Comparison of destructive and nondestructive DNA extraction methods for the metabarcoding of arthropod bulk samples

Ameli Kirse | Sarah J. Bourlat | Kathrin Langen | Björn Zapke | Vera M. A. Zizka

LIB/Zoological Research Museum Alexander Koenig (ZFMK), Centre for Biodiversity Monitoring, Bonn, Germany

Correspondence Ameli Kirse, LIB/Zoological Research Museum Alexander Koenig (ZFMK), Centre for Biodiversity Monitoring, Bonn, Germany. Email: a.kirse@leibniz-lib.de

Funding Information Bundesministerium für Bildung und Forschung, Grant/Award Number: 01LC1903A

Handling Editor: Pierre Taberlet

Abstract

DNA metabarcoding is routinely used for biodiversity assessment, in particular targeting highly diverse groups for which limited taxonomic expertise is available. Various protocols are currently in use, although standardization is key to its application in large-scale monitoring. DNA metabarcoding of arthropod bulk samples can be conducted either destructively from sample tissue, or nondestructively from sample fixative or lysis buffer. Nondestructive methods are highly desirable for the preservation of sample integrity but have yet to be experimentally evaluated in detail. Here, we compare diversity estimates from 14 size-sorted Malaise trap samples processed consecutively with three nondestructive approaches (one using fixative ethanol and two using lysis buffers) and one destructive approach (using homogenized tissue). Extraction from commercial lysis buffer yielded comparable species richness and high overlap in species composition to the ground tissue extracts. A significantly divergent community was detected from preservative ethanol-based DNA extraction. No consistent trend in species richness was found with increasing incubation time in lysis buffer. These results indicate that nondestructive DNA extraction from incubation in lysis buffer could provide a comparable alternative to destructive approaches with the added advantage of preserving the specimens for postmetabarcoding taxonomic work but at a higher cost per sample.

Keywords: arthropods, biodiversity assessment, DNA extraction, environmental DNA, insects, metabarcoding

1 | INTRODUCTION

Biodiversity is declining rapidly worldwide, but the full extent is yet unknown. In particular for terrestrial arthropods, dramatic data have been published (Sánchez-Bayo & Wyckhuys, 2021). In Germany, a decline in flying insect biomass of more than 75% over a 27-year period was documented (Hallmann et al., 2017), while in the rain forests of Puerto Rico ground-dwelling arthropod biomass dropped by up to 98% over a period of 36 years (Lister & Garcia, 2018). Even if biomass already provides a useful indicator of insect declines, detailed understanding of changes in community composition and their potential causes and consequences needs to be based on high-resolution diversity assessments. For insects, this has long been limited to a small number of groups which are comparatively easy to identify based on morphological characteristics. Hampered by the shortage in taxonomic expertise this is also referred to as taxonomic...
impediment (Wheeler et al., 2004). Since Malaise trap samples can contain up to several thousand species (Geiger et al., 2016), this lack of taxonomic expertise has hindered large-scale diversity studies targeting flying insect diversity using conventional methods. Ongoing developments in DNA-based approaches and high-throughput sequencing (HTS) techniques have enabled diversity assessments of complex bulk samples, requiring comparably less time and effort. For example, in metabarcoding, DNA from specimen mixtures is bulk extracted, a specific marker gene is PCR-amplified, sequenced using HTS techniques and subsequently assigned to a species by matching against a reference database (Compson et al., 2020).

However, choices made at every step of the metabarcoding protocol can directly affect the resulting species list, such as primer choice (Elbrecht et al., 2019; Elbrecht & Leese, 2017; Hajibabaei et al., 2019; Marquina, Andersson, & Ronquist, 2019), extraction method (Deiner et al., 2015; Dopheide et al., 2019; Kirse et al., 2021b; Majaneva et al., 2018) and sequencing depth (Alberdi et al., 2018), but also bioinformatic analysis of sequencing data (Brandon-Mong et al., 2015; Brandt et al., 2021; Kopylova et al., 2016), emphasizing that the trade-offs associated with different protocols should be considered (Bruce et al., 2021; Kirse et al., 2021b). While laboratory protocol optimization has focused on postextraction steps such as primer choice (Elbrecht et al., 2019), less attention has been paid to the choice of DNA extraction method. DNA extraction from homogenized sample tissue, a method which is currently widely used (Elbrecht et al., 2021), results in sample destruction and thereby prevents all subsequent morphological investigation of the sample (Buchner et al., 2021; Elbrecht et al., 2019; Kocher et al., 2017; Mata et al., 2020). Consequently, storage of the complete sample is not possible, preventing postmetabarcoding morphological analysis, and unexpected diversity patterns or rare and potentially undescribed species which could help to complete reference databases cannot be re-examined (Carew et al., 2018). Nondestructive extraction methods are therefore highly desirable to enable the long-term preservation of samples and retrospective analysis. Various nondestructive DNA barcoding and metabarcoding protocols have been tested on single specimens or bulk samples, including either a chemical lysis step prior to extraction (Giebner et al., 2020; Ji et al., 2020; Kirse et al., 2021a; Svenningsen et al., 2021; Vesterinen et al., 2016) or the isolation of DNA directly from the preservative ethanol (Marquina, Esparza-Salas et al., 2019; Zenker et al., 2020; Zikka et al., 2018). While Nielsen et al. (2019) demonstrated the efficiency of nondestructive DNA extraction from mock communities, studies on complex bulk samples such as Malaise traps resulted in different assessments. In particular, assessments from highly diverse terrestrial insect samples seemed incomplete or resulted in different diversity patterns when DNA extracts from preservative ethanol were directly compared to tissue extracts, which highlights the need for further experimental testing of nondestructive DNA metabarcoding approaches (Marquina, Esparza-Salas et al., 2019; Zenker et al., 2020). Despite these methodological inconsistencies, DNA metabarcoding is already widely used in various large-scale studies as the tool of choice for diet studies, biodiversity assessment and monitoring (Bonato et al., 2021; Cabodevilla et al., 2021; Ingala et al., 2021; Lozano Mojica & Caballero, 2021; Svenningsen et al., 2021). The processing of large sample numbers requires fast, reliable and cost-efficient protocols. In particular, bulk insect samples from Malaise traps are often dried and homogenized in a bead mill or mixer mill. This is a time-consuming procedure creating a fine powder which needs to be handled carefully to avoid cross-contamination. Next to the maintenance of sample integrity, nondestructive approaches would circumvent this elaborative handling step and can therefore significantly speed up the metabarcoding process and reduce the risk of cross-contamination.

In this study, we compare four extraction methods consecutively from the same 14 Malaise trap bulk samples (Figure 1), obtained from two habitat types. One destructive method (1) extraction from homogenized tissue) is compared to three nondestructive approaches (2) extraction from commercial ATL lysis buffer [Qiagen]; [3] extraction from home-made lysis buffer [Vesterinen et al., 2016, modified from Aljanabi & Martinez, 1997]; and [4] extraction from sample preservative ethanol [fixative]). In addition, we investigate the effect of different incubation times (2, 4, 8, 12 h) on DNA yield and species detection from commercial ATL lysis buffer. Alpha diversity (number of arthropod species obtained) and overlap in species composition are used to assess the efficacy of different methods. In addition, workload, handling time and costs are considered in the analysis and discussion of trade-offs between alternative methods.

2 | MATERIAL AND METHODS

2.1 | Sample collection

Twelve Malaise trap samples (1000 ml collection bottles filled with 96% denatured ethanol [1% MEK, type 641]) were collected on an agricultural field near Hennef, North Rhine Westphalia (Germany, experimental setup campus Wiesengut, 50.7869°N, 7.2756°E, referred to as WG samples, WG 1–12) using automated and nonautomated Malaise traps (Wägele et al., 2022). Malaise traps were set up between the August 7th and November 10th, 2020 and collection bottles were changed every second week. For the present study, six samples collected on September 10th and six samples collected on September 24th were processed. In addition, two Malaise trap samples were collected at the Bislicher Insel nature reserve, North Rhine Westphalia (Germany, 51.6529°N, 6.5231°E, referred to as BI samples, BI 1–2). Sampling was conducted on October 28, 2020. After collection, samples were stored at −20°C in a freezing chamber until further processing.

2.2 | Extraction from sample preservative ethanol

Two weeks after collection, the total volume of ~800 ml preservative ethanol from samples WG1–WG12 were filtered through 0.45-μm nitrocellulose filters (Nalgene Sterile Analytical Filter Units,
0.45 μm, 150 ml, Thermo Scientific) connected to a minimembrane-gas-vacuum pump (VWR, Type PM20405-86.18). Insect samples were covered with new 96% denatured ethanol and stored at −20°C until further processing. Filters were torn into small pieces with fine tweezers and dried until the ethanol was completely evaporated. Subsequently, DNA was extracted from the filters with the DNeasy 96 Blood and Tissue Kit (Qiagen) following the manufacturer’s instructions. Laboratory bench and equipment were sterilized with ethanol, DNA-AWAY (Thermo Scientific Molecular BioProduct) and UV radiation for 5 min before use. Ethanol filtering was carried out in a clean room free of DNA amplicons and DNA extraction was carried out under a PCR workstation sterilized with UV surface irradiation (Labcaire Systems). Extraction success and DNA quality was checked on a 1% agarose gel.

2.3 Extraction from commercial buffer and column-based extraction

Samples WG 1–6 and BI 1 were divided into two size fractions by wet sieving through a 4 × 4-mm mesh sized stainless steel sieve with a wire diameter of 0.5 mm (Elbrecht et al., 2021). From here on, the size fractions are referred to as S (small <4 mm) and L (large ≥4 mm). Each size fraction was extracted and further processed separately. Specimens were left to air dry for 5 min before proceeding with the protocol.

In the next step, 45 ml of ATL buffer (Qiagen) mixed with 400 μg Proteinase K per ml buffer was added to both size fractions of six WG (WG 1–6) and one BI (Bl 1) sample and placed in a shaking incubator (INCU Line ILS6, shaking incubator, VWR) at 56°C and 200 rpm. Ten millilitres of lysate was taken from each sample and placed into a new 50 ml Falcon tube (Corning Life Sciences) after 2, 4, 8 and 12 h respectively and immediately stored at −20°C. At each time point, a negative control was included (10 ml purified water). After the removal of lysis buffer, specimens were carefully washed with 96% denatured ethanol before drying in an incubator for 3 days at 50°C to prepare for tissue-based DNA extraction. Lysis buffer removed at each time point (2, 4, 8 and 12 h for the seven samples WG 1–6, Bl 1) was processed in two centrifugation steps. As shown in several studies, a simple differential centrifugation protocol can be used to isolate DNA (Djafarzadeh & Jakob, 2017; Macher et al., 2018). Cell debris were pelleted in a first centrifugation step at 12,000 g for 2 min. The supernatant was transferred to a new 50 ml Falcon tube and centrifuged at 12,000 g for 60 min to achieve DNA enrichment. The supernatant was discarded and pelleted DNA was resuspended in 180 μl ATL buffer with 20 μl Proteinase K and further processed using the Qiagen DNeasy Blood and Tissue kit following the manufacturer’s protocol. Extraction success, DNA quality and negative controls were checked on a 1% agarose gel.

2.4 Extraction with the home-made buffer (HM) and salt precipitation

Samples (WG 7–12 and Bl 2) were divided into two size fractions by wet sieving through a 4 × 4-mm mesh sized stainless steel sieve with a wire diameter of 0.5 mm (Elbrecht et al., 2021). From here on the size fractions are referred to as S (small <4 mm) and L (large
>4 mm). Each size fraction was extracted and further processed separately. In the following step, 45 ml of a home-made lysis buffer from Vesterinen 2016, modified from Aljanabi and Martínez (1997): 0.4 M NaCl, 10 mM Tris–HCl pH 8.0, 2 mM EDTA pH 8.0% and 2% SDS were added to both size fractions of six WG samples and one BI sample. Subsequently, 400 μg Proteinase K per ml buffer was added to the sample. Samples were placed in a shaking incubator at 56°C. 10 ml of solution was taken from each sample and transferred into a new 50 ml Falcon tube (Corning Life Sciences) after 2, 4, 8 and 12 h respectively, and immediately stored at −20°C. At each time point, a negative control was included (10 ml purified water). In the following step, a 6 M NaCl saturated salt solution was added to the lysate to a final concentration of 4 mmol and the mixture was vortexed for 30 s, to precipitate the proteins and cell membranes. Tubes were centrifuged at 3556 × g for 15 min. The supernatant was transferred to a new Falcon tube and an equal volume of isopropanol was added to precipitate the DNA in solution. After mixing by inversion, tubes were placed at −20°C for 1 h and subsequently centrifuged at 3556 × g for 60 min to pellet the DNA. The supernatant was discarded, and the resulting DNA pellet was washed with 20 ml ice cold 70% ethanol, then centrifuged at 3556 × g for 15 min. The remaining ethanol was discarded, and the pellet was left to dry at room temperature overnight. The next day, the pellet was resuspended in 1 ml of sterile H2O and stored at −20°C until further processing. Extraction success, DNA quality and negative controls were checked on a 1% agarose gel.

2.5 Extraction from the sample tissue

Both size fractions of the 14 samples (WG 1–12, BI 1–2) were dried in a shaking incubator at 50°C for up to 3 days until ethanol was completely evaporated. The dried samples were homogenized for 3 min in a Turax mixer mill (Tube Mill 100 Control) at 20,000 rpm. Approximately 25 mg of homogenized tissue powder was transferred per sample to a 1.5 ml Eppendorf tube, where 180 μl ATL buffer and 400 mg proteinase K were added. DNA was extracted for each size fraction separately using the DNeasy 96 Blood and Tissue Kit (Qiagen) including six negative controls following the manufacturer’s instructions. Extraction success and DNA quality were checked on a 1% agarose gel.

2.6 Library preparation

Mitochondrial cytochrome oxidase 1 (COI) metabarcoding was carried out using a two-step PCR protocol. The first PCR (amplicon PCR) is carried out using amplicon-specific primers with Illumina adapter overhangs and the second (index PCR) allows the incorporation of Illumina index adapters (Bourlat et al., 2016). The amplicon PCR was set up as follows: 12.5 μl of PCR Multiplex Plus Mastermix (Qiagen), 1 μl of template DNA, 0.2 μM of the fwhF2 forward primer (GGDACWGGWTAACWGWTAYCCCHCC; Vamos et al., 2017) and 0.2 μM of the Fol_degen_rev reverse primer (TANACYTCNGGRTGCRAARAAAYC; Yu et al., 2012) with 10.5 μl ddH2O for a 25 μl final reaction volume. PCR was run on an Applied Biosystems 2720 thermocycler with the following programme: initial denaturation at 95°C for 5 min; 25 cycles of 30 s at 95°C, 30 s at 50°C and 50 s 72°C; final extension of 5 min at 72°C. The resulting PCR product was used for the index PCR, set up as follows: 1 μl DNA template from PCR 1, 0.2 μM of each tagging primer (Nextera, Illumina), 12.5 μl PCR Multiplex Plus Mastermix (Qiagen) and 10.5 μl H2O. The PCR programme was as above, running for 15 cycles instead of 25. PCRs were set up on a 96-well plate including eight extraction negative controls. PCR success was evaluated on a 1% agarose gel before normalization to a final concentration of 25 ng per sample using a SequalPrep normalization plate (Thermo Fisher Scientific) following the manufacturer’s instructions. Ten microliters of each sample was pooled and two left-sided size selections were carried out on the sample pool using magnetic beads at a ratio of 0.76x to remove primer dimers (SPRIselect, Beckman Coulter). Library concentration was measured with a Quanta Fluorometer with the QuantiFluor dsDNA System (Promega) and on a Fragment Analyser (Agilent Technologies). The pooled library was sent for sequencing on one lane of an Illumina HiSeq covering 2 × 250 bp at Macrogen Europe.

2.7 Bioinformatic analysis

Demultiplexing was carried out by the sequencing company. Before loading demultiplexed samples into qiime2 version 2020.11 (Bolyen et al., 2019), primer pairs were removed using cutadap 3.5. (Martin, 2011) with the following settings: maximum error rate (−e): 0.1, minimum overlap (−O): 20, minimum sequence length (−m): 150. Only sequences with both forward and reverse primers were retained for further analysis. In qiime2, sequences were truncated to 175 bp for the forward reads and 170 bp for the reverse reads, respectively. DADA2 (Callahan et al., 2016) was used for merging paired-end reads, quality filtering and denoising, resulting in a total of 24,393 amplicon sequence variants (ASVs). An initial Blast search of ASV representative sequences against each other was conducted with blastn (version 2.9.0). The following parameter settings were chosen: “query coverage high-scoring sequence pair percent” (−qcov_hsp_perc) was set to 80 and minimum percent identity (−perc_identity) was set to 84. The resulting ASV match list was uploaded into R (version 3.5) and the r-package “lulu” (version 0.1.0) was used to perform postclustering curation using standard settings (Froslev et al., 2017). As an additional filtering step, ASVs found in the negative controls were extracted from the samples. After all filtering steps, 6728 ASVs were retained in total. Taxonomic assignment was carried out against the BOLD database (https://www.boldsystems.org) using boldigger (Buchner & Leese, 2020). The output list was filtered using the JAMP-Pipeline option implemented in boldigger. In detail, assignments to different taxonomic levels were conducted after the following similarity thresholds: 98% species, 95% genus, 90% family, 85% order, <85% class (e.g., for a 96% hit the species-level assignment will be discarded and
genus-level information will be used as the lowest taxonomic level. Only ASVs identified to the species level were included for further analysis. If several ASVs were assigned to the same species indicating intraspecific diversity, ASVs and assigned reads were merged at the species level (Table S5). In addition, for the samples separated into two size fractions of <4 and >4 mm in the laboratory, the data sets were merged in silico.

UpsetR plots were made using the R packages UpSetR (version 1.4.0) (Conway et al., 2017), ComplexUpset (version 1.4.1) (Krassowski, 2021) and ggplot2 (version 3.3.3) (Wickham, 2016) for visualization of the number of shared arthropod species between extraction methods as well as between incubation times. Resulting plots were further modified using Microsoft PowerPoint (Microsoft Corporation). To analyse dissimilarities between assessed communities depending on extraction methods, permutational multivariate analysis of variance (PERMANOVA) using Jaccard distance matrices for incidence data of detected arthropod species (blastID ≥98%) were performed using dplyr (version 0.8.3) (Wickham et al., 2015), betapart (version 1.5.1) (Baselga & Orme, 2012) and vegan (version 2.5-7) (Dixon, 2003).

Boxplots showing differences in mean number of assessed species depending on extraction method and insect order were prepared using the R package ggplot2 (version 3.3.3) and the package rstatix (version 1.4.0) (Kassambara, 2021). A t-test was conducted to check for significant differences in the number of assessed species between extraction methods.

Differences in assessed species communities depending on incubation time were visualized with a principal coordinates analysis (PCOA) plot using the R package vegan (version 2.5-7). To statistically analyse dissimilarities between assessed communities depending on incubation time, PERMANOVAs using Jaccard distance matrices for incidence data of detected arthropod species (blastID ≥98%) were performed using dplyr (version 0.8.3), betapart (version 1.5.1) and vegan (version 2.5-7). Subsequently, Kruskal–Wallis and t tests were conducted to test for significant differences in number of assessed species depending on incubation time using the R package rstatix (version 0.7.0).

Samples were rarefied to even sequence depth (same number of sequences per Malaise trap sample with each extraction method) using the phyloseq command rarefy_even_depth (McMurdie & Holmes, 2013). Rarefaction curves, prepared with the R-package iNext (Hsieh et al., 2016), can be found in Figure S2.

3 | RESULTS

On average, 178,000 reads were recovered per sample. We observed that sequencing depth was slightly higher for the tissue and HM extraction with 207,000 and 217,000 reads respectively in comparison to the extraction from ethanol (153,000 reads) and ATL (134,000 reads). While only a single extraction per sample was conducted from the ethanol, for the extraction from tissue and lysis buffer, samples were size sorted into two fractions that were processed and sequenced separately. The reads from both size fractions were then merged in silico, resulting in twice the sequencing depth for the tissue, HM and ATL samples in comparison to the ethanol samples. Subsequent rarefaction to the same sequencing depth resulted in no change in the observed results presented here (Tables S2–S5, Figure S1).

Extraction from HM lysis buffer was more successful in terms of DNA yield than extraction from commercial ATL buffer (Table S1). However, PCR amplification of HM buffer samples was less successful. Even if a clear band was visible on the DNA extraction gel, 14 of the 56 samples could not be amplified by PCR (Table S1). Gel electrophoresis and analysis of the 260/280-nm ratio did not show significant differences in purity of DNA extracts between the two lysis buffers (Table S1). Because fewer PCR amplifications were obtained with the HM buffer, our comparison and statistical analysis focus on the latter three DNA extraction approaches (ATL, ethanol and tissue).

3.1 | Species number, composition and overlap between different extraction methods

The number of species detected within the highly diverse insect orders Coleoptera, Hemiptera, Hymenoptera and Lepidoptera did not vary significantly between extraction methods. Dipterans and other arthropods not included within the above-mentioned insect orders (Arachnida, Chilopoda, Collembola and Malacostraca as well as the insect orders Trichoptera, Neuroptera, Ephemeroptera, Orthoptera, Raphidioptera, Psocodea, Dermaptera, Thysanoptera, Mecoptera, Megaloptera, Blattodea and Zygentoma) showed a significantly higher diversity when samples were extracted using ATL buffer in comparison to extraction directly from the preservative ethanol. However, no significant differences in numbers of assessed species were detected between the ATL and tissue-based extraction for any of the target groups (Figure 2).

From 811 arthropod species identified in total, 224 (27.6%) were shared between all three extraction methods (Figure 3). An additional 149 (18.4%), 99 (12.2%) and 61 (7.5%) species were exclusively found in extraction from ethanol, ATL and tissue respectively. The number of species shared between tissue and ATL buffer-based extraction was 176 (21.7%), higher than the number of shared species between the ethanol and ATL-based extraction (88 species/10.9%) and the number of shared species between extraction from tissue and ethanol (14 species/1.7%).

3.1.1 | Coleoptera

The total number of coleopterans detected with ATL buffer extraction was 66 species, higher than detection with the other two extraction methods (ethanol: 61 species; tissue: 52 species). The number of unique species was highest in the ethanol (21 species), followed by the tissue (16 species) and the ATL buffer-based extraction (nine species) (Figure 4a). In addition to 17 species detected with all three methods, 22 species were overlapping between ATL and ethanol-based extraction.
3.1.2 | Diptera

Overall, the highest number of dipterans was detected when DNA was extracted from the ATL buffer (278 species) followed by extraction from the tissue (225 species), and the lowest species number was retrieved through extraction from the ethanol (209 species) (Figure 4b). In total 139 dipteran species were detected with all three extraction methods (Figure 4b). An additional 72 species were overlapping between the lysis buffer and tissue-based extraction. A high number of species were exclusively found in only one of the extraction methods, accounting for 31 species in the ethanol extracts, 34 species in the ATL lysis buffer extracts and eight species when DNA was extracted from the homogenized tissue.

3.1.3 | Hemiptera

The number of hemipterans identified when DNA was extracted from the ATL buffer (33 species) and the tissue-based approach (33 species) was higher compared with detection from the ethanol-based...
approach (21 species) (Figure 4c). Additionally, a high number of species were shared between extraction from ATL and tissue (19). However, 13 species were exclusively detected when DNA was extracted from the ethanol, while nine and six species were only found with the ATL and tissue methods respectively.

3.1.4 Hymenoptera

The highest number of hymenopteran species was found when DNA was extracted from the ethanol (98 species) while with the ATL and tissue-based extraction 94 and 84 species were detected respectively (Figure 4d). From the 98 species detected with the ethanol extraction, 57 were not found with the two other extraction methods. However, 24 and 22 species were uniquely found with the tissue and ATL-based extraction respectively. An overlap of 35 species was found between extraction from tissue and ATL buffer while 19 species were detected with all three extraction methods (Figure 4d). Overlap between the ethanol and the other two approaches was comparatively low (ATL-ethanol: 18 species; ethanol-tissue: four species) (Figure 4d).

3.1.5 Lepidoptera

In total, the highest number of species was found when DNA was extracted from the ATL buffer (52 species). In total, 43 and 38 species were detected with the ethanol and tissue-based extraction method respectively. Twenty-two lepidopterans were found with all three extraction methods (Figure 4e). Additionally, 15 species intersected with the ATL- and tissue-based extraction while eight species were exclusively found with the extraction from ATL buffer. Fourteen species were exclusively detected with the ethanol-based extraction, while seven additional species were shared between ethanol and ATL buffer.

3.1.6 Others

The category “Others” includes species assigned to the arthropod classes Arachnida, Chilopoda, Collembola and Malacostraca as well as the insect orders Trichoptera, Neuroptera, Ephemeroptera, Orthoptera, Raphidioptera, Psocodea, Dermaptera, Thysanoptera, Mecoptera, Megaloptera, Blattodea and Zygentoma, which are infrequently collected with Malaise trapping devices and constitute low overall species numbers in the traps. The greatest number of species were picked up with the DNA extraction from ATL buffer (64 species), while 47 and 43 species were detected with the tissue- and ethanol-based extraction respectively. Twenty-four species were detected with all three extraction methods and an overlap of a further 17 taxa could be detected between ATL and tissue. In total, 17, 13 and six species were uniquely found with ATL, ethanol and tissue-based extraction (Figure 4f).
3.2 | Incubation time

No significant effect of incubation time on the total number of identified species was observed (Kruskal-Wallis $p = .78$) (Figure 5). However, for three samples there was a slightly positive relationship between species detection and increasing incubation time (WG 1-WG 3). Species number randomly varied across incubation time for the other samples (WG 4-WG 6, BI 1) (Figure 6; see Table S1 for a list of sample names and treatments). Out of the seven samples treated with the home-made (HM) buffer, DNA amplification was possible for three, and these samples showed only small variations in the number of detected species for the different incubation times. Also, arthropod community composition did not show major differences depending on incubation time (Figures 5 and 6).

For four of five most abundant orders, no significant relationship was observed between incubation time and species richness (Figures 6 and 7). The highest species richness of all orders was detected after 12 h of incubation; however, this observation was not significant. The numbers of detected species within Diptera, Hemiptera and Lepidoptera varied only slightly across incubation times (Figure 7a-c,e) whereas coleopterans had a significantly lower number of species detected after 4 h of incubation and the number of detected hymenopteran species increased with incubation time (Figure 7d).

4 | DISCUSSION

By comparing several DNA extraction approaches sequentially on the same bulk samples, we demonstrate that nondestructive extraction from lysis buffer produces comparable results in terms of species number and composition as destructive extraction from sample tissue (Figures 1 and 2). Here it needs to be considered that extraction from ATL lysis buffer included four different time points (2, 4, 8 and 12 h) with four different extractions, compared to only one extraction in the tissue-based approach, resulting in significant differences in sequencing depth. However, incubation time showed no impact on total species number nor on number of shared species with the tissue-based extraction, indicating that for most insect groups one replicate is sufficient to produce comparable results to the destructive approach (Figure 6). Additionally, observed patterns did not change after rarefying data sets to the same read number.

4.1 | Sample integrity after incubation in lysis buffer

Sample integrity and long-term storage is crucial in biodiversity assessment through DNA metabarcoding, to ensure retrospective analysis and therefore enable methodological refinements and the complementation of reference databases (Carew et al., 2018; Martoni et al., 2019; Zizka, Koschorreck et al., 2022). While sample integrity is fully given when using ethanol as the source for DNA extraction, incubation in lysis buffer directly influences the quality of preserved specimens. In a subsequent experiment, specimens were incubated in lysis buffer for 2, 4, 8 and 12 h respectively before the buffer was drained off and specimens were carefully washed with 70% ethanol before returning in 97% ethanol. The samples were subsequently checked by highly skilled taxonomists specialized in the insect orders Hymenoptera, Lepidoptera and Diptera to evaluate the potential for morphological determination after lysis. While after 2 and 4 h of incubation no decrease in...
specimen integrity was observed, internal structures in the abdomen of very small and soft specimens (e.g., Nematocera) started to dissolve after 8 h of incubation. However, the majority of specimens were still identifiable to the species level after 12 h of incubation (Figure S2).

### 4.2 Trade-offs of different methods and potential for large-scale biodiversity assessments

In addition to preserved sample integrity, decreased handling time and low risk of cross-contamination associated with nondestructive approaches provide good potential for large-scale biodiversity assessments or biodiversity monitoring (Nielsen et al., 2019). However, choice of lysis buffer and extraction method should be carefully considered. Here, we observed that the two lysis buffers and extraction methods compared (ATL and column-based extraction vs. HM and salt-based extraction) performed qualitatively very differently. While the DNA extraction from commercial ATL buffer solution and Blood & Tissue DNA Extraction Kit (Qiagen) was successful, processing of samples treated with a home-made lysis buffer and a salt precipitation protocol (HM) was more challenging. Here, several extracts required special treatment, such as dilution and/or an increase in number of PCR cycles to retrieve a sufficiently high amplicon concentration for library preparation and sequencing. However, for several HM samples amplification was not successful and only three out of seven samples could be included with all four incubation times, pointing to an issue with inhibition or DNA purity. Several strategies are known to counteract inhibition (Aylagas et al., 2016; Elbrecht et al., 2017; Majaneva et al., 2018). In previous experiments similar issues have been observed with this protocol and could be resolved by repeating the standard protocol with specific adjustments: (i) dilution series of extraction product to counteract inhibition, (ii) increase in PCR cycles and (iii) further DNA purification of the samples (DNeasy PowerClean Pro Cleanup Kit; Qiagen), but resulting in increasing handling time, cost and potentially reducing total DNA amount of the template.

Overall material costs for the extraction of one sample with the tested lysis buffers were as follows (based on the exchange rate of the euro against the US dollar on December 6, 2022): while a single extraction with ATL buffer (50 ml per sample) costs around $57, $63 had to be budgeted for a single extraction with the home-made buffer. The large volume of Proteinase K added (400 μg Proteinase K per ml buffer) to counteract the activity of DNase during lysis is the costliest reagent in the protocol ($21.47 per ml). While complete omission of Proteinase K can lead to a decrease in DNA yield due to
DNA degradation (Tsuji et al., 2017), a reduced volume has already been successfully tested (Kirse et al., 2021a). Additionally, the extraction from ATL buffer has the added advantage of using a ready-made solution, potentially giving more consistent results.

### 4.3 Discussion of observed differences between methods

Aside from extraction from ATL lysis buffer, the extraction directly from ethanol recovered similar species numbers compared with the tissue-based approach and required a shorter processing time. However, the species composition differed greatly compared to ATL buffer and tissue-based assessments (Figure 3). This is congruent with a previous study investigating Malaise traps (Marquina, Esparza-Salas et al., 2019) and can be explained by the different sclerotization levels of species present in the samples, but also by the different DNA sources upon which the approaches are based. Body structure but also size are the main parameters defining the amount of DNA released into the fixative ethanol, leading to the underrepresentation of highly sclerotized taxa in final sequencing results (Erdozain et al., 2019; Marquina, Esparza-Salas et al., 2019; Zizka et al., 2018). Conversely, DNA extracted from bulk tissue constitutes a highly concentrated source of DNA, termed community DNA (Deiner et al., 2017), which also comes with its own biases. The grinding of specimens of different biomass means that specimens of low biomass, rare taxa and DNA traces (e.g., stomach contents) may be underrepresented in sequencing results, potentially leading to inconsistent detection (Iwaszkiewicz-Eggebrecht et al., 2022). DNA extraction from sample fixative ethanol and lysis buffer targets the DNA released from the specimens into the processing liquid and can therefore be considered as intermediate between community DNA and environmental DNA metabarcoding. Extraction of DNA from preservative ethanol facilitates in addition the detection of DNA traces from, for example, stomach contents or endoparasites due to the frequently observed regurgitation of arthropods when fixed alive in ethanol (Linard et al., 2018; Tiede et al., 2017; Zizka et al., 2018).

As a result, it is likely that metabarcoding from preservative ethanol

---

**FIGURE 7** Total number of identified species (blastID ≥98%) with the ATL lysis buffer at different incubation times. Analysis includes both size classes per sample (S+L).

| Species | ATL 12H | ATL 8H | ATL 4H | ATL 2H | Tissue |
|---------|---------|--------|--------|--------|--------|
| Coleoptera | 47 | 40 | 27 | 49 | 52 |
| Diptera | 235 | 223 | 226 | 225 |
| Hemiptera | 16 | 9 | 3 | 2 |
| Hymenoptera | 26 | 13 | 6 | 4 |
| Lepidoptera | 33 | 5 | 2 | 2 |
| Others | 30 | 6 | 5 | 4 |

---
or lysis buffer not only reflects the insect community caught but also gives a glimpse of associated species, for example stomach contents or parasites. Our approach of size sorting the sample before incubation in lysis buffer also increases the detection probability for small and low-biomass taxa, as has been shown previously for destructive extraction methods (Elbrecht et al., 2021). Remarkably, the highest number of hymenopterans was detected through DNA extraction from the ethanol, although this group is difficult to recover with metabarcoding (Elbrecht et al., 2019). Various representatives of this highly diverse insect order perform important ecological functions in ecosystems and are of particular interest in ecological studies. However, to draw further conclusions about composition and ecological function of communities, a more detailed analysis of species’ traits detected with the different extraction methods needs to be logical function of communities, a more detailed analysis of species’ traits detected with the different extraction methods needs to be conducted (Anderson et al., 2011; Gallai et al., 2009). While all three extraction methods reveal unique taxa and also come with their own biases, a combination of the different approaches can further increase resolution for a single sample.

4.4 | Effect of subsampling, replication and incubation time

The different number of extraction replicates might further enhance the observed differences in the species lists between the ethanol extraction and the ATL and tissue samples respectively. Studies have already shown that biological and technical replication or an increase in processed sample volume can increase species detection rates in metabarcoding studies (Beentjes et al., 2019; Buchner et al., 2021; Ficetola et al., 2015; Zizka, Geiger et al., 2022). Here we show that although combining different time replicates increased species detection rates with the ATL buffer-based extraction, a single extraction yields comparable species numbers to the tissue-based approach. Except for the Hymenoptera, incubation time during lysis had no significant influence on the total number of detected species nor on assessed community composition. While for Lepidoptera, Diptera and Hemiptera results reveal more than 50% of all species detected at all incubation times, this was only observed for ~30% of coleopterans and hymenopterans. Hymenopterans are one of the most difficult groups to target with metabarcoding, which could be explained by less conserved primer binding sites inducing primer mismatches when universal primers are used (Brandon-Mong et al., 2015). Additionally, several representatives of low biomass are often hard to detect, contributing only low amounts to the DNA mixture for sequencing.

Here we found that after 4 h of incubation DNA concentration significantly increases until a peak was reached at 12h of incubation time. It is likely that relative proportions of DNA originating from the larger specimens increases with incubation time, causing a change in lysis DNA soup composition, resulting in shifts in DNA proportions, and probably resulting in overrepresentation of the large specimens (Iwaskiwicz-Eggebrecth et al., 2022). However, our approach of size sorting the samples into two size fractions (>4 and <4 mm) prior to incubation in lysis buffer seems to have remedied some of the biomass biases and contributed to comparable species lists to the ground tissue extracts.

It cannot be excluded that the observed random fluctuations in number of assessed species but also the species composition during incubation time are artefacts of the subsampling strategy used. At each time point, 10 ml of lysis buffer was removed from the sample. Although samples were thoroughly mixed throughout the experiment, it cannot be guaranteed that DNA from each specimen was contained in each subsample. As this effect would be enhanced with lower subsample volumes (Batovska et al., 2021; Giebner et al., 2020; Martins et al., 2019), we would recommend using the total volume of lysate for the DNA extraction.

Overall, further studies are needed to evaluate the effect of using a higher number of subsamples of ground tissue extract and the observed overlap with lysis buffer extracts. Regardless, a higher number of extraction replicates will increase costs. While with the ATL buffer a single extraction (50 ml extraction buffer, one size fraction) costs around $57, extraction from 25 mg tissue (a complete ground single Malaise trap bulk sample often weighs more than 10 g) will cost between $8 and $18 depending on the homogenization approach used.

5 | CONCLUSION

We demonstrate that using nondestructive lysis buffer-based DNA extraction methods on size-sorted arthropod malaise trap samples deliver comparable results in terms of species richness and composition as destructive, tissue-based approaches. In agreement with previous studies, DNA extraction from ethanol results in high numbers of species but yields different community compositions than lysis buffer and tissue-based extractions. Considering sample integrity, time-efficiency and a low contamination risk, nondestructive extraction from lysis buffer is a promising alternative to sample tissue homogenization, especially in large-scale projects where sample handling needs to be kept to a minimum. Using a combination of extraction methods can strongly increase assessed species richness especially in highly diverse samples. This is consistent with previous studies which have already shown that combining several methods for the extraction of DNA from the same sample type can increase species detection rates.

AUTHOR CONTRIBUTIONS

A.K. and V.Z. conceived and planned the experiments. A.K., V.Z., B.Z. and K.L. carried out the experiments. A.K., V.Z. and S.B. contributed to the interpretation of the results. A.K. took the lead in writing the manuscript with contributions from V.Z. and S.B. All authors provided critical feedback and helped shape the research, analysis and manuscript.

ACKNOWLEDGEMENTS

This project was carried out in the context of AMMOD (https://ammod.de) and funded by the German Federal Ministry of Education...
and Research (BMBF). V.M.A.Z. is part of the DNA (Diversity of Insects in Nature protected Areas) funded by the BMBF. We thank the Krefeld Entomological Society for help with the Malaise traps and sampling permits at the Bislicher Insel nature reserve. We thank Marianne Espeland, Björn Rulk, Ralph Peters and Ximo Mengual for examination of the specimens following lysis.

CONFLICT OF INTEREST
All authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT
Raw sequence data for this project have been submitted to NCBI's SRA archive under accession no. PRJNA817517.

OPEN RESEARCH BADGES

This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at [provided NCBI's SRA archive under accession no. PRJNA817517].

BENEFIT-SHARING STATEMENT
Benefits from this research accrue from the sharing of our data and results on public databases as described above.

ORCID

Ameli Kirse https://orcid.org/0000-0002-7118-9328
Sarah J. Bourlat https://orcid.org/0000-0003-0218-0298

REFERENCES

Alberdi, A., Alipazienza, O., Gilbert, M. T. P., & Bohmann, K. (2018). Scrutinizing key steps for reliable metabarcoding of environmental samples. Methods in Ecology and Evolution, 9(1), 134–147.

Aljanabi, S. M., & Martinez, I. (1997). Universal and rapid salt-extraction acids for PCR-based techniques. Nucleic Acids Research, 25(22), 4692–4693.

Anderson, A., McCormack, S., Helden, A., Sheridan, H., Kinsella, A., & Purvis, G. (2011). The potential of parasitoid Hymenoptera as bioindicators of arthropod diversity in agricultural grasslands. Journal of Applied Ecology, 48(2), 382–390. https://doi.org/10.1111/j.1365-2664.2010.01937.x

Aylagas, E., Borja, A., Irigoien, X., & Rodríguez-Espeleta, N. (2016). Benchmarking DNA metabarcoding for biodiversity-based monitoring and assessment. Frontiers in Marine Science, 3, 96.

Baselga, A., & Orme, C. D. L. (2012). Betapart: An R package for the study of beta diversity. Methods in Ecology and Evolution, 3(5), 808–812.

Batovska, J., Piper, A. M., Valenzuela, I., Cunningham, J. P., & Blacket, M. J. (2021). Developing a non-destructive metabarcoding protocol for detection of pest insects in bulk trap catches. Scientific Reports, 11(1), 1–14.

Beentjes, K. K., Speksnijder, A. G., Schilthuizen, M., Hoogeveen, M., & van der Hoorn, B. B. (2019). The effects of spatial and temporal replicative sampling on eDNA metabarcoding. PeerJ, 7, e7335.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislaw, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., ... Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology, 37(8), 852–857.

Bonato, L., Peretti, E., Sandionig, A., & Bortolin, F. (2021). The diet of major predators of forest soils: A first analysis on syntopic species of Chilopoda through DNA metabarcoding. Soil Biology and Biochemistry, 158, 108264.

Bourlat, S. J., Haenel, Q., Finnman, J., & Leray, M. (2016). Preparation of amplicon libraries for metabarcoding of marine eukaryotes using Illumina MiSeq: The dual-PCR method. In Marine genomics (pp. 197–207). Springer.

Brandon-Mong, G. J., Gan, H.-M., Sing, K.-W., Lee, P.-S., Lim, P.-E., & Wilson, J.-J. (2015). DNA metabarcoding of insects and allies: An evaluation of primers and pipelines. Bulletin of Entomological Research, 105(6), 717–727.

Brandt, M. I., Troupe, B., Quintric, L., Günther, B., Wincker, P., Poulain, J., & Arnaud-Haond, S. (2021). Bioinformatic pipelines combining denoising and clustering tools allow for more comprehensive prokaryotic and eukaryotic metabarcoding. Molecular Ecology Resources, 21, 1904–1921.

Bruce, K., Blackman, R., Bourlat, S. J., Hellström, A. M., Bakker, J., Bista, I., Bohmann, K., Bouchez, A., Brys, R., Clark, K., Elbrecht, V., Fazi, S., Fonseca, V., Häning, B., Leese, F., Mächler, E., Mahon, A. R., Meisssner, K., Panksep, K., ... Deiner, K. (2021). A practical guide to DNA-based methods for biodiversity assessment. Advanced Books. https://doi.org/10.3897/ab.e68634

Buchner, D., Haase, P., & Leese, F. (2021). Wet grinding of invertebrate bulk samples – A scalable and cost-efficient protocol for metabarcoding and metagenomics. Metabarcoding and Metagenomics, 5, e67533.

Buchner, D., & Leese, F. (2020). BOLDGigger – A python package to identify and organise sequences with the barcode of life data systems. Metabarcoding and Metagenomics, 4, e53535.

Cabodavilla, X., Mougeot, F., Bota, G., Mañosa, S., Cuscó, F., Martínez-Garcia, J., Arroyo, B., & Madeira, M. J. (2021). Metabarcoding insights into the diet and trophic diversity of six declining farmland birds. Scientific Reports, 11(1), 1–13.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature methods, 13(7), 581–583. https://doi.org/10.1038/nmeth.3869

Carew, M. E., Coleman, R. A., & Hoffmann, A. A. (2018). Can non-destructive DNA extraction of bulk invertebrate samples be used for metabarcoding? PeerJ, 6, e4980. https://doi.org/10.7717/peerj.4980

Compson, Z. G., McLenaghan, B., Singer, G. A., Fahner, N. A., & Hajibabaei, M. (2020). Metabarcoding from microbes to mammals: Comprehensive bioassessment on a global scale. Frontiers in Ecology and Evolution, 8, 379.

Conway, J. R., Lex, A., & Gehlenborg, N. (2017). UpSetR: An R package for the visualization of intersecting sets and their properties. Bioinformatics, 33(18), 2938–2940.

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D. M., de Vere, N., Pfender, M. E., & Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. Molecular Ecology, 26(21), 5872–5895. https://doi.org/10.1111/mec.14350

Deiner, K., Walser, J.-C., Mächler, E., & Altermatt, F. (2015). Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. Biological Conservation, 183, 53–63.

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. Journal of Vegetation Science, 14(6), 927–930.
Ingala, M. R., Simmons, N. B., Wultsch, C., Krampis, K., Provost, K. L., & Hsieh, T., Ma, K., & Chao, A. (2016). INEXT: An R package for rarefaction.

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, Giebner, H., Langen, K., Bourlat, S. J., Kukowka, S., Mayer, C., Astrin, J. J., Geiger, M. F., Moriniere, J., Hausmann, A., Haszprunar, G., Wägele, W., Hajibabaei, M., Porter, T. M., Wright, M., & Rudar, J. (2019). COI metabarcoding primer choice affects richness and recovery of indicator taxa in freshwater systems.

Elbrecht, V., Bourlat, S. J., Hörren, T., Lindner, A., Mordente, A., Noll, N. W., Schäffler, L., Sorg, M., & Ziska, V. M. A. (2021). Pooling size sorted malaise trap fractions to maximize taxon recovery with metabarcoding.

Elbrecht, V., Braunsk, T. W. A., Ivanova, N. V., Prosser, S. W. J., Hajibabaei, M., Wright, M., Zahkarov, E. V., Hebert, P. D. N., & Steinke, D. (2019). Validation of COI metabarcoding primers for terrestrial arthropods.

Elbrecht, V., & Leese, F. (2017). Validation and development of COI metabarcoding primers for freshwater macroinvertebrate bioassessment.

Elbrecht, V., Vamos, E. E., Meissner, K., Arovíita, J., & Leese, F. (2017). Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring.

Erdozain, M., Thompson, D. G., Porter, T. M., Kidd, K. A., Kreutzweiser, D. P., Sibley, P. K., Swystun, T., Chartrand, D., & Hajibabaei, M. (2019). Metabarcoding of storage ethanol vs. conventional morphometric identification in relation to the use of stream macroinvertebrates as ecological indicators in forest management.

Ficetola, G. F., Pansu, J., Bonin, A., Coissac, E., Giguët-Covex, C., De Barba, M., Gielly, L., Lopes, C. M., Boyer, F., Pompanon, F., Rayé, G., & Taberlet, P. (2015). Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data.

Fraser, T. G., Kjeller, R., Bruun, H. H., EJneas, R., Brunbjerg, A. K., Pietroni, C., & Hansen, A. J. (2017). Algorithm for post-clustering curatin of DNA amplicon data yields reliable biodiversity estimates.

Gallai, N., Salles, J.-M., Settele, J., & Vaisseir, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline.

Geiger, M. F., Moriniere, J., Hausmann, A., Haszprunar, G., Wägele, W., Hebert, P. D., & Rulik, B. (2016). Testing the global malaise trap program—how well does the current barcode reference library identify flying insects in Germany?

Giebner, H., Langen, K., Bourlat, S. J., Kukowka, S., Mayer, A., Astrin, J. J., Misof, B., & Fonseca, V. G. (2020). Comparing diversity levels in environmental samples: DNA sequence capture and metabarcoding approaches using 185 and COI genes.

Hajibabaei, M., Porter, T. M., Wright, M., & Rudar, J. (2019). COI metabarcoding primer choice affects richness and recovery of indicator taxa in freshwater systems.

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stemmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D., & de Kroon, H. (2017). More than 75 percent decline over 27 years in total taxa in freshwater systems.

Hajibabaei, M., Porter, T. M., Wright, M., & Rudar, J. (2019). COI metabarcoding primer choice affects richness and recovery of indicator taxa in freshwater systems.

Hajibabaei, M., Porter, T. M., Wright, M., & Rudar, J. (2019). COI metabarcoding primer choice affects richness and recovery of indicator taxa in freshwater systems.

Hajibabaei, M., Porter, T. M., Wright, M., & Rudar, J. (2019). COI metabarcoding primer choice affects richness and recovery of indicator taxa in freshwater systems.

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stemmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D., & de Kroon, H. (2017). More than 75 percent decline over 27 years in total taxa in freshwater systems.

Hajibabaei, M., Porter, T. M., Wright, M., & Rudar, J. (2019). COI metabarcoding primer choice affects richness and recovery of indicator taxa in freshwater systems.

Hajibabaei, M., Porter, T. M., Wright, M., & Rudar, J. (2019). COI metabarcoding primer choice affects richness and recovery of indicator taxa in freshwater systems.

Kassambabara, A. (2021). Rstatix: Pipe-friendly framework for basic statistical tests. R package version 0.7.0. (Version 0.7.0). https://CRAN.R-project.org/package=rstatix

Kirse, A., Bourlat, S. J., Langen, K., & Fonseca, V. G. (2021a). Metabarcoding malaise traps and soil eDNA reveals seasonal and local arthropod diversity shifts.

Kirse, A., Bourlat, S. J., Langen, K., & Fonseca, V. G. (2021b). Unearthing the potential of soil eDNA metabarcoding—Towards best practice advice for invertebrate biodiversity assessment.

Kocher, A., Gantier, J. C., Gaborit, P., Zinger, L., Holota, H., Valiere, S., Dusfour, I., Girod, R., Bañuls, A. L., & Murienne, J. (2017). Vector soup: High-throughput identification of neotropical phlebotomine sand flies using metabarcoding.

Kopylova, E., Navas-Molina, J. A., Mercier, C., Xu, Z. Z., Mahé, F., He, Y., Zhou, H. W., Rognes, T., Caporaso, J. G., & Knight, R. (2016). Open-source sequence clustering methods improve the state of the art.

Linter, B. C., & Garcia, A. (2018). Climate-driven declines in arthropod abundance restructure a rainforest food web.

Lozano-Mojica, J. D., & Caballero, S. (2021). Applications of eDNA metabarcoding for vertebrate diversity studies in northern Colombian water bodies.

Macher, J., Zizka, V. M. A., Weigand, A. M., & Leese, F. (2018). A simple centrifugation protocol for metagenomic studies increases mitochondrial metagenomics to large-scale data mining and phylogenetic analysis of coleoptera.

Ovaskainen, O. (2020). SPIKEPIPE: A metagenomic pipeline for the accurate quantification of eukaryotic species occurrences and intraspecific abundance change using DNA barcodes or mitogenomes.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads.

Iwaszkiewicz-Eggebrecht, E., Granqvist, E., buczech, M., Prus, M., Roslin, T., Tack, A. J., Andersson, A. F., Miraldo, A., Ronquist, F., & Lukasik, P. (2022). Optimizing insect metabarcoding using replicated mock communities.

Ji, Y., Huotari, T., Roslin, T., Schmidt, N. M., Wang, J., Yu, D. W., & Ovaskainen, O. (2020). SPIKEPIPE: A metagenomic pipeline for the accurate quantification of eukaryotic species occurrences and intraspecific abundance change using DNA barcodes or mitogenomes.

Kassambabara, A. (2021). Rstatix: Pipe-friendly framework for basic statistical tests. R package version 0.7.0. (Version 0.7.0). https://CRAN.R-project.org/package=rstatix

Kirse, A., Bourlat, S. J., Langen, K., & Fonseca, V. G. (2021a). Metabarcoding malaise traps and soil eDNA reveals seasonal and local arthropod diversity shifts.

Kirse, A., Bourlat, S. J., Langen, K., & Fonseca, V. G. (2021b). Unearthing the potential of soil eDNA metabarcoding—Towards best practice advice for invertebrate biodiversity assessment.

Kocher, A., Gantier, J. C., Gaborit, P., Zinger, L., Holota, H., Valiere, S., Dusfour, I., Girod, R., Bañuls, A. L., & Murienne, J. (2017). Vector soup: High-throughput identification of neotropical phlebotomine sand flies using metabarcoding.

Kopylova, E., Navas-Molina, J. A., Mercier, C., Xu, Z. Z., Mahé, F., He, Y., Zhou, H. W., Rognes, T., Caporaso, J. G., & Knight, R. (2016). Open-source sequence clustering methods improve the state of the art.

Linter, B. C., & Garcia, A. (2018). Climate-driven declines in arthropod abundance restructure a rainforest food web.

Lozano-Mojica, J. D., & Caballero, S. (2021). Applications of eDNA metabarcoding for vertebrate diversity studies in northern Colombian water bodies.

Macher, J., Zizka, V. M. A., Weigand, A. M., & Leese, F. (2018). A simple centrifugation protocol for metagenomic studies increases mitochondrial DNA yield by two orders of magnitude.

Majaneva, M., Diserud, O. H., Eagle, S. H., Hajibabaei, M., & Ekrem, T. (2018). Choice of DNA extraction method affects DNA metabarcoding of unsorted invertebrate bulk samples.

Marquina, D., Andersson, A. F., & Ronquist, F. (2019). New mitochondrial primers for metabarcoding of insects, designed and evaluated using in silico methods.

Marquina, D., Espanar-Salas, R., Roslin, T., & Ronquist, F. (2019). Establishing arthropod community composition using metabarcoding: Surprising inconsistencies between soil samples and preservative ethanol and homogenate from malaise trap catches.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads.
Martins, F. M. S., Galhardo, M., Filipe, A. F., Teixeira, A., Pinheiro, P., Paupério, J., Alves, P. C., & Beja, P. (2019). Have the cake and eat it: Optimizing nondestructive DNA metabarcoding of macroinvertebrate samples for freshwater biomonitoring. Molecular Ecology Resources, 19(4), 863–876. https://doi.org/10.1111/1755-0998.13012

Martoni, F., Valenzuela, I., & Blacket, M. J. (2019). Non-destructive DNA extractions from fly larvae (Diptera: Muscidae) enable molecular identification of species and enhance morphological features. Austral Entomology, 58(4), 848–856. https://doi.org/10.1111/aen.12419

Mata, V. A., Ferreira, S., Campos, R. M., da Silva, L. P., Verissimo, J., Corley, M. F. V., & Beja, P. (2020). Efficient assessment of nocturnal flying insect communities by combining automatic light traps and DNA metabarcoding. Environmental DNA, 3(2), 398–408. https://doi.org/10.1002/edn3.125

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One, 8(4), e61217.

Nielsen, M., Gilbert, M. T. P., Pape, T., & Bohmann, K. (2019). A simplified DNA extraction protocol for unsorted bulk arthropod samples that maintains exoskeletal integrity. Environmental DNA, 1(2), 144–154. https://doi.org/10.1002/edn3.16

Sánchez-Bayo, F., & Wyckhuys, K. A. (2021). Further evidence for a global decline of the entomofauna. Austral Entomology, 60(1), 9–26.

Svenningsen, C. S., Frøslev, T. G., Bladt, J., Pedersen, L. B., Larsen, J. C., Ejrnæs, R., Flejgaard, C., Hansen, A. J., Heilmann-Clausen, J., Dunn, R. R., & Tøttrup, A. P. (2021). Detecting flying insects using car nets and DNA metabarcoding. Biology Letters, 17(3), 20200833.

Tiede, J., Scherer, C., Mutschler, J., McMahon, K. D., & Gratton, C. (2017). Gut microbiomes of mobile predators vary with landscape context and species identity. Ecology and Evolution, 7(20), 8545–8557. https://doi.org/10.1002/ece3.3390

Tsujii, S., Yamanaka, H., & Minamoto, T. (2017). Effects of water pH and proteinase K treatment on the yield of environmental DNA from water samples. Limnology, 18(1), 1–7. https://doi.org/10.1007/s10201-016-0483-x

Vamos, E. E., Elbrecht, V., & Leese, F. (2017). Short COI markers for freshwater macroinvertebrate metabarcoding (No. 2167–9843). PeerJ Preprints.

Vesterinen, E. J., Ruokolainen, L., Wahlberg, N., Peña, C., Roslin, T., Laine, V. N., Vasko, V., Sääksjärvi, I. E., Norrdahl, K., & Lilley, T. M. (2016). What you need is what you eat? Prey selection by the bat Myotis daubentonii. Molecular Ecology, 25(7), 1581–1594.

Wägele, J. W., Bodesheim, P., Bourlat, S. J., Denzler, J., Diepenbroek, M., Fonseca, V. G., Frommolt, K., Geiger, M. F., Gemeinholzer, B., Glöckner, F. O., Haucke, T., Kirse, A., Kölpin, A., Kostadinov, I., Kühl, H. S., Kurth, F., Lasseck, M., Liedke, S., Losch, F., ... Wildermann, S. (2022). Towards a multisensor station for automated biodiversity monitoring. Basic and Applied Ecology, 59, 105–138.

Wheeler, Q. D., Raven, P. H., & Wilson, E. O. (2004). Taxonomy: Impediment or expedient? Science, 303(5656), 285. https://doi.org/10.1126/science.303.5656.285

Wickham, H. (2016). ggplot2: Elegant graphics for data analysis. Springer.

Wickham, H., Francois, R., Henry, L., & Müller, K. (2015). dplyr: A grammar of data manipulation. R Package Version 0.8.3, 3.

Yu, D. W., Ji, Y., Emerson, B. C., Wang, X., Ye, C., Yang, C., & Ding, Z. (2012). Biodiversity soup: Metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. Methods in Ecology and Evolution, 3(4), 613–623. https://doi.org/10.1111/j.2041-210X.2012.00198.x

Zenker, M. M., Specht, A., & Fonseca, V. G. (2020). Assessing insect biodiversity with automatic light traps in Brazil: Pearls and pitfalls of metabarcoding samples in preservative ethanol. Ecology and Evolution, 10(5), 2352–2366.

Zizka, V., Koschorreck, J., Khan, C. C., & Astrin, J. J. (2022). Long-term archival of environmental samples empowers biodiversity monitoring and ecological research. Environmental Sciences Europe, 34(1), 1–8.

Zizka, V. M., Geiger, M. F., Hörrsen, T., Kirse, A., Noll, N. W., Schäffler, L., Scherges, A. M., & Sorg, M. (2022). Recommendations for tissue homogenisation and extraction in DNA metabarcoding of malaise trap samples. BioRxiv. https://doi.org/10.1101/2022.01.25.477667

Zizka, V. M., Leese, F., Peinert, B., & Geiger, M. F. (2018). DNA metabarcoding from sample fixative as a quick and voucher-preserving biodiversity assessment method. Genome, 62(3), 122–136.

SUPPORTING INFORMATION

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

How to cite this article: Kirse, A., Bourlat, S. J., Langen, K., Zapke, B., & Zizka, V. M. A. (2023). Comparison of destructive and nondestructive DNA extraction methods for the metabarcoding of arthropod bulk samples. Molecular Ecology Resources, 23, 92–105. https://doi.org/10.1111/1755-0998.13694