1-1-2023

Edna metabarcoding of avocado flowers: 'Hass' it got potential to survey arthropods in food production systems?

Joshua H. Kestel
Edith Cowan University

Philip W. Bateman

David L. Field
Edith Cowan University

Nicole E. White

Rose Lines

See next page for additional authors

10.1111/1755-0998.13814
Kestel, J. H., Bateman, P. W., Field, D. L., White, N. E., Lines, R., & Nevill, P. (2023). Edna metabarcoding of avocado flowers: 'Hass' it got potential to survey arthropods in food production systems?. Molecular Ecology Resources. Advance online publication. https://doi.org/10.1111/1755-0998.13814

Authors
Joshua H. Kestel, Philip W. Bateman, David L. Field, Nicole E. White, Rose Lines, and Paul Nevill

Received: 14 December 2022 | Revised: 26 April 2023 | Accepted: 12 May 2023

DOI: 10.1111/1755-0998.13814

RESOURCE ARTICLE

MOLECULAR ECOLOGY RESOURCES WILEY

eDNA metabarcoding of avocado flowers: 'Hass' it got potential to survey arthropods in food production systems?

Joshua H. Kestel[1,2] | Philip W. Bateman[1,3] | David L. Field[2] | Nicole E. White[1] | Rose Lines[1,4] | Paul Nevill[1]

[1]Trace and Environmental DNA (TrEnD) Laboratory, School of Molecular and Life Sciences, Curtin University, Perth, Western Australia, Australia

[2]Molecular Ecology and Evolution Group (MEEG), School of Science, Edith Cowan University, Joondalup, Australia

[3]Behavioural Ecology Laboratory, School of Molecular and Life Sciences, Curtin University, Perth, Western Australia, Australia

[4]Department of Primary Industries and Regional Development, Perth, Western Australia, Australia

Correspondence
Joshua H. Kestel, Trace and Environmental DNA (TrEnD) Laboratory, School of Molecular and Life Sciences, Curtin University, Perth 6102, WA, Australia.
Email: joshua.kestel@postgrad.curtin.edu.au

Funding information
Hort Innovation; South West Catchments Council, Grant/Award Number: eDNA for classification – PH19007

Handling Editor: Carla Martins Lopes

Abstract

In the face of global biodiversity declines, surveys of beneficial and antagonistic arthropod diversity as well as the ecological services that they provide are increasingly important in both natural and agro-ecosystems. Conventional survey methods used to monitor these communities often require extensive taxonomic expertise and are time-intensive, potentially limiting their application in industries such as agriculture, where arthropods often play a critical role in productivity (e.g. pollinators, pests and predators). Environmental DNA (eDNA) metabarcoding of a novel substrate, crop flowers, may offer an accurate and high throughput alternative to aid in the detection of these managed and unmanaged taxa. Here, we compared the arthropod communities detected with eDNA metabarcoding of flowers, from an agricultural species (*Persea americana*—'Hass' avocado), with two conventional survey techniques: digital video recording (DVR) devices and pan traps. In total, 80 eDNA flower samples, 96 h of DVRs and 48 pan trap samples were collected. Across the three methods, 49 arthropod families were identified, of which 12 were unique to the eDNA dataset. Environmental DNA metabarcoding from flowers revealed potential arthropod pollinators, as well as plant pests and parasites. Alpha diversity levels did not differ across the three survey methods although taxonomic composition varied significantly, with only 12% of arthropod families found to be common across all three methods. eDNA metabarcoding of flowers has the potential to revolutionize the way arthropod communities are monitored in natural and agro-ecosystems, potentially detecting the response of pollinators and pests to climate change, diseases, habitat loss and other disturbances.

KEYWORDS
agroecosystem, arthropod, environmental DNA, flower-visitor and pollinator, metabarcoding

1 | INTRODUCTION

Effective management of food production systems requires detailed knowledge of both their abiotic (e.g. climate) and biotic features (e.g. ecosystem services), herein referred to as agroecosystems (Lippert et al., 2021; Savary et al., 2019; Wada et al., 2016). Of the many biotic features which make up agroecosystems, animal-mediated pollination is one of the most critical, with at least 75% of cultivated plant species relying on this ecosystem service to improve both the quantity and quality of crop yield (Aizen et al., 2009; Garibaldi et al., 2013; Ricketts et al., 2008). Consequently, pollination services have considerable value for countries with large agriculture sectors, including the United States ($US30 billion in 2012; Jordan et al., 2021), China (US$106 billion in 2010; Mashilingi et al., 2021) and Australia ($US1.1 billion in 2003; Hein, 2009). Unfortunately, the majority of pollination services within agroecosystems are largely reliant on pollinating taxa that are increasingly threatened by climate change and pathogens.

Currently, the majority of animal-mediated pollination services in agroecosystems are reliant on managed insects, primarily the European honeybee (*Apis mellifera*), to facilitate cross-pollination (Potts et al., 2016). However, the focus is increasingly shifting towards unmanaged insects (e.g. native bees, flies and moths), which are now recognized as important contributors to global crop pollination (Cook et al., 2020; Garibaldi et al., 2013; Rader et al., 2016). This shift has become necessary as *A. mellifera* hives and the pollination services they provide are increasingly threatened by mites and fungal infections (e.g. *Varroa destructor* and *Ascosphaera apis*), and associated diseases (e.g. Varroosis and Chalkbrood disease; see Goulson & Hughes, 2015; Sammataro et al., 2000). These biotic pressures weaken hives and increase the likelihood of colony collapse, a phenomenon which is currently estimated to affect over 30% of hives annually in the USA, Canada and many European nations (see Biesmeijer et al., 2006; Gray et al., 2020; Steinhauer et al., 2021). In response, practitioners and researchers alike are increasingly promoting the value of unmanaged pollinators, although surveys to detect such taxa remain relatively uncommon in agroecosystems.

Surveying arthropod diversity to determine the presence or absence of beneficial (e.g. pollinators, predators) and antagonistic species (e.g. herbivorous pests, arthropod vectors) is critical for managing the health of agroecosystems and increasing food security (Barrios, 2007; Kestel et al., 2022; Letourneau et al., 2011; Senapathi et al., 2021). To date, identifying these taxa has largely relied upon passive trapping (e.g. pan, Malaise and vane traps), visual observation and active survey techniques (e.g. sweep netting; Gervais et al., 2018; Kearns & Inouye, 1993; Prado et al., 2017; Shi et al., 2022). Indeed, pan, Malaise and vane traps are some of the most commonly used methods to measure bee diversity in agroecosystems, largely because they provide a low-cost means to sample multiple sites simultaneously (McCravy, 2018; Prado et al., 2017; Spafford & Lortie, 2013). Studies using these passive survey methods have, for example, demonstrated the benefit of adjacent natural habitats for pollinator abundance and crop yield (see Klein et al., 2012; Morandin & Winston, 2006), and identified the inverse relationship between cultivated land use and wild pollinator diversity, particularly for wild bee species (see Bergholz et al., 2022; Zou et al., 2017). Unfortunately, passive sampling techniques often require extensive time commitments and increasingly rare specialist taxonomic expertise to morphologically identify the arthropod taxa collected or observed (Brown, 2020; Pardo & Borges, 2020; Shi et al., 2022). Furthermore, even when morphological identifications are possible, passive sampling techniques often have intrinsic biases in the taxa that are captured. For instance, pan traps capture a range of taxa that share an attraction to the trap (e.g. bees attracted to blue pan traps; see Joshi et al., 2015) but are not necessarily ecologically relevant to the system under study (e.g. not all captured pan trap insects are flower-visitors or pollinators; see Popic et al., 2013). Pan traps also disproportionately capture small insect taxa (see Prado et al., 2017) and suffer from variable capture rates due to placement position (e.g. sampling under forest canopies can reduce capture rates for pan traps; see Abrahamczyk et al., 2010). As a consequence of these limitations, passive sampling techniques are often complemented by visual observations and active surveys to provide more accurate measures of arthropod diversity (see Prendergast et al., 2020) and overcome the biases of each individual technique.

Visual observations and interpretation of the relationships between arthropods and the plants they pollinate have been a part of scientific inquiry since insect pollination was first documented in the 18th Century (Baker, 1979; Kolreuter, 1761; Sprengel, 1793). Detailed observations of flower visits can be difficult to achieve however, as the process is generally time-consuming and often limited in sample size (Bosch et al., 2009; Waser et al., 1996). Further, visual identification of each flower visitor requires specialist taxonomic expertise, which may become increasingly inaccurate as more species visit (Bosch et al., 2009; Ebeling et al., 2008; Van Zandt et al., 2020). Observational-based studies may also fail to capture irregular movement patterns typically shown by floral-visiting insects, increasing the potential of misclassification of generalist and specialist relationships (Pornon et al., 2017; Thomsen & Sigsgaard, 2019; Waser et al., 1996). In the context of agroecosystems, these issues can reduce the accuracy and effectiveness of arthropod surveys. As a result, new survey methods are being used that complement visual observations, one of the most notable being digital video recording (DVR) devices. DVR devices have gained attention as a means to monitor flower-visitor interactions because they can capture multiple flower visits simultaneously across many plants, the recordings can then be watched to obtain taxonomic and behavioural data (e.g. animal identity, stigma contact; see Krauss et al., 2017). Previous studies have shown that DVRs of *A. mellifera* foraging on to *Lavandula angustifolia* provided significantly similar visit rates to visual observations, while also showing that this technology can capture over four times the number of interactions between Hymenoptera species and flowering plants than with visual observations alone (Gilpin et al., 2017; Naqvi et al., 2022). Despite such promise, DVR devices are often limited by their resolution and the size of the visiting arthropod, both of which can limit the number of detections possible

(although see Droissart et al., 2021; Steen, 2017), and prevent accurate taxonomic identifications below the family level (e.g. Bonelli et al., 2020). As such, DVR devices do not currently provide a 'silver bullet' for monitoring flower-visits and alternative technologies and methods are still required.

Recently, environmental DNA (eDNA) metabarcoding has been added to the biodiversity survey tool kit, whereby DNA barcodes from multiple organisms can be sequenced in parallel eDNA metabarcoding of environmental samples (e.g. soil, water and now air). It has been widely used to monitor aquatic and terrestrial systems (e.g. Capo et al., 2021; Clare et al., 2021; van der Heyde et al., 2020), but studies of plant–animal interactions using eDNA extracted from flowers are rare (Gamonal Gomez et al., 2023; Johnson et al., 2023; Newton et al., 2023; Thomsen & Sigsgaard, 2019), and few have systematically compared metabarcoding of arthropod DNA on flowers to other survey methods, despite alternative approaches potentially detecting different taxa (Gamonal Gomez et al., 2023; Newton et al., 2023). We compared two commonly used arthropod survey methods—pan traps and DVR devices—with two common eDNA barcoding assays, to detect a wide range of arthropods. We applied eDNA metabarcoding as it would be deployed in many agroecosystems around the world, by using existing arthropod metabarcoding assays (see Clarke et al., 2014; Vamos et al., 2017), and without comprehensive arthropod DNA barcode reference libraries for the study region (Rasmussen et al., 2021; Young et al., 2021). Our aim was to understand the extent to which different arthropod survey methods complement one another, and ultimately improve the monitoring of plant–animal interactions in agroecosystems.

2 | METHODS

2.1 | Field site

For this study, inflorescences were collected from a *Persea americana* ('Hass' Avocado) orchard, Marron Brook Farm (34°18′52 S, 116°08′36 E), located in the avocado production region of Manjimup-Pemberton in south-west Western Australia (SWWA) (Mccarthy & McCauley, 2020). DVRs and pan trap sampling were carried out at the same time that inflorescences were collected from the study orchard. In the Manjimup-Pemberton region, the dominant land uses are pasture and orchards, interspersed with remnants of native karri forest (*Eucalyptus diversicolor*). Orchards in this region are largely reliant on hiring managed *A. mellifera* hives to facilitate cross-pollination (Mccarthy & McCauley, 2020), although the importance of unmanaged arthropods to complement these services remains unclear (Ish-Am, 2005; Ish-Am & Eisikowitch, 1998; Mccarthy & McCauley, 2020). Marron Brook Farm sits approximately 200 m above the sea level and is dominated by 'Hass' trees interspersed with 'Fuerte' pollinisers. Unlike many other orchards in the region, Marron Brook Farm cultivates an understorey of wild radish (*Raphanus raphanistrum*), which grows to a height of 1 m, and aims to encourage avocado pollinator presence. We randomly selected eight 'Hass' trees between eight columns of 41 trees within this orchard, all of which were 8 years old and of heights between 3 and 5 m. The final three columns and rows were excluded from sampling in both orchards to help reduce the impact of edge effects. For each sample tree, ten *P. americana* inflorescences were removed for eDNA analysis during the peak *P. americana* flowering season in 2020 (October 30th and 31st) (Figure 1a).

2.2 | eDNA surveys

2.2.1 | Sample collection and DNA extraction

Prior to sampling, a pilot study determined that more arthropod eDNA detections (fwhF2/fwhR2n assay; Vamos et al., 2017) were obtained for flowers ground in a mortar and pestle than

FIGURE 1 Three methods used to measure flower-visiting arthropods for *Persea americana* at Marron Brook Farm in Pemberton, Western Australia. (a) Inflorescences were removed from upper and lower storey of *P. americana* trees for eDNA metabarcoding. Lower understorey inflorescences were removed using sterilized hand secateurs (not pictured), while the upper storey inflorescences were removed using extended secateurs which were captured in net lined with a sterilized plastic bag (pictured). Inflorescences were then placed on ice until they could be stored at −20°C. (b) Two inflorescences per tree were monitored for 6 h over 2 days using GoPro Hero 7 Silver cameras. (c) Three pan traps (white, blue and yellow) were deployed for 16 h over 2 days to capture flying insects. Images captured by Diana Adorno.

metabarcoding MilliQ wash water from entire inflorescences (results not shown). For eDNA analysis, five inflorescences were collected from both the upper (>2 m) and lower canopy (<2 m) of each *P. americana* tree ($N = 10$ inflorescences per tree, $N = 80$ inflorescence total). Six inflorescences were collected from each tree on the 30th of October 2020 (three upper canopy, three lower canopy) and four inflorescences (two upper canopy and two lower canopy) were collected from each tree on the 31st of October 2020 (Figure 1a). Both days were sunny with low winds and no rain (Table S1). To minimize sampling bias, inflorescences were sampled randomly from both the upper and lower canopy while walking around the full circumference of each *P. americana* study tree (collection method adapted from Howlett et al., 2018). Inflorescences were removed from the lower canopy using sterilized hand secateurs, and inflorescences in the upper canopy were sampled using a net covered with a clean plastic bag replaced after each sample, and sterilized extended secateurs. To minimize cross-contamination, all equipment was sprayed with 10% bleach solution and wiped down after each inflorescence was collected. Once removed, each inflorescence was placed into a thick plastic bag, zip-tied and kept on ice until the samples could be stored at −20°C. Frozen inflorescences were processed in the TrEnD laboratory at Curtin University. For inflorescence processing, open florets of each inflorescence were removed with doubled-gloves (changed after every inflorescence) and placed in a mortar and pestle where the plant material was ground into a fine paste. Mortars and pestles were soaked in 10% bleach solution, rinsed with reverse osmosis water, and placed in a UV oven for 15 min to prevent cross contamination between samples. In total, 140–190 mg of ground material was weighed out and transferred into a 2 mL safe-lock Eppendorf tube with 540 μL ATL buffer and 60 μL Proteinase K (QIAGEN). Samples were digested in a slow-rotating hybridisation oven at 56°C overnight (~12 h). Following digestion, DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN) using a QIAcube Connect automated DNA extraction platform (QIAGEN). The final elution volume was 100 μL, and extraction controls (blanks) were carried out for every batch of DNA extractions.

2.2.2 | PCR amplification

Quantitative polymerase chain reaction (qPCR; Applied Biosystems) was used to assess the quality of each eDNA sample targeting the cytochrome oxidase 1 (CO1) and 16S ribosomal subunit genes. Inhibitors in the PCR reactions and low copy number can impact metabarcoding data (Murray et al., 2011, 2015), therefore each eDNA extract was assessed with a qPCR dilution series (neat, 1/10, 1/100) under the following conditions: 25 μL reaction volumes containing 2.5 μL of 10 × PCR Gold Buffer, 2 μL of 2.5 mM $MgCl_2$, 1 μL of 0.4 mg/mL BSA, 0.25 μL of dNTPs, 0.5 μL of each primer, 0.2 μL AmpliTaq Gold, 2 μL of DNA and the remaining volume supplemented with DNase/RNase-Free Distilled Water. Two PCR assays were used: assay fwhF2/fwhR2n (Vamos et al., 2017), targeting the CO1 gene, herein referred to as CO1, and assay Ins_16S_shortF/Ins_16S_shortR (Clarke et al., 2014), targeting the 16S ribosomal subunit gene, herein referred to as 16S. The forward primer sequence for CO1 was 5′-GGDACWGGWTGAACWGTWTAYCCHCC-3′ and reverse primer sequence 5′-GTRATWGCHCCDGCTARWACWGG-3′. The forward sequence for 16S was 5′-TRRGACGAGAAGACCCTATA-3′ and reverse sequence 5′- ACGCTGTTATCCCTAAGGTA-3′. Amplicons for each assay were ~205 bp and ~167 bp for CO1 and 16S, respectively. Extracts were amplified on a StepOnePlus Real-Time PCR System (Applied Biosystems) under the following conditions for CO1: initial denaturation at 95°C for 5 min, followed by 50 cycles of 30 s at 95, 50°C for 30 s and 2 min at 72°C, with a final extension for 10 min at 72°C. For 16S the conditions were as follows: initial denaturation at 95°C for 5 min, followed by 50 cycles of 30 s at 95, 51°C for 30 and 45 s at 72°C, with a final extension for 10 min at 72°C. Extraction and non-template controls were included in each qPCR assay. DNA extracts that showed inhibition were diluted using MilliQ water and the optimum quantity of DNA input was determined for fusion tagging.

Environmental DNA that were of sufficient quality and free of inhibition, as determined from the initial qPCR screen (qPCR dilution series), were assigned a unique (6–8 bp in length) multiplex identifier tag (MID-tag) for both the CO1 and 16S assays. To reduce the likelihood of contamination, chimera production and MID-tag jumping (Esling et al., 2015), DNA was amplified in a single round of qPCR for each assay using MID-tag primers consisting of either the CO1 or 16S primers coupled to Illumina flow cell adaptors, custom sequencing primers and MID-tag combinations unique to this study. All fusion-tagged qPCR reactions were prepared in dedicated clean room facilities at the TrEnD Laboratory, Curtin University designed for ancient DNA work using an automated QIAgility robotics platform (QIAGEN) and were carried out in 25 μL reactions containing 2.5 μL of 10 × PCR Gold Buffer, 2 μL of 2.5 mM $MgCl_2$, 1 μL of 0.4 mg/mL BSA, 0.25 μL of dNTPs, 0.5 μL of each primer, 0.2 μL AmpliTaq Gold, 4–8 μL of DNA and the remaining volume supplemented with DNase/RNase-Free Distilled Water. MID-tag PCR amplicons were carried out in duplicate reactions to control for PCR stochasticity and all fusion-tagged qPCRs were processed using the same parameters as the initial qPCR screens described above.

2.2.3 | DNA library preparation and sequencing

Replicate MID-tag amplicons were pooled at approximately equimolar concentrations (e.g. minipool) based on their respective qPCR DRn values and were measured under a high-resolution capillary electrophoresis system (QIAxcel; QIAGEN) and the final library was size-selected (160–425 bp) using a PippinPrep (Millennium Science Pty Ltd) with a 2% ethidium bromide cassette (Sage Science, Beverly) to remove any off-target amplicons and primer dimer. The final library was purified using the QIAquick PCR Purification Kit (QIAGEN), quantified using a Qubit 4.0 Fluorometer (Invitrogen) and diluted to 2 nM prior to sequencing. Sequencing by synthesis was performed on an Illumina MiSeq platform (Illumina) located in the Trace and Environmental DNA lab at Curtin University and as

per Illumina's protocol for single-end sequencing with a 300 cycle MiSeq®V2 reagent kit and standard flow cell for environmental metabarcoding.

2.3 | Data processing

Sequenced multiplex identifier-tagged amplicons were inputted to a containerized workflow (eDNAFlow; Mousavi-Derazmahalleh et al., 2021) and run through the Pawsey Supercomputing Centre in Kensington, Western Australia. Here, the sequences were filtered, formed into zero-radius operational taxonomic units (ZOTUs) and assigned taxonomic identifications. Sequences were quality checked using FASTQC (Andrews, 2010) and quality filtered (Phred quality score < 20), before the multiplex identifiers were trimmed from the sequence reads using AdapterRemoval v2 (Schubert et al., 2016). Subsequently, the filtered reads were demultiplexed using OBITOOLS (Boyer et al., 2016) and sequences shorter than the minimum length of 120 bp were filtered out. Sequences were then dereplicated into ZOTUs with a minimum sequence abundance of 5 (see van der Heyde et al., 2020) using the USEARCH Unoise3 algorithm (Edgar, 2016). A database of ZOTUs was then generated and queried against the GenBank (NCBI) nucleotide database with 100% query coverage and 95% identity using BLASTN (Altschul et al., 1990). Erroneous ZOTUs with a sequence similarity below the 95% threshold were removed using the LULU post clustering curation method (Frøslev et al., 2017). Finally, a custom Python script (eDNAFlow; Mousavi-Derazmahalleh et al., 2021) was used to assign taxonomic identifications to the curated ZOTUs using the lowest common ancestor (LCA) approach. Taxonomic identification was assigned to a ZOTU when the percentage identity of two or more queried sequences with ≤1% difference had 100% query coverage and 97% sequence similarity. For the purposes of this study, we set the minimum threshold count of 5 reads for ZOTUs to classify a taxa as present within a sample.

2.4 | DVR and pan trap surveys

Two GoPro cameras (Hero 7 Silver) were mounted on 1.5 m wooden stands to observe two of the ten sample inflorescences per study tree (Figure 1b). Due to the limited number of DVR devices and the complexity of building and transporting taller stands, only inflorescences in the lower canopy (<2 m) were monitored. Each DVR device was set to time-lapse mode (one image every 0.5 s) to maximize battery life. During video observations, 3 h of arthropod visits per day for the two sample days were recorded and condensed into two 11 min 50 s videos ($N = 64$ DVRs). On the 30th of October DVRs commenced between 11:25 AM–12:25 PM and recorded a total 3 h of footage, while on the 31st of October the DVRs commenced between 8:35 AM–9:23 AM and recorded a total of 3 h of footage. DVRs were deployed when temperatures were warm enough for bee activity (clear skies, wind speed <30 km/h, and >17°C; Prendergast et al., 2020). DVRs were downloaded at the end of each sampling day. One researcher watched each DVR on 0.4× speed to allow for individual visitations to be classified (method adapted from Gilpin et al., 2017). A visit was noted when an arthropod made contact with a flower on the inflorescence, subsequent flower contacts were not noted (method adapted from Sakamoto et al., 2012). If an arthropod flew out of frame and then revisited the same inflorescence, this was counted as a new visit. Arthropod images were grouped into morphotypes and identified to the species level, where possible, using photographic reference material and descriptions from Zborowski and Storey (2017).

Pan traps offer a non-invasive, efficient and cost effective means to measure arthropod diversity without observer bias (Westphal et al., 2008; Wilson et al., 2008). In contrast to other active sampling techniques, pan traps are effective at capturing arthropod communities independent of floral resource availability (see Popic et al., 2013), and may better capture beneficial (e.g. predators) and antagonistic (e.g. pests) taxa relevant to agricultural systems. In the present study, standard pan trapping procedures were followed, whereby three 4.8 cm × 10 cm polypropylene picnic bowls were painted either yellow, blue or white using waterproof enamel-based paint (Kearns & Inouye, 1993; Saunders et al., 2013; Zou et al., 2017). Each set of three bowls were deployed near the base of the eight chosen *P. americana* trees within the study orchard. Each bowl was filled with ~250 mL of ultrapure water and one drop of detergent. The three bowls were attached to a piece of chipboard with adhesive putty to prevent them from blowing over (Figure 1c). Pan traps were set up between 10:30 AM–12:40 PM on the 30th of October and remained in the orchard for 5–6 h, while on the 31st of October, pan traps were set up between 9:00 AM and 10:00 AM and remained in the orchard for 5–6 h. Pan traps were set up when temperatures were warm enough for bee activity (clear skies, wind speed <30 km/h, and >17°C; Prendergast et al., 2020). At the end of each sampling day, the arthropods collected in each pan trap were transferred using plastic tweezers into 15 mL falcon tubes (one per pan) filled with 20% Dimethyl sulfoxide (DMSO) with saturated salt (NaCl). Arthropod samples were identified morphologically by an entomologist, David Knowles, to provide taxonomic identifications to species-level where possible.

2.5 | Statistical analysis

All statistical analyses were performed on R 3.5.1 (R Core Team, 2018). For all three survey methods, taxa not resolved to the species level were grouped into morphotype at the family level (e.g. Chironomidae sp.) and these morphotypes were used as a proxy for species (method adapted from D'Souza et al., 2021). In the eDNA dataset, samples with low sequencing depth and ZOTUs with 5 or more reads found in the negative controls were removed. Read counts were transformed to presence-absence to reduce the effects of PCR amplification and primer biases (Elbrecht & Leese, 2015). Shapiro–Wilk and non-parametric correlation tests were used to verify that no correlation existed between arthropod

species size and eDNA detection frequency. eDNA species counts per inflorescence were then calculated for all arthropods, as well as the two dominant flower-visiting cohorts determined by the DVRs: Diptera and Hymenoptera. The Diptera species cohort contained seven families, representing seven unique species: Drosophilidae sp., *Hydrellia tritici* (Ephydridae), Sciaridae sp., Chironomidae sp., *Simosyrphus grandicornis* (Syrphidae), *Aedes notoscriptus* (Culicidae), and *Musca domestica* (Muscidae). While the Hymenoptera species cohort was comprised of two families, representing two unique species: *Apis mellifera* (Apidae) and a Braconidae sp. Generalized linear models (GLMs) with Poisson distributions were then generated for each of the three eDNA datasets (all arthropods, Diptera and Hymenoptera).

The co-variates included in these initial GLMs were: sample tree (1–8), inflorescence location (upper; sampled >2 m in the canopy or lower; sampled <2 m in the canopy), and sampling date (30/10/2020 or 31/10/2020), all three co-variates were listed as categorical. Dispersion statistics were calculated for each GLM and 100,000 simulated datasets were run to confirm that the models could account for the high frequency of zeros, a common phenomenon in eDNA datasets (Song et al., 2017; Spear et al., 2021). Akaike information criterion (AIC) frequentist testing was then used to assess the quality of each model and select the most appropriate GLMs for each dataset. AIC frequentist values were then recalculated and the final models were rerun in the 100,000 dataset simulations. To determine the significance of the co-variates in the final models, we re-ran the final GLMs using robust standard errors and compared the output to the pan trap and DVR co-variate significance results. Cameron and Trivedi (2009) recommended using robust standard errors for estimating parameters derived from GLMs to control for instances when the distribution assumption that the variance equals the mean have minor violations.

For DVR and pan trap datasets, the same taxa pooling procedures were followed to create three datasets for each method; all arthropods, Diptera and Hymenoptera. For the DVR dataset, the Diptera species cohort comprised seven families totalling 17 species: *Calliphora albifrontalis* (Calliphoridae), *Lucilia cuprina* (Calliphoridae), Calliphoridae spp., Chloropidae spp., Drosophilidae spp., Ephydridae sp., *Musca domestica* (Muscidae), *Musca vetustissima* (Muscidae), Sarcophagidae sp., Syrphidae sp., and unclassified Diptera sp. The Hymenoptera species cohort detected on DVRs comprised eight families representing seven species: *Apis mellifera* (Apidae), Bethylidae sp., Braconidae sp., Formicidae sp., Halictidae sp., Pompilidae sp., and *Polistes humilis* (Vespidae). For the pan trap dataset, all arthropods, Diptera and Hymenoptera species cohorts were pooled per set of three coloured pan traps (blue, yellow and white). The pan trap Diptera species cohort comprised 11 families representing 20 species: Agromyzidae sp., *Calliphora varifrons* (Calliphoridae), Chaemaeyiidae sp., Chironomidae spp., Chloropidae spp., Dolichopodidae sp., Drosophilidae spp., Phoridae sp., Sciaridae sp., *Melangyna viridiceps* (Syrphidae), and unclassified Diptera sp. The pan trap Hymenoptera species cohort comprised six families totalling 10 species: *Apis mellifera* (Apidae), Bethylidae spp., Braconidae sp., Formicidae sp., *Lasioglossum hapsidium* (Halictidae), *L. castor* (Halictidae), *Lipotriches flavoviridis* (Halictidae), Mutillidae sp., and unclassified Hymenoptera sp. Both the DVR and pan trap datasets were tested for Skewness and Kurtosis values using the Skewness and Kurtosis function in the 'e1071' package. A Shapiro–Wilk normality test and a non-parametric Kruskal–Wallis test were used to examine if all arthropod, Diptera and Hymenoptera species counts for the DVR and pan traps datasets varied according to sample tree. Mann–Whitney tests were then used to see if DVR and pan trap Diptera and Hymenoptera species counts varied according to the date of collection.

Sampling effort was examined using rarefaction curves, in the package 'vegan' in R, to determine if the number of samples collected were enough to fully capture the diversity for all arthropod families and species for each survey method. The differences in richness and identity detected by all three methods were quantified using a Jaccard Index; this required all three datasets to be standardized into presence-absence data. The Jaccard index analysis was undertaken using the package 'Vegan' in R. Subsequently, non-metric multidimensional scaling (NMDS) ordination was used to visualize the similarity in species communities generated by each method using one dimension to minimize NMDS stress. An analysis of similarity (ANOSIM) was then used with the Jaccard dissimilarity matrix and 9999 permutations to test if the communities differed significantly between the methods. We compared alpha diversity between the three survey methods using Chao2 alpha diversity indices at both the family and species level with collection dates pooled, Chao2 was calculated using the package 'fossil' in R. The Chao2 index returns an estimate of richness based on incidence data (Chao, 1984). A Shapiro–Wilk normality test and a non-parametric Kruskal-Wallis test were used to examine if the Chao2 diversity indices for arthropod families and species differed between the three methods. A non-parametric Kruskal-Wallis test was used to compare the means for all arthropods, Diptera and Hymenoptera species counts per tree across the three survey methods. We tested the differences for family and species counts between survey methods for all arthropods, as well as for the dominant flower-visiting Diptera and Hymenoptera cohorts using Kruskal-Wallis tests and non-parametric post-hoc Dunn tests with the Benjamini–Hochberg adjustment method.

3 | RESULTS

3.1 | eDNA surveys

We generated 15,366,374 raw sequence reads from the 80 inflorescence samples and 10 hybrid, extraction and PCR controls. In total, 13,392,006 quality-filtered sequences were generated with a mean sequencing depth of 148,800 per sample. One ZOTU, *Agrotis ipsilon*, was found in the hybrid control (five reads) and was removed from the entire dataset. An additional 27 ZOTUS corresponding to fungi spp., *Homo sapiens* and *Canis* sp. were also removed from the eDNA dataset. After removal, the mean number of

reads per sample was 2758 (±556 SE) for CO1 and 3391 (±1391 SE) for 16S, and between the two assays five samples failed to amplify.

3.2 | Taxonomic composition

Overall, 24 families were represented in the eDNA dataset, of which Thripidae (*Thrips australis*, *T. tabaci*, *Frankliniella* sp. and *Megalurothrips* sp.: 80% of inflorescence samples), Apidae (*Apis mellifera*: 26% of inflorescence samples), as well as Sciaridae (*Lycoriella castanescens* and Sciaridae sp.: 25% of inflorescence samples) were the most common. In total, 38 taxa were identified by eDNA, with 10 (26%) resolved to genus level, 23 (61%) to species level, while 5 (13%) could not be resolved beyond family level. Taxa included potential pollinators (e.g. *Apis mellifera* and *Simosyrphus grandicornis*), pests (e.g. Helicidae and Limacidae sp.) and parasites (e.g. Eriophyidae sp.). Average arthropod size was not found to correlate with eDNA detection frequency ($p = .98$). For GLM testing, the species counts for all arthropods, Diptera and Hymenoptera returned dispersion values of 1.16, 0.98 and 0.87 respectively, potentially indicating overdispersion for all arthropods and underdispersion for Hymenoptera. The percentage of zeros was found to be low for all arthropods (6%), but large for both the Diptera (57%) and Hymenoptera (72%) datasets. When simulated however, we found that the majority of the zeros for both Diptera and Hymenoptera GLMs could be explained (Figure S1) and concluded that our models were capable of accounting for the zeros in both groups. AIC testing indicated that sample tree was a non-significant co-variate for all three models and was removed (Tables S2–S4). Subsequently, the dispersion values for all three models were found to be close to 1 (Figure S2). With robust standard errors, we found that for all arthropods and Hymenoptera, inflorescence location was a significant explanatory co-variate ($p = .05$ in both instances) (Table S5). Indicating that samples collected from the understorey (<2 m) yielded more arthropod species overall and more Hymenoptera species than samples collected from the upper storey (>2 m), while date of collection was not found to be a significant co-variate for either all arthropods or Hymenoptera ($p = .35$ and .92). For Diptera, neither inflorescence location ($p = .76$) nor sampling day ($p = .25$) were found to be significant explanatory co-variates (Table S5).

3.3 | DVR monitoring

Of the 14,032 flower visits observed across 96 h of DVRs, 35 taxa were identified: 18 (52%) to family level, 12 (34%) to species level and 5 (14%) could not be resolved beyond the level of order. In total, the DVR dataset comprised 23 families, of which hoverflies (*Simosyrphus grandicornis* and *Melangyna viridiceps*) were the most numerous visitors (89% of all flower visits with 130 ± 15.5 SE visits per hour), followed by the European honeybee (*Apis mellifera*) (7% of all flower visits with 10 ± 1.1 SE visits per hour) and non-syrphid Diptera species (Calliphoridae sp. and Muscidae sp.) (3% of all flower visits with 4 ± 1.1 SE visits per hour). Flower visits by moth species (*Phrissogonus laticostata* and *Plutella xylostella*) and native wasp species (*Polistes humilis* and Bethylidae sp.) were rare (<1 flower visit per hour). The percentage of zeros was found to be zero for all arthropods, Diptera and Hymenoptera. The skewness values generated were 0.63 for all arthropods, 0.54 for Diptera, and 0.76 for Hymenoptera, indicating a mild positive skews. The kurtosis values were −0.31 for all arthropods, −1.24 for Diptera and −0.24 for Hymenoptera, indicating platykurtic distributions. As with the pan trap dataset, we employed non-parametric testing and found that neither sample tree nor date of collection significantly influenced all arthropods, Diptera or Hymenoptera species counts (Table S5).

3.4 | Pan traps

A total of 499 individual arthropods were collected from the pan traps, with 35 taxa identified of which 21 (60%) were resolved to family level, 6 (17%) to species level, and 8 (23%) could not be resolved beyond order level. In total, 28 families were represented in the pan trap dataset and among these, the three most common taxa were all members of Diptera; Drosophilidae sp. (33%), Phoridae sp. (22%) and Dolichopodidae sp. (6%). Unlike the eDNA results, the pan traps also showed the presence of three native bee species: *Lipotriches flavoviridis* (Halictidae), *Lasioglossum hapsidum* (Halictidae), and *L. castor* (Halictidae). The data for all arthropods, Diptera, and Hymenoptera were all found to be non-normally distributed. The percentage of zeros was found to be zero for all three datasets. The skewness values were 1.58 for all arthropods, −1.01 for Diptera and 1.02 for Hymenoptera, indicating a positive skew for both all arthropods and Hymenoptera and a negative skew for Diptera. All arthropods had a leptokurtic tail shape (kurtosis value = 2.99), while both Diptera and Hymenoptera had mesokurtic tail shapes (kurtosis values = 0.43 and 0.24, respectively). Using non-parametric testing, the species counts for all three pan trap datasets did not show significant variation between sample trees or collection date (Table S5).

3.5 | Three method comparison

Overall, eDNA collected from flowers detected the greatest diversity of arthropod families of all three collection methods (Figure 2a and Table 1). Although none of the three methods alone appeared to capture the total arthropod diversity present within the orchard (Figure S3). Arthropod family composition recorded by each survey method showed clear partitioning (Figure 2b; ANOSIM, $p < .01$). Alpha diversity index values at both the family and species level did not differ significantly between the three survey methods (Figure 2c; Kruskal–Wallis test, $p = .10$). While the number of all arthropod species detected per tree did not vary significantly between the three survey methods, the number of Diptera and Hymenoptera species captured by eDNA were significantly lower

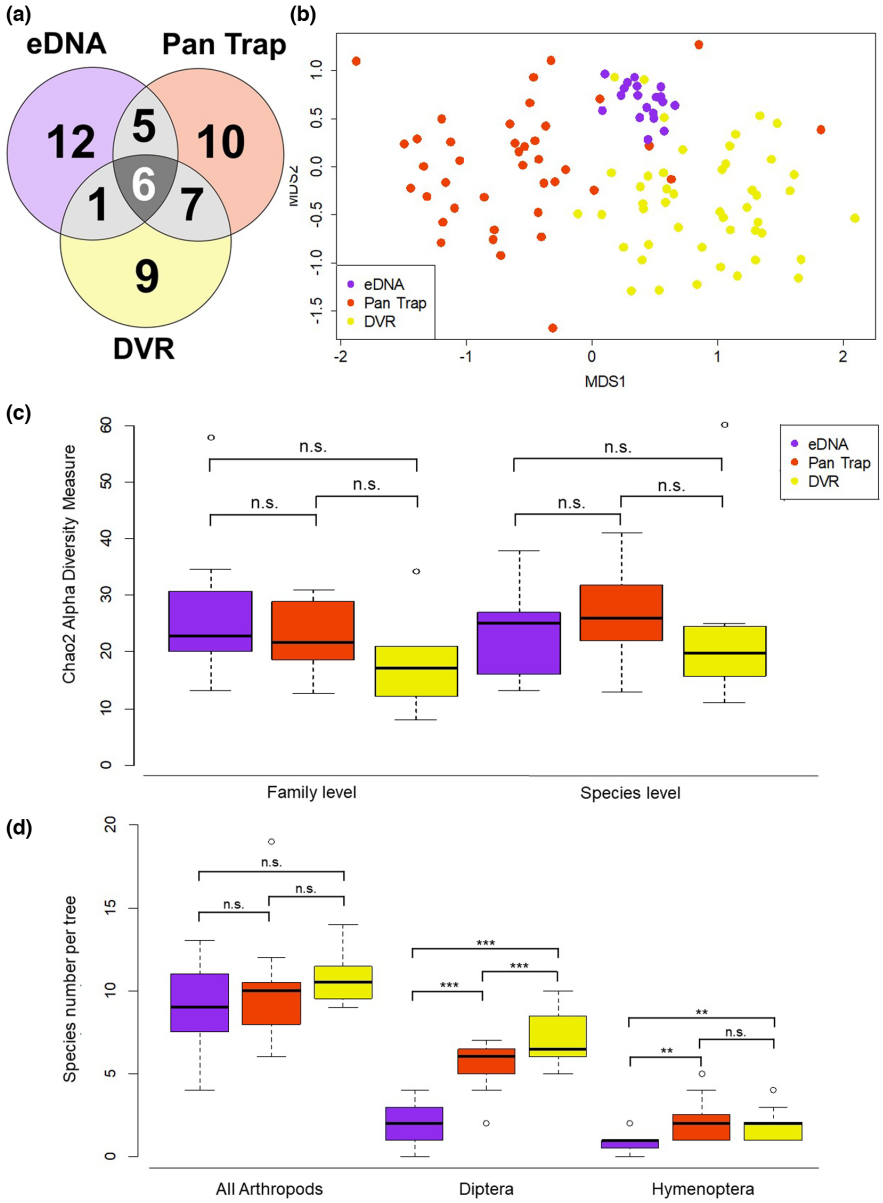

FIGURE 2 (a) Number of families identified for each survey method; eDNA ($N=24$), DVR ($N=23$) and Pan Trap ($N=28$). (b) Non-metric multidimensional scaling ordination (Stress value = 0.1098) showing the relationship between arthropod family assemblage and survey method based on a Jaccard dissimilarity matrix for factor method. (c) Chao2 alpha diversity measures based on presence-absence data for arthropod families and species. Chao2 values were calculated, per survey method, by pooling all samples over both collection dates for each tree (eDNA; $N=10$ per tree, DVR; $N=4$ per tree, pan trap; $N=6$ per tree) and calculated using the package 'fossil' in R. (d) Dunn Tests generated for all arthropod species collected per tree and both major flower-visiting arthropod groups for *Persea americana* (Diptera and Hymenoptera) for the three methods (eDNA; $N=80$; Pan trap $N=48$; DVR $N=32$). *p*-Values were adjusted with the Benjamini-Hochberg method to correct for Type 1 errors. Significance values; n.s. = $p > .05$, * = $p \leq .05$, ** = $p \leq .01$ and *** = $p \leq .001$.

when compared to pan traps and DVRs (Figure 2d; Dunn tests, $p < .01$). Collection method was also found to have a significant effect on the number of taxa recorded for both Diptera ($p < .01$) and Hymenoptera ($p < .01$), but not for all arthropod taxa ($p = .24$). Per sample, DVRs captured the greatest diversity of arthropod families (6 ± 0.35 SE), followed by pan traps (4 ± 0.27 SE), and eDNA (3 ± 0.19 SE) (Dunn tests, $p < .001$). Similarly at the level of species, DVRs captured the greatest diversity (7 ± 0.39 SE), followed by pan traps (4 ± 0.27 SE), and eDNA (3 ± 0.21 SE) (Dunn tests, $p < .001$). A post-hoc test for Diptera showed that the three collection methods differed significantly for the number of species collected, with DVRs detecting the largest number of Diptera species (7 ± 0.39 SE), followed by pan traps (6 ± 0.33 SE), and eDNA (1 ± 0.35 SE). For Hymenoptera, the species counts between eDNA (1 ± 0.27 SE) and DVRs (2 ± 0.21 SE) as well as DVRs and pan traps (3 ± 0.30 SE) were comparable, with the only significant difference found between eDNA and pan traps (Figure 2d).

4 | DISCUSSION

Globally, ~40% of all insect species, both managed and unmanaged, could be at risk of extinction in coming decades as a consequence of climate change, loss of habitat, pesticide use, as well as vulnerability to emerging pests and pathogens (Sánchez-Bayo & Wyckhuys, 2019). Improved arthropod survey methods are therefore increasingly necessary to complement current survey techniques and allow reliable and timely detections of both beneficial and antagonistic arthropod species (D'Souza et al., 2021; Evans & Kitson, 2020; Newton et al., 2023). Here, we show that when combined with traditional survey methods, metabarcoding of eDNA collected from flowers can increase the number of arthropod families detected by 25%. Consistent with previous studies, eDNA metabarcoding allowed efficient and reliable detections of potential pollinators, as well as plant pests and parasites without the need for extensive taxonomic expertise (Gamonal Gomez et al., 2023;

TABLE 1 Taxonomic identifications of the 49 arthropod families found between the three survey methods (eDNA, Pan Trap and DVR) at Marron Brook Farm between 30/10/2020 and 31/10/2020.

Order	Family	eDNA	Pan trap	DVR
Diptera 	Agromyzidae	□	■	□
	Calliphoridae	□	■	■
	Chamaemyiidae	□	■	□
	Chironomidae	■	■	□
	Chloropidae	■	■	■
	Culicidae	■	□	□
	Dolichopodidae	□	■	□
	Drosophilidae	■	■	■
	Ephydridae	□	□	■
	Muscidae	■	□	■
	Phoridae	□	■	□
	Sarcophagidae	□	□	■
	Sciaridae	■	■	□
	Syrphidae	■	■	■
Hymenoptera 	Apidae	■	■	■
	Bethylidae	□	■	■
	Braconidae	■	■	■
	Formicidae	□	■	■
	Halictidae	□	■	■
	Mutillidae	□	■	□
	Pompilidae	□	□	■
	Vespidae	□	□	■
Other	Acrididae	□	■	■
	Bourletiellidae	■	■	□
	Caeciusidae	■	■	□
	Chrysopidae	■	□	□
	Cicadellidae	□	■	□
	Coccinellidae	□	□	■
	Curculionidae	□	■	□
	Ectopsocidae	■	□	□
	Eriophyidae	■	□	□
	Geometridae	■	■	■
	Helicidae	■	□	□
	Latridiidae	■	□	□
	Limacidae	■	□	□
	Miridae	■	■	■
	Nitidulidae	■	□	□
	Noctuidae	■	□	□
	Phlaeothripidae	■	□	□
	Plutellidae	■	□	■
	Staphylinidae	□	■	□
	Thomisidae	□	□	■
	Thripidae	■	■	□
	Tydeidae	■	□	□
	Unknown families (Number)	□ (0)	■ (3)	■ (3)

Note: The main flower-visiting orders, as determined by DVRs; Diptera and Hymenoptera, are highlighted. Shaded boxes indicate presence. The unknown families for Pan Traps were: Termite sp. (order Isoptera), elongated fly sp. (order Nematocera) and unclassified fly spp. (order Diptera). While the unknown families for DVR were: beetle spp. (order Coleoptera), unclassified fly spp. (order Diptera) and unclassified sp. (order unknown).

Johnson et al., 2023; Newton et al., 2023). The accuracy of this molecular method is, however, dependent on the quantity and quality of arthropod eDNA, the presence of non-target DNA contamination, the sequence analysis method chosen, and the availability of arthropod sequences in online databases (Evans & Kitson, 2020; Ficetola et al., 2016; Valentin et al., 2021). Importantly, this study demonstrates that no single survey method can capture the complete range of taxa foraging on the avocado orchards. For instance, eDNA detected the highest levels of arthropod family diversity overall, but family diversity levels for the main *P. americana* flower visitors—Diptera and Hymenoptera—were significantly higher in pan traps and DVR devices, respectively, compared to the eDNA results. While eDNA metabarcoding of flowers has the potential to revolutionize the way arthropod communities are monitored, as applications continue to diversify, it is crucial that novel uses are validated against longstanding survey methods, and that the relative strengths and weaknesses of each are evaluated.

4.1 | Complementarity of three survey methods

Using three survey methods, we detected a wide range of arthropod taxa, and while alpha diversity was similar, each method characterized a different arthropod community from one another, and only represented a fraction of the total diversity. However, the most common flower-visiting insects - hoverflies (Syrphidae spp. 130 ± 15.5 SE visits per hour) and European honey bees (*Apis mellifera* 10 ± 1.1 SE visits per hour)—were detected by all three methods.

It is unclear why each survey method detected a different arthropod community, but it is likely that different detection biases are associated with each method. For example, Drosophilidae sp. were the most common insects found in the pan traps (62% of samples), but they were not observed by DVR devices (likely due to camera resolution), and only detected once by eDNA metabarcoding (Figure S4). These findings complement those of Newton et al. (2023), where only two of the total 57 arthropod taxa were shared between eDNA metabarcoding of flowers and visual surveys. Indeed, compared to the arthropod communities described by DVRs and eDNA, the pan trap dataset likely represents a broader community of both Diptera and Hymenoptera than just flower-visiting arthropods (e.g. only pan traps detected members from Dolichopodidae, the adults and larvae of which are generally insectivorous; see Zborowski & Storey, 2017), thereby potentially increasing the dissimilarity between the three methods (Kelly et al., 2017; Leempoel et al., 2020). Similarly, the presence of DNA from non-flower-visiting pest and a parasite taxa likely increased the dissimilarity between the three survey methods. For instance, only eDNA detected herbivorous pests belonging to Helicidae, Limacidae and Eriophyidae (see Berlander & Baker, 2007), which, although not nectivorous, may have left traces of DNA while moving across floral tissue or during feeding (see Kudoh et al., 2020). As well as highlighting the differences within each method, the three-survey method approach also helped to identify the taxonomic gaps in the eDNA dataset.

The inclusion of DVRs highlighted the presence of false negatives in the eDNA metabarcoding dataset. For example, some families (e.g. Calliphoridae, Sarcophagidae, and Pompilidae) were observed by DVRs visiting flowers, but were absent in the eDNA dataset. A previous study by Thomsen and Sigsgaard (2019) also found that metabarcoding of arthropod eDNA on flowers failed to detect common flower visitors, possibly because of poor efficacy of available assays for some taxa. For instance, the AT rich mitochondrial genomes of Hymenoptera and Hemiptera form homopolymer regions which are difficult to amplify and sequence (Hebert et al., 2016). Additionally, given that most crop flower visits are brief (e.g. Rader et al., 2012), the opportunities for arthropod DNA deposition that is detectable by metabarcoding may be limited. As a consequence, we stress that eDNA metabarcoding is currently unable to show which taxa are the most frequent flower-visitors, and potentially the most likely to affect pollination, due to the lack of abundance data (see Mathon et al., 2021), as well as the presence of incidental non-target DNA (e.g. herbivorous pest taxa; see Kudoh et al., 2020). We therefore recommend a combined approach of eDNA metabarcoding with DVR devices to utilize the significant taxonomic resolution afforded by eDNA with the abundance and behavioural data available from DVRs.

4.2 | Assay, study design and database considerations

Metabarcoding of arthropod DNA on flowers is a nascent method for measuring arthropod diversity, but one that holds great promise for the future. As an emerging monitoring tool however, we identify potential areas that can be incorporated into future studies to help improve its accuracy and reliability in agricultural systems. First, further development of eDNA metabarcoding assays that detect a broader range of agriculturally important flower-visiting taxa (e.g. Diptera, Lepidoptera and Hymenoptera sp.), or amplify shorter amplicons in regions that minimize sequencing errors (Leese et al., 2021; Marquina et al., 2019), would improve the robustness of eDNA-based surveys by reducing the frequency of false positive and negative species identifications. Second, incorporating additional complementary methods with eDNA surveys can help overcome the limitations of presence-absence data (Johnson et al., 2023; Newton et al., 2023). Third, we recommend that detection thresholds, established with pilot studies, are incorporated into eDNA studies to determine DNA deposition and degradation rates from different arthropod species, as well as quantifying the size range of fragments that successfully amplify (Valentin et al., 2021). These studies could incorporate locally relevant abiotic and biotic factors (e.g. UV light or microbial activity; see Strickler et al., 2015) to provide temporal caveats for species assemblages generated from eDNA detections (Macher et al., 2016). An a priori understanding of these caveats would help improve the accuracy of eDNA survey techniques because they could account for rapid DNA degradation with either more frequent sampling (see Krehenwinkel et al., 2018), or targeting of shorter amplicons (see Saito & Doi, 2021). By incorporating these recommendations, metabarcoding of arthropod DNA on flowers

could generate reliable and accurate classifications of flower-visitor communities, while also accounting for the biotic (e.g. DNA deposition) and abiotic (e.g. UV levels) factors that can often influence the accuracy of eDNA-based surveys.

Robust eDNA metabarcoding based detection of arthropods important to agricultural systems have, to date, required combinations of assays targeting CO1 and 16S, paired with a custom reference library to achieve broad taxonomic coverage (Clarke et al., 2014; Magoga et al., 2022; Thomsen & Sigsgaard, 2019). This approach maximized the amount of recoverable taxa because ribosomal genes show less taxonomic bias (Deagle et al., 2014), while the reference libraries developed for CO1 are more extensive and offer higher taxonomic resolution (Elbrecht et al., 2019). In the present study, we found that the addition of an assay targeting 16S helped to identify three insect families that were not identified with CO1 (Figure S5). Compared to CO1, the 16S region has a limited number of reference sequences for arthropods, meaning that many of the potentially flower-visiting taxa present in a given agricultural system may erroneously appear as absent (Ficetola et al., 2015; Thomsen & Sigsgaard, 2019). In the context of monitoring agricultural systems, false negatives for flower-visitors could misinform management decisions and increase expenditure (e.g. unnecessarily hiring more managed bee hives to increase pollination services; see Ish-Am & Eisikowitch, 1998). This problem is notable because only ~20% of all insect taxa have been morphologically described (Stork, 2018). Hence, in countries like Australia, with more than 70,000 insect species, only a small proportion of specimens have been vouchered and barcoded (Rougerie et al., 2014; Zborowski & Storey, 2017), although globally the completeness of reference libraries is improving (Kjærandsen, 2022; Magoga et al., 2022; Taylor et al., 2018). While it remains possible to ignore this issue and analyse metabarcoding data without a custom reference library (see Aizpurua et al., 2017; Moran et al., 2019), we reason that, despite using two assays, the lack of barcode reference sequences in our study decreased the diversity of floral visitors detected by eDNA. In practice, without complementary surveys or prior verification (e.g. pilot surveys; see Goldberg et al., 2018), eDNA surveys may underestimate the diversity of both beneficial and antagonistic agricultural arthropod taxa. Therefore, we recommend using active and/or passive survey techniques, such as sweep netting and pan traps, to capture local arthropod specimens (Saunders & Luck, 2013; Spafford & Lortie, 2013). These specimens can then be identified morphologically and compared against the NCBI and BOLD databases to determine if barcode regions from these species have already been sequenced.

4.3 | Potential applications of eDNA metabarcoding of flowers

Either complementing long-standing survey approaches, or used on its own, eDNA metabarcoding offers many opportunities to improve the characterization of plant–animal visitor networks within agroecosystems. For example, unmanaged non-bee flower-visitors have historically been omitted from crop pollination studies, in part because observing flower-visitors and identifying pollen grains are time-intensive and require specialist expertise that is not always readily available, and becoming rarer (Bell et al., 2016; Bosch et al., 2009; Rader et al., 2016). As a consequence, the pollination services offered by these unmanaged taxa as well as the food resources they require remains largely unexplored (Potts et al., 2016). By targeting pollen accumulated on the bodies of arthropods (arthropod-centric sampling), previous eDNA studies have been able to classify unobserved nocturnal pollen transport networks (Macgregor et al., 2019), as well as detecting broader foraging resources for an economically damaging pest species—the turnip moth (*Agrotis segetum*)—than formerly reported in the literature (see Chang et al., 2018). If combined with eDNA metabarcoding of flowers (plant-centric sampling), researchers could target arthropod DNA on floral structures significant for pollination (e.g. stigma or anthers) to help determine if these unmanaged taxa are providing neutral (no effect), facilitative (e.g. pollination) or resource parasitism (e.g. only harvesting pollen) interactions (Evans & Kitson, 2020; Rathcke, 1983). Such targeted eDNA sampling could be incorporated with single visit deposition (SVD) (see King et al., 2013), often used for crop pollination studies, to help improve the accuracy of morphological classifications for both flower visitors and the pollen they deposit (Pornon et al., 2017). Furthermore, eDNA metabarcoding of flowers could be used to compare arthropod diversity at different flowering stages (e.g. dichogamy), or between flowering populations in separate geographical locations. The information generated from such studies could then be used by practitioners to encourage or suppress these unmanaged species, depending on their relationship to the crop under study, potentially helping increase the resilience of plant-pollinator networks within these agroecosystems (Kestel et al., 2022). The use of eDNA metabarcoding of flowers also holds potential in natural systems to both accurately identify flower-visiting species for previously undocumented plant species (see Newton et al., 2023) and assist in the detection of pest taxa before they become established in natural habitats, especially those in remote or difficult to access locations.

5 | CONCLUSION

We compared the arthropod communities detected with eDNA metabarcoding of flowers from *P. americana* with two commonly used survey techniques: DVR devices and pan traps. In total, 80 eDNA flower samples, 96 h of DVRs showing 14,032 flower visits, and 48 pan trap samples containing 499 arthropods were collected. This study confirms that eDNA metabarcoding of flowers can increase the number of arthropod families detected by 25% when combined with conventional DVR devices and pan traps. When comparing family diversity levels for the main *P. americana* flower visitors – Diptera and

Hymenoptera—we found significantly higher diversity levels with pan traps and DVR devices, respectively, compared to eDNA. We suggest that the accuracy and reliability of eDNA metabarcoding of flowers could be improved by: (i) further development of eDNA metabarcoding assays to target agriculturally important flower-visiting taxa, (ii) including complementary methods to generate behavioural and count data for the flower-visiting species of interest (e.g. DVR devices), (iii) pilot studies to establish locally relevant eDNA detection thresholds, and (iv) sequencing of barcode regions from key taxa missing from the NCBI and BOLD databases. Overall, we have demonstrated that eDNA metabarcoding of flowers has the potential to transform the way arthropod communities are monitored in general, potentially detecting the response of pollinators and pests to climate change, diseases, habitat loss and other disturbances.

AUTHOR CONTRIBUTIONS

Joshua H. Kestel: Conceptualization, methodology, visualization, writing—original draft. David L. Field: Conceptualization, writing—original draft, writing—review & editing. Philip W. Bateman: Conceptualization, methodology, writing—original draft. Rose Lines: Conceptualization, writing—original draft, methodology, writing—review & editing. Nicole E White: Writing—original draft, methodology, writing—review & editing. Paul Nevill: Conceptualization, writing—original draft, methodology, writing—review & editing.

ACKNOWLEDGEMENTS

We acknowledge the traditional owners of the land on which the research presented here was undertaken, the Whadjuk Noongar and Bibulmun/Piblemen people, and pay our respects to Elders past, present and emerging. We are also indebted to Jo-Anne Kestel, Terry Kestel and Diana Adorno for generously helping with the field work. We also thank David Knowles for identifying the arthropod specimens and Doug Pow for allowing us to access his orchard during the 2020 flowering season. Open access publishing facilitated by Curtin University, as part of the Wiley - Curtin University agreement via the Council of Australian University Librarians.

The development and application of this project "eDNA for the classification and management of plant pollinator networks in an agroecosystem" is funded by the Hort frontiers Pollination fund, part of the Hort frontiers partnership initiative developed by Hort Innovation, with co-investment from the Avocado Research and Development levy, Curtin University, and through funding from the Australian Government in partnership with South West Catchments Council.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

Sequencing and sample data and DADA2 script are available at https://datadryad.org/stash/share/wjeCMSIu431ckJBO7U1k8P6Ip_kBnrAxtkUO_II_E1s on the Dryad Digital repository.

ORCID

Joshua H. Kestel https://orcid.org/0000-0002-3159-5559
Philip W. Bateman https://orcid.org/0000-0002-3036-5479
David L. Field https://orcid.org/0000-0002-4014-8478
Nicole E. White https://orcid.org/0000-0002-0068-6693
Paul Nevill https://orcid.org/0000-0001-8238-0534

REFERENCES

Abrahamczyk, S., Steudel, B., & Kessler, M. (2010). Sampling hymenoptera along a precipitation gradient in tropical forests: The effectiveness of different coloured pan traps. *Entomologia Experimentalis et Applicata*, *137*(3), 262–268. https://doi.org/10.1111/j.1570-7458.2010.01063.x

Aizen, M. A., Garibaldi, L. A., Cunningham, S. A., & Klein, A. M. (2009). How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. *Annals of Botany*, *103*(9), 1579–1588. https://doi.org/10.1093/aob/mcp076

Aizpurua, O., Budinski, I., Georgiakakis, P., Gopalakrishnan, S., Ibanez, C., Mata, V., Rebelo, H., Russo, D., Szodoray-Parádi, F., Zhelyazkova, V., Zrncic, V., Gilbert, M. T. P., & Alberdi, A. (2017). Agriculture shapes the trophic niche of a bat preying on multiple pest arthropods across Europe: Evidence from DNA metabarcoding. *Molecular Ecology*, *27*(3), 815–825. https://doi.org/10.1111/mec.14474

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Andrews, S. (2010). *FastQC: A quality control tool for high throughput sequence data*. Babraham Bioinformatics, Babraham Institute.

Baker, H. (1979). Anthecology: Old testament, New testament, Apocrypha (Banquet address, 8 February 1979). *New Zealand Journal of Botany*, *17*(4), 431–440. https://doi.org/10.1080/0028825X.1979.10432563

Barrios, E. (2007). Soil biota, ecosystem services and land productivity. *Ecological Economics*, *64*(2), 269–285. https://doi.org/10.1016/j.ecolecon.2007.03.004

Bell, K. L., De Vere, N., Keller, A., Richardson, R. T., Gous, A., Burgess, K. S., & Brosi, B. J. (2016). Pollen DNA barcoding: Current applications and future prospects. *Genome*, *59*(9), 629–640. https://doi.org/10.1139/gen-2015-0200

Bergholz, K., Sittel, L. P., Ristow, M., Jeltsch, F., & Weiss, L. (2022). Pollinator guilds respond contrastingly at different scales to landscape parameters of land-use intensity. *Ecology and Evolution*, *12*(3), e8708. https://doi.org/10.1002/ece3.8708

Berlander, F., & Baker, G. (2007). Oilseeds: Winter oilseeds. In P. Bailey (Ed.), *Pests of field crops and pastures: Identification and control* (1st ed., pp. 135–154). CSIRO Publishing.

Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G., Kleukers, R., Thomas, C. D., Settele, J., & Kunin, W. E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and The Netherlands. *Science*, *313*(5785), 351–354. https://doi.org/10.1126/science.1127863

Bonelli, M., Melotto, A., Minici, A., Eustacchio, E., Gianfranceschi, L., Gobbi, M., Casartelli, M., & Caccianiga, M. (2020). Manual sampling and video observations: An integrated approach to studying flower-visiting arthropods in high-mountain environments. *Insects*, *11*(12), 881. https://doi.org/10.3390/insects11120881

Bosch, J., Martín González, A. M., Rodrigo, A., & Navarro, D. (2009). Plant-pollinator networks: Adding the pollinator's perspective. *Ecology Letters*, *12*(5), 409–419. https://doi.org/10.1111/j.1461-0248.2009.01296.x

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., & Coissac, E. (2016). Obitools: A unix-inspired software package for DNA metabarcoding. *Molecular Ecology Resources*, *16*(1), 176–182. https://doi.org/10.1111/1755-0998.12428

Brown, B. (2020). Sampling methods for adult flies (Diptera). In J. Santos (Ed.), *Measuring arthropod biodiversity: A handbook of sampling methods* (pp. 187–204). Springer Nature Switerland. https://doi.org/10.1007/978-3-030-53226-0

Cameron, C., & Trivedi, P. (2009). *Microeconomics using stata*. Stata Press. https://scholar.google.com/scholar?hl=en&q=Cameron+and+Trivedi+%282009%29+%22Microeconometrics+Using+Stata&btnG=&as_sdt=1%2C47&as_sdtp=#1

Capo, E., Spong, G., Koizumi, S., Puts, I., Olajos, F., Königsson, H., Karlsson, J., & Byström, P. (2021). Droplet digital PCR applied to environmental DNA, a promising method to estimate fish population abundance from humic-rich aquatic ecosystems. *Environmental DNA*, *3*(2), 343–352. https://doi.org/10.1002/edn3.115

Chang, H., Guo, J., Fu, X., Liu, Y., Wyckhuys, K. A. G., Hou, Y., & Wu, K. (2018). Molecular-assisted pollen grain analysis reveals spatiotemporal origin of long-distance migrants of a noctuid moth. *International Journal of Molecular Sciences*, *19*(2), 567. https://doi.org/10.3390/ijms19020567

Chao, A. (1984). Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics*, *11*(4), 265–270.

Clare, E. L., Economou, C. K., Faulkes, C. G., Gilbert, J. D., Bennett, F., Drinkwater, R., & Littlefair, J. E. (2021). eDNAir: Proof of concept that animal DNA can be collected from air sampling. *PeerJ*, *9*, e11030. https://doi.org/10.7717/peerj.11030

Clarke, L. J., Soubrier, J., Weyrich, L. S., & Cooper, A. (2014). Environmental metabarcodes for insects: In silico PCR reveals potential for taxonomic bias. *Molecular Ecology Resources*, *14*(6), 1160–1170. https://doi.org/10.1111/1755-0998.12265

Cook, D. F., Voss, S. C., Finch, J. T. D., Rader, R. C., Cook, J. M., & Spurr, C. J. (2020). The role of flies as pollinators of horticultural crops: An Australian case study with worldwide relevance. *Insects*, *11*, 23–31. https://doi.org/10.3390/insects11060341

Deagle, B. E., Jarman, S. N., Coissac, E., Pompanon, F., & Taberlet, P. (2014). DNA metabarcoding and the cytochrome c oxidase subunit I marker: Not a perfect match. *Biology Letters*, *10*(9), 20140562. https://doi.org/10.1098/rsbl.2014.0562

Droissart, V., Azandi, L., Onguene, E. R., Savignac, M., Smith, T. B., & Deblauwe, V. (2021). PICT: A low-cost, modular, open-source camera trap system to study plant–insect interactions. *Methods in Ecology and Evolution*, *12*(8), 1389–1396. https://doi.org/10.1111/2041-210X.13618

D'Souza, M. L., van der Bank, M., Shongwe, Z., Rattray, R. D., Stewart, R., van Rooyen, J., Govender, D., & Hebert, P. D. N. (2021). Biodiversity baselines: Tracking insects in Kruger National Park with DNA barcodes. *Biological Conservation*, *256*, 109034. https://doi.org/10.1016/j.biocon.2021.109034

Ebeling, A., Klein, A. M., Schumacher, J., Weisser, W. W., & Tscharntke, T. (2008). How does plant richness affect pollinator richness and temporal stability of flower visits? *Oikos*, *117*(12), 1808–1815. https://doi.org/10.1111/j.1600-0706.2008.16819.x

Edgar, R. C. (2016). UNOISE2: Improved error-correction for Illumina 16S and ITS amplicon sequencing. *BioRxiv*, 081257. https://doi.org/10.1101/081257

Elbrecht, V., Braukmann, T., Ivanova, N., Prosser, S., Hajibabaei, M., Wright, M., Zakharov, E. V., Hebert, P. D. N., & Steinke, D. (2019). Validation of COI metabarcoding primers for terrestrial arthropods. *PeerJ*, *7*, e7745. https://doi.org/10.7717/peerj.7745

Elbrecht, V., & Leese, F. (2015). Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass – sequence relationships with an innovative metabarcoding protocol. *PLoS One*, *10*(7), e0130324. https://doi.org/10.1371/journal.pone.0130324

Esling, P., Lejzerowicz, F., & Pawlowski, J. (2015). Accurate multiplexing and filtering for high-throughput amplicon-sequencing. *Nucleic Acids Research*, *43*(5), 2513–2524. https://doi.org/10.1093/nar/gkv107

Evans, D. M., & Kitson, J. J. (2020). Molecular ecology as a tool for understanding pollination and other plant–insect interactions. *Current Opinion in Insect Science*, *38*, 26–33. https://doi.org/10.1016/j.cois.2020.01.005

Ficetola, G., Pansu, J., Bonin, A., Coissac, E., Giguet-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. *Molecular Ecology Resources*, *15*(3), 543–556. https://doi.org/10.1111/1755-0998.12338

Ficetola, G. F., Taberlet, P., & Coissac, E. (2016). How to limit false positives in environmental DNA and metabarcoding? *Molecular Ecology Resources*, *16*(3), 604–607. https://doi.org/10.1111/1755-0998.12508

Frøslev, T. G., Kjøller, R., Bruun, H. H., Ejrnæs, R., Brunbjerg, A. K., Pietroni, C., & Hansen, A. J. (2017). Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nature Communications*, *8*(1), 1188. https://doi.org/10.1038/s41467-017-01312-x

Gamonal Gomez, N., Sørensen, D. H., Chua, P. Y. S., & Sigsgaard, L. (2023). Assessing flower-visiting arthropod diversity in apple orchards through metabarcoding of environmental DNA from flowers and visual census. *Environmental DNA*, *5*(1), 117–131. https://doi.org/10.1002/edn3.362

Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A., Kremen, C., Carvalheiro, L. G., Harder, L. D., Afik, O., Bartomeus, I., Benjamin, F., Boreux, V., Cariveau, D., Chacoff, N. P., Dudenhöffer, J. H., Freitas, B. M., Ghazoul, J., Greenleaf, S., … Klein, A. M. (2013). Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science*, *339*(6127), 1608–1611. https://doi.org/10.1126/science.1230200

Gervais, A., Chagnon, M., & Fournier, V. (2018). Diversity and pollen loads of flower flies (Diptera: Syrphidae) in cranberry crops. *Annals of the Entomological Society of America*, *111*(6), 326–334. https://doi.org/10.1093/aesa/say027

Gilpin, A. M., Denham, A. J., & Ayre, D. J. (2017). The use of digital video recorders in pollination biology. *Ecological Entomology*, *42*(4), 383–388. https://doi.org/10.1111/een.12394

Goldberg, C. S., Strickler, K. M., & Fremier, A. K. (2018). Degradation and dispersion limit environmental DNA detection of rare amphibians in wetlands: Increasing efficacy of sampling designs. *Science of the Total Environment*, *633*, 695–703. https://doi.org/10.1016/J.SCITOTENV.2018.02.295

Goulson, D., & Hughes, W. O. H. (2015). Mitigating the anthropogenic spread of bee parasites to protect wild pollinators. *Biological Conservation*, *191*, 10–19. https://doi.org/10.1016/j.biocon.2015.06.023

Gray, A., Adjlane, N., Arab, A., Ballis, A., Brusbardis, V., Charrière, J. D., Chlebo, R., Coffey, M. F., Cornelissen, B., da Costa, C. A., Dahle, B., Danihlík, J., Dražić, M. M., Evans, G., Fedoriak, M., Forsythe, I., Gajda, A., de Graaf, D. C., Gregorc, A., … Brodschneider, R. (2020). Honey bee colony winter loss rates for 35 countries participating in the COLOSS survey for winter 2018–2019, and the effects of a new queen on the risk of colony winter loss. *Journal of Apicultural Research*, *59*(5), 744–751. https://doi.org/10.1080/00218839.2020.1797272

Hebert, P. D. N., Ratnasingham, S., Zakharov, E. V., Telfer, A. C., Levesque-Beaudin, V., Milton, M. A., Pedersen, S., Jannetta, P., & Dewaard, J. R. (2016). Counting animal species with DNA barcodes: Canadian insects. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1702), 20150333. https://doi.org/10.1098/rstb.2015.0333

Hein, L. (2009). The economic value of the pollination service, A review across scales. *The Open Ecology Journal*, *2*, 74–82. https://doi.org/10.2174/1874213000902010074

Howlett, B. G., Evans, L. J., Kendall, L. K., Rader, R., McBrydie, H. M., Read, S. F. J., Cutting, B. T., Robson, A., Pattemore, D. E., &

Willcox, B. K. (2018). Surveying insect flower visitors to crops in New Zealand and Australia. *BioRxiv*, 373126. https://doi.org/10.1101/373126

Ish-Am, G. (2005). *Avocado pollination: A review. New Zealand and Australia Avocado Grower's Conference '05*, *9*. Avocado Source.

Ish-Am, G., & Eisikowitch, D. (1998). Low attractiveness of avocado (*Persea americana* Mill.) flowers to honeybees (*Apis mellifera* L.) limits fruit set in Israel. *The Journal of Horticultural Science and Biotechnology*, *73*(2), 195–204. https://doi.org/10.1080/14620316.1998.11510965

Johnson, M. D., Katz, A. D., Davis, M. A., Tetzlaff, S., Tomczyk, S., Edlund, D., Tomczyk, S., Molano-Flores, B., Wilder, T., & Sperry, J. H. (2023). Environmental DNA metabarcoding from flowers reveals arthropod pollinators, plant pests, parasites, and potential predator-prey interactions while revealing more arthropod diversity than camera traps. *Environmental DNA*, *5*(3), 551–569. https://doi.org/10.1002/edn3.411

Jordan, A., Patch, H. M., Grozinger, C. M., & Khanna, V. (2021). Economic dependence and vulnerability of United States agricultural sector on insect-mediated pollination service. *Environmental Science and Technology*, *55*(4), 2243–2253. https://doi.org/10.1021/acs.est.0c04786

Joshi, N. K., Leslie, T., Rajotte, E. G., Kammerer, M. A., Otieno, M., & Biddinger, D. J. (2015). Comparative trapping efficiency to characterize bee abundance, diversity, and community composition in apple orchards. *Annals of the Entomological Society of America*, *108*(5), 785–799. https://doi.org/10.1093/aesa/sav057

Kearns, C. A., & Inouye, D. W. (1993). In D. W. Inouye (Ed.), *Techniques for pollination biologists* (1st ed.). University Press of Colorado.

Kelly, R. P., Closek, C. J., O'Donnell, J. L., Kralj, J. E., Shelton, A. O., & Samhouri, J. F. (2017). Genetic and manual survey methods yield different and complementary views of an ecosystem. *Frontiers in Marine Science*, *3*, 283. https://doi.org/10.3389/FMARS.2016.00283

Kestel, J. H., Field, D. L., Bateman, P. W., White, N. E., Allentoft, M. E., Hopkins, A. J. M., Gibberd, M., & Nevill, P. (2022). Applications of environmental DNA (eDNA) in agricultural systems: Current uses, limitations and future prospects. *Science of the Total Environment*, *847*, 157556. https://doi.org/10.1016/j.scitotenv.2022.157556

King, C., Ballantyne, G., & Willmer, P. G. (2013). Why flower visitation is a poor proxy for pollination: Measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution*, *4*(9), 811–818. https://doi.org/10.1111/2041-210X.12074

Kjærandsen, J. (2022). Current state of DNA barcoding of Sciaroidea (Diptera)—Highlighting the need to build the reference library. *Insects*, *13*(2), 147. https://doi.org/10.3390/insects13020147

Klein, A.-M., Brittain, C., Hendrix, S. D., Thorp, R., Williams, N., & Kremen, C. (2012). Wild pollination services to California almond rely on semi-natural habitat. *Journal of Applied Ecology*, *49*, 723–732. https://doi.org/10.1111/j.1365-2664.2012.02144.x

Kolreuter, J. (1761). *Vorlaufige Nachricht von einigen das Geschlecht der Pflanzen betreffenden Versuchen und Baobachtung*. In der Gleditschischen Handlung.

Krauss, S. L., Phillips, R. D., Karron, J. D., Johnson, S. D., Roberts, D. G., & Hopper, S. D. (2017, May 1). Novel consequences of bird pollination for plant mating. In *Trends in plant science* (Vol. *22*, pp. 395–410). Elsevier Current Trends. https://doi.org/10.1016/j.tplants.2017.03.005

Krehenwinkel, H., Fong, M., Kennedy, S., Huang, E. G., Noriyuki, S., Cayetano, L., & Gillespie, R. (2018). The effect of DNA degradation bias in passive sampling devices on metabarcoding studies of arthropod communities and their associated microbiota. *PLoS One*, *13*(1), e0189188. https://doi.org/10.1371/journal.pone.0189188

Kudoh, A., Minamoto, T., & Yamamoto, S. (2020). Detection of herbivory: eDNA detection from feeding marks on leaves. *Environmental DNA*, *2*(4), 627–634. https://doi.org/10.1002/edn3.113

Leempoel, K., Hebert, T., & Hadly, E. A. (2020). A comparison of eDNA to camera trapping for assessment of terrestrial mammal diversity. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1918), 20192353. https://doi.org/10.1098/RSPB.2019.2353

Leese, F., Sander, M., Buchner, D., Elbrecht, V., Haase, P., & Zizka, V. M. A. (2021). Improved freshwater macroinvertebrate detection from environmental DNA through minimized nontarget amplification. *Environmental DNA*, *3*(1), 261–276. https://doi.org/10.1002/edn3.177

Letourneau, D. K., Armbrecht, I., Rivera, B. S., Lerma, J., Carmona, E. J., Daza, M. C., Escobar, S., Galindo, V., Gutiérrez, C., López, S. D., Mejía, J. L., Rangel, A. M., Rangel, J. H., Rivera, L., Saavedra, C. A., Torres, A. M., & Trujillo, A. R. (2011). Does plant diversity benefit agroecosystems? A synthetic review. In *Ecological Applications* (Vol. *21*, pp. 9–21). John Wiley & Sons, Ltd. https://doi.org/10.1890/09-2026.1

Lippert, C., Feuerbacher, A., & Narjes, M. (2021). Revisiting the economic valuation of agricultural losses due to large-scale changes in pollinator populations. *Ecological Economics*, *180*, 106860. https://doi.org/10.1016/j.ecolecon.2020.106860

Macgregor, C. J., Kitson, J. J. N., Fox, R., Hahn, C., Lunt, D. H., Pocock, M. J. O., & Evans, D. M. (2019). Construction, validation, and application of nocturnal pollen transport networks in an agro-ecosystem: A comparison using light microscopy and DNA metabarcoding. *Ecological Entomology*, *44*(1), 17–29. https://doi.org/10.1111/een.12674

Macher, J. N., Salis, R. K., Blakemore, K. S., Tollrian, R., Matthaei, C. D., & Leese, F. (2016). Multiple-stressor effects on stream invertebrates: DNA barcoding reveals contrasting responses of cryptic mayfly species. *Ecological Indicators*, *61*, 159–169. https://doi.org/10.1016/j.ecolind.2015.08.024

Magoga, G., Forni, G., Brunetti, M., Meral, A., Spada, A., De Biase, A., & Montagna, M. (2022). Curation of a reference database of COI sequences for insect identification through DNA metabarcoding: COins. *Database*, *00*, 7. https://doi.org/10.1093/database/baac055

Marquina, D., Andersson, A. F., & Ronquist, F. (2019). New mitochondrial primers for metabarcoding of insects, designed and evaluated using in silico methods. *Molecular Ecology Resources*, *19*(1), 90–104. https://doi.org/10.1111/1755-0998.12942

Mashilingi, S. K., Zhang, H., Chen, W., Vaissière, B. E., Garibaldi, L. A., & An, J. (2021). Temporal trends in pollination deficits and its potential impacts on Chinese agriculture. *Journal of Economic Entomology*, *114*(4), 1431–1440. https://doi.org/10.1093/jee/toab100

Mathon, L., Valentini, A., Guérin, P. E., Normandeau, E., Noel, C., Lionnet, C., Boulanger, E., Thuiller, W., Bernatchez, L., Mouillot, D., Dejean, T., & Manel, S. (2021). Benchmarking bioinformatic tools for fast and accurate eDNA metabarcoding species identification. *Molecular Ecology Resources*, *21*(7), 2565–2579. https://doi.org/10.1111/1755-0998.13430

Mccarthy, A., & McCauley, D. (2020). *Avocados in Western Australia – Overview*. https://www.agric.wa.gov.au/avocados/avocados-western-australia---overview

McCravy, K. W. (2018). A review of sampling and monitoring methods for beneficial arthropods in agroecosystems. *Insects*, *9*(4), 170. https://doi.org/10.3390/insects9040170

Moran, A., Prosser, S., & Moran, J. (2019). DNA metabarcoding allows non-invasive identification of arthropod prey provisioned to nestling rufous hummingbirds (*Selasphorus rufus*). *PeerJ*, *7*, e6596. https://doi.org/10.7717/peerj.6596

Morandin, L. A., & Winston, M. L. (2006). Pollinators provide economic incentive to preserve natural land in agroecosystems. *Agriculture, Ecosystems and Environment*, *116*(3–4), 289–292. https://doi.org/10.1016/j.agee.2006.02.012

Mousavi-Derazmahalleh, M., Stott, A., Lines, R., Peverley, G., Nester, G., Simpson, T., Zawierta, M., De La Pierre, M., Bunce, M. F., & Christophersen, C. T. (2021). eDNAFlow, an automated, reproducible

and scalable workflow for analysis of environmental DNA sequences exploiting Nextflow and Singularity. *Molecular Ecology Resources*, *21*(5), 1697–1704. https://doi.org/10.1111/1755-0998.13356

Murray, D., Coghlan, M., & Bunce, M. (2015). From benchtop to desktop: Important considerations when designing amplicon sequencing workflows. *PLoS One*, *10*(4), e0124671. https://doi.org/10.1371/journal.pone.0124671

Murray, D. C., Bunce, M., Cannell, B. L., Oliver, R., & Houston, J. (2011). DNA-based Faecal dietary analysis: A comparison of qPCR and high throughput sequencing approaches. *PLoS One*, *6*(10), 25776. https://doi.org/10.1371/journal.pone.0025776

Naqvi, Q., Wolff, P. J., Molano-Flores, B., & Sperry, J. H. (2022). Camera traps are an effective tool for monitoring insect–plant interactions. *Ecology and Evolution*, *12*(6), e8962. https://doi.org/10.1002/ece3.8962

Newton, J., Bateman, P. W., Heydenrych, M., Kestel, J. H., Dixon, K. W., Prendergast, K. S., White, N. E., & Nevill, P. G. (2023). Monitoring the birds and the bees: Environmental DNA metabarcoding from flowers detects plant-animal interactions. *Environmental DNA*, *5*(3), 488–502. https://doi.org/10.1002/edn3.399

Pardo, A., & Borges, P. A. V. (2020). Worldwide importance of insect pollination in apple orchards: A review. *Agriculture, Ecosystems and Environment*, *293*, 106839. https://doi.org/10.1016/j.agee.2020.106839

Popic, T. J., Davila, Y. C., & Wardle, G. M. (2013). Evaluation of common methods for sampling invertebrate pollinator assemblages: Net sampling out-perform pan traps. *PLoS One*, *8*(6), e66665. https://doi.org/10.1371/journal.pone.0066665

Pornon, A., Andalo, C., Burrus, M., & Escaravage, N. (2017). DNA metabarcoding data unveils invisible pollination networks. *Scientific Reports*, *7*(1), 16828. https://doi.org/10.1038/s41598-017-16785-5

Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Biesmeijer, J. C., Breeze, T. D., Dicks, L. V., Garibaldi, L. A., Hill, R., Settele, J., & Adam, J. (2016). *Summary for policymakers of the thematic assessment on pollinators, pollination and food production Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Key messages Values of pollinators and pollination* (pp. 847–36). Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services.

Prado, S. G., Ngo, H. T., Florez, J. A., & Collazo, J. A. (2017). Sampling bees in tropical forests and agroecosystems: A review. *Journal of Insect Conservation*, *21*, 753–770. https://doi.org/10.1007/s10841-017-0018-8

Prendergast, K. S., Menz, M. H. M., Dixon, K. W., & Bateman, P. W. (2020). The relative performance of sampling methods for native bees: An empirical test and review of the literature. *Ecosphere*, *11*(5), e03076. https://doi.org/10.1002/ecs2.3076

R Core Team. (2018). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Rader, R., Bartomeus, I., Garibaldi, L. A., Garratt, M. P. D., Howlett, B. G., Winfree, R., Cunningham, S. A., Mayfield, M. M., Arthur, A. D., Andersson, G. K., Bommarco, R., Brittain, C., Carvalheiro, L. G., Chacoff, N. P., Entling, M. H., Foully, B., Freitas, B. M., Gemmill-Herren, B., Ghazoul, J., … Woyciechowski, M. (2016). Non-bee insects are important contributors to global crop pollination. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(1), 146–151. https://doi.org/10.1073/pnas.1517092112

Rader, R., Howlett, B. G., Cunningham, S. A., Westcott, D. A., & Edwards, W. (2012). Spatial and temporal variation in pollinator effectiveness: Do unmanaged insects provide consistent pollination services to mass flowering crops? *Journal of Applied Ecology*, *49*(1), 126–134. https://doi.org/10.1111/j.1365-2664.2011.02066.x

Rasmussen, J., Nielsen, M., Mak, S. S. T., Döring, J., Klincke, F., Gopalakrishnan, S., Dunn, R. R., Kauer, R., & Gilbert, M. T. P. (2021). eDNA-based biomonitoring at an experimental German vineyard to characterize how management regimes shape ecosystem diversity. *Environmental DNA*, *3*(1), 70–82. https://doi.org/10.1002/edn3.131

Rathcke, B. (1983). Competition and facilitation among plants for pollination. In L. Real (Ed.), *Pollination biology* (1st ed., pp. 305–329). Academic Press INC.

Ricketts, T. H., Regetz, J., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Bogdanski, A., Gemmill-Herren, B., Greenleaf, S. S., Klein, A. M., Mayfield, M. M., Morandin, L. A., Ochieng, A., Potts, S. G., & Viana, B. F. (2008). Landscape effects on crop pollination services: Are there general patterns? *Ecology Letters*, *11*(5), 499–515. https://doi.org/10.1111/j.1461-0248.2008.01157.x

Rougerie, R., Kitching, I., Haxaire, J., Miller, S., Hausmann, A., & Hebert, P. (2014). Australian Sphingidae – DNA barcodes challende current species boundaries and distributions. *PLoS One*, *9*(7), e101108. https://doi.org/10.1371/journal.pone.0101108

Saito, T., & Doi, H. (2021). A model and simulation of the influence of temperature and amplicon length on environmental DNA degradation rates: A meta-analysis approach. *Frontiers in Ecology and Evolution*, *9*, 623831. https://doi.org/10.3389/fevo.2021.623831

Sakamoto, R. L., Morinaga, S. I., Ito, M., & Kawakubo, N. (2012). Fine-scale flower-visiting behavior revealed by using a high-speed camera. *Behavioral Ecology and Sociobiology*, *66*(5), 669–674. https://doi.org/10.1007/s00265-011-1314-z

Sammataro, D., Gerson, U., & Needham, G. (2000). Parasitic mites of honey bees: Life history, implications, and impact. *Annual Review of Entomology*, *45*(1), 519–548. https://doi.org/10.1146/annurev.ento.45.1.519

Sánchez-Bayo, F., & Wyckhuys, K. A. G. (2019). Worldwide decline of the entomofauna: A review of its drivers. *Biological Conservation*, *232*, 8–27. https://doi.org/10.1016/j.biocon.2019.01.020

Saunders, M. E., & Luck, G. W. (2013). Pan trap catches of pollinator insects vary with habitat. *Australian Journal of Entomology*, *52*(2), 106–113. https://doi.org/10.1111/aen.12008

Saunders, M. E., Luck, G. W., & Mayfield, M. M. (2013). Almond orchards with living ground cover host more wild insect pollinators. *Journal of Insect Conservation*, *17*(5), 1011–1025. https://doi.org/10.1007/s10841-013-9584-6

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology and Evolution*, *3*(3), 430–439. https://doi.org/10.1038/s41559-018-0793-y

Schubert, M., Lindgreen, S., & Orlando, L. (2016). AdapterRemoval v2: Rapid adapter trimming, identification, and read merging. *BMC Research Notes*, *9*, 88. https://doi.org/10.1186/s13104-016-1900-2

Senapathi, D., Fründ, J., Albrecht, M., Garratt, M. P. D., Kleijn, D., Pickles, B. J., Potts, S. G., An, J., Andersson, G. K. S., Bänsch, S., Basu, P., Benjamin, F., Bezerra, A. D. M., Bhattacharya, R., Biesmeijer, J. C., Blaauw, B., Blitzer, E. J., Brittain, C. A., Carvalheiro, L. G., … Klein, A. M. (2021). Wild insect diversity increases inter-annual stability in global crop pollinator communities. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1947), 20210212. https://doi.org/10.1098/rspb.2021.0212

Shi, X., Fu, D., Xiao, H., Hodgson, J. A., Yan, D., & Zou, Y. (2022). Comparison between window traps and pan traps in monitoring flower-visiting insects in agricultural fields. *Bulletin of Entomological Research*, *1–6*, 691–696. https://doi.org/10.1017/s0007485322000104

Song, J. W., Small, M. J., & Casman, E. A. (2017). Making sense of the noise: The effect of hydrology on silver carp eDNA detection in the Chicago area waterway system. *Science of the Total Environment*, *605–606*, 713–720. https://doi.org/10.1016/j.scitotenv.2017.06.255

Spafford, R. D., & Lortie, C. J. (2013). Sweeping beauty: Is grassland arthropod community composition effectively estimated by sweep netting? *Ecology and Evolution*, *3*(10), 3347–3358. https://doi.org/10.1002/ece3.688

Spear, M. J., Embke, H. S., Krysan, P. J., & Vander Zanden, M. J. (2021). Application of eDNA as a tool for assessing fish population abundance. *Environmental DNA*, *3*(1), 83–91. https://doi.org/10.1002/edn3.94

Sprengel, C. K. (1793). *Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der.* Bei Friedrich Vieweg dem ælteren.

Steen, R. (2017). Diel activity, frequency and visit duration of pollinators in focal plants: in situ automatic camera monitoring and data processing. *Methods in Ecology and Evolution*, *8*(2), 203–213. https://doi.org/10.1111/2041-210X.12654

Steinhauer, N., Aurell, D., Bruckner, S., Wilson, M., Rennich, K., VanEngelsdrop, D., & Williams, G. (2021). *United States Honey Bee Colony Losses 2020–2021: Preliminary Results*. https://beeinformed.org/2021/06/21/united-states-honey-bee-colony-losses-2020-2021-preliminary-results/

Stork, N. E. (2018). How many species of insects and other terrestrial arthropods are there on earth? *Annual Review of Entomology*, *63*, 31–45. https://doi.org/10.1146/annurev-ento-020117-043348

Strickler, K. M., Fremier, A. K., & Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation*, *183*, 85–92. https://doi.org/10.1016/j.biocon.2014.11.038

Taylor, G. S., Braby, M. F., Moir, M. L., Harvey, M. S., Sands, D. P. A., New, T. R., Kitching, R. L., McQuillan, P. B., Hogendoorn, K., Glatz, R. V., Andren, M., Cook, J. M., Henry, S. C., Valenzuela, I., & Weinstein, P. (2018). Strategic national approach for improving the conservation management of insects and allied invertebrates in Australia. *Austral Entomology*, *57*, 124–149. https://doi.org/10.1111/aen.12343

Thomsen, P. F., & Sigsgaard, E. E. (2019). Environmental DNA metabarcoding of wild flowers reveals diverse communities of terrestrial arthropods. *Ecology and Evolution*, *9*(4), 1665–1679. https://doi.org/10.1002/ece3.4809

Valentin, R. E., Kyle, K. E., Allen, M. C., Welbourne, D. J., & Lockwood, J. L. (2021). The state, transport, and fate of aboveground terrestrial arthropod eDNA. *Environmental DNA*, *3*(6), 1081–1092. https://doi.org/10.1002/edn3.229

Vamos, E. E., Elbrecht, V., & Leese, F. (2017). Short CO1 markers for freshwater macroinvertebrate metabarcoding. *Metabarcoding and Metagenomics*, *1*, 14625. https://doi.org/10.3897/mbmg.1.14625

van der Heyde, M., Bunce, M., Wardell-Johnson, G., Fernandes, K., White, N. E., & Nevill, P. (2020). Testing multiple substrates for terrestrial biodiversity monitoring using environmental DNA metabarcoding. *Molecular Ecology Resources*, *20*(3), 732–745. https://doi.org/10.1111/1755-0998.13148

Van Zandt, P. A., Johnson, D. D., Hartley, C., LeCroy, K. A., Shew, H. W., Davis, B. T., & Lehnert, M. S. (2020). Which moths might be pollinators? Approaches in the search for the flower-visiting needles in the lepidopteran haystack. *Ecological Entomology*, *45*(1), 13–25. https://doi.org/10.1111/een.12782

Wada, Y., Florke, M., Hanasaki, N., Eisner, S., Fischer, G., Tramberend, S., Satoh, Y., van Vliet, M. T. H., Yillia, P., Ringler, C., Burek, P., & Wiberg, D. (2016). Modeling global water use for the 21st century: The water futures and solutions (WFaS) initiative and its approaches. *Geoscience Model Development*, *9*(1), 175–222. https://doi.org/10.5194/gmd-9-175-2016

Waser, N. M., Chittka, L., Price, M. V., Williams, N. M., & Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, *77*(4), 1043–1060. https://doi.org/10.2307/2265575

Westphal, C., Bommarco, R., Carré, G., Lamborn, E., Morison, N., Petanidou, T., Potts, S. G., Roberts, S. P. M., Szentgyörgyi, H., Tscheulin, T., Vaissière, B. E., Woyciechowski, M., Biesmeijer, J. C., Kunin, W. E., Settele, J., & Steffan-Dewenter, I. (2008). Measuring bee diversity in different European habitats and biogeographical regions. *Ecological Monographs*, *78*(4), 653–671. https://doi.org/10.1890/07-1292.1

Wilson, J., Griswold, T., & Messingery, O. (2008). Sampling bee communities (hymenoptera: Apiformes) in a desert landscape: Are pan traps sufficient? *Journal of the Kansas Entomological Society*, *81*(3), 288–300.

Young, R., Milián-García, Y., Yu, J., Bullas-Appleton, E., & Hanner, R. (2021). Biosurveillance for invasive insect pest species using an environmental DNA metabarcoding approach and a high salt trap collection fluid. *Ecology and Evolution*, *11*, 1558–1569. https://doi.org/10.1002/ece3.7113

Zborowski, P., & Storey, R. (2017). *A field guide to insects in Australia (fourth).* New Holland Publishers.

Zou, Y., Bianchi, F. J. J. A., Jauker, F., Xiao, H., Chen, J., Cresswell, J., Luo, S., Huang, J., Deng, X., Hou, L., & van der Werf, W. (2017). Landscape effects on pollinator communities and pollination services in smallholder agroecosystems. *Agriculture, Ecosystems and Environment*, *246*, 109–116. https://doi.org/10.1016/j.agee.2017.05.035

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: Kestel, J. H., Bateman, P. W., Field, D. L., White, N. E., Lines, R., & Nevill, P. (2023). eDNA metabarcoding of avocado flowers: 'Hass' it got potential to survey arthropods in food production systems? *Molecular Ecology Resources*, *00*, 1–16. https://doi.org/10.1111/1755-0998.13814