect community composition turnover of both rare and common species.

Discussion
Marked declines in insect abundance, biomass and species richness have recently been quantified in Europe. The causes of insect decline are multifactorial with rapid climate change identified as one of the major drivers. Here we investigated whether forest disturbances such as drought-induced forest decline and subsequent salvage logging have an impact on flying insects. Surprisingly, insect richness remained stable regardless of the extent of dieback or salvage logging. However, insect species composition changed...
significantly with the level of dieback, driven mainly by turnover among rare insect species. Comparable changes in insect species composition but stable insect species richness have been observed in response to pest-induced forest dieback of other tree species,\(^22,33\), and the fact that similar observations have been made in aquatic insect communities over time\(^13\) suggests that our findings are indicative of a broad ecological pattern.

While species richness remained similar across the forest dieback gradient, community composition of the orders Lepidoptera, Hemiptera, Diptera and Hymenoptera changed significantly. Furthermore, all functional groups studied (i.e. floricolous/non-floricolous and parasitoids/non-parasitoids) also showed significant compositional turnover driven by forest dieback. In line with previous study, we demonstrate how widely impacted biodiversity can be from dieback regarding both taxonomic and functional assemblages\(^34\). Only Coleoptera had no detected effect of disturbances in general on the composition of their communities. This is surprising as Coleoptera have been shown to be significantly affected by both forest diebacks and salvage logging\(^35\). But while they were the sole group bearing significant change in species richness across districts, the absence of community change across dieback gradient had been already observed on saproxylic beetles in the same sampling area\(^36\) but may also be an artefact of the use of Malaise traps which do not collect many Coleoptera and a biased or lower sampling efficiency of Coleoptera in Sault plateau.

In contrast to forest diebacks, salvage logging practices had no impact on species richness nor on community composition in our studied area of the Pyrenees. This result has to be taken with caution since the salvage logging intensity and the associated amount of deadwood removed from our plots might have been too small to significantly impact insect fauna. Because the slopes of the sampled montane forest plots were steep and logging mechanically performed using skidders and cables, we suppose that both reasoned management practices and the difficulty to harvest deadwood mitigated salvage logging impacts on insect biodiversity. In addition, winning and losing taxa from salvage logging\(^13\) may have had overall counter-balancing effects. Indeed, we found a weak but expected negative effect of salvage logging on the prevalence of Coleoptera similar to a meta-analysis\(^13\). We think the first explanation of low salvage logging intensity is more plausible and supported by the fact that we did not detect any impact of salvage logging on deadwood amount either in our study sites\(^36\). Furthermore, as sampling occurred in non-old-growth forests, salvage logging may have left a sufficient amount of coarse woody debris on-site to prevent severe impacts on insect diversity. Insect communities in managed stands such as those we studied may be poorer, more homogenous and have fewer taxa associated with deadwood than in more mature forests. This may have reduced our ability to detect changes in species richness. In addition, our sampling was carried out more than 10 years after the onset of climate-induced tree dieback and subsequent logging, perhaps providing time for a recovery of species richness, even though extinction debt (i.e. a time-delayed negative response of a taxon to an environmental disturbance) was observed in Diptera after 29 years in Quercus spp. dominated forests of Southern France\(^37\).

The lack of variation of species richness of insects with dieback level found in our study contrasts with other studies that show a positive effect of forest dieback on insect biodiversity\(^14,15\). However, those studies are based on the response of well-studied groups, i.e. "those insects that we love and cherish"\(^38\). Indeed, Moretti et al.\(^13\) highlighted that insect diversity could respond both negatively and positively to fire-induced forest diebacks after examining the datasets at lower taxonomic level. They found that the winners were among the most studied groups of insects.

**Fig. 5** Variation in community composition across dieback gradient, stand types and geographical districts. Gaussian copula ordination plots representing variations in insect community assemblages in regards to: a dieback level conditions (i.e. low, medium and high) and stand types (i.e. healthy, disturbed and salvaged), and b to the two different geographical districts sampled. Mvabund analyses of community dissimilarities were performed using 999 bootstraps. Each black line represents the resultant of both factors 1 and 2 of the traitGLM Gaussian copula ordination for each studied plot reduced to 95% of the 2.5 set alpha-ratio. Ordination a highlighted higher similarity in community composition between low dieback level plots than the other two dieback levels, more similar to each other but expressing high variability in community composition. No grouping of salvaged-logged plots in terms of community composition could be distinguished. Ordination b highlighted the greater dissimilarity in terms of community composition within plots of Aure valley compared with Sault plateau that hosted species assemblages more similar to each other.
(i.e. hoverflies, bees, social wasps and ground beetles) while only one losing insect group was identified (weevils)\textsuperscript{15}. This implies that other, unidentified groups were potentially “losers” but less likely to be reported. Similar bias toward these well-known insect groups is also identifiable in studies on pest-induced forest dieback with an additional focus on red-listed species\textsuperscript{16}, or in the studies considered in Thom and Seidl’s meta-analysis\textsuperscript{14}. From this detailed taxonomic view\textsuperscript{15} and recent comprehensive studies\textsuperscript{22,33}, an observation of a positive response of insects to disturbances must be taken cautiously as it could be biased towards the response of the better studied insect groups. Previously reported global patterns may thus not be fully representative. Here, by including hyper-diverse and understudied groups (“dark taxa”)\textsuperscript{32}, the overall stable species richness that we found across both the dieback gradient and stand types highlights a nuanced and idiosyncratic response of insect orders and functional groups such as floricolous/non-floricolous and parasitoid/non-parasitoid insects to forest disturbances. In addition, our results in both insect species richness and compositional turnover emphasize the limitation inherent to the sole use of species richness as a metric\textsuperscript{39} and the need to look more closely at community composition and functional changes to detect the response of insects to disturbance\textsuperscript{40}. Indeed, we show that various dieback levels would rather promote different insect community assemblages and be part of natural succession dynamics (i.e. ecological change occurring in a predictable way after disturbance), with equally weighted response of species either winning or losing throughout the dieback intensity gradient. Hence, management policies based

Fig. 6 Effect of dieback on community composition for insect orders and functions. Representation of the species retention rate (i.e. zeta ratio) per plot (i.e. zeta order) following nearest-neighbour plot combinations scheme (NN) with parameter sample set to 5000 and Monte-Carlo (mc) sampling for low, medium and high dieback levels, respectively (a–c) for the five main insect Orders (Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera) and (d–f) for the four main ecological functions recovered from taxonomic assignment (floricolous/non-floricolous and parasitoid/non-parasitoid species). Green line with plain dots represents mean species retention rate of the total dataset in each respective dieback category. Increasing curves express that common MOTUs are more likely to be retained in additional samples than rare ones (with presence of common species over all plots if zeta ratio = 1) and decreasing curves indicate species turnover. In our study, no core of common species could be sampled for Coleoptera at low dieback level as well as for Lepidoptera and Hemiptera throughout the entire dataset for each dieback category, respectively. For both Diptera and Hymenoptera, common core of species was observed within all dieback level plots except between high dieback level ones. Similarly, drop and lack of structure in common species was detected for floricolous and parasitoid functional assemblages within high dieback level plots while stable for the other two dieback levels, with this functional turnover being driven by dipteran and hymenopteran species turnovers at high dieback.
only on species richness metrics and letting be forest diebacks to favour biodiversity may hold potential risks by driving a catastrophic decline of insects through a potential homogenization of dieback levels and associated species assemblages with more generalist species. Albeit Borges and collaborators highlighted the issue in the very distinct island ecosystem, their concern can be extended to freshwater streams and disturbed forest environments as in the present case.

Looking at community compositional changes in a more specific way, zeta decline fitting a power-law regression implied a compositional turnover of the total dataset driven by environmental factors rather than stochastic events. The zeta ratio analyses showed that species are indeed retained differently within each dieback category. Thus, the lack of core set of species observed for Lepidoptera may be attributed to a relatively low moth diversity associated with silver fir-dominated forests with community composition mainly shaped by turnover processes. On the other hand, sampling efficiency could explain the absence of common species retained for Hemiptera for the different dieback categories or stand types tested. Nevertheless, the prevalence (i.e. the presence probability of a taxa within all the plots grouped in a given environmental category) of these two orders remained positively correlated with dieback in general, in accordance with previous observation in which open canopy deriving from diebacks favours heliophilous and flower-visiting insects. Interestingly, the prevalence of Coleoptera was favoured by low dieback level while species richness across the dieback gradient was similar and the species retention rate decreased. Even though we could not finely assess feeding guilds, these observations may support high turnover processes and competition deriving from specialist species' population decrease as factors shaping Coleoptera communities. However, salvage logging
had a slight but significant negative impact on Coleoptera prevalence, in line with previous observations and consistent with the fact that a large proportion of forest-dwelling Coleoptera are saproxylic13,36,42. In addition, each dieback gradient hosted a specific core set of one or more common species composed of Diptera and Hymenoptera at both low and medium dieback levels. The reduced openness associated with healthy stands—hence low dieback level—might also support the reduced prevalence of both Diptera and Hymenoptera observed in these environments, as most of the species recovered for these orders are considered floricolous. Dieback might also increase environment complexity and support the positive effect of medium dieback level that we observed on Hymenoptera, especially parasitoid ones23, rather than a negative bottom-up effect of the host condition on parasitoid species24.

A compelling observation in terms of functional diversity is that while both Diptera and Hymenoptera displayed strong compositional turnover at high dieback level, their drastic changes in community composition drove in turn the turnover observed for floricolous and parasitoid functional assemblages. It is known that both floricolous and parasitoid Hymenoptera can indeed be significantly impacted by structural changes in silver fir-dominated montane forests43. However, our results emphasize the ecological importance of some species-rich yet often overlooked taxa on wide functional groups. Furthermore, it highlights the need for more work to investigate whether climate-induced forest dieback can deeply impact functional efficiency and ecosystem services through these taxa3. While these broad functions remain, the observed complete change in their respective species cohorts through the dieback gradient might impact trophic relationship at finer scales and species-dependent interactions.

In a more detailed view, within each studied order or functional group, the winner and loser taxa were relatively rarely occurring species in our dataset. This pattern is comparable to previously reported taxonomic dissimilarities driven by rare species34 and consistent with models showing that selective perturbations directed towards rare species generate more dynamical effects on the ecosystem34. As rare species have a positive impact on ecosystem multifunctionality15,46, these rare taxa have high patrimonial value and thus their importance in forest ecosystems functioning should be further investigated34.

To investigate for environmental features in an attempt to explain these complex compositional changes, we first found that geographic distance—regardless of the zeta order considered—played a significant role as a macroecological factor shaping the different insect communities of the two districts and, as for the aforementioned winner and loser taxa, mostly taxa with low prevalence shaped compositional specificities of each district. As expected, many other forest features tested (i.e. altitude, density of large trees, canopy openness, total volume of deadwood and both TreM diversity and density) also had a significant effect in shaping insect community composition, indicating a complex response to disturbances. Remarkably, environmental factors explaining most of the compositional variance differed according to the observed zeta ratio. At low scale (zeta order = 2), community changes were mainly driven by canopy openness, density of large trees and altitude. This result supports the role of distance and stand structure heterogeneity in favour of biodiversity47 and is in line with previous observations on these features affecting the rare species composition at low zeta order22. In addition, slope trends for both altitude and canopy openness at low zeta orders reflect previous observation, with respectively continuous (full range) and discontinuous (between 0 and 20% of its range) impacts on community compositional changes22. However, these environmental features at large scale (zeta order >10) were no longer the main drivers of biodiversity changes. Instead, both TreM diversity and density, as well as the amount of deadwood were the main factors influencing community composition when considering the whole dataset. Interestingly, we highlight that TreM diversity, TreM density and amount of deadwood impacting ranges on community changes at high zeta orders have similar impacts as canopy openness and altitude at low zeta orders, respectively. This finding may result in various management strategies to promote biodiversity, with both canopy openness and TreM diversity manipulated in similarly limited way (i.e. around 20% increase maximum) in accordance with the size of the managed area, but without threshold in regards to deadwood amount on-site for wide areas. Here, we also highlight that TreM diversity had more impact than TreM density on insect diversity48, similarly to deadwood diversity and amount in other studies47,49. However, as TreM diversity is partly linked to TreM density, it indirectly also has a great influence on biodiversity48. Furthermore, and contrary to previous report47, we found deadwood amount to be as valuable as stand structure for biodiversity conservation, depending on the geographic scale managed42, which further supports the

**Fig. 8 Insect orders winning and losing from environmental conditions.** Heatmap representation based on traitGLM analyses with LASSO penalty of insect orders’ prevalence according to environmental conditions: a forest dieback level (i.e. low, medium and high) and b stand types (i.e. healthy, disturbed and salvaged). Negative and positive interactions and their intensity in terms of biodiversity response to environmental conditions for each of the five most represented orders are highlighted by a continuous colour gradient spanning from blue to red, respectively. We found specific responses for each insect orders’ diversity in regards to dieback levels and stand types. For instance, the low prevalence of dipterans and hymenopterans observed at low dieback level while relatively high prevalence for coleopterans. However, hymenopteran prevalence was greater in forests of medium dieback level. Coleoptera was the only order with prevalence negatively impacted by salvage logging. Finally, disturbed plots showed a relatively high prevalence of both Lepidoptera and Hymenoptera.
general call on acknowledging and considering the importance of deadwood to biodiversity. Interestingly, a previous study successfully linked hoverfly diversity to plot connectivity and the combined effect of environmental factors from both stand and landscape scales. The shift in environmental variables driving community assemblages according to the zeta order considered here support such geographic scale effect and help disentangling the key drivers in action. This may potentially be extended to most aerial insects with higher dispersal ability, which are the main representatives of our Malaise-trapped dataset. Hence, while species-specific response to environmental factors must be accounted for, geographic scale of the conservation area, especially through stand and landscape scales, as well as dieback intensity should also be considered when managing stand heterogeneity and environmental factors to favour forest insect biodiversity.

Finally, our study confirms DNA metabarcoding of Malaise-trapped samples to be an efficient approach for biomonitoring...
changes in species-rich insect communities that include “dark taxa”, which collectively represent the bulk of forest insect diversity. While read-counts may not provide accurate abundance estimates for species, promising developments such as the spike-in method may improve this capability. Nevertheless, our metacounting pipeline coupled with recent efforts to complete the DNA barcoding reference library of European insect fauna allowed us to inventory nearly 3000 insect species, more than half of which were identified to species level, including hyper-diverse taxa such as Diptera and Hymenoptera. These two taxonomic groups were the most diverse in our study, like in other Malaise trap environmental surveys. Although Malaise-trapped samples are biased toward flying insects, the zeta decline analyses indicated that our dataset incorporated rare species as non-stochastic events and tracked changes in both species richness and community composition at both large and small spatial scales while overcoming sampling district discrepancies. Our study is limited to a single year but provides a detailed account of insect diversity for the studied area and may serve for future monitoring of ecosystem recovery and biodiversity changes over time. Indeed, scaling up our approach to national and continental levels would help to monitor insect biodiversity and potential decline, to provide an understanding of the environmental drivers of biodiversity loss in a rapidly changing climate, and allow for regular assessment of the efficiency of conservation and forest management policies.

Methods

Study sites, stand description and insect sampling. Fifty-six natural forest plots were selected for our study in 2017 in silver fir (Abies alba) forests in the Central (Aure valley) and Eastern (Sault plateau) French Pyrenees (see Supplementary Data 2 for the complete plot list). In each plot, one Malaise trap was placed with the rear aperture facing south. Each trap was equipped with a commercial lightemitting diode (LED) body and a cover that was open to capture all insects flying above the canopy (Fig S1). Malaise traps were filled with a mixture of 20% monopropylene glycol and 80% pure ethanol. Samples were retrieved once per month, resulting in a total of four samples per plot over the sampling period (124 trap days), for a total of 222 samples (two samples were lost). After collection, all samples were stored at 4 °C until laboratory processing.

Insect sampling was conducted from late spring to early autumn in 2017 (May 15 to September 15) using 56 Townes-style Malaise traps with black walls and a white roof. One Malaise trap was placed at the center of each of the 56 one-ha plots. Malaise trap sample-bottles were filled with a mixture of 20% monopropylene glycol and 80% pure ethanol. Samples were retrieved once per month, resulting in a total of four samples per plot over the sampling period (124 trap days), for a total of 222 samples (two samples were lost). After collection, all samples were stored at 4 °C until laboratory processing.

Laboratory processing and DNA extraction. Insects were first filtered from the trapping solution and rinsed with ultrapure Milli-Q water to remove monopropylene glycol residue. Insects were then placed within sterile and disposable Petri dishes, and cleaned absorbing paper to dry overnight at ambient temperature.

Once dried, insects were size-sorted using decontaminated forceps. Insects larger than a European honey bee (Apis mellifera Linnaeus, 1758) were removed and only the head or a part of the abdomen was retained in order to reduce the biomass and improve the detection of rare or small species. Insect bulk samples were then ground and homogenized into fine powder using disposable BMT-50-S-M gamma sterile tubes (IKA) with 10 steel beads with an Ultra Turrax Tube Drive grinder (IKA). Ground bulk samples were then conserved at −21 °C.

DNA extraction of 25 mg (±2 mg) of insect powder was performed on silica columns using a standard DNeasy Blood & Tissue extraction kit (QIAGEN): (see www.qiagen.com/handbooks for further information). For each sample, we took 25 mg (±2 mg) of insect powder in a 1.5-mL microcentrifuge tube using spatula previously decontaminated with 4% Decon 90 solution and autoclaved. During powder sampling, empty 1.5-mL microcentrifuge tubes containing 200 µL ATL buffer were left open and changed every nine samples to control for potential cross-contamination with volatile contaminant powder and deadwood extraction controls (EC). Mock communities of 248 Asian insect species were used as positive controls. Negative (NC), extraction (EC) and positive (PC) controls were processed down to sequencing. Lysis was performed with horizontal shaking overnight at 56 °C in 180 µL ATL buffer and 20 µL proteinase K. All vortex steps were preceded by handshaking. Reduced eluates were diluted to 80 µL of AE buffer following 15 min incubation on the silica column at ambient temperature, and a second elution with the previous eluate after 5 min incubation on the silica column. Each sample was quantified using Qubit 2.0 fluorometer dsDNA High Sensitivity kit (Invitrogen) and eluate sub-samples were diluted with dH2O to a final dilution of 180 µL to a concentration of 2 ng/µL. Three different dilutions of each sample were used for each qPCR reaction, in 25 µL reaction volume as follows: 200 ng/µL (v1), 20 ng/µL (v2), and 2 ng/µL (v3). Each v3 sample was subjected to analysis. 

To amplify DNA, we ran a qPCR reaction on 25 µL of each sample. The qPCR conditions were as follows: 10 min at 95 °C for enzyme activation, followed by 40 cycles (1 cycle = 30 s at 95 °C, 30 s at 60 °C, and 1 min at 72 °C) of PCR amplification and a final melting step of 95 °C for 60 s, 40 °C for 60 s, and an acquisition gradient from 65 to 97 °C. The qPCR reactions with a final volume of 15 µL were prepared with 7.5 µL of QAPA qPCR mix (2X), 0.3 µL of each forward and reverse primers (10 mM), 3.9 µL of extra pure molecular grade water and 1.6 µL of template DNA (at 10 ng/µL). One microliter of each template was added to the qPCR master mix and cycled on the thermal cycler: 3 min denaturation at 95 °C, 40 cycles of 15 s at 95 °C, 30 s at 60 °C, and 1 min at 72 °C, and a high-resolution melting step of 95 °C for 60 s, 40 °C for 60 s, and an acquisition gradient from 65 to 97 °C. The qPCR reactions with a final volume of 15 µL were prepared with 7.5 µL of QAPA qPCR mix (2X), 0.3 µL of each forward and reverse primers (10 mM), 0.2 µL of Metabion mi-Taq (5 U/µL), 16.8 µL of extra pure molecular grade water and 3 µL of DNA template at 2 ng/µL. Each sample was PCR-amplified three times in different well-plates with three different twin-tag sequences, to allow independent tracking of the three PCR processes after amplification. PCR conditions were as follows: initial denaturation at 94 °C for 15 min, followed by 30 cycles of 30 s at 94 °C, 30 s at 60 °C, 1 min at 72 °C, and a final extension of 10 min at 72 °C.

Shifting was in accordance with green/red light balance on v2/v3 Illumina MiSeq technology. Neither proof-reading nor hot-start Taq polymerases were used for PCR amplification due to too high level of failure.

To prior PCR amplification, we tested the optimal number of cycles and the quantity of DNA added to the PCR mix by quantitative PCR (qPCR) using a LightCycler® 96 Instrument (Roche) with the KAPA Library Quantification Kit (Illumina). The qPCR conditions were the same as those used for the qPCR cycle reaction and were run for 25 cycles under the same conditions as the qPCR cycle, with 25 µL of 1:100 dilution reaction as follows: 2.5 µL of buffer green (10 mM), 0.2 µL of Metabion mi-Taq (5 U/µL), 16.8 µL of extra pure molecular grade water and 3 µL of DNA template at 2 ng/µL. Each sample was PCR-amplified three times in different well-plates with three different twin-tag sequences, to allow independent tracking of the three PCR processes after amplification. PCR conditions were as follows: initial denaturation at 94 °C for 15 min, followed by 30 cycles of 30 s at 94 °C, 30 s at 60 °C, 1 min at 72 °C, and a final extension of 10 min at 72 °C.

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TruSeq DNA PCR-free Library Prep kit for PCR-free ligation (Illumina) with 18 different indices (one per 96-well plate) following the protocol given in Leray et al.45. Libraries consisting of the total 222 samples were sequenced on five MiSeq runs – v3 (Illumina) with 600 cycles.

Use of positive controls and blanks in demultiplexing. Mock communities of known species composition with DNA barcode sequences available were used as PCRs. The 248 species used in the mock community all have distributions restricted to east Asia, and hence allow for the examination of tag-jumping in the Illumina sequencing process. PCRs were sequenced and bioinformatically treated the same way as bulk samples to ensure comparability. We focused on a restrictive approach, to test the taxonomic richness, and hence allow for the examination of tag-jumping in the Illumina PCs. The 248 species used in the mock community all have distributions restricted to east Asia, and hence allow for the examination of tag-jumping in the Illumina PCs.

Reads demultiplexing, taxonomic and functional group assignments. AdapterRemoval ver. 2.2.250 was used to trim the twin-taged adaptors of the reads and we employed sickle ver. 1.331 to perform paired-end quality trimming. Error correction using Bayes Hamner was done via SPADEs ver. 3.12.0, followed by paired-end error correction using Pandana. Reads were clustered into Miller Operational Taxonomic Units (MOTUs) with a 97% similarity threshold78 using CLARK (ver. 0.9.0). Clustering quality was controlled via R, using the LULU approach80. Taxonomic assignment was performed by comparing nucleotide sequences with the BOLD System database27 in April 2019 using a 97% threshold similarity22 with bold System database. Our twin tagging approach allowed us to easily identify errors and problems at this infection stage. Renkonen Similarity Indices (RSI value) were calculated for unique sequences found in each of the three PCR replicates and represented by at least three reads (see section ‘Use of positive controls and blanks in demultiplexing’ for arbitration of required numbers of PCR replicates and reads). Average read size was plotted in R to ensure filtering and trimming quality, and VSEARCH ver. 2.8.1 was run to remove chimeras.

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We first assigned each of the 258 recovered insect families to different trophic guilds (i.e. saproxylic, zoophagous...) based on the known ecological functions of both immature and adult stages. Then, each family was assigned or not to two main functional groups: florivorous and parasitoids. This was done based on the capability of each family to give rise to functionally florivorous or parasitoid (or none) during at least one part of its life cycle (therefore fulfilling the given function in the ecosystem overall) (see Supplementary Data 2 for functional assignment of insect families). We identified the insect families with parasitoid and/or florivorous species based on the literature62-84 and with the help of expert taxonomists.

Statistics and reproducibility. All statistical analyses were carried out in R ver. 3.6.173. Read-sequence data were transformed in incidence-based data to account for presence/absence of MOTUs given that abundance data from metabarcoding can be misleading for metazoans85. We generated accumulation curves using INEX package ver. 2.0.209 with incidence-based frequency dataset parameters and Hill number q value set to 0 to reflect rare-invariant results. Extending the effect of the dieback gradient, stand types, and MOTUs below 97% of NC and EC were checked for reads way as bulk samples to ensure comparability. We focused on a restrictive approach, to test the taxonomic richness, and hence allow for the examination of tag-jumping in the Illumina PCs. The 248 species used in the mock community all have distributions restricted to east Asia, and hence allow for the examination of tag-jumping in the Illumina PCs.

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calculations were performed with all sites kept for each category, including those without focal taxa.

Estimations of environmental variables contribution to insect species assemblage changes were performed with zeta multi-site generalised dissimilarity modelling (zeta.msgdm in zetadiv R package ver. 1.2.0) at different zeta order, ranging from 2 to 50. As for zeta-diversity analyses, zeta.msgdm model was run with the number of samples fixed to 5000. After checking for non-colinearity, a total of nine environmental variables were tested with zeta.msgdm: geographic distance to the nearest plot, altitude, canopy openness, total amount of deadwood in m² per ha, the basal area per ha, the tree diversity per ha, the density of very large trees (Ø > 67.5 cm) per ha and both the TreM diversity and density per ha. zeta.msgdm was calculated using I-spline models. Zeta.msgdm model was performed using Sørensen-equivalent metric and run over 30 rounds to obtain stable I-spline response curves of those predictors.

**Reporting summary.** Further information on research reporting is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

All datasets used for analyses are publicly available on Zenodo (https://doi.org/10.5281/zenodo.5653307) or at the following GitHub repository: https://github.com/Lucasire/Malaise_FR_2017. Supplementary Data are publicly available on Figshare (at the following)^10^ https://doi.org/10.6084/m9.figshare.1697563.v1. Raw sequencing data are available on NCBI at the following accession number: PRJNA702908.

**Code availability**

All scripts used for bioinformatic demultiplexing and analyses are publicly available on Zenodo (https://doi.org/10.5281/zenodo.5653307) or at the following GitHub repository: https://github.com/Lucasire/Malaise_FR_2017. Received: 8 May 2021; Accepted: 7 December 2021; Published online: 18 January 2022

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Author contributions
This study was conceptualized and designed by C.L.V., E.A.H. and C.B. Forest plot selection and sampling were designed by L.L. and C.B. Sample processing, wet-lab experiments and sequencing were performed by L.S., P.S.Y. with the help of A.B., B.C. and M.T.M. Bioinformatic analyses were done by L.S. and P.S.Y. with the help of D.W.Y. and C.W. Ecological and environmental analyses were conducted by L.S. and C.W., with the help of J.C., C.B., D.W.Y. and D.F. L.S. led the writing of the manuscript. All authors contributed substantially to the interpretation and discussion of the results, with M.T.M., D.W.Y., S.T., J.M., E.A.H. and C.L.V. contributing substantially to the revision of the article. All authors approved the submitted version.

Competing interests
The authors declare no competing interests.

Additional information
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