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Harnessing Environmental DNA to Explore Frugivorous Interactions: A Case Study in Papaya (*Carica papaya*) and Pineapple (*Ananas comosus*)

Pritam Banerjee^{1,2}  | Jyoti Prakash Maity^{3,4}  | Nalonda Chatterjee⁵  | Sven Weber¹  | Gobinda Dey⁶  |
Raju Kumar Sharma^{7,8}  | Chien-Yen Chen^{6,8,9} 

¹Department of Environmental Science, Policy, and Management, University of California, Berkeley, California, USA | ²Department of Biomedical Sciences, Graduate Institute of Molecular Biology, National Chung Cheng University, Minxiong, Chiayi County, Taiwan | ³Department of Chemistry, School of Applied Sciences, Kalinga Institute of Industrial Technology Deemed to be University, Bhubaneswar, Odisha, India | ⁴Centre for Water Research and Climate Change (CWRCC), under Centers for Innovations & Research (COIR), Kalinga Institute of Industrial Technology Deemed to be University, Bhubaneswar, Odisha, India | ⁵Department of Aquatic Ecology, Eawag: Swiss Federal Institute of Aquatic Science and Technology, Kastanienbaum, Switzerland | ⁶Doctoral Program in Science, Technology, Environment, and Mathematics, Department of Earth and Environmental Sciences, National Chung Cheng University, Minxiong, Chiayi County, Taiwan | ⁷Department of Chemistry and Biochemistry, National Chung Cheng University, Minxiong, Chiayi County, Taiwan | ⁸Center for Nano Bio-Detection, AIM-HI, National Chung Cheng University, Minxiong, Chiayi, Taiwan | ⁹Department of Earth and Environmental Sciences, National Chung Cheng University, Minxiong, Chiayi County, Taiwan

Correspondence: Pritam Banerjee (pritam8683@gmail.com) | Chien-Yen Chen (chien-yen.chen@oriel.oxon.org)

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ABSTRACT

Plant–animal interactions (PAIs) are critical in natural and agricultural ecosystems, mediating energy flow with both positive and negative interactions. Traditional methods of tracking PAIs, such as morphological identification and camera trapping, are limited in speed and scalability, posing challenges for comprehensive biodiversity monitoring. Recently, environmental DNA (eDNA) metabarcoding has emerged as a promising technique for detecting species interactions non-destructively. This pilot study explores the application of eDNA metabarcoding to investigate frugivorous interactions involving 18 partially consumed and three intact fruits of each *Carica papaya* and *Ananas comosus*. Metabarcoding of mitochondrial COI gene fragments generated 796,234 paired-end reads representing 117 ASVs spanning diverse taxonomic groups, including Metazoans, Protozoans, Algae, Fungi, and Bacteria. After filtering for animal taxa, 41 ASVs were retained, dominated by Arthropoda (~97%). Major frugivores included *Drosophila*, *Zaprionus*, and *Bactrocera* species. Additional detections included beetles, ants, parasitoid wasps, and vertebrates such as *Acridotheres javanicus*, *Callosciurus erythraeus*, and *Bandicota indica*. Moreover, consumed fruits showed high insect (~90%–95%) and mammal (~4%–5%) DNA, while intact fruits were dominated by rotifers (~75%–80%). Distinct communities were found between pineapple and papaya, with 15 and 11 unique ASVs, respectively, and only one ASV was unique to intact fruits. Alpha and beta diversity analyses confirmed the differences in community structure between fruit types. Despite the limited sample size, our findings demonstrate the potential of fruit-surface eDNA to monitor frugivory and species interactions. Future studies should scale this approach across seasons and crop types to assess its potential in long-term biodiversity and pest management monitoring.

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1 | Introduction

Species interactions play a central role in energy flow within ecosystems, with both positive and negative interactions simultaneously contributing to ecosystem functioning (Åkesson et al. 2021; Valiente-Banuet et al. 2015). Plant–animal interactions (PAIs) are key drivers of energy flow and ecosystem processes, occurring in various forms such as predation, frugivory, herbivory, parasitism, and mutualism, encompassing both positive and antagonistic relationships (Thompson 1991; Schaefer et al. 2004; Strauss and Irwin 2004; Herrera and Pellmyr 2009). However, in the current era of increasing anthropogenic pressures, such as environmental pollution, climate change, and species introduction, it is becoming increasingly difficult to track the rapid changes occurring in both natural and agricultural systems (Fletcher et al. 2024; Mazor et al. 2018; Cardinale et al. 2012). Understanding species interactions within agricultural systems is essential for maintaining ecological balance by supporting beneficial organisms while preventing the establishment and spread of invasive and agricultural pest species (Swift and Anderson 1994; Shennan 2008; Ren et al. 2014).

Monitoring species diversity (native and/or introduced), community composition, and interactions using traditional methods such as morphological screening and camera trapping is effective but time-consuming (Deiner et al. 2017; Thomsen and Sigsgaard 2019; Johnson et al. 2021; Banerjee et al. 2022a; Banerjee et al. 2022b). Over the past few decades, biomonitoring has advanced across multiple disciplines, including morphological screening with high-resolution microscopy, chemical taxonomy, radar-based remote sensing, automated acoustic monitoring, artificial intelligence, robotic monitoring, and high-throughput DNA/RNA sequencing, all of which are becoming standard practices globally (Evans and Kitson 2020; Silvestro et al. 2022; Bell et al. 2023; Chua et al. 2023; van Klink et al. 2024; Geckeler et al. 2025). Recent developments in metabarcoding and metagenomics have enabled deeper exploration of environmental genomics, ranging from presence/absence data to abundance estimation, species detection to species interactions, population genetics, and even gene expression analysis (Evans and Kitson 2020; Stevens and Parsley 2023; Blackman et al. 2024).

Environmental DNA (eDNA)-based biodiversity monitoring has gained substantial attention due to its non-destructive nature, high accuracy, and cost-effectiveness compared to traditional methods (Thomsen and Willerslev 2015; Deiner et al. 2017; Blackman et al. 2024). To date, eDNA has been successfully applied to address a wide range of ecological questions, including species presence/absence (Ficetola et al. 2008), relative abundance (Lacoursière-Roussel et al. 2016), habitat preference (Stoeckle et al. 2017), population genetics (Shum and Palumbi 2021; Andres et al. 2023), and species interactions (Banerjee et al. 2022a; Banerjee et al. 2022b; Newton et al. 2023; Weber et al. 2024). Although still a newly developed approach, species interaction detection using environmental DNA has already been explored in studies recovering traces of DNA from flowers (Thomsen and Sigsgaard 2019; Johnson et al. 2023; Kestel et al. 2023), leaves (Kudoh et al. 2020; Valentin et al. 2020; Krehenwinkel et al. 2022), tree bark (Valentin

et al. 2020; Allen et al. 2023), honey (Utzeri et al. 2018), and even from tea (Krehenwinkel et al. 2022).

Given the notable success of eDNA in evaluating species interactions from various substrates, we propose that fruits can serve as a potential source of eDNA for understanding different levels of frugivory and the identification of agricultural pests. Fruits, particularly those that are fleshy and consumed by animals, offer a unique substrate for detecting species interactions through eDNA (Monge et al. 2020). Fruits are often directly bitten, chewed, or licked by frugivores, which increases the likelihood of saliva, skin cells, and other biological material being deposited on their surface. Additionally, fruits are readily collectable, naturally accumulate interaction signals over time, and are ecologically central to PAI networks, making them a promising but underexplored target for eDNA-based studies. In a species-specific study, Monge et al. (2020) successfully amplified Macaw DNA from partially consumed tropical almond fruits, suggesting that DNA traces from the fruits can be used to identify the interacting taxa. However, the potential of fruits as a source of eDNA to understand the community dynamics of frugivores remains unexplored. Thus, we present the first attempt to apply eDNA metabarcoding on fruits to understand the frugivorous community. As a pilot study, we selected partially consumed and intact fruits of two economically important plants, papaya (*Carica papaya*) and pineapple (*Ananas comosus*), from a locally cultivated agricultural site in Chiayi County, Taiwan.

Based on our understanding, we hypothesized that (i) eDNA metabarcoding of fruits can detect signatures of PAIs; (ii) partially consumed fruits will contain stronger interaction signatures due to prolonged or repeated contacts than intact fruits with limited interaction marks; and (iii) species composition will differ between papaya (*Carica papaya*) and pineapple (*Ananas comosus*). We argue that understanding these interactions is critical for conservation biology, biosecurity, and agricultural pest management. As a rapidly advancing technique with multiple methodological choices at each step, we propose the development of a detailed, easy-to-follow workflow for implementing eDNA in such species monitoring and interaction studies. This approach could facilitate the rapid monitoring of frugivores in natural habitats and provide a quick method for identifying pest issues in agricultural systems.

2 | Material and Methods

2.1 | Study Area and Sampling

Samples were collected from agricultural farms within a 5 km radius of National Chung Cheng University (23.558754° N, 120.472276° E), Minxiong Township, Chiayi County, Taiwan. Fruits were divided into two categories: (i) partially consumed/damaged fruits, and (ii) intact fruits without feeding marks (controls). Minxiong Township is known as the “Home of Pineapple” due to its prominent pineapple production, and a popular agro-tourism site, “Pineapple Hill,” is located near the University. While pineapple is the dominant crop, papaya is also cultivated year-round on a smaller scale in farms surrounding the campus. Several such fields were identified

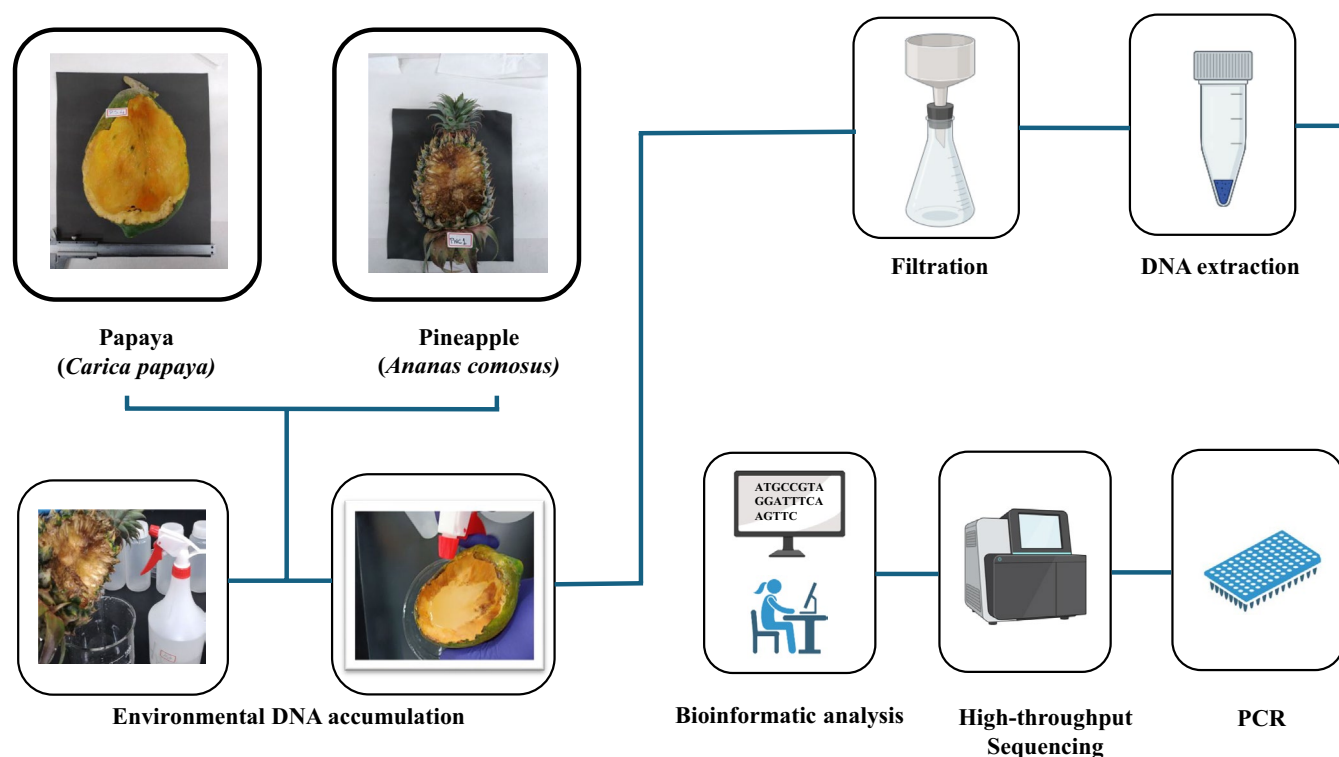


FIGURE 1 | Diagrammatic workflow of environmental DNA metabarcoding from fruits of papaya (*Carica papaya*) and pineapple (*Ananas comosus*).

within a 5 km radius of the University. Due to difficulties in sampling partially consumed fruits, we conducted frequent farm visits between March and May 2022, collecting 36 partially consumed and six intact fruits. During this period, Minxiong Township experienced average daily temperatures of 16°–28°C and frequent rainfall (100–167 mm per month across 14–15 rainy days), coinciding with the early plum rain season (Retrieved from <https://www.cwa.gov.tw/>). Each sample was collected using nitrile gloves, placed in separate Whirl-Pak bags, and transported in a cooler box with dry ice to the laboratory. Three bottles containing 300 mL of distilled water each were taken to the field and left open for 5 min to serve as field blank samples. During our multiple visits, we observed the presence of common mynas, pallas's squirrels, and various smaller insects such as fruit flies. Additionally, through conversations with local farmers, we learned that the greater bandicoot rat is a common pest in these agricultural habitats and that farmers actively implement measures to mitigate crop damage.

2.2 | Accumulation of eDNA From Fruits

Thirty-six partially consumed fruits and six intact fruits of papaya (*Carica papaya*) and pineapple (*Ananas comosus*) were processed for eDNA collection (Figure 1). To dislodge eDNA from the fruit surfaces, we sprayed the fruits with double-distilled water using a plastic spray bottle and collected the runoff in a glass beaker (Figure 1). For each fruit, 300 mL of water was sprayed, and the collected water was filtered through a 0.45 µm pore-size mixed cellulose ester filter paper (GN-6 Metrcel, Pall Corporation, USA) with a diameter of 47 mm. The spray volume

was selected based on preliminary trials to ensure thorough surface coverage. After filtration, the filter papers were divided into two halves: one half was preserved in Longmire's buffer (Longmire et al. 1997) at –20°C for long-term archiving, while the other half was stored in ATL buffer (Qiagen, Germany) at 4°C for subsequent DNA extraction. Filter papers were handled with sterilized forceps and processed individually to avoid cross-sample contamination. Field, accumulation, and filtration blanks were prepared by processing 300 mL of double-distilled water per replicate, with three replicates for each blank type to monitor potential contamination. To minimize cross-contamination, all equipment and work surfaces were frequently decontaminated with 10% bleach and 75% ethanol, and gloves were changed between handling each sample.

2.3 | DNA Extraction and PCR Amplification

DNA extraction and PCR were conducted separately in a dedicated clean area at the Department of Biomedical Science and the Department of Earth and Environmental Sciences, National Chung Cheng University, designated for handling low-DNA samples. We added 20 µL of Proteinase K to a tube containing 180 µL of ATL buffer and filter paper. DNA extraction was performed using the DNeasy Blood & Tissue Kit (Qiagen, Germany) with the following modifications: (i) the filter paper was chopped into small pieces before lysis, (ii) lysis was carried out overnight at 60°C, and (iii) the spin column was incubated twice with 30 µL of AE buffer at 37°C for 10 min each. DNA quality and quantity were assessed using a NanoPhotometer (Implen, Germany) and an Invitrogen Qubit 3.0 fluorometer (Thermo Fisher Scientific, USA). All samples, including field,

accumulation, and extraction blanks, were extracted individually. Subsequently, we pooled three samples from the same or nearby plants (based on species) into a single composite sample to increase detection sensitivity while minimizing processing and sequencing costs (Melcher et al. 2024). Thus, the 36 partially consumed fruit samples were pooled into 12 samples (labeled as papaya: PP1–PP6 and pineapple: PN1–PN6), and the six intact fruits into two samples (labeled as papaya: PPC and pineapple: PNC). These sample names were used consistently throughout the manuscript, including in figures and tables. All extracted DNA was stored at -20°C .

We amplified a ~313 bp fragment of mitochondrial cytochrome c oxidase subunit I (COI) gene using universal degenerate primers: mICoIintF-5'-GGWACWGGWTGAACGWTWTAYCCYCC-3' and jgHCO2198-5'-TAIACYTCIGGRTGICCRAARAAYCA-3', specially designed for metazoans (Leray et al. 2013). Three PCR replicates were carried out for each sample (including blanks), and the final reaction mixture of 25 μL was prepared by mixing 5 μL of 5 $\times$ Fast-Run Taq Master Mix with Dye (Protech Technology Enterprise, Taiwan), 0.5 μL each of 10 pmol forward and reverse primers (Genomics, Taiwan), 3 μL of the eDNA sample, with UltraPure DNase/RNase-Free Distilled Water (Thermo Fisher Scientific, USA) added to reach the final volume. PCR was performed using a CLUBIO PCR system (CB series, Scientific Biotech Corp., Taiwan), and the protocol included an initial denaturation step at 94°C for 5 min, followed by 36 cycles of amplification: denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. A final extension was performed at 72°C for 5 min. The PCR replicates were then pooled in equimolar amounts based on band intensities observed on a 1.5% agarose gel using the Gel Doc XR system with the QUANTITY ONE software (Bio-Rad, USA). No visible amplification was observed in any blank samples (field, filtration, or extraction blanks); these were nevertheless pooled into a single negative control library and sequenced alongside the true samples to detect and control for potential low-level contamination (Ficetola et al. 2016). In addition, intact fruits were treated as control samples. The PCR products were then purified using 1.5 $\times$ SPRI beads (AMPure XP, Beckman Coulter, USA). A total of 20 μL of PCR products was purified with 30 μL of SPRI beads, following the manufacturer's protocol. Purified amplicons were quantified using an Invitrogen Qubit 3.0 fluorometer and submitted for library preparation and sequencing at Genomics Bioscience and Technology Co. Ltd., Taiwan.

2.4 | Library Preparation and Next-Generation Sequencing

The library was prepared using the TruSeq Nano DNA Library Prep Kit (Illumina, USA), and its quality was assessed using an Invitrogen Qubit 3.0 fluorometer (Thermo Fisher Scientific, USA) and an Agilent Bioanalyzer 2100 system (Agilent Technologies, USA). The target inserts (~313 bp of the mitochondrial COI gene fragment) were derived from the PCR amplification described earlier. The final library size, including Illumina adapters, was approximately 450 bp, as measured by Bioanalyzer. Libraries were individually indexed (barcoded) during preparation and pooled (multiplexed) in equimolar concentrations before sequencing. Library quantification was performed using qPCR to

ensure accurate equimolar pooling. All qualified libraries were sequenced using 300 bp paired-end reads on an Illumina MiSeq platform (Illumina, USA), operated by Genomics Bioscience and Technology Co. Ltd., Taiwan. Sequencing was carried out on a multiplexed standard v3 MiSeq flow cell, with an expected sequencing depth of ~240,000 reads per composite sample (~80,000 reads per single fruit).

2.5 | Data Processing

Raw sequencing data were demultiplexed using an in-house script developed by Genomics Bioscience and Technology Co. Ltd., Taiwan, to assign reads to their corresponding samples based on barcode sequences. Adapter sequences and low-quality bases (QV < 3) were subsequently removed using Trimmomatic (v0.39) with the following parameters: ILLUMINACLIP:adapter:2:30:10:1:true LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15. This configuration removes Illumina adapter sequences (allowing up to two seed mismatches), trims low-quality bases from both ends of the reads, and applies sliding window trimming when the average Phred quality score within the window falls below 15 (Bolger et al. 2014). Sequences corresponding to both ends of the mICoIintF and jgHCO2198 primers were trimmed using Cutadapt (v3.4) (Martin 2011). Primer trimming was performed with a minimum read length cutoff of 150 bp to remove short, low-information reads, and reads lacking primer sequences were discarded. The trimming allowed a minimum overlap of 3 bp and an error rate of 0.1 for primer matching. Denoising, chimera removal, and assignment to Amplicon Sequence Variants (ASVs) were performed using DADA2 (Callahan et al. 2016), following the approach of Sigsgaard et al. (2021). After error removal, to eliminate contamination from fields and laboratory sources, we removed all ASVs from samples if they were detected with more than 50 reads in any negative control. This cutoff was selected to eliminate likely contaminants while minimizing the loss of true low-abundance taxa. All remaining sequences were then BLASTed against the GenBank nucleotide database (Sayers et al. 2022) (as of September 2022) using BLASTN, and taxa were selected based on the Lowest Common Ancestor (LCA) method (<https://github.com/timkahlke/BASTA>). The criteria for sequence alignment included a maximum of 500 aligned sequences per query, with a $\geq 90\%$ query cover and 70%–100% sequence similarity. For taxonomic assignments, we applied similarity thresholds as follows: 80%–84% for class, 85%–89% for order, 90%–94% for family, 95%–97% for genus, and $>98\%$ for species (Sigsgaard et al. 2021). In cases where multiple taxonomic matches occurred at a given level, we assigned the ASV to the next higher taxonomic rank. For example, if an ASV was identified up to genus but had overlapping similarities with two or more genera, we assigned it at the family level. Additionally, sequences assigned to genus and species were manually BLASTed against the NCBI database to confirm their identity, following Sigsgaard et al. (2021).

2.6 | Statistical Analysis

All statistical analyses were performed using R version 4.4.3 (2024). To account for variation in sequencing depth, we rarefied

ASV richness to the minimum sample size using the *rarefy()* function (vegan package) with 999 permutations (Schloss 2024). To examine differences in taxonomic composition between fruit types, we conducted nonmetric multidimensional scaling (NMDS) based on Jaccard similarity matrices using the *metaMDS()* function from the *vegan* package (Oksanen et al. 2022). The significance of compositional differences between fruit types was tested using PERMANOVA based on Bray–Curtis dissimilarity with 999 permutations. To detect differential abundance of individual ASVs between fruit types, we applied *DESeq2* (Love et al. 2014), which models count data using a negative binomial distribution and accounts for differences in sequencing depth. Visualization of taxonomic distributions and sample overlaps was performed using the *ggplot2* and *ggvenn* packages (Wickham 2016, 2018; Yan and Yan 2021; Hamilton and Ferry 2018).

3 | Results

3.1 | Taxonomic Composition

After filtering and denoising, a total of 1,692,642 single-end reads, corresponding to 796,234 paired-end reads, were generated from 42 fruit samples, sequenced as 14 pooled composite samples. On average, each fruit produced $56,873 \pm 15,733$ reads (mean $\pm$ SD). Blank controls generated 1350 reads from eight ASVs, primarily from non-target organisms. The most abundant ASV in blanks was of human origin, followed by a known laboratory contaminant, *Pomacea canaliculata*. All ASVs with > 50 reads in blank samples were excluded from downstream analysis.

Given the use of universal degenerate primers that amplify a broad range of taxa, approximately 33.32% of reads from nontarget groups (Fungi: 33.14%, Plants: 0.17%, Bacteria: 0.01%) were excluded from downstream analysis (Tables S1–S2). Additionally, 21.75% of the total reads were classified only at the domain level (Eukaryota), 16.77% remained unassigned, and 1.59% exhibited < 80% sequence similarity; these were also excluded (Tables S1–S2). From the 14 pooled samples, a total of 117 ASVs spanning diverse taxonomic groups, including Metazoans, Protozoans, Algae, Fungi, and Bacteria, were identified (Table S2).

Ultimately, 196,869 reads representing 41 ASVs classified exclusively as animal taxa were retained for further analysis (Table S3). The dominant phylum was Arthropoda, accounting for 97.61% of all animal reads, followed by Rotifera (1.52%), Chordata (0.79%), Nematoda (0.06%), Amoebozoa (~0.01%), and Euglenozoa (~0.01%) (Table S4). Rotifera was represented by five ASVs, classified into two orders: Bdelloida and Philodinida (Table S3). One ASV of Euglenozoa was assigned to the family Trypanosomatidae, and one Amoebozoa ASV was assigned to *Acanthamoeba* sp. Three ASVs from Chordata were matched to distinct vertebrates: *Acridotheres javanicus* (Javan myna), *Callosciurus erythraeus* (Pallas's squirrel), and *Bandicota indica* (greater bandicoot rat). Within Arthropoda, the dominant class was Insecta (53.79%), followed by Arachnida (0.19%), Malacostraca (0.04%), and Collembola (0.01%). Notably, 45.98% of Arthropoda reads were assigned only at the phylum level (Table S5). Arachnida included five ASVs: two at

the family level (Phytoseiidae and Acaridae), two at the order level (Sarcoptiformes and Trombidiformes), and one at the class level (Arachnida). One ASV in Malacostraca was identified as *Burmoniscus* sp., and one ASV in Collembola was identified to species level as *Homidia sinensis*.

Among insects, Diptera dominated with 97.01% of reads, followed by Coleoptera (2.31%) and Hymenoptera (0.13%), with the remainder (0.55%) identified only at the class level (Table S6). Within Coleoptera, two beetle species were identified: *Araecerus fasciculatus* and *Carpophilus marginellus*. In Hymenoptera, one ant (*Tapinoma* sp.) and one parasitoid wasp (*Leptopilina* sp.) were detected (Table S3). Within Diptera, three fruit fly genera, such as *Drosophila* sp., *Zaprionus* sp., and *Bactrocera* sp., were identified, along with *Chironomus javanus* (one nonbiting midge species). The *Drosophila* genus includes six species: *D. takahashii*, *D. bipectinata*, *D. malerkotliana*, *D. ananassae*, *D. immigrans*, and *D. eugracilis*. Other flies included *Bactrocera dorsalis* and *Zaprionus* sp.

3.2 | Consumed Pineapple Versus Consumed Papaya Versus Intact Samples

Relative abundance data indicated that consumed fruit samples were dominated by insects (~90%–95%) and mammals (~4%–5%), while intact fruit samples were dominated by rotifers (75%–80%) and insects (20%–25%) (Figure 2A,B). The top three frugivore taxa were *Drosophila* sp., *Zaprionus* sp., and *Callosciurus* sp. (Figure 2C,D), with *Drosophila* spp. being the most abundant across all samples. Alpha and beta diversity analyses suggested clear differences in taxonomic composition between fruit types (Figure 3A,B). However, statistical power was limited due to the small sample size. Pineapple samples contained 15 unique ASVs, papaya samples had 11 unique ASVs, and 15 ASVs were shared between the two (Figure 4). Most taxa, such as *Zaprionus* sp., *D. takahashii*, *D. eugracilis*, *C. javanus*, and *C. erythraeus*, were detected in both the fruits (Figure 5). However, taxa such as *D. ananassae*, *D. bipectinata*, and *D. malerkotliana* were specific to pineapple, while *Tapinoma* sp., *A. javanicus*, *A. fasciculatus*, and *Burmoniscus* sp. were found in papaya (Figure 5). These findings warrant further investigation using expanded sampling and complementary methods.

The taxonomic composition between consumed and intact samples also differed. Specifically, 15 and 11 unique ASVs were detected in consumed pineapple and papaya samples, respectively, with an additional four ASVs shared exclusively between the two consumed fruit types. Seven ASVs were common across all samples, while only one unique ASV was detected in the intact samples (Figure 4). Arthropod orders such as Coleoptera, Diptera, and Sarcoptiformes were predominantly associated with consumed pineapple samples, whereas the mammalian order Rodentia was more closely associated with consumed papaya. In contrast, the intact fruit samples showed a noteworthy detection of the order Rotifera (Figure 6). Despite the limited number of pooled replicates, NMDS analysis revealed two distinct clusters for papaya and pineapple samples, supporting the hypothesis of fruit-specific frugivore communities (Figure 3). PERMANOVA confirmed this separation as statistically significant ($p = 0.026$). To identify the taxa driving these differences,

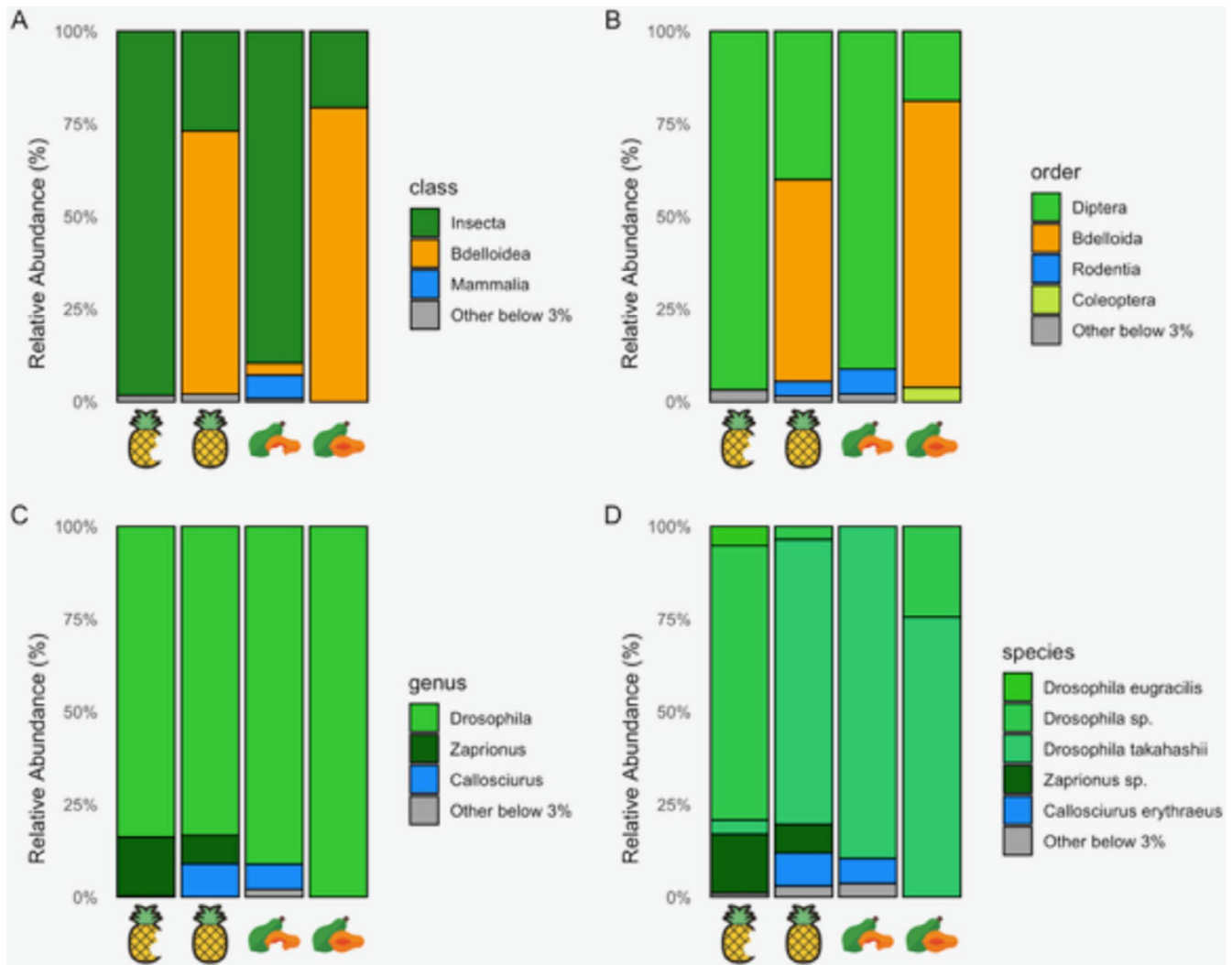


FIGURE 2 | Top relative abundant taxa detected on consumed and intact fruits, categorized by (A) Class, (B) Order, (C) Genus, and (D) Species.

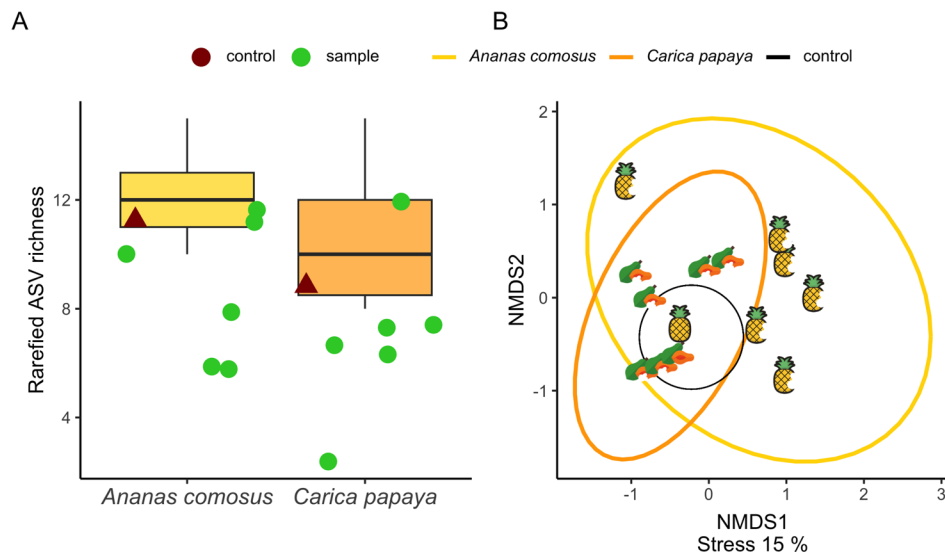


FIGURE 3 | Sample orientation and richness from the fruits of papaya (*Carica papaya*) and pineapple (*Ananas comosus*), (A) Boxplot of ASV richness across consumed papaya (orange), consumed pineapple (yellow), intact fruits are represented with brown triangles. (B) Nonmetric multidimensional scaling showing clear clusters between eaten fruits (yellow pineapple; orange papaya).

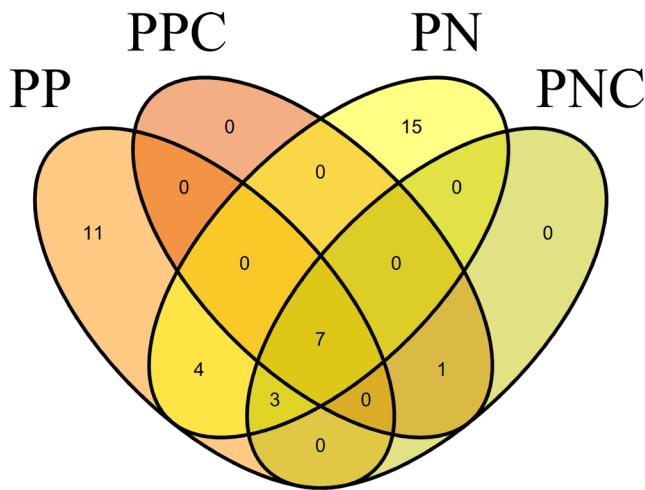


FIGURE 4 | Venn diagram of overlapping ASVs between consumed fruits (PP: Papaya; PN: Pineapple) and intact fruits (PPC: Papaya; PNC: Pineapple).

differential abundance analysis (DESeq2) was performed. After correcting multiple testing ($p_{adj} < 0.05$), ASV 1 (*D. takahashii*) was significantly more abundant in papaya compared to pineapple (Table S7). ASV 116 (*C. erythraeus*) showed a trend toward significance but did not meet the threshold (Table S7).

4 | Discussion

In this study, we successfully detected eDNA signatures from various species interacting with consumed and intact fruits, supporting our first hypothesis. Signals of eDNA have previously been recovered from different plant parts to study interactions, including flowers (Thomsen and Sigsgaard 2019; Johnson et al. 2023; Kestel et al. 2023), leaves (Valentin et al. 2020; Krehenwinkel et al. 2022), and tree bark (Valentin et al. 2020; Allen et al. 2023); however, comparable investigations focused on fruits remain limited or largely unexplored. In addition to the species-specific study by Monge et al. (2020), this study

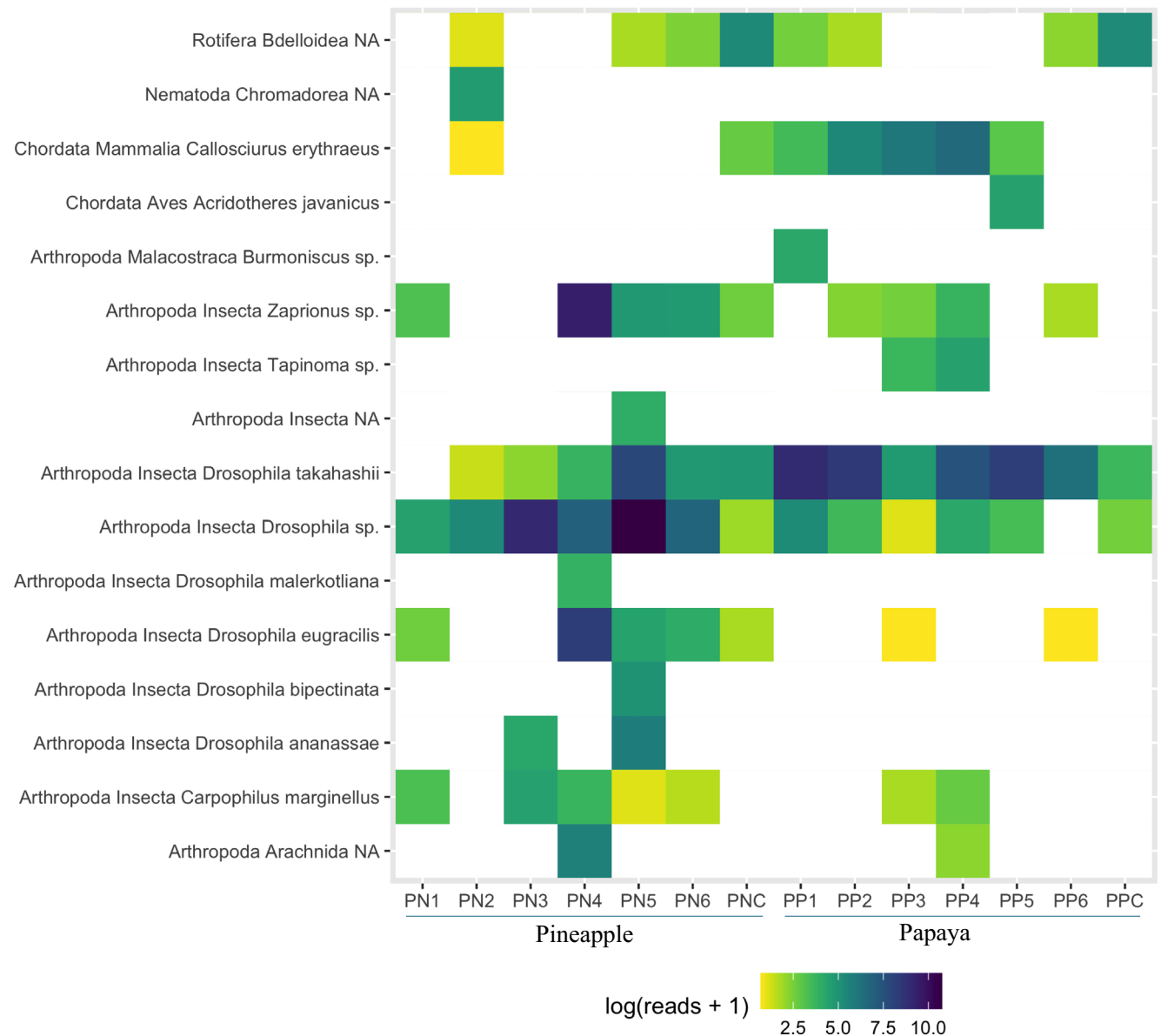


FIGURE 5 | Heatmap of top 20 ASVs and their relative abundance in fruits of papaya (*Carica papaya*) and pineapple (*Ananas comosus*).

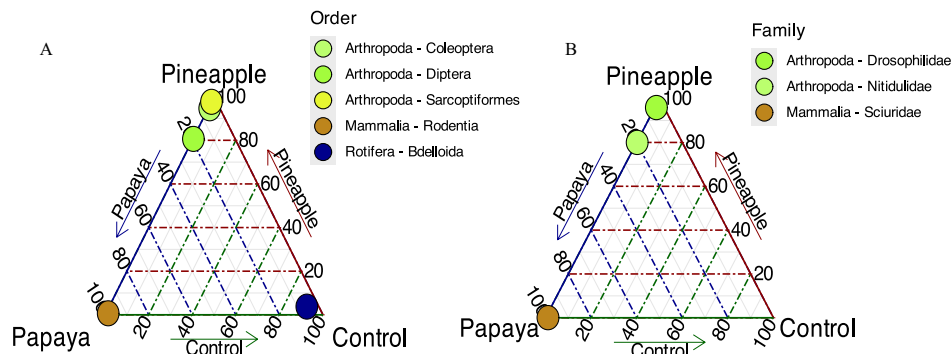


FIGURE 6 | Ternary plot illustrating the distribution of individual ASVs in consumed pineapple (PP), consumed papaya (PAP), and control intact samples (PPC and PNC), represented by (A) order and (B) family.

represents the first eDNA metabarcoding-based effort to recover eDNA signals directly from fruit surfaces. The study was specifically designed to compare eDNA recovered from consumed and intact fruits to evaluate their relative effectiveness for future biodiversity surveys. Signals from insects, birds, squirrels, rats, and other organisms detected through eDNA metabarcoding enabled the capture of a broad taxonomic spectrum from a limited sampling effort, revealing insights into species composition and ecological interactions, particularly among frugivorous species. Furthermore, the rapid generation of species inventories facilitated the timely identification of frugivorous taxa, offering practical application for agricultural stakeholders. Traditionally, these interactions have been assessed using morphological observations and camera trapping (Li et al. 2022); however, our streamlined eDNA-based approach offers a more efficient alternative for elucidating species interactions, requiring less time and effort, as supported by findings from other recent studies (Thomsen and Sigsgaard 2019; Johnson et al. 2023; Newton et al. 2023; Allen et al. 2023; Kestel et al. 2023). Moreover, eDNA methods may outperform individual tissue-based barcoding or bulk-scale tissue-based metabarcoding (malaise traps, pan traps), which involve destructive sampling (Banerjee et al. 2022a; Banerjee et al. 2022b; Kestel et al. 2023). Integrating traditional and eDNA-based approaches could maximize the species detection capacity, although eDNA may be more informative for detecting species interactions (Weber et al. 2024). Our results provide a comprehensive profile of the taxonomic diversity associated with fruits in both consumed and intact states, with consumed fruits showing higher eDNA signal intensity likely due to increased species interactions (Figures 2, 3, 4 and 6). As a proof-of-concept, these observed patterns provide valuable insight into fruit insect interaction dynamics. However, future studies incorporating larger-scale sampling and complementary methodological approaches will be essential for validation and broader applicability.

4.1 | Taxonomic Composition and Insect Community Structure

The taxonomic composition detected in our samples highlights the dominance of the phylum Arthropoda, particularly the class Insecta, which comprised the majority of ASVs (Table S4), primarily from fruit-associated species (Figures 2, 5, and 6). However, approximately 33.32% of reads originated

from non-target taxa (e.g., Fungi, Algae, Bacteria), indicating a potential need for more taxon-specific primers in future studies (Table S1–S2). A substantial proportion of ASVs could not be taxonomically assigned, likely due to limitations in current reference DNA databases (Table S2). Additionally, reads from Rotifera (1.52%), Nematoda (0.06%), Amoebozoa (~0.01%), and Euglenozoa (~0.01%) may reflect low-level secondary contamination, despite stringent contamination control. Notably, nematode reads were detected only in pineapple samples, which are cultivated closer to the soil, suggesting a possible environmental source (Figure 5).

The high proportion of fruit flies (*Drosophila* spp. and *Bactrocera dorsalis*) and beetles (*Araecerus fasciculatus* and *Carpophilus marginellus*) suggests these taxa are abundant frugivores and decomposers (Figure 5) (Deconninck et al. 2024). Moreover, Taiwan's unique biogeographic position contributes to its rich fruit fly and beetle diversity (Chang et al. 2001; Hu et al. 2024; Doorenweerd et al. 2019). Additionally, the detection of vertebrate frugivores such as Javan myna, Pallas's squirrel, and greater bandicoot rat corroborated field observations and farmer reports. These species likely act as early fruit colonizers, breaching the pericarp and facilitating access for smaller taxa and decomposers. A gradual succession study could offer further insights into these dynamics. The presence of multiple *Drosophila* species supports their abundance in Taiwan (Robert Liu et al. 2015) (Figure 2, Table S3) and aligns with studies showing their adaptability to diverse fruit types and decay stages (Deconninck et al. 2024). Despite low read counts, the detection of *Homidia sinensis* (springtails), *Burmoniscus* sp. (isopods), and *Chironomus javanus* (non-biting midges) was tentative and warrants caution. *Chironomus javanus* is typically associated with aquatic habitats and decaying plant materials (Al-Shami et al. 2012), and *Burmoniscus* sp. is primarily found in leaf litter and the soil environment (Kwon and Jeon 1993; Karasawa and Goto 2014). Although *Homidia sinensis* exhibits a broad habitat range (Xiang et al. 2022), there are no known reports of its presence on fruits. It is possible that some of the consumed fruit samples were in early stages of decay, subject to secondary contamination by other species, or exposed to contamination during rainfall, which could support the occurrence of these species; however, this explanation remains hypothetical and not fully supported by the current data. Further investigation is required to explore the potential ecological roles of these organisms, ranging from detritivores to participants in nutrient

cycling within decaying fruit microhabitats. Our findings of genera such as *Leptopilina*, a known parasitoid of *Drosophila*, underscore the presence of complex trophic interactions within the fruit environment. This suggests parasitoid-host dynamics, typically observed in natural settings, may also occur within these more transient microhabitats. This co-occurrence was also previously studied by researchers, where those parasites were used as regular natural biological control of *Drosophila* (Chabert et al. 2012; Ibouh et al. 2019).

4.2 | Comparative Analysis of Pineapple and Papaya Communities

The relative abundance of interacting taxa revealed a stronger number of eDNA signals from consumed fruits than from intact fruits (Figure 2). This supports our second hypothesis, suggesting that partially consumed fruits contain stronger interaction signatures than intact fruits. Our alpha and beta diversity analyses revealed distinct differences in taxonomic composition between pineapple and papaya samples (Figure 3A,B). The unique ASVs associated with each fruit type suggest certain taxa may exhibit specialization or preferences based on fruit characteristics such as sugar content, texture, or chemical/odor profile (Rodríguez et al. 2013; Cunningham et al. 2016) (Figures 4 and 5). This partially supports our third hypothesis, however, species-specific interactions could not be conclusively determined for either pineapple or papaya samples, likely due to the limited number of sampling events. For instance, species like *Drosophila ananassae* and *Drosophila malerkotiana* were primarily associated with pineapple, while other *Drosophila* species were predominantly found in both fruits. Also, taxa such as *Tapinoma* sp. (ants) and the introduced bird species, *Acridotheres javanicus* (Javan myna), were predominantly found in papaya samples. However, these data are preliminary, and we suggest a more in-depth study with a large sampling effort coupled with some traditional sampling (morphological identification, camera records, and tissue-derived barcoding), to study species interactions and changes in the communities. Moreover, these findings suggest designing experiments to understand the specific ecological traits of the fruits that may drive niche differentiation among taxa, leading to specialized interactions and potential competitive exclusion in these microhabitats.

4.3 | Differences Between Consumed and Intact Fruits

A notable difference in taxonomic composition was observed between consumed and intact fruits, with consumed fruits exhibiting higher ASV richness and more diverse arthropod assemblages (Figures 2 and 4). This pattern likely reflects increased accessibility and nutrient availability due to the open pericarp and ripening status of consumed fruits, which attract a greater diversity of decomposers, frugivores, and opportunistic species (Rodríguez et al. 2013; Cunningham et al. 2016). The significant association of arthropod and mammalian orders with consumed fruits and rotifers with intact fruits suggests that our method can effectively reveal actual ecological interactions from fruit surfaces (Figure 6). The Rotifera reads found in intact fruits, possibly due to secondary contamination, imply minimal

ecological interaction. This likely results from the physical barrier of intact pericarp and non-ripening status, which deters colonization and limits DNA transfer. These findings support our second hypothesis.

The distinct arthropod associations with consumed fruits, as depicted in the ternary plot (Figure 6), highlight the attraction of these organisms to damaged or partially decayed fruits, which provide an enriched microenvironment relative to intact counterparts (Rodríguez et al. 2013). This suggests that open or decayed fruits attract a more complex community structure, facilitating ecological interactions across trophic levels (Rodríguez et al. 2013). Here, eDNA serves as an effective method for detecting such ecological interactions with precision and timeliness (Thomsen and Sigsgaard 2019; Banerjee et al. 2022a; Banerjee et al. 2022b).

4.4 | Identification of Agricultural Pests, and Implications for Invasion Ecology

The diverse array of detected species, including frugivorous, sap-feeding, and omnivorous, highlights the role of fruits as critical interaction sites. The presence of pest species such as sap-feeding beetle, *Carpophilus marginellus*, and storage product pest, *Araecerus fasciculatus* suggested their interactions with fruits, and they are abundant in that particular place in Taiwan (Lin 1976; Kirejtshuk 2005). Furthermore, other agricultural pests such as *Acridotheres javanicus*, *Bandicota indica*, and *Bactrocera dorsalis* were identified within the fruit samples, indicating their capacity to exploit agricultural products (Lin 2001; Doorenweerd et al. 2019; Lin et al. 2024; Sridhara 2025). *Bactrocera dorsalis* is a highly invasive pest species around the Pacific-Asia Region that was first reported from Taiwan (Doorenweerd et al. 2019; Wan et al. 2012). This species is responsible for enormous economic loss in agriculture as the larvae feed on fruits and destroy the cultivation (Hardy 1973; Wan et al. 2012). Additionally, *Acridotheres javanicus* (invasive) and *Bandicota indica* (native) are also responsible for crop damage in agricultural ecosystems. Although the seed dispersal role of *Callosiurus erythraeus* (native), they are also involved in agricultural damage throughout Taiwan (Kuo and Liao 1986). Besides, the presence of several *Drosophila* sp. and *Zaprionus* sp. may be a contributing factor to fruit damage.

4.5 | Limitations and Future Perspectives

The present study has some limitations that require further evaluation. As a pilot study, the limited sample size constrained our ability to draw broader conclusions. To address resource constraints, we pooled three samples from the same or nearby plants (per species) into a single composite sample, 36 partially consumed fruit samples into 12 samples, and the six intact fruits into two samples. While necessary, this approach may have affected data variability and statistical power (Sato et al. 2017). Given the limited sampling events and sequencing depth, detecting clear divergence among communities based on fruit type remains challenging. Expanding the number and diversity of fruit samples, along with broader sampling locations, will strengthen the robustness of our findings.

Additionally, the use of more specific primers targeting different taxonomic groups, such as those designed for birds (e.g., MiBird; Ushio et al. 2018), mammals (e.g., Mimammals; Ushio et al. 2017), and vertebrates (e.g., Mod_RepCOI_F/VertCOI_7216_R; Reeves et al. 2018; VertU; Wang et al. 2023), could enhance taxonomic resolution. Furthermore, while no method is flawless, integrating eDNA with traditional survey techniques and developing a comprehensive reference database will be critical for its effective application in ecological management.

Overall, this study sheds light on the ecological complexity and taxonomic diversity associated with fruit habitats with eDNA metabarcoding, emphasizing the importance of monitoring fruit-associated communities for maintaining ecological balance and species interactions. This method could also be potentially useful for monitoring agricultural pest species in a short time and with limited effort. Future research should explore the temporal dynamics of these communities and assess how fruit decomposition stages further shape taxonomic composition. Also, exploring the species interactions study with eDNA for conservation and invasion biology can aid in proper management decisions.

Implementation of eDNA in PAI studies offers significant potential for conservation as well as agricultural management. By enabling non-destructive, cost-effective, high-throughput monitoring of species interactions, this approach could advance policies related to pest control, disease monitoring, invasive species control, biodiversity assessments, and habitat restoration. In agricultural landscapes, the ability to detect pest species early can facilitate targeted pest management strategies, minimizing economic losses (Kestel et al. 2022). Moreover, integrating eDNA methods with traditional ecological monitoring could enhance the accuracy of biodiversity assessments and guide conservation policies aimed at preserving native pollinators and other beneficial species (Evans et al. 2016; Johnson et al. 2023). Comparing our findings with other eDNA-based studies on PAIs could provide deeper insights into the effectiveness of this approach across different ecosystems. Previous research has demonstrated the utility of eDNA in detecting pollinators, seed dispersers, and herbivores, highlighting its potential for broad ecological applications (Thomsen and Sigsgaard 2019; Monge et al. 2020; Johnson et al. 2023; Weber et al. 2024). Future studies incorporating cross-system comparisons and validation with traditional survey methods would further strengthen the case for eDNA as a robust tool for studying species interactions in both natural and managed ecosystems.

5 | Conclusions

Our work highlighted that the eDNA-based method is capable of detecting PAIs from fruits by unraveling the taxonomic composition. Signals from various organisms detected through eDNA metabarcoding from consumed fruits allowed us to capture a wide array of taxa, revealing insights into species interactions and ecological relationships. However, intact fruits also carried eDNA signatures from interacting taxa, but significantly less compared to the consumed fruits due to limited interactions.

The unique ASVs associated with each fruit type suggest that certain taxa may show preferences based on fruit characteristics such as sugar content, texture, or chemical/odor profile (Rodríguez et al. 2013; Cunningham et al. 2016). However, to understand the details of the species-specific interactions or traits, a wide-scale sampling and implementation of multidisciplinary methods are needed. The eDNA method needs limited taxonomic expertise, less labor, and fast and effective monitoring, which can be implemented in monitoring ecological and economical species interactions. Moreover, this biomonitoring method can be used for regular monitoring assays in wildlife as well as agricultural fields for suitable management decisions. Future work should aim to validate these findings through controlled experiments, time-series sampling, and integration with visual or camera-based observations.

Author Contributions

P.B., J.P.M., and C.-Y.C. designed the study. P.B., N.C., R.K.S., and G.D. performed the experiments. P.B. and S.W. conducted the data analysis. P.B. wrote the first draft of the manuscript. All authors provided extensive edits and contributed to the revision of the manuscript.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing supporting the study will be openly available in Dryad (<https://doi.org/10.5061/dryad.k98sf7mhh>).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1–S7:** edn370196-sup-0001-TableS1-S7.docx.