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A comparison of different Malaise trap types

Johannes Uhler[1] | Peter Haase[2,3] | Lara Hoffmann[3,4] | Torsten Hothorn[5] |
Jürgen Schmidl[6] | Stefan Stoll[3,4] | Ellen A. R. Welti[2,7] | Jörn Buse[8] | Jörg Müller[1,9]

[1]Field Station Fabrikschleichach, Department of Animal Ecology and Tropical Biology, Julius-Maximilians-University Würzburg, Würzburg, Germany

[2]Department of River Ecology and Conservation, Senckenberg Research Institute and Natural History Museum Frankfurt, Gelnhausen, Germany

[3]Faculty of Biology, University of Duisburg-Essen, Essen, Germany

[4]University of Applied Sciences Trier, Environmental Campus Birkenfeld, Hoppstädten-Weiersbach, Germany

[5]Epidemiology, Biostatistics and Prevention Institute, University Zürich, Zürich, Switzerland

[6]Department of Developmental Biology/ Institute of Biology, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany

[7]Conservation Ecology Center, Smithsonian's National Zoo and Conservation Biology Institute, Front Royal, Virginia, USA

[8]Seebach, Germany

[9]Bavarian Forest National Park, Grafenau, Germany

Correspondence
Johannes Uhler, Field Station Fabrikschleichach, Department of Animal Ecology and Tropical Biology, Julius Maximilians-University Würzburg, Am Hubland, 97074 Würzburg, Germany.
Email: johannes.uhler@uni-wuerzburg.de

Abstract

1. Recent reports on insect decline have highlighted the need for long-term data on insect communities towards identifying their trends and drivers.

2. With the launch of many new insect monitoring schemes to investigate insect communities over large spatial and temporal scales, Malaise traps have become one of the most important tools due to the broad spectrum of species collected and reduced capture bias through passive sampling of insects day and night. However, Malaise traps can vary in size, shape, and colour, and it is unknown how these differences affect biomass, species richness, and composition of trap catch, making it difficult to compare results between studies.

3. We compared five Malaise trap types (three variations of the Townes and two variations of the Bartak Malaise trap) to determine their effects on biomass and species richness as identified by metabarcoding.

4. Insect biomass varied by 20%–55%, not strictly following trap size but varying with trap type. Total species richness was 20%–38% higher in the three Townes trap models compared to the Bartak traps. Bartak traps captured lower richness of highly mobile taxa but increased richness of ground-dwelling taxa. The white roofed Townes trap captured a higher richness of pollinators.

5. We find that biomass, total richness, and taxa group specific richness are all sensitive to Malaise trap type. Trap type should be carefully considered and aligned to match monitoring and research questions. Additionally, our estimates of trap type effects can be used to adjust results to facilitate comparisons across studies.

KEYWORDS
Bartak, biodiversity, insect communities, insect monitoring, Malaise trap, Townes, trap selectivity

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INTRODUCTION

In recent years, several studies have reported significant declines of insect communities (Hallmann et al., 2017; Seibold et al., 2019; van Klink et al., 2020) mainly in temperate regions highlighting the importance of long-term monitoring. As insects provide many essential services and are vital for our ecosystems (Costanza et al., 1997; Dainese et al., 2019; Reid, 2005), it is critical to continuously monitor insect communities in order to determine when and where populations are declining, identify drivers of declines, and develop targeted mitigation measures. While an increasing number of long-term monitoring programs began in the last decade, many studies lack comparability as a result of variation in sampling methods. Even within monitoring programs, methods can vary over time and space (Welti, Joern, et al., 2021). Assessments of how different monitoring methods affect insect estimates are needed to facilitate comparisons.

The Malaise trap is one of the most used traps for insect sampling (Vårdal & Taeger, 2011) and has gained popularity especially in the last two decades (Figure S1). They are versatile passive net-traps capable of catching a large spectrum of insect taxa (Karlsson et al., 2020; Malaise, 1937; Skvarla et al., 2021). Malaise traps primarily catch flying insects but can also capture ground-dwelling species. They are effective for studies aiming to collect a representative snapshot of the local insect community and are convenient for long-term monitoring as they require low field maintenance. In addition, samples collected by Malaise traps are usually stored in ethanol and can therefore be readily analysed using metabarcoding, which is especially useful for large-scale monitoring studies, producing a large number of samples that are otherwise very labour-intensive to handle.

Malaise traps were originally developed by the Swedish entomologist Rene Edmund Malaise (1937). He designed multiple versions of his trap, each for a specific purpose. However, the design from which traps today are based, was developed further by Townes (1962). This design allows for a lightweight and versatile trap, which is easy to handle and setup. One vertical net serves as a barrier, intercepting insects flying perpendicular to the trap. Since most flying insects have a positive phototropic reaction, they will attempt to escape upwards and are then funnelled by a roof section, leading to a capture bottle, which is usually filled with ethanol. In addition to flying insects, non-flying arthropods, like bush-crickets and spiders, that crawl or jump up the trap from the ground or nearby vegetation are frequently captured (Karlsson et al., 2020). Overall, there are various factors that determine catch, both in terms of number of individuals caught and catch composition (Matthews & Matthews, 1983; Townes, 1972). The most important is the trap placement, followed by trap characteristics such as trap design and mesh size (van Achterberg, 2009). A net with a broad mesh forms as a less visible barrier, especially for larger insects, particularly ones with good vision like dragonflies or larger Aculeata, whereas a smaller mesh sizes enable the capture of smaller species like Microhymenopterans (Darling & Packer, 1988; van Achterberg, 2009). Most trap variants lean towards smaller mesh size to better capture the hyper-diverse taxa of Hymenoptera and Diptera.

Another and much debated selecting factor is the choice between a black or white roof. White roofs were initially used to strengthen the phototrophic reaction of insects, thereby reducing the chance of them escaping to the sides (Townes, 1972). However, white-roofed traps may increase the catch of flower-visiting taxa that are attracted to white, a common flower colour (van Achterberg, 2009); this effect could result in higher overall trap catch, but would mean white-roofed traps are not an entirely passive sampling method. The use of a certain trap or trap variant can thus influence estimates of flying insect biomass, species richness and composition. However, despite the increasing popularity of Malaise traps and attempts to standardise approaches (Montgomery et al., 2021), systematic comparisons of these effects on total biomass, total richness, and the richness of specific taxa are not available, hampering comparisons among studies using different Malaise trap types.

Here, we setup an experiment in three regions of Germany to compare five widely used Malaise traps, three Townes variants, and two Bartak variants in two different open habitats, meadows and forest gaps. We test three hypotheses: (i) a larger trap collects more insect biomass than a smaller trap of the same type, (ii) the longer and lower Bartak traps catch more ground-dwelling insects, while the higher Townes traps catch more highly mobile flying insects, and (iii) the white-roofed Townes trap catches more flower-visiting taxa than its black-roofed counterpart.

MATERIALS AND METHODS

Study design

This study was conducted in 2020 at three German Long-Term Ecological Research (LTER) sites (Steigerwald, Spessart, Hunsrück). For each LTER site, two suitable patches were identified, one meadow and one forest clearing. In each patch, we established a strip of 50 × 3 m with a north–south orientation. Two different habitats were chosen to control for specific effects of habitat on the capture characteristics of individual trap types.

In forests, we selected gaps of similar size as in meadows, so that sunlight was able to reach the ground for most of the day and minimise bias in activity trapping (Hallmann et al., 2017). Clearings were located at least 50 m from a forest edge. In each strip, we established five plots with different Malaise trap types (Figure S2).

Arthropod sampling

The five Malaise traps (Figure 1; Figure S3) were placed in a row with 5 m distance to each other along the strip, arranged in parallel so that the capture area of one trap did not impair that of another and with the capture bottle facing south. A distance of 10 m was kept to any nearby trees or shrubs (Figure S2). The order of the placed traps in each plot was randomly selected and differed for all patches. After
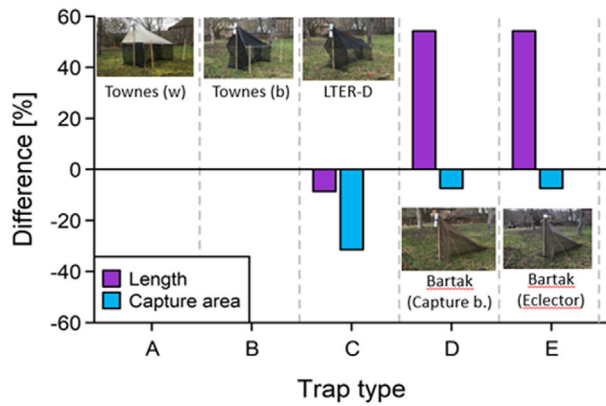
FIGURE 1 Comparison of length and capture area of five Malaise trap types. For details on trap types, see Figure S3. Capture area of a trap = area of trap opening, see also Table S1

half of the season, we changed the order of the trap types once. Growing vegetation in close proximity to the trap was kept low throughout the sampling period (<20 cm).

Three variants of the Townes style Malaise trap were used: a trap with a white roof (A) and with a black roof (B), both manufactured closely to the guidelines of Hallmann et al. (2017) and a smaller variant (also completely black), which is used in the German Malaise trap program coordinated by the German LTER network (Welti, Zajicek, et al., 2021) (C). Additionally, two variants of the Bartak style trap were used, one equipped with a conventional capture bottle (D) and the other with an eclector bottle (E). The latter capture device has a clear plastic cover at the top, which lets more sunlight through with the aim of increasing the phototrophic reaction of insects caught in the trap. Trap A was selected as a benchmark trap in all results visualisations.

Traps A and B are the largest traps with a capture area of 1.75 m^2, followed by the two Bartak traps with an area of 1.62 m^2, and then the German LTER trap with an area of 1.2 m^2 (Figure 1). The Bartak design is longer, and the capture area is extended to the ground (Table S1).

Ethanol (80%) was used to ensure DNA preservation for metabarcoding. Sampling began in April and traps were emptied every 2 weeks until early October for a total of 13 arthropod biomass samples (referred to in the following as 'insects') per trap across the growing season. The individual sampling periods were variable in terms of the number of days sampled (mean: 14.09 days min: 12 days max: 17 days). Biomass was measured for all samples, but due to financial restrictions only three sampling periods (second half of May, late July to early August and second half of August) were analysed using DNA metabarcoding. Of 390 possible samples, 16 could not be obtained due to collapsed traps, vandalism, or other complications (see Table S6 for further information). Therefore, 374 samples were used for the analysis of insect biomass and 88 samples for the analysis of species richness.

In order to measure insect biomass, all samples were placed in a fine sieve until the time between drops of ethanol reached 10 s and then weighed on a precision scale to 0.1 g. To improve DNA metabarcoding results and address potential bias due to insect body size

differences and therefore variable amounts of DNA per individual, the samples were divided into a smaller and larger fraction. This was done using an 8-mm sieve. Insect species were identified using mitochondrial cytochrome oxidase 1 gene (CO1-5P) DNA metabarcoding according to the laboratory and bioinformatic pipelines following Hausmann et al. (2020), for more information see Supplementary Text S1. We used Barcode Index Numbers (BINs) instead of operational taxonomic units (OTUs) to measure richness for all taxonomic groups, since the accuracy of OTUs can vary between taxonomic groups. For a detailed explanation of the benefits of using BINs, see Hausmann et al. (2013). The BIN system was developed by the Barcoding of Life Data Systems (BOLD) and is based on a chain of algorithms, which cluster similar barcode sequences and check their taxonomic integrity. The BIN clusters match the actual taxonomically identified species at different levels (90%–99% COI genetic similarity), varying with taxa, and allowing comparisons with studies based on morphological determination.

Statistical analysis

Analyses were performed using R v. 3.6.2 (R Core Team, 2019). We fitted generalised additive models using the package mgcv (Wood, 2004) to test for the effects of trap type and plot type on insect biomass and total species richness, and the richness of major taxonomic groups. For biomass, the mean day of a trap-specific sampling period was modelled using a smoothed non-linear spline of time to account for seasonality, an offset of sampling length to control for variation in individual sampling periods, the trap position on the respective study site and the region, as random effects (Uhler et al., 2021). To account for repeated measurements on a specific trap position, we also included Region_plotnumber as additional random effect. For BIN richness, the sampling period was used as a fixed effect instead of a smooth of the mean day of sampling, since only three sampling rounds were analysed. For insect biomass, a Gaussian distribution with a log-link was used, and for richness a negative binomial distribution, allowing extraction of the relative values compared to the benchmark trap type A. Significant differences between trap types were assessed by multiple post hoc comparisons using the function glht (package multcomp; Hothorn et al., 2008).

To assess the importance of trap type as well as region, habitat and sampling campaign for species composition on two scales of taxonomic resolution, we used multiple regression of distance matrices (MRM) using the function MRM (R package ecodist; Goslee & Urban, 2007). Morisita–Horn index (Horn, 1966) was used to calculate the dissimilarity matrices of insect composition on BIN-level and aggregated on family level and Euclidean distances for the dissimilarity matrices of the predictor sets. Predictor sets were standardised by using decostand (method = 'standardise', R package vegan; Oksanen et al., 2020).

For the richness of groups frequently used as indicators for biodiversity or ecosystem service studies, we used the richness of bees (Apiformes), hoverflies (Diptera: Syrphidae), and diurnal butterflies (families: Lycaenidae, Riodinidae, Hesperiidae, Papilionidae,

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Nymphalidae, and Pieridae). For detailed R code and data to reproduce all analyse and figures, see Data availability.

RESULTS

Based on 374 samples from 13 sampling periods, traps caught an average of ~3.05 g/day of insects, with a strong humped-shaped pattern over the growing season and a peak in late July/early August (Figure S4). In total, 88 samples of three campaigns (mid-May/early August/late August) were DNA metabarcoded, resulting in the identification of ~3,887 taxonomically described species.

Trap A collected the most biomass, followed sequentially by Trap B (−23%), C (−28%), E (−54%), and D (−56%). Total BIN richness was closely matched for Traps A, B, and C but much lower for Traps D (−21%) and E (−37%) (Figure 2).

BIN richness of flying insects (Diptera, Hymenoptera and Lepidoptera) was highest for Traps A, B, and C and much lower for Traps D and E (Figure 3a). BIN richness of ground-dwelling insects (Hemiptera, Coleoptera and Orthoptera) was highest for Trap D, followed by Trap B and lowest for Traps A, C, and E (Figure 3b). BIN richness of groups frequently used as indicators for biodiversity or ecosystem service studies (hoverflies, bees and butterflies) was highest for Trap A and much lower for all other trap types (Figure 3c). Overall, biomass and BIN richness were comparable for forest clearing and meadow sites (Figure S5). Regarding species composition, region, habitat type and sampling campaign were of much greater importance than trap type on species level (Figure 4a). However, when rerunning the analysis on family level (number of BINs aggregated per family), which removes regional differences of the species level, trap type was of equal importance as region and sampling
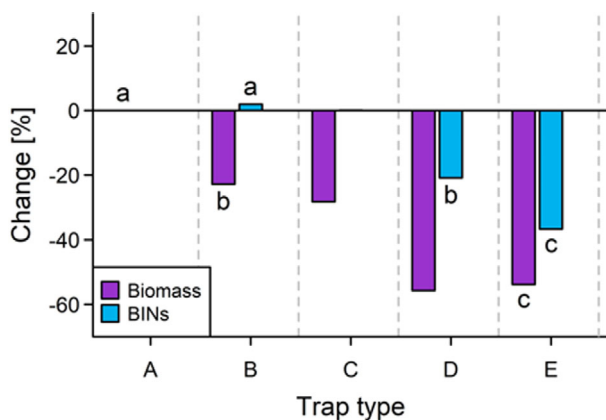

FIGURE 2 Partial effects of trap type on insect biomass and diversity (total BIN richness). The displayed values are based on comparisons with trap type A. Significance was tested by multiple post hoc comparisons using the function *glht* in the R package multcomp (Hothorn et al., 2008). Different letters indicate significant differences (*p* < 0.05) between categories. Note that only the first significant entry for a predictor is shown, subsequent entries between trap types, even if significant, were omitted for clarity. For detailed model results, see Table S2

campaign (Figure 4b). This indicates a difference in the coarse composition of the samples depending on trap type.

DISCUSSION

We provide guidelines for future biodiversity studies using Malaise traps with varying goals and improve comparability of estimates of insect biomass and richness across studies. Our results did not support our first hypothesis that a larger Townes trap collects more biomass than its smaller variant (i). The smaller Townes variant C did catch less biomass than A, but so did Trap B of exactly the same size as A, and the similar sized Traps D and E collected even lower amounts of biomass. However, since Trap A also differed in roof colour and Traps D and E were of different size and shape, the only valid comparison was between Traps B and C, which differed only in size and showed no difference in caught biomass or species richness. The first part of our second hypothesis (ii) that the lower and longer Bartak traps catch more ground-dwelling insects compared to the Townes traps was supported by results from Trap D, but not from E; this is likely a result of DNA denaturation which we discussed in more detail below. Trap B (Townes) additionally caught many Coleoptera and Orthoptera. Contrastingly, the overall higher Townes style traps caught more highly mobile flying insects than the lower Bartak traps, supporting the second part of our second hypothesis (ii).

Both Bartak Traps (D/E) which are similar in size to the Townes Traps A/B, although overall longer and less high, caught significantly less biomass and species. The Bartak Trap D caught significantly more ground-dwelling insects than the Townes Traps A and C, whereas the higher Townes Traps (A, B, C) were more effective at capturing highly mobile insects in comparison to the Bartak Trap D, but not the second Bartak Trap (E). Trap E featured an alternative type of capture bottle than the rest, an eclector bottle. This eclector bottle, which is used in other types of insect traps, like tree emergence traps (Hagge et al., 2019; Müller et al., 2020), has the benefit of letting more light through the top and was designed to enhance capture effectiveness through increasing insect photo-attraction. However, this also leads to increased evaporation of the ethanol, used to capture and preserve insects. In our study, this type of bottle was unable to hold ethanol for more than 1 week, even early in the year, with even worse results in June/July when light exposure and temperature was higher. This can increase the risk of degenerating the DNA of captured organisms, biasing results when comparing the otherwise equal Traps D and E (Figure 2/3). Finally, we found support for our third hypothesis (iii) that a Townes trap with a white roof catches more flower-visiting taxa than its all-black counterpart.

Compared to the otherwise identical Trap B, we found that the white roof of trap A had a positive effect on insect biomass and altered species composition but not total species richness. Trap A showed increased capture of indicator flower visiting species, specifically hoverflies, bees (Apiformes), and diurnal butterflies. Additionally, Orthoptera and Coleoptera were caught at reduced rates. This suggests that the white roof increases trap catch of certain groups, either by strengthening the phototrophic reaction or by attracting individuals due to its colour
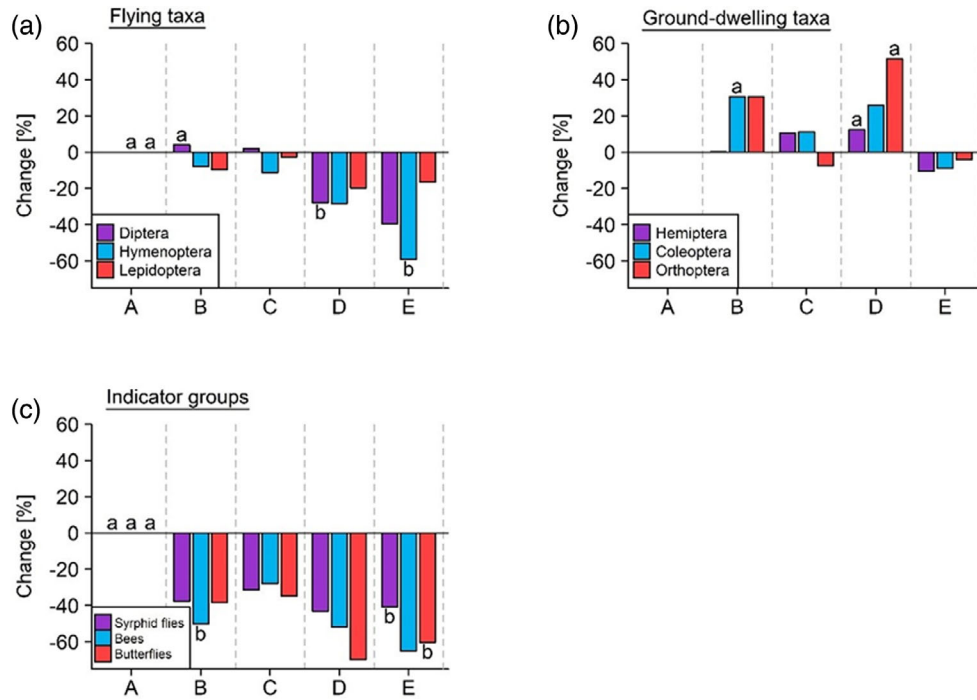
FIGURE 3 Partial effects of trap type on species richness of taxonomic groups: (a) richness of taxonomic groups using predominantly flying as locomotion, (b) richness of predominantly ground-dwelling taxonomic groups, (c) richness of groups frequently used as indicator groups for biodiversity studies. The displayed values are based on comparisons with trap type A. Significance was tested by multiple post hoc comparisons using *glht* (R package multcomp; Hothorn et al., 2008). Different letters indicate significant differences ($p < 0.05$) between categories. Note that only the first significant entry for a predictor is shown, subsequent entries between trap types, even if significant, were omitted for clarity. BIN richness of all other taxa was <5%. For detailed model results, see Tables S3-S5
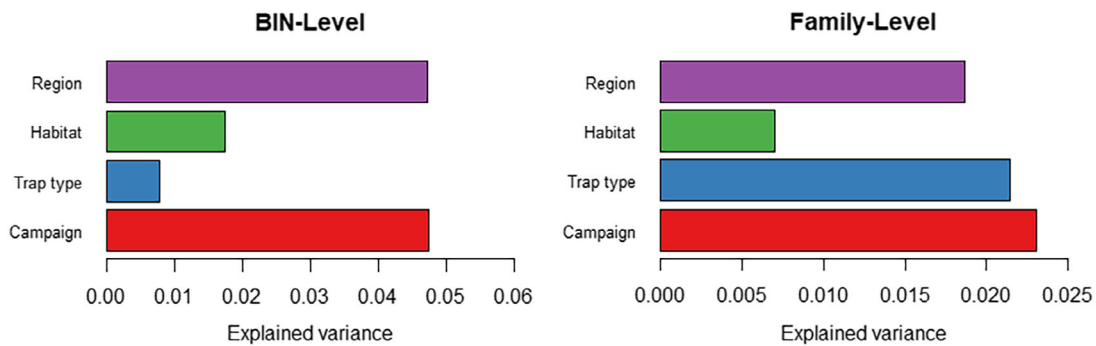

FIGURE 4 Results of multiple regression on distance matrices (MRM) of 30 Malaise traps. Regression coefficients of insect dissimilarity matrices related to region, habitat, trap type and sampling campaign. Insect dissimilarities were calculated using Morisita–Horn indices and predictor dissimilarity matrices were calculated using Euclidean distances.

(van Achterberg, 2009). Due to this increased capture of flower visiting insects, it is possible that white roofs serve a similar role to coloured pan traps placed inside Malaise traps to increase capture efficiency (Darling & Packer, 1988). Having Malaise traps attract insects however, can be undesirable when the goal is to passively capture an unbiased representation of the local flying insect community.

As expected, variation in overall species composition was much better explained by region, habitat type and sampling campaign than trap type (Figure 4a). However, when analysing the differences in

composition on family level and thereby removing regional differences on species level, trap type proved to be of equal significance to the afore mentioned environmental predictors, which underlines our findings regarding the selective trapping of different trap variants.

The effort needed to maintain field sampling and the practicability of the methods used are one of the most important factors when designing a study, especially for long-term monitoring projects. Therefore, several additional trap characteristics should be considered. A trap with a white roof is much easier to spot than a completely black

TABLE 1 Multiplicative-partial effect coefficients of trap types relative to the benchmark trap type A

	Type A	Type B	Type C	Type D	Type E
Biomass	0	0.77	0.72	0.44	0.46
BINs	0	1.02	1.00	0.79	0.63
Diptera	0	1.04	1.02	0.72	0.61
Hymenoptera	0	0.92	0.89	0.72	0.41
Lepidoptera	0	0.90	0.97	0.80	0.83
Hemiptera	0	1.00	1.10	1.12	0.89
Coleoptera	0	1.31	1.11	1.26	0.91
Orthoptera	0	1.30	0.93	1.51	0.96
Syrphid flies	0	0.62	0.69	0.57	0.59
Bees	0	0.50	0.72	0.48	0.35
Butterflies	0	0.62	0.65	0.30	0.40

Results of generalised additive mixed models for insect biomass (13 sampling campaigns), richness of Barcode Index Numbers (BINs, 3 sampling campaigns) using trap type and plot type as fixed effects and trap position and the region as random effects. For insect biomass, mean sampling day was used as a smoothed effect, for BIN richness this was replaced by sampling round as a fixed effect. To control for differences in the sampling periods, log(sampling days) was used as an offset, family = Gaussian(link = 'log') for biomass, and family = negative binomial for richness was used.

or green/brown trap. Due to this higher visibility, the risk of human interference, such as vandalism, may increase. In remote areas, trap weight and ease of setup become important factors. This is where the big advantage of Bartak traps lies. A Bartak trap can be carried and setup much easier, even in difficult terrain. Only one short wooden pole is required to set up this trap and due to its lower height, the pole does not need to be buried as deep into the ground for stability compared to other traps, making it easier to use in rocky areas or areas with dry, hardened soil. Once set up, a Malaise trap only needs to be emptied once every 1–2 weeks, depending on the size of the capture bottle, making the trap relatively easy to maintain throughout the season. However, this seems to be the main drawback of the Bartak trap with an eclector capture bottle (Trap E). It is more difficult to empty in the field, quicker to dry out and less reliable when used for metabarcoding due to possible degeneration of DNA. Both for this difficulty of use, general functionality, and the integrity of the sample DNA, we do not recommend the use of eclector capture bottles. A final key factor, especially for large-scale studies, is the cost of a trap. This can vary depending on the quality of materials used, particularly the resistance to UV light, which chiefly determines the longevity of the trap. In general, the Krefeld style Townes traps are the most expensive and can cost significantly more than the Bartak traps.

In sum, Malaise traps are versatile passive net-traps capable of catching a large spectrum of insect taxa (Aguiar & Santos, 2010; Skvarla et al., 2021; Ssymank et al., 2018), mainly capturing flying insects, but also catching ground-dwelling species. They are low maintenance and therefore convenient for long-term monitoring and effective when the study goal is to sample a representative and sustainable snapshot of the local insect community. The samples can be stored in ethanol and analysed effectively by DNA metabarcoding. We find that the choice of Malaise trap type can greatly affect the estimates of insect biomass, species richness and composition, thereby confirming previous indications with empirical data while also quantifying the magnitude of differences. We do not provide a general recommendation for the use of one trap type as, like any study methodology, the type of trap needs to be selected to fit the research question. Townes style traps with their overall larger height, capture more flying insects, whereas the elongated, but lower Bartak traps catch higher numbers of ground-dwelling insects. Trap characteristics illustrated in this study, including the colour of the trap or the type of capture bottle, should be applicable to other Malaise trap types, not explicitly reviewed here. Finally, if the results of several studies using different trap types are to be compared, we provide guidelines to correct the data generated with a respective trap (Table 1).

AUTHOR CONTRIBUTIONS

Johannes Uhler: Conceptualization (equal); formal analysis (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Peter Haase:** Conceptualization (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Lara Hoffmann:** Conceptualization (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Torsten Hothorn:** Formal analysis (supporting); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Jürgen Friedrich Schmidl:** Conceptualization (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Stefan Stoll:** Conceptualization (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Ellen A.R. Welti:** Visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Jörn Buse:** Conceptualization (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Jörg Müller:** Conceptualization (equal); formal analysis (supporting); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting).

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

Data and annotated R script needed to reproduce the statistical analyses and generate the graphs are publicly available on Zenodo: https://doi.org/10.5281/zenodo.6982995.

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SUPPORTING INFORMATION

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

Appendix S1 Supporting Information.

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