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ORIGINAL ARTICLE

Evaluating the Effects of Preservation Method and Storage Duration on Lysis Buffer–Based Non-Destructive DNA Metabarcoding in Insect Mock Communities

Sophie de Becquevort | Zoe Withey | Nadia Barsoum

Forest Research, Alice Holt Lodge, Farnham, UK

Correspondence: Sophie de Becquevort (sophie.debecquevort@gmail.com)

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ABSTRACT

Suspended traps such as Lindgren funnel traps and vane traps are increasingly used in combination with DNA metabarcoding to characterise canopy arthropod communities. Despite their wide use, it remains unclear how different preservatives and storage conditions affect DNA integrity in samples destined for molecular analysis. Ethanol, while commonly used as a preservative, poses several challenges for field deployment: it is volatile, expensive and unsuitable for passive trapping because it is known to attract some insects. Monopropylene glycol (MPG) has emerged as a practical alternative: it is non-toxic, affordable, easy to handle and transport and preserves specimen morphology for identification. However, its effect on DNA preservation, especially when diluted by rainwater in the field or during storage, remains poorly understood. This study uses insect mock communities of low (6 species) and high (12 species) diversity to test the effects of preservation method, storage duration and ethanol transfer on species detection via lysis buffer-based non-destructive DNA metabarcoding. Mock communities were processed with metabarcoding over time after being stored in undiluted MPG stored at 5°C, diluted MPG stored at 5°C and absolute ethanol stored at −18°C as the optimal reference treatment. Community composition recovered was significantly influenced by preservation method and the diversity of the mock community. On the other hand, the effects of storage time and transfer were significant only in low diversity mock communities, and detection rates varied across species. Overall, results confirmed that MPG is a suitable and effective fixative for short- to medium-term storage at 5°C, even when diluted, maintaining DNA integrity for metabarcoding applications. These findings are consistent with prior studies and provide further support for MPG as a viable alternative to ethanol in arthropod biomonitoring and research.

1 | Introduction

Originally developed for the research, monitoring and mass trapping of scolytid beetles, Lindgren funnel traps have since been recognised as effective tools for capturing a broad range of tree canopy arthropods, including bark and ambrosia beetles (Scolytinae), longhorn beetles (Cerambycidae) and various other saproxylic and phytophagous taxa (e.g., Lindgren 1983; Petersen et al. 2004; Barringer 2015; Skvarla et al. 2016; Young 2021; Madden et al. 2022). Together with other trap types such as vane traps (e.g., Knuff et al. 2019), they are widely used for monitoring tree canopy arthropod biodiversity and for targeted forest pest assessment projects (Brockerhoff et al. 2023). For example, Forest Research (FR) uses Lindgren funnel traps to monitor canopy arthropod communities as part of the national-scale terrestrial Natural Capital and Ecosystem Assessment (tNCEA) programme, and in several recent research projects (e.g., the 'Ecological Function of Trees Outside Woodland' and 'Ancient woodlands and development-related threats' projects; Forest Research 2026a; Forest Research 2026b).

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Lindgren funnel traps consist of a series of cone-shaped plastic funnels stacked vertically, typically in arrays ranging from 5 to 12. They are generally suspended from a sturdy branch or strung between trees using rope and positioned to intercept insects in the lower or upper canopy where many target species are most active (e.g., Miller and Crowe 2011; Rassati et al. 2019). The funnels guide intercepted insects downward into a collection cup at the base of the trap. Depending on research objectives, this cup may be deployed in a 'dry' configuration (without preservative) or 'wet' configuration, using preservatives such as monopropylene glycol (MPG), ethanol, or saturated salt solution to kill and preserve specimens (Miller and Duerr 2008; British Columbia Ministry of Forests 2016). Consequently, Lindgren multiple-funnel traps can support both traditional entomological surveys and DNA-based arthropod community assessments, including non-destructive DNA metabarcoding workflows (Young et al. 2021; Brockerhoff et al. 2023; Dodds et al. 2024).

When Lindgren funnel traps are used in combination with non-destructive DNA metabarcoding for arthropod species identification, selecting an appropriate solution for trapping, killing and preserving samples is critical. Effective preservation of DNA relies on rapid inhibition of nuclease activity and the prevention of chemical degradation (Quicke et al. 1999). Despite being the standard solution for trapping terrestrial invertebrates, >95% molecular-grade ethanol has significant limitations (Liu et al. 2020; Marquina et al. 2021). It is costly, highly volatile and challenging to obtain and transport (Robinson et al. 2021; Weigand et al. 2021). In open traps like funnel traps, it is known to attract some insect species and is therefore not suitable for passive monitoring (Fiala et al. 2023). Finally, absolute ethanol makes specimens brittle (Marquina et al. 2021), which hinders post-processing tasks such as identifying rare or invasive species and assessing species abundance. Monopropylene glycol is an ethanol substitute that is used in many invertebrate research studies. It is non-flammable, inexpensive, non-toxic, and can be widely purchased and easily transported (Liu et al. 2020; Weigand et al. 2021). Importantly, MPG preserves both DNA integrity and specimen morphology, enabling its use in non-destructive molecular identification and subsequent morphological analysis (Patrick et al. 2016; Weigand et al. 2021).

Despite its promising properties, the effectiveness of MPG as a DNA preservative remains insufficiently understood, particularly when diluted by rainwater, as is common under field conditions, and when used in combination with non-destructive DNA metabarcoding for arthropod identification (Nakamura et al. 2020; Weigand et al. 2021). Further research is needed to clarify these uncertainties and optimise the use of MPG for biodiversity monitoring applications. This study investigates how preservation methods and storage durations affect the effectiveness of lysis buffer-based non-destructive DNA metabarcoding, using mock communities. Specifically, this study compares the performance of three preservation methods: undiluted MPG stored at 5°C, diluted MPG stored at 5°C and absolute ethanol stored at −18°C as the optimal reference treatment. It also evaluates the effects of short- and medium-term storage durations, and tests whether transferring samples from MPG to absolute ethanol after collection enhances species recovery. By assessing how different preservation strategies and handling protocols influence the success of lysis buffer-based non-destructive DNA metabarcoding, this research provides critical insights to inform best practices for researchers and monitoring agencies using Lindgren funnel traps in large-scale canopy arthropod biodiversity surveys.

2 | Methods

2.1 | Insect Mock Communities

Low and high diversity insect mock communities were assembled using similar proportions of insect orders as have been typically recovered in Lindgren canopy traps in English forests. High diversity mock communities were made up of 12 species and 38 individuals. Low diversity mock communities were made up of 6 species and 16 individuals (Table 1). Mock communities were constructed using commercially available insects. All specimens were received alive and maintained under appropriate holding conditions until the start of the experiment, which occurred within 3 days of receipt for all species. Specimens were briefly immobilised by freezing prior to transfer into preservative. For each species, only a single life stage was included and individuals showing injuries or abnormal body sizes (unusually large or small) were excluded. Information on body size, level of sclerotisation and supplier for each species included in the mock communities is provided in Table S1.

2.2 | Preservation Method, Storage Duration and Transfer From MPG to Ethanol

Three different preservation methods were tested to evaluate their comparative influence on species detection using lysis buffer-based non-destructive DNA metabarcoding. These included: (1) absolute ethanol (99.9%) stored at −18°C, (2) 100% MPG stored at 5°C and (3) 'rainwater'-diluted MPG (DMPG: 40% MPG and 60% distilled water) stored at 5°C. Storage temperatures were selected to represent conditions commonly used in large-scale field studies, where canopy trap samples preserved in MPG may become diluted by rainwater and are unsuitable for freezing. These were compared to ideal storage conditions, represented by ethanol stored at −18°C. Preservation treatments (preservative × storage temperature) are hereafter referred to as DMPG, MPG and ethanol.

The samples were stored in 150 mL of preservative for 2 weeks, 2 months, or 4 months until processing, which involved an initial transfer to 100% MPG as per standard operating procedure at the sequencing facility (NatureMetrics) (Figure 1A). A total of 54 samples were prepared, comprising three biological replicates for each combination of preservation methods, storage duration and community size. Additionally, to test the effect of transferring samples from a first storage solution (e.g., representing the trapping solution) into a second storage solution (i.e., ethanol), 24 samples (12 low diversity and 12 high diversity) were stored in 150 mL of MPG or DMPG for 2 weeks at 5°C and then transferred into 150 mL of absolute ethanol (99.9%). These mock community insect samples were then left in cool storage at −18°C for either 2 or 4 months until processing (Figure 1B).

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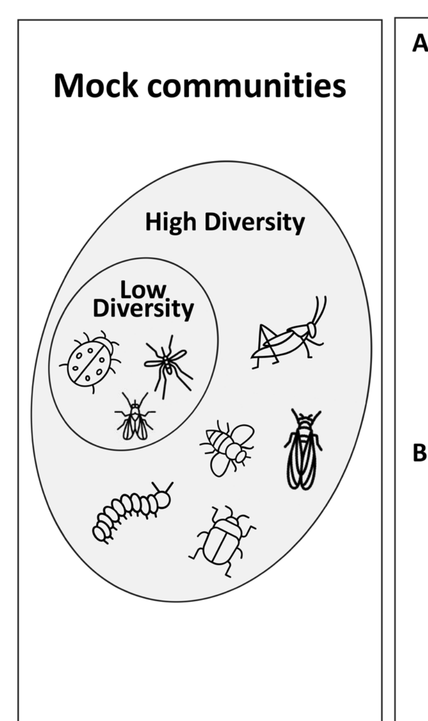
TABLE 1 | Taxonomic composition and abundance of species included in the insect mock communities. For each species, the order, life stage and number of individuals included in the low and high diversity community samples are shown.

Order	Species	Life stage	Number of individuals—low diversity community	Number of individuals—high diversity community
Coleoptera	*Adalia bipunctata*	Adult	0	2
	Cryptolaemus montrouzieri	Adult	2	2
Diptera	*Drosophila hydei*	Adult	4	4
	Drosophila melanogaster	Adult	4	4
	Eupeodes corollae	Adult	2	4
	Hermetia illucens	Adult	0	4
Hemiptera	*Macrolophus pygmaeus*	Adult	2	4
	Orius laevigatus	Adult	0	4
Hymenoptera	*Aphidius colemani*	Pupae (aphid mummies)	0	4
	Dacnusa sibirica	Adult	2	4
Neuroptera	*Chrysoperla carnea*	Larvae	0	1
Orthoptera	*Gryllodes sigillatus*	Adult	0	1



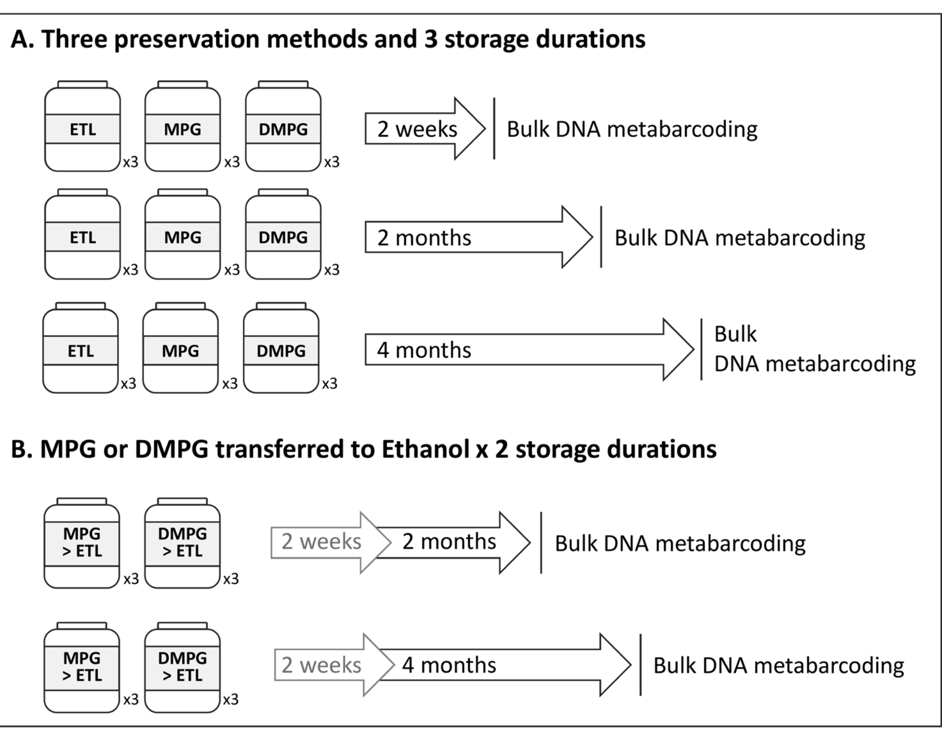


FIGURE 1 | Overview of the experimental design. (A) Samples were stored in monopropylene glycol (MPG), diluted MPG (DMPG), or ethanol, and processed after 2 weeks, 2 months, or 4 months. (B) Samples stored in MPG or DMPG were transferred to ethanol (ETL) after 2 weeks and then processed after 2 or 4 months.

2.3 | DNA Extraction, Library Preparation and Sequencing

Samples were taken out of MPG, rinsed with ethanol and dried at 30°C overnight. They were then incubated in up to 300 mL of lysis buffer (volume dependent on invertebrate volume; Ivanova et al. 2006) at 56°C for up to 4 h. DNA was extracted from a 200 μL aliquot of the lysate using the DNeasy Blood and Tissue Kit (Qiagen), following the manufacturer's protocol. Each batch was extracted alongside a negative control of lysis buffer to detect contamination.

A ~313 bp fragment of the cytochrome *c* oxidase subunit I (COI) gene was amplified using the forward primer

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mlCOIintF-XT (5′-GGWACWRGWTGRACWITITAYCC YCC-3′; Wangensteen et al. 2018) and the reverse primer jgHCO2198 (5′-TAIACYTCIGGRTGICCRAARAAYCA-3′; Geller et al. 2013). Each sample was amplified by replicate PCRs in a two-step process with adapters added (Forward overhang: 5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG; Reverse overhang: 5′ GTCTCGTGGGCTCGGAGATGTGTA TAAGAGACAG).

PCR amplifications were performed in 25 μL reactions containing 12.5 μL of 2× DreamTaq HS Master Mix, 0.1 μL each of forward and reverse primers (100 μM), 1 μL of bovine serum albumin (20 mg/mL), 10.3 μL of nuclease-free water and 1 μL of non-normalised template DNA. The PCR cycling conditions consisted of an initial denaturation at 95°C for 10 min, followed by 35 cycles of 95°C for 60 s, 45°C for 60 s and 72°C for 90 s, with a final elongation step at 72°C for 5 min. Every plate was amplified alongside a negative control of PCR-grade water and a positive control of artificial sequences to verify amplification performance. PCR amplicons were resolved by gel electrophoresis and purified using MagBind TotalPure NGS magnetic beads (Omega Biotek, USA), normalised and indexed. No negative extraction controls were sequenced, as none showed amplification. A pooled library was created prior to sequencing according to Illumina's 16S Metagenomic Sequencing Library Preparation protocol. The final library was sequenced on an Illumina MiSeq system using a V3 600 cycle reagent kit (Illumina, USA).

2.4 | Bioinformatics

Sequences were demultiplexed using bcl2fastq software (Illumina). Paired-end FASTQ sequences with at least 80% overlap were merged using USEARCH (Edgar 2010). Sequences with error rates greater than 0.01 were dereplicated, and primers and sequences between 300 and 330 bps were identified and retained using cutadapt (Martin 2011). UNOISE (Edgar 2016) was used to denoise unique sequences, requiring retained zOTUs (zero-radius Operational Taxonomic Units) to have a minimum abundance of 8 in at least one sample. zOTUs were checked against NCBI nucleotide (NCBI nt) and BOLD databases (Ratnasingham and Hebert 2007) using blastn (Altschul et al. 1990; Camacho et al. 2009), with hits requiring a minimum of 90% of the query sequence cover, an e-score of 1e-20, and with similarity requiring 98%, 95% and 92% for species, genus and family level respectively. The taxonomic identification associated with all hits was converted to match the GBIF taxonomic backbone. ZOTUs were clustered at 97% similarity with USEARCH UPARSE (Edgar 2013) to obtain OTUs and remove chimeric sequences.

2.5 | Statistical Analyses

Species detection patterns across community type, preservative method, storage time and ethanol transfer were visualised using ggplot2 (Wickham 2016). False negatives were calculated as the proportion of species present in the samples but failed to be detected by the DNA metabarcoding workflow. To assess how these factors influenced variation in recovered species composition, Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted using the adonis2 function from the vegan package (version 2.6–4; Oksanen et al. 2022). Euclidean distance was used as the similarity metric. Although Jaccard similarity is commonly applied to presence–absence data, it ignores joint absences, which are informative in this study (e.g., when the same species is undetected across multiple treatments). In contrast, Euclidean distance accounts for joint absences, making it more appropriate for the analyses conducted here. Pairwise differences between groups were assessed using the pairwise.adonis2 function from the pairwiseAdonis package (version 0.4; Arbizu 2020).

The data were analysed using two complementary approaches to account for the experimental design. First, results from both community types were analysed together, including samples that were transferred to ethanol. For this combined analysis, only the six species from the low diversity community (shared across both mock communities) were considered. The model included storage time, community type and the interaction between community type and the nested factors preservation method and transfer (with transfer nested within each preservation method). Second, the two community types were analysed separately, both with and without the inclusion of ethanol-transferred samples. These analyses considered all 12 species in the high diversity community and six species in the low diversity community. In both separate analyses, storage time, preservation method and transfer (nested within preservation method) were included as predictor variables. When transferred samples were excluded, the models assessed both main effects and interactions between storage time and preservation method.

3 | Results

DNA amplified in all but one sample (i.e., a low diversity community stored for 2 weeks in DMPG). For the remaining 77 samples, the total read count prior to removing non-target taxa was 5,769,278, with an average read count of 74,926 per sample. After removing non-target taxa, the total read count was 5,684,499 with an average read count of 73,825 per sample (minimum: 15,049; maximum: 109,310). Table S2 summarises sequencing read counts (maximum, minimum, average and total) for each dataset, as well as the minimum and maximum number of species recovered, grouped by community type and transfer into preservative.

3.1 | Presence-Absence of Species in Both High and Low Diversity Communities

When results from both community types were analysed together and only the six species shared across both mock communities were considered, PERMANOVA results indicated that both community type and preservation method had a significant effect on the composition of the community retrieved ($R^2=0.254$, $p=0.001$ and $R^2=0.070$, $p=0.002$, respectively; Table S3). Dispersion differed significantly between community types (PERMDISP, $p=0.001$). Two-way and three-way interactions involving community type, preservation method and transfer were statistically significant (PERMANOVA: $R^2=0.038$, $p=0.038$; $R^2=0.038$, $p=0.030$, respectively), but

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their explanatory power was small relative to the main effects. Dispersion differed significantly among the corresponding interaction groupings (PERMDISP, $p=0.002$). Given their low R^2 values and the observed differences in dispersion, these interactions contributed only marginally to overall compositional variation. On the other hand, sample storage time and the interaction between preservation method and transfer were not significant (PERMANOVA: $p=0.150$ and $p=0.082$).

3.2 | Presence-Absence of Species in High Diversity Communities

All 12 species included in the high diversity community samples were detected in at least one sample (both transferred and non-transferred, Figure 2). However, the detection rate varied by species, with some being detected in most of samples (*Gryllodes sigillatus, Hermetia illucens*) and others being detected only in a few samples (*Dacnusa sibirica*, *Aphidius colemani* and *Chrysoperla carnea*). PERMANOVA results indicated that preservation method had a significant effect on the high diversity community ($R^2=0.131$, $p=0.002$; Table S4) unlike storage time or the interaction between preservation method and transfer to ethanol ($p=0.564$ and $p=0.322$). For transferred samples, the average percentage of false negatives was lower for MPG (33%) than DMPG (44%). When the transferred samples were excluded from the analysis, preservation method also had a significant effect on the detection of species in the high diversity community ($R^2=0.183$, $p=0.003$; Table S5) unlike storage time or the interaction between storage time and preservation method ($p=0.647$ and $p=0.361$). For non-transferred samples, the average percentage of false negatives was lower for DMPG (33%) and ethanol (43%) compared to MPG (46%). Pairwise comparisons revealed significant differences between ethanol and DMPG ($p=0.001$) and ethanol and MPG ($p=0.014$) but not between DMPG and MPG ($p=0.174$). Across preservation methods (transferred and non-transferred samples), false negatives remained relatively stable with processing time, averaging 37% at 2 weeks, 42% at 2 months and 40% at 4 months (Table 2).

3.3 | Presence-Absence in Low Diversity Communities

All six species included in the low diversity community samples were detected in at least one sample (both transferred and non-transferred, Figure 3). However, the detection rate varied by species. Most species were detected in most of the samples they were added to. Two false positives (*Gryllodes sigillatus* and *Hermetia illucens*) were detected in two of the low

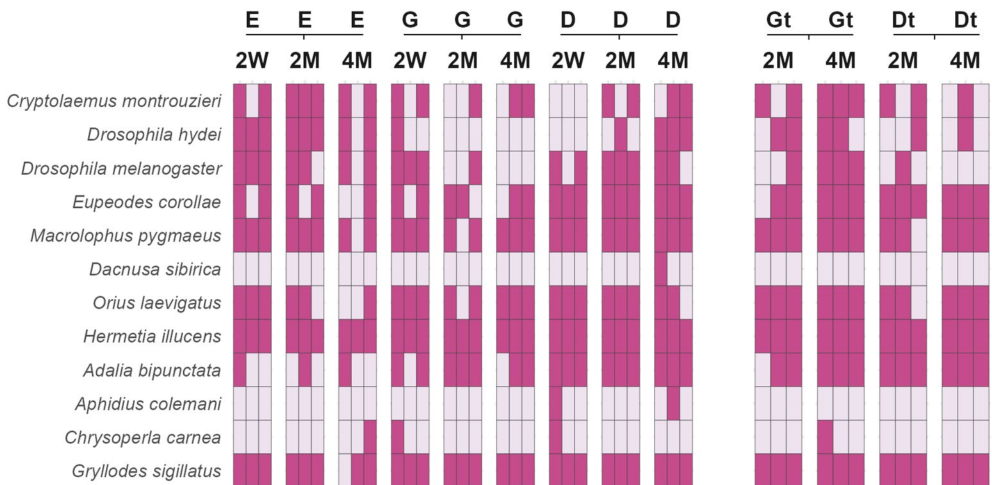

FIGURE 2 | Detection of the 12 species comprising the high diversity community in each sample. Dark square indicates species detection; light square indicates non-detection. Preservatives: E = ethanol; G = MPG; D = diluted MPG; Gt = MPG transferred to ethanol; Dt = diluted MPG transferred to ethanol. Storage durations: 2 W = 2 weeks; 2 M = 2 months; 4 M = 4 months. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 | Percentage of false negatives for each preservation treatment in high diversity communities across 12 species, assessed at 2 weeks, 2 months and 4 months (with and without transfer).

Transfer into ethanol after 2 weeks?	Preservative type	False negatives after 2 weeks (%)	False negatives after 2 months (%)	False negatives after 4 months (%)
No	DMPG	38.89	33.33	27.78
	Ethanol	36.11	38.89	52.78
	MPG	36.11	52.78	50.00
Yes	DMPG	n/a	44.44	44.44
	MPG	n/a	41.67	25.00

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diversity community samples, despite having only been added to the high diversity community samples. PERMANOVA results revealed that storage time, preservation method, and the interaction between preservation method and transfer significantly influenced community composition ($R^2=0.131$, $p=0.004$; $R^2=0.201$, $p=0.001$; $R^2=0.102$, $p=0.004$, respectively; Table S6). Dispersion did not differ for storage time or preservation method (PERMDISP, $p=0.339$ and $p=0.242$) but differed among preservation × transfer groupings ($p=0.001$). For transferred samples, the average percentage of false negatives is null for all samples across all preservation methods and processing time. When analysed without the transferred samples, preservation method also had a significant effect on the species detected in the low diversity community ($R^2=0.283$, $p=0.001$; Table S7) unlike storage time or the interaction between storage time and preservation method ($p=0.06$ and $p=0.424$). Pairwise comparisons revealed significant differences between ethanol and DMPG ($p=0.001$), DMPG and MPG ($p=0.03$) but not between ethanol and glycol ($p=0.052$). For non-transferred samples, the average percentage of false negatives was highest for DMPG (22%), followed by ethanol (13%) and lowest for MPG (6%). Across preservation methods (transferred and non-transferred samples), false negatives decreased with processing time, averaging 19% at 2 weeks, 8% at 2 months and 6% at 4 months (Table 3).

4 | Discussion

In this study, both preservation method and mock community diversity influenced the composition of insect communities recovered using lysis buffer-based non-destructive DNA metabarcoding. Community diversity had a substantial effect on species detection, with higher false-negative rates observed in the more diverse mock communities. Differences among preservation methods were more clearly resolved in the low diversity communities. Notably, storage time and ethanol transfer influenced community composition recovery only in the low diversity datasets. Detection rates also varied across species, further highlighting that recovery patterns reflect the combined influence of community diversity, preservation strategy, sample handling and species-specific biases.

4.1 | Community Diversity

Community diversity significantly influenced the detection of arthropods in this study. Samples containing more diverse communities had a higher average number of false negatives compared to samples containing less diverse communities, regardless of whether insect samples had been transferred or not into ethanol after 2 weeks in MPG or DMPG. The limited recovery of four species within the high-diversity samples likely contributed substantially to this pattern.

Aphidius colemani and *Chrysoperla carnea*, added exclusively to the diverse community samples, were only detected in a small fraction of samples. For the parasitoid wasp *A. colemani*, limited recovery may reflect methodological constraints as individuals were introduced in the form of unemerged aphid mummies. Although previous studies have successfully extracted DNA from aphid mummies using destructive protocols (e.g., Varennes et al. 2014; Zhu et al. 2019; Slusher et al. 2024), these methods physically disrupt the mummy, enabling DNA

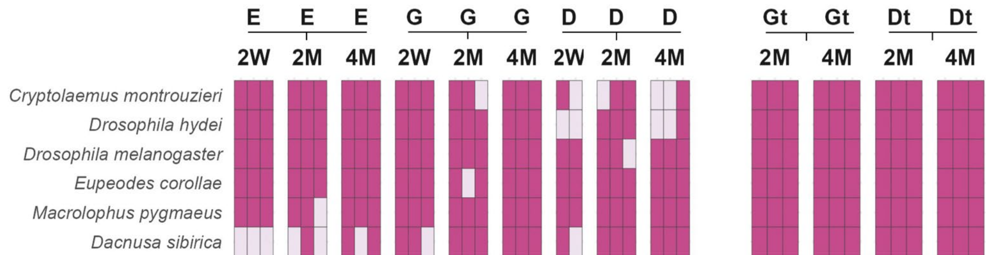

FIGURE 3 | Detection of the 6 species comprising the low diversity community in each sample. Dark square indicates species detection; light square indicates non-detection. Preservatives: E = ethanol; G = MPG; D = diluted MPG; Gt = MPG transferred to ethanol; Dt = diluted MPG transferred to ethanol. Storage durations: 2 W = 2 weeks; 2 M = 2 months; 4 M = 4 months. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 | Percentage of false negatives for each preservation treatment in low diversity communities across 6 species, assessed at 2 weeks, 2 months and 4 months (with and without transfer).

Transfer into ethanol after 2 weeks?	Preservative type	False negatives after 2 weeks (%)	False negatives after 2 months (%)	False negatives after 4 months (%)
No	DMPG	33.33	11.11	22.22
	Ethanol	16.67	16.67	5.56
	MPG	5.56	11.11	0.00
Yes	DMPG	n/a	0	0
	MPG	n/a	0	0

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release from both the parasitoid and the aphid tissues. In contrast, the non-destructive extraction approach used here may have limited DNA release from the enclosed parasitoid. For *C. carnea*, comparable detection limitations have previously been reported under non-destructive metabarcoding approaches (Marquina et al. 2021, 2022), suggesting that certain taxa or life stages may inherently yield lower recovery under such protocols.

Drosophila hydei and *Dacnusa sibirica*, were added to both the high and low diversity community samples but their detection rate was higher in the latter. This discrepancy may reflect several sources of PCR-related bias, including effects associated with the chosen annealing temperature, the number of PCR cycles, and primer–template mismatches (Sipos et al. 2007; Bru et al. 2008; Krehenwinkel et al. 2017). The PCR conditions used in this study reflect commonly applied conditions for DNA metabarcoding (45°C annealing temperature and 35 cycles; e.g., Earth Microbiome Project, Thompson et al. 2017) and were identical for both the high and low diversity mock communities. Under such conditions, primer–template mismatches, particularly near the 3′ end of COI metabarcoding primers, can strongly influence amplification efficiency among taxa (Piñol et al. 2015, 2018). In mixed template metabarcoding, templates with better primer matches are preferentially amplified during early cycles and may quickly dominate the resulting amplicon pool. These effects may have been exacerbated in the high diversity samples, where a greater number of competing templates are present.

Although PCR amplification biases can strongly affect amplification efficiency, they do not necessarily prevent the detection of taxa, particularly when sequencing depth is sufficient (Shirazi et al. 2021). In this study, sequencing depth was considered adequate for the mock community samples, with an average of 73,825 reads per sample and a minimum of 15,049 reads following the removal of non-target taxa. Overall, the variability in species detection is expected to remain an obstacle in accurately characterising insect communities through metabarcoding (Marquina et al. 2021), and a trade-off is often needed when choosing protocols. Regardless, PCR conditions were identical across treatments, meaning any PCR bias should affect all treatments equally rather than driving the observed differences in preservation methods within the high or low diversity community results.

4.2 | Preservation Methods

Significant differences in species detection were observed in both high and low diversity community samples among the different preservation methods. This was regardless of whether the two communities were analysed separately, combined and with or without transferred samples. However, the effectiveness of the preservation method, as well as the significance of differences and patterns, depended on community diversity. The diverse mock community samples exhibited unexpected patterns, with the DMPG preservation method showing a lower false negative rate than the ethanol preservation method. On the other hand, the low diversity mock community samples revealed more expected trends, with the MPG preservation method significantly and consistently showing the lowest false negative rate across all storage durations, followed by the ethanol and the DMPG preservation methods. Because of the limited recovery of four species within the high diversity samples, preservation effects may be more readily detectable in simpler communities, where reduced taxonomic competition allows methodological differences to emerge more clearly. Repeating the present study with a larger sample size would help clarify the observed patterns, considering that results can be inconsistent across biological replicates for studies using metabarcoding and non-destructive DNA extraction from lysates (Iwaszkiewicz-Eggebrecht et al. 2023).

Despite variations between communities, MPG, even when diluted, was found to be effective at preserving bulk samples stored at 5°C prior to lysis buffer-based non-destructive DNA extraction. In this study, MPG performed as well as or better than ethanol in preserving DNA, which aligns with findings of studies using destructive DNA extraction (Nakamura et al. 2020). On the other hand, DMPG performed moderately well in this study. In the high diversity mock community, false-negative rates after 2 weeks were comparable to those observed for ethanol and MPG, and at longer storage durations DMPG showed lower false-negative rates than both alternatives. However, this pattern was not consistent in the low diversity mock community, where DMPG generally performed less well than ethanol and MPG overall. Given the water content of DMPG, higher false-negative rates were initially expected due to the increased potential for hydrolytic degradation of nucleic acids. Nevertheless, the study results suggest that DMPG does not necessarily compromise COI fragment amplification under the conditions tested. This is broadly consistent with previous studies demonstrating successful amplification of mitochondrial markers from specimens stored in MPG or DMPG at various concentrations prior to destructive DNA extraction (Ferro and Park 2013; Ruppert et al. 2023). Nonetheless, the DNA recovered from highly diluted MPG may not be suitable for certain HTS applications (e.g., Whole Genome Sequencing), particularly when samples are stored at higher temperatures (Ferro and Park 2013).

4.3 | Storage Time and Transfer

Storage time had a significant impact on species recovery in low diversity community samples, although none of the pairwise comparisons were statistically significant. Interestingly, false negative rates decreased over time across all preservation methods for the non-transferred samples (from 18.52% to 9.26%). Prolonged exposure to preservatives may gradually trigger cell lysis, improving the efficiency of non-destructive DNA extraction methods. This is consistent with evidence that arthropod DNA can be successfully extracted from storage solution (Shokralla et al. 2010; Hajibabaei et al. 2012; Zizka et al. 2019; Couton et al. 2021). In MPG and ethanol, this likely occurs through cell dehydration and membrane weakening whereas in DMPG it may result from cell swelling and rupture.

In this study, the detection probability of *D. sibirica* increased significantly with storage duration ($p = 0.036$), rising from 18% at 2 weeks to 43%–50% after 2–4 months across all preservation methods (see additional statistical analyses in the Supporting Information). In contrast, two more heavily sclerotised coccinellids showed only modest, non-significant increases in detection

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over time across all preservation methods (*Cryptolaemus montrouzieri*: ~65% to ~77%, $p = 0.38$; *Adalia bipunctata*: ~35% to ~40%, $p = 0.75$). This pattern is more consistent with progressive intracellular membrane permeability than with cuticular degradation and indicates that storage-time effects on DNA accessibility are species-dependent. Overall, this study suggests that within a 4-month storage period at low temperatures, DNA degradation is not substantial enough to impede DNA amplification and species detection through non-destructive DNA metabarcoding.

Transfer significantly improved the detection of species in low diversity community samples with the average percentage of false negatives being null for all transferred samples across all preservation methods and storage time periods. This trend was also present for the high diversity community samples across all preservation methods but was not significant. However, while transferring samples upon receipt may improve species recovery through non-destructive DNA extraction and metabarcoding, it also incurs additional costs, including staff time and preservative expenses and adds the risk of sample cross-contamination due to extra handling. Further research should evaluate the performance of DMPG as a trapping and storage solution under realistic field conditions, including rain, temperature fluctuations and UV exposure. Under such conditions, environmental contaminants such as plant debris, pollen, fungal spores, soil particles, pollutants and decomposed wood compounds may accumulate and act as PCR inhibitors (Sire et al. 2023). The higher viscosity of MPG (i.e., the absolute viscosity of MPG (~0.042) is approximately 40 times higher than that of ethanol (~0.001095); Martoni et al. 2021) may facilitate the adhesion of such materials to the insect cuticle, potentially increasing the co-extraction of inhibitory substances during non-destructive DNA workflows. While these contaminants may have a more limited impact on lysis buffer–based non-destructive DNA extraction than on workflows where DNA is extracted directly from the preservative solution, the influence of such environmental contamination on metabarcoding results remains to be investigated.

In this study, non-destructive lysis-based DNA metabarcoding outcomes were influenced by community diversity, preservation method (temperature × preservative), storage time and sample transfer from a first storage solution (e.g., representing the trapping solution) into a second storage solution. Detection rates were lower in high diversity samples, likely reflecting species-specific challenges. The MPG preservation method performed as well as or better than optimal ethanol preservation method, even when diluted. Transferring samples to ethanol improved detection rates, particularly in low diversity communities, but adds cost and handling risks. Overall, MPG shows strong potential for field use, though further testing under more realistic conditions is recommended.

Author Contributions

Sophie de Becquevort: funding acquisition, methodology, writing – original draft, visualization, investigation, conceptualization, formal analysis, writing – review and editing, project administration. **Nadia Barsoum:** conceptualization, funding acquisition, methodology, writing – review and editing, supervision. **Zoe Withey:** writing – review and editing.

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Conflicts of Interest

We confirm that this has not been published elsewhere, nor is it currently under consideration for publication elsewhere. We have no conflicts of interest to disclose.

Data Availability Statement

All raw sequence reads generated from the metabarcoding analyses have been deposited in the European Nucleotide Archive (ENA) under accession number PRJEB91249.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Taxonomic composition and abundance of species included in the mock insect communities. For each species, the order, life stage, and number of individuals included in the low- and high-diversity community samples are shown. **Table S2:**. Summary of sequencing results across different experimental groups (mock community species only). LD = Low diversity; HD = High diversity. **Table S3:** Results of PERMANOVA analysis: effects of storage time, community type, and preservation method on arthropod DNA metabarcoding results (including transferred samples). **Table S4:** Results of PERMANOVA analysis: effects of storage time and preservation method on arthropod DNA metabarcoding results (high diversity samples with transferred samples). **Table S5:** Results of PERMANOVA analysis: effects of storage time and preservation method on arthropod DNA metabarcoding results (high diversity samples without transferred samples). **Table S6:** Results of PERMANOVA analysis: effects of storage time and preservation method on arthropod DNA metabarcoding results (low diversity samples with transferred samples). **Table S7:** Results of PERMANOVA analysis: effects of storage time and preservation method on arthropod DNA metabarcoding results (low diversity samples without transferred samples).

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