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Received: 13 July 2021 | Revised: 22 April 2022 | Accepted: 23 April 2022

DOI: 10.1002/edn3.308

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REVIEW

Plant–animal interactions in the era of environmental DNA (eDNA)—A review

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Abstract

Plant–animal interactions (PAI) represent major channels of energy transfer through ecosystems, where both positive and antagonistic interactions simultaneously contribute to ecosystem functioning. Monitoring PAI therefore increases the understanding of environmental health, integrity, and functioning, and studying complex interactions through accurate, cost-effective sampling can aid in the management of detrimental anthropogenic impacts. Environmental DNA (eDNA)-based monitoring represents an increasingly common, nondestructive approach for biodiversity monitoring, which could help to elucidate PAI. Here, we aim to provide an overall discussion on the potential of using eDNA to study PAI. We assessed the existing literature on this subject from 2009 to 2021 using a freely accessible web search tool. The search was conducted by using keywords involving eDNA and PAI, including both species-specific and metabarcoding approaches, recovering 43 studies. We summarized the advantages and current limitations of such approaches, and we outline research priorities to improve future eDNA-based methods for PAI analysis. Among the 43 studies identified using eDNA to measure PAI such as pollination, herbivory, mutualistic, and parasitic relationships, they have often identified higher taxonomic diversity in several direct comparisons with DNA-based gut/bulk sampling and conventional survey methods. Research needs include the following: better understanding of the influencing factors of eDNA detection involved in PAI (e.g., eDNA degradation, origin, and types), methodological standardization (sampling methods and primer development), and more inclusive sequence reference databases. If these research priorities are addressed, it will have a significant impact to enable PAI biodiversity monitoring with eDNA. In the future, the implementation of eDNA methods to study PAI can particularly benefit the scalability of environmental biomonitoring surveys that are imperative for ecosystem health assessments.

KEYWORDS
biodiversity loss, biodiversity sampling, conservation management, ecosystem functioning, environmental DNA (eDNA), molecular ecology, nondestructive, plant–animal interactions (PAI)

1 | INTRODUCTION

More than one million species are at risk of becoming threatened with extinction (IPBES, 2019), heralding the Anthropocene as the sixth mass extinction event (Myers, 1990; Román-Palacios & Wiens, 2020). The loss of species interactions may occur well before the actual extinction of individual species, thereby initiating deleterious effects on species functionality and its service to the ecosystem (Valiente-Banuet et al., 2015). This in turn further accelerates species extinction rates (Simmons et al., 2020), which is especially pertinent for specialist species (Colles et al., 2009). In fact, given that the loss of successive interactions provides an early warning system for the deterioration of ecosystem health (Valiente-Banuet et al., 2015), documenting, monitoring, and conserving such complex interactions is critical to retain ecosystem functioning.

One of the principal means by which taxa are interconnected in nature is via plant–animal interactions (PAI). These interactions can play pivotal ecological roles and materialize in multiple combinations of positive and antagonistic relationships (e.g., predation, frugivory and herbivory, parasitism, and mutualism). For example, frugivory contributes to propagation and thus facilitates plant restoration (Chama et al., 2013; Monge et al., 2020) and gene flow (Robledo-Arnuncio & Garcia, 2007). Without such mutualistic relationships, some plants may not be able to complete their life cycles, and the animals may starve due to resource deficiency. Herbivory leads to defoliation or root removal, which can regulate or diminish overall phytomass but can also increase species diversity and influence plant distribution (Castagneyrol et al., 2017; Milchunas & Lauenroth, 1993), thereby regulating ecosystem stability (Castagneyrol et al., 2017; Schallhart et al., 2012; Wirth et al., 2008). In pollinator-plant mutualisms, the former acquires feeding from the latter and in return serves as an agent of plant propagation and a vector for gene flow (Ellis & Johnson, 2012). Studies documenting the food habits of pollinators and their interactive role in sustaining ecosystems have already shed light on the complex network of species-specificity, habitat preference, and coevolution between plants and their pollinators (Sargent & Ackerly, 2008). Mutualisms also assist with growth and offer protection from pathogens (e.g., plant–insect associations; Rasmussen et al., 2021). In contrast, antagonistic interactions (e.g., parasites and parasitoids) can affect the growth of plants and result in economical and ecological loss (Derocles et al., 2015). Thus, PAI underpins many of the fundamental processes related to ecosystem structure and functioning (Pacini et al., 2008).

However, studying these multifaceted interactions using conventional methods (e.g., field observation, camera, malaise, pitfall traps, and gut content analysis) is often difficult and laborious (Thomsen & Sigsgaard, 2019). Alternatively, molecular advancements with the analysis of trace DNA from environmental samples (i.e., environmental DNA or "eDNA") have provided researchers and managers the ability to scale up documentation and monitoring of such relationships, and to do so at increased spatiotemporal frequencies with more cost-effectiveness (see Figure 1).

Methodological development for the application of eDNA has rapidly evolved from the presence/absence detection of organisms (Ficetola et al., 2008) and abundance quantification of eDNA signals (Taberlet et al., 2012), to the detection of whole communities (Deiner et al., 2021) and even their trophic interactions (D'Alessandro & Mariani, 2021; Thomsen & Sigsgaard, 2019). Indeed, eDNA-based methods have experienced a sharp adoption in different fields such as conservation biology (e.g., detection of endangered or invasive species; Piaggio et al., 2014; Stewart et al., 2017), ecological biomonitoring in the terrestrial and aquatic ecosystem (e.g., environmental health monitoring; Xie et al., 2017), wildlife forensics (Allwood et al., 2020), wildlife disease monitoring (Barnes et al., 2020), and animal behavior (Nichols et al., 2015). The application of eDNA methods to investigate a myriad of ecological interactions such as pollination (e.g., plant-insects, plant–animal), predation (e.g., herbivory, frugivory), and mutualism (e.g., plant–nematode, plant–insect, plant–animals) (Rasmussen et al., 2021; Thomsen & Sigsgaard, 2019; van Beeck Calkoen et al., 2019) further demonstrates the application of eDNA as a multidisciplinary approach (Deiner et al., 2021; Veilleux et al., 2021) poised to tackle complex ecological questions regarding inter-taxa relationships. However, as with every newly developed method, the use of eDNA for PAI studies remains limited.

To understand the current use and limitations of eDNA for studying PAI, we reviewed the current literature. We discuss the advantages and current limitations of such methods, and propose research priorities that may improve future eDNA-based methods for PAI analysis. Within this context, our goal is to highlight for both researchers and managers, the potential utility of noninvasive/destructive eDNA-based methods, but we also aim to identify and clarify uncertainties and research needed to advance these methods for broader application.

2 | METHODS

In order to understand the state-of-the-art in using eDNA detections of species to study PAI and enable an effective discussion of this application, we qualitatively reviewed studies incorporating eDNA methods into PAI research (targeted and metabarcoding approaches) by searching the literature published between 2009 to 2021 using Google Scholar (https://scholar.google.com/) with the following search conditions: (1) "[eDNA] AND [plant-animal

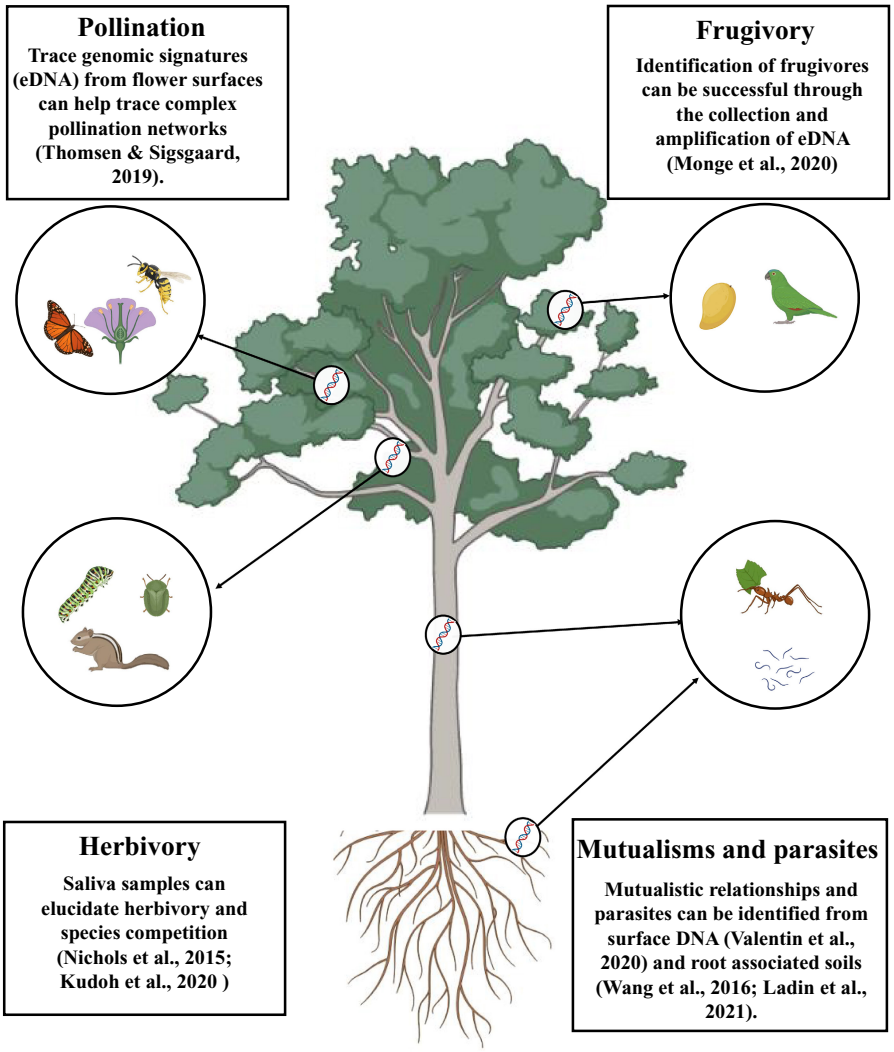

FIGURE 1 Biological signatures in the form of eDNA or eRNA can be detected from plants noninvasively to trace out complex interactions. Illustration presents hypothetical examples of PAI (e.g., pollination, herbivory, frugivory, and mutualism) including representative examples in the literature

interactions]"; (2) "[eDNA] AND [pollination]"; (3) "[pollen metabarcoding]"; (4) "[eDNA] AND [herbivory]"; (5) "[herbivory metabarcoding]"; and (6) "[fecal metabarcoding]". The content of the top 100 publications on Google Scholar was manually verified. We selected papers that worked on nondestructive eDNA-based methods for detection. The selected publications were then evaluated to understand the potential advantages and limitations of eDNA-based methods for the study of PAI.

2.1 | Why use eDNA-based methods for studying PAI?

Conventional methods such as field observation, histological and biochemical analysis, various cameras, malaise, or pitfall traps, etc. have proved their utility for identifying and expanding knowledge on PAI across numerous species groups, research questions, and intended outcomes (ecological or evolutionary fundamental knowledge, agricultural/horticultural production, conservation management, and action plans). Yet historically, most studies on PAI generally focus on pairs of species (e.g., plant–insect) (Herrera & Pellmyr, 2009), and thus, the ecologically complex interactions between species groups (e.g., plant–nematode–insect) remain less understood (Luna & Dáttilo, 2021). Indeed, numerous animal species are interconnected with plants, they may coexist or not but still have potential impacts on each other across their network (Luna & Dáttilo, 2021). These interactions are dynamic processes, and thus, their subsequent observation is often difficult using discrete means of data collection.

In direct comparison with conventional methods, for example, DNA metabarcoding has a greater ability to detect closely related taxa (Macgregor et al., 2019), is time-efficient and cost-effective, whereas the application of conventional methods may be difficult for large-scale sampling due to their larger time investment and costs. Additionally, conventional methods may be unable to resolve diverse yet morphologically conserved groups (e.g., Nematodes; Derycke et al., 2010) and particularly cryptic species (Sheth & Thaker, 2017). Thus, the implementation of DNA-based methods may help us to understand how ecological mechanisms shape different PAIs and the assembly of communities. Studying PAI in a community context would therefore require sampling methods that provide broad spatiotemporal inference, involving a wide range of species.

Indeed, DNA-based methods offer broad output with the capability of identifying multiple PAI simultaneously, and the ease at which DNA is collected and analyzed also affords multiple sampling events for an integrative approach (Evans & Kitson, 2020). DNA metabarcoding methods (e.g., metabarcoding of gut contents or bulk samples) contain several organisms from different taxonomic groups together (e.g, Kick-Net sampling or insect trap, that amalgamate entire organisms into a single sample, Taberlet et al., 2018) and have already proved useful in elucidating complex species and trophic interactions (García-Robledo et al., 2013). For instance, DNA metabarcoding analysis from gut content or bulk samples has illuminated different nodes across various food webs and reconstructed the trophic links in terrestrial (Gogarten et al., 2020; Wirta et al., 2014, 2016; Wirta, Vesterinen, et al., 2015; Wirta, Weingartner, et al., 2015), aquatic (Leray et al., 2012; Leray et al., 2015), and often inaccessible environments, such as deep-sea beds, hydrothermal vents, and cold-seeps (Olsen et al., 2014). Several reviews to date have summarized the history, achievements, and current applications of studying species interaction using DNA metabarcode methods across multiple fields (Clare, 2014; Evans et al., 2016; Kress et al., 2015; Pompanon et al., 2012; Symondson, 2002; Valentini et al., 2009). These methods, however, are still a direct DNA-based method requiring tissue from single individuals or from bulk samples like a pitfall trap and gut content samples. All of these sample types are destructive and invasive sometimes requiring the sacrifice of focal organisms, which is not ideal or practical for species of conservation concern. Both conventional and these direct DNA-based methods further (generally) focus on animal interactions with different plants, whereas the converse (plant interactions with multiple animal species) remains less understood.

Novel sampling techniques such as the collection of DNA from soil, water, or air environments (eDNA) offer a method for studying PAI with the potential capability for simultaneously identifying multiple PAI, while also facilitating a conservation-friendly, and for many species noninvasive and nondestructive (including nontarget taxa) alternative. In fact, advancements including DNA collections from the surface of organisms (e.g., DNA from leaf surfaces; Valentin et al., 2020) prevented the sacrifice of organisms and highlighted the nondestructive advantages of eDNA for measuring complex PAI.

2.2 | Current advancements in eDNA for the study of PAI

Although still in its infancy, species-specific assays and metabarcoding of eDNA have demonstrated a great application for understanding PAI in nature (see Table 1 and Table 2). Here, we summarize the ways in which various PAI (i.e., pollination, predation, and mutualism) can be documented for whole communities using the collection and analysis of eDNA.

Pollination is one of the most well studied PAI since it brings about gene recombination (Faegri & Pijl, 1979), and exemplifies a myriad of central ecological and evolutionary principles and theories. In pollinator PAI, the loss of even a singular plant species can trigger the rapid extinction of specialist pollinators, a process of serious ecological and economical concern (Klein et al., 2007; UK National Ecosystem Assessment, 2011). To date, researchers have taken advantage of eDNA-based analysis to detect and monitor pollinators, their feeding preferences, species-specificity, niche separation, and coevolution (Table 1). In particular, eDNA metabarcoding of honey samples has been demonstrated to detect more taxa than conventional methods, where species-specificity (i.e., identification of generalists and specialists), foraging activity, and complex interactions have been analyzed rapidly and cost-effectively (De Vere et al., 2017; Hawkins et al., 2015). Interestingly, eDNA from honey samples can also help to identify other entomological signatures within forests or agricultural fields, such as those from plant-sucking insects whose "honeydew" droplets are incorporated in honey reserves (Utzeri et al., 2018). Bovo et al. (2018) further demonstrated the utility of eDNA tools to understand the micro-ecosystem within honey bee colonies by detecting the eDNA signals from five distinct groups (i.e., arthropods, plants, fungi, bacteria, viruses). Although not strictly PAI, this study further exemplifies eDNA-based methods as a potential avenue for information regarding wildlife diseases and epidemics.

While complex pollinator networks are typically difficult to identify and discriminate using conventional sampling, eDNA collections taken directly from flowers or leaves have further shown promise to gain an in-depth understanding of dynamic pollinator and herbivore interactions (Kudoh et al., 2020; Thomsen & Sigsgaard, 2019). For example, Thomsen and Sigsgaard (2019) detected eDNA signatures from 135 arthropod species originating from diverse ecological groups deposited on wildflowers (e.g., pollinators, parasitoids, gall inducers, predators, and phytophagous species) and suggested the potential use of eDNA approaches for estimating interactive species compositions, deducing the effects of environmental change, and monitoring endangered, cryptic and invasive species (Thomsen & Sigsgaard, 2019).

Understanding the complex interactions between frugivores and plants also remains a challenge, but recent strides using eDNA traces to detect specific interactions of fruit-eaters have now made this prospect more convenient. For example, Monge et al. (2020) successfully amplified the salivary eDNA of frugivorous birds (*Ara macao*) from tropical almond (*Terminalia catappa*) fruit remains. Albeit with limited success, this study further provided proof of concept for the use of eDNA in nondestructive sex identification, potentially ushering in a new frontier for studying sex-specific differences in PAI.

Herbivores often prefer a certain plant or group of species, which may cause shifts in plant composition. Thus, it would be beneficial to identify the diversity of plant taxa eaten by particular herbivores and the number of herbivores visiting focal plants. For herbivory, eDNA-based methods have been shown to detect large numbers of taxa more efficiently than other sampling methodologies (e.g., microscopic analysis of fecal samples, bulk DNA metabarcoding; Tournayre et al., 2021). In fact, eDNA

TABLE 1 Number of PAI studies through eDNA metabarcoding approach from 2009 to 2021 separated for different types of PAI

Types of PAI	Organisms involved	Applications	Reference
Positive			
Mutualism/symbiosis	Arthropods and plants	Sustainable agricultural methods	Rasmussen et al. (2021)
Pollination	Arthropods and plants	Environmental integrity and pest management	Thomsen and Sigsgaard (2019)
	Bats and plants	Identification of plant-feeding behavior, pollen transport, and seed dispersions	Bell et al. (2021); Edwards et al. (2019)
	Honey bees and plants	Pollinator–plant preference and interactions	Bovo et al. (2018); De Vere et al. (2017); Hawkins et al. (2015); Keller et al. (2015); Milla et al. (2021); Oliver et al. (2021)
	Insects and plants	Pollen–transport interactions; ecosystem monitoring	Baksay et al. (2020); Pornon et al. (2016); Utzeri et al. (2018)
	Moths and plants	Identification of plant-feeding behavior and pollen transport networks	Chang et al. (2018); Macgregor et al. (2019)
Frugivory	Birds and plants	Species interaction and potential use in population genetics	Monge et al. (2020)
Antagonistic			
Parasite/parasitoid	Parasitoid and plants	Identification of plant–parasitoid interaction, environmental integrity, and pest management	Derocles et al. (2015); Thomsen and Sigsgaard (2019)
Predation/herbivory	Deers and plants	Forest management, foraging behaviors, dietary assessment for conservation purposes	van Beeck Calkoen et al. (2019); Iacolina et al. (2020); Nakahama et al. (2021)
	European bison and plants	Dietary assessment	Kowalczyk et al. (2019)
	Gazelle and plants	Dietary assessment for conservation purposes	Ait Baamrane et al. (2012)
	Grouse and plants	Dietary assessment for conservation purposes	Chua et al. (2021)
	Idaho ground squirrel and plants	Dietary assessment for conservation purposes	Goldberg et al. (2020)
	Insects and plants	Mesocosm validation of insect–plant interactions	Kudoh et al. (2020)
	Italian hare and plants	Dietary assessment for conservation purposes	Buglione et al. (2018)
	Lambs and plants	Dietary assessment and feeding selectivity	Pegard et al. (2009)
	Lemmings and plants	Dietary assessment and foraging behaviors	Soininen et al. (2017)
	Lemur and plants	Dietary assessment for conservation purposes	Quéméré et al. (2013)
	Mammals and plants	Dietary assessment, foraging behaviors, trophic interactions, and niche partitioning	Boukhdoud et al. (2021); Kartzinel et al. (2015); Meyer et al. (2020); ter Schure et al. (2021)
	Moose and plants	Dietary assessment for conservation purposes	Iacolina et al. (2020)
	Mouse and plants	Dietary assessment for conservation purposes	Iwanowicz et al. (2016)
	Nematodes and plants	Change to Identification of Plant-nematodes interactions	Wang et al. (2016)
	Orthoptera and plants	Dietary assessment and study the impact of climatic variation	Pitteloud et al. (2020)
	Tapir and plants	Dietary assessment and trophic interactions	Hilbert et al. (2013)
	Turtles and lotus roots	Dietary assessment and feeding activity	Koizumi et al. (2016)
	Ungulates and plants	Dietary assessment, foraging behaviors, and ecological interactions	Nichols et al. (2012); Nichols et al. (2015)
	Woodland caribou and plants	Dietary assessment for conservation purposes	Newmaster et al. (2013)

Note: Key expressions were used for study inclusion via Google Scholar: "eDNA and plant-animal interactions," "eDNA and herbivory," "eDNA and pollination," "eDNA and symbiosis," "eDNA and predation," "eDNA and parasitism," and "fecal DNA."

metabarcoding has also been applied to understand the dietary overlap and competition among domestic and wild herbivores (ter Schure et al., 2021). Notably, sampling matter may be a restricted application to large organisms with detectable fecal deposits. To overcome this limitation, salivary samples can be collected to identify herbivores that have fed upon specific plants, even

TABLE 2 Number of PAI studies through eDNA metabarcoding approach from 2009 to 2021 separated for different types of PAI

Type of PAI	Number of peer-reviewed articles (2009–2021)
Mutualism/symbiosis	1
Parasite/parasitoid	2
Pollination	17
Predation/herbivory	25
Total	43

from small taxa (e.g., from browsed twigs, Valentin et al., 2020; or leaves, Nichols et al., 2012). For example, Nichols et al. (2015) applied eDNA analysis across a large forest landscape, proving the utility of this method for studying cryptic browsing behavior. Salivary eDNA signatures can also be used to assess foraging preferences and niche separation among species (e.g., van Beeck Calkoen et al., 2019). Impressively, salivary eDNA signals from insect herbivores within mesocosms have also shown a positive correlation between rim length (i.e., total outer edge) of feeding marks and eDNA concentration, implying eDNA signatures may be able to quantitatively delineate the amount of herbivory (Kudoh et al., 2020).

Detecting plant-pathogen/parasite interactions through eDNA has also recently become possible. Derocles et al. (2015) for example, successfully amplified trace DNA from plants-leaf miners-parasitoid interactions and Thomsen and Sigsgaard (2019) detected numerous phytophagous species, parasitoids, gall inducers, and predator insects through the metabarcoding of flowers. Although in these studies nontarget taxa were used to isolate eDNA, this also can be done in a completely nondestructive manner using newly developed surface-sampling methods (see Valentin et al., 2020). Cumulatively, these studies provide a foundation for detecting antagonistic and cryptic plant-arthropod interactions with applications for disease monitoring and pest management.

Mutualistic relationships between plants and animals (e.g., insects and nematodes) assist plant growth and development, and these relationships can also be studied effectively through eDNA analysis (Ladin et al., 2021). For example, Rasmussen et al. (2021) used eDNA metabarcoding to explore how the diversity of fungi and arthropods was affected by different agricultural management practices. For a more historical perspective of mutualistic relationships, Gous et al. (2019) applied eDNA methods to investigate pollinator interactions that had occurred over a century ago via ancient honey samples, highlighting eDNA's potential to reveal a time series of species interactions.

Certainly, eDNA methods have advanced our ability to accurately detect the occupancy of species (Deiner et al., 2021) and are highly cost and time-efficient (Qu & Stewart, 2019). Indeed, they have even outperformed conventional methods of biodiversity sampling in several comparisons (Fediajevaite et al., 2021; McElroy et al., 2020), including their ability to capture increased taxonomic diversity compared with conventional methods, which can be applicable for large-scale monitoring (Macher et al., 2018). Thus, eDNA-based methods have gradually overcome some limitations associated with conventional monitoring techniques (e.g., field identifications). Perhaps most importantly for the assessment of ecological integrity and functionality, eDNA can rapidly detect entire communities.

2.3 | Current limitations

There remains a need to understand the current limitations of eDNA analysis, especially when it pertains to PAI detection and interpretation. Limitations are found at each step of the collection-analysis-interpretation process (Figure 2). The existing limitations of this method are:

I The complex, and often idiosyncratic, ecology of eDNA. In effect, practitioners may sample different sources of eDNA (cellular, extracellular, extraorganismal, etc.) (Rodriguez-Ezpeleta et al., 2021; Stewart, 2019), which may lead to different PAI interpretations. For example, pollen and spores (extraorganismal DNA) are ubiquitous in the atmosphere, travel long distances (through wind or water), and contain adaptations to persist in dormant stages for long periods of time. These transport mechanisms of eDNA, when settled and collected on nontargeted and noninteracting organisms, can lead to misinterpretation of interaction when there is not one. Alternatively, extracellular DNA and cellular DNA are generally specific to places where organisms recently moved and are subject to easy degradation. Thus, clear differentiation of eDNA in different states and their behavior may help to draw more precise conclusions about when a species was present (Mauvisseau et al., 2022).

II The production and release of eDNA into the environment can also occur at different rates, where eDNA concentration can depend on many variables such as life stage, metabolic activity, or breeding season (Stewart, 2019). What's more, the production rate of eDNA is most likely influenced by species interactions themselves (e.g., competition between/among species) (Stewart, 2019). In fact, mixed-species populations have been shown to increase eDNA production rates when housed together compared with single-species populations (Sassoubre et al., 2016). Besides the aforementioned characteristics, the persistence of eDNA (Barnes & Turner, 2016; Deiner et al., 2017; Kudoh et al., 2020), and its transport in and between environmental media (air, water, soil) should also be considered (Barnes & Turner, 2016; Lacoursière-Roussel & Deiner, 2021), especially given that these parameters have yet to be standardized for many taxa.

III Translating eDNA quantification metrics to organismal abundance has been controversial (Marshall et al., 2021), although recent research has advanced the possibility of absolute quantification (Hoshino et al., 2021; Tillotson et al., 2018) and even predicting the dispersion time of eDNA within the environment (Marshall et al., 2021).

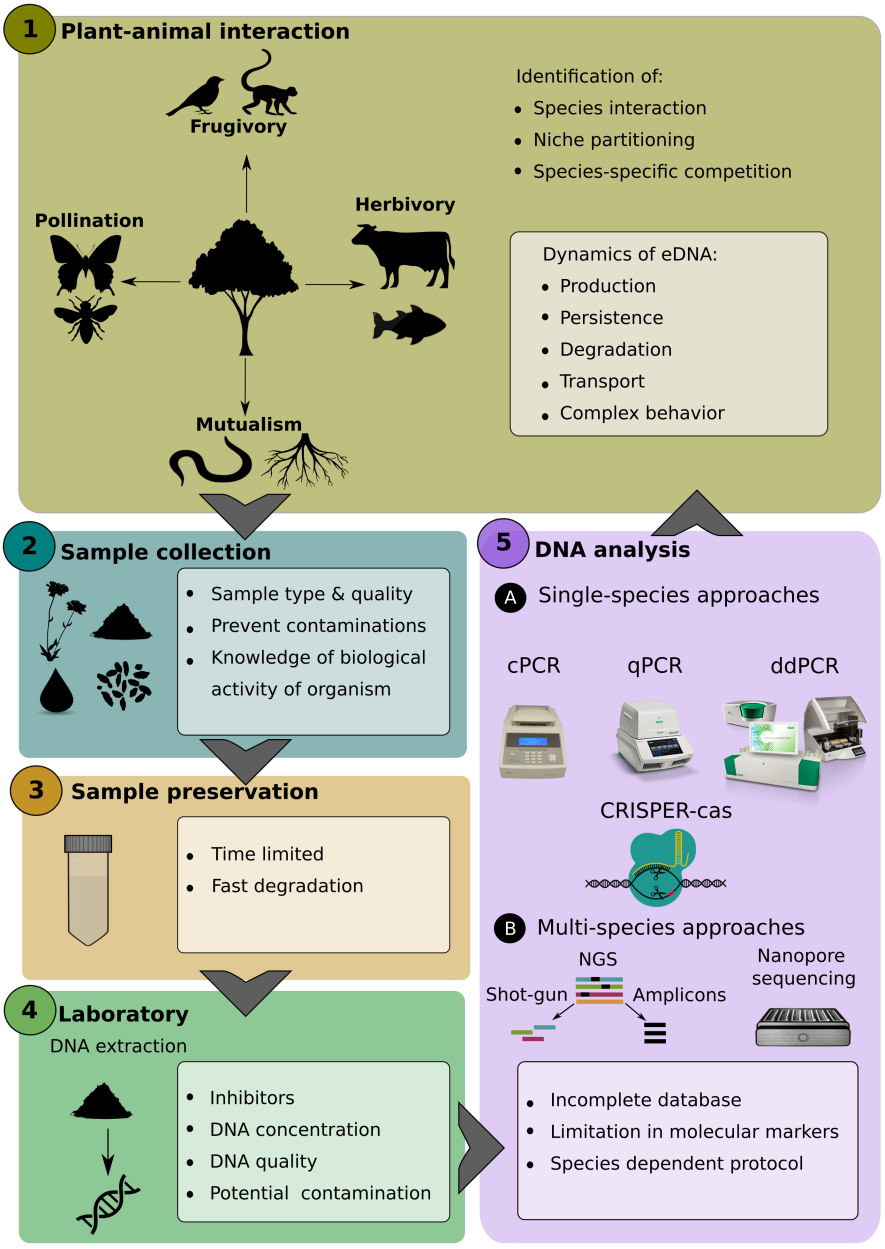

FIGURE 2 Workflow including potential limitations (inserted box) in each step for eDNA analysis in plant–animal interactions (PAI) detection (cPCR, conventional PCR; CRISPR-cas, clustered regularly interspaced short palindromic repeats—CRISPR-associated protein; ddPCR, droplet digital PCR; NGS, next generation sequencing; qPCR, quantitative PCR)

IV A universal limitation to any genetic-based species identification reliant on databases, is certainly missing species sequences, sequencing error, cloning vector contamination, and the redundancy of data (Singh, 2015). These issues may cause species misidentification, which may also lead to the failure in decrypting accurate PAI (Roslin & Majaneva, 2016; Sheppard et al., 2005).

V As eDNA methods sometimes struggle with low detection rates, more comparisons are needed between eDNA and conventional surveys (e.g., camera, malaise traps) for translation of results and inferences between these different methods.

VI The detection of niche partitioning using eDNA-based methods is only just beginning (ter Schure et al., 2021) and fine-scale partitioning (e.g, different herbivory behavior on the same plant) may be difficult with current eDNA analysis techniques.

VII Unsurprisingly, and similar to conventional approaches, eDNA methods also encounter some technical field and laboratory challenges. This is often because eDNA samples frequently contain PCR inhibitors thereby further reducing already low DNA concentrations (McKee et al., 2015). Besides this, false-positive and false-negative detections are also a matter of concern.

VIII Laboratory protocols, including the method of standardization, are directly dependent on sampling procedures, sample quality, environmental factors, and molecular markers design. Although recent studies show evidence of overcoming some technical limitations, such as primer development, chloroplast and nuclear primer for plants (rbcL, matK, trnH-psbA, ITS2, etc.), group-specific primers for animals (MiFish, MiBird, etc.), protocol standardization, and removing the barrier of inhibitors (Burian et al., 2021), collection, and analysis, optimization may still be required. Mitochondrial COI is the most common universal barcode for animals demonstrating good species discrimination (Che et al., 2012), but in plants, no single universal barcode

provides suitable taxonomic resolution (Jones et al., 2021). For plants, multiple primers from two primary plastid regions in the chloroplast (e.g., rbcl and matK), frequently combined with nuclear regions (e.g., ITS2), have been used for barcoding, but none of these have been found to be suitable across all species due to rampant introgressive hybridization and polyploidy (Jones et al., 2021).

2.4 | Future perspectives

The advent of eDNA quantification has offered an exciting but as yet untapped future in discovering the complex and dynamic pattern of species interactions. Implementation of eDNA analysis has thus far proved helpful in studying rapid changes in ecosystems (e.g., diversity and species interaction changes due to anthropogenic pressure; DiBattista et al., 2020) and may also advance our understanding of the effect of habitat fragmentation, sudden natural calamities, or rapid climatic changes (Bartlett et al., 2016). Environmental DNA may even demonstrate utility in assessing how range or phenological shifts via climate change alter PAI. For example, will climate change maintain or dismantle entire networks of integrated species? We envision research into the congruence or discordance of plant flowering time and their pollinators. Certainly, the ease of collecting eDNA is a major advantage to questions requiring successive time-series data (e.g., coevolution or niche separation), and we expect this to be a major avenue for investigation in the near future.

The ease and rapidity of eDNA analysis particularly lend itself to the monitoring of invasive species (Kim et al., 2018), and here too, eDNA methodology may illuminate how invasive species change complex species interactions on an ecosystem scale. While it is true that invasive species, at least initially, add to the net biodiversity of a region, will these species also add to species interactions, weaken specialized species interactions, or break them altogether? Here, eDNA analysis may be especially important for these assessments early during colonization events, when invasive species removal and thus their impact on well-established species interactions, may be circumvented.

Recent methodological developments to collect and extract environmental RNA (eRNA) might also be leveraged to understand changes in gene expression with physical and biological pressure (functional genomics; Tsuri et al., 2021), with possibilities of expansion into ecological epigenetics, ecosystem health, functional metagenomics, population-level inference, or even the interface of species-species interactions (e.g., Stewart & Taylor, 2020; Veilleux et al., 2021). Unlike eDNA, eRNA can go beyond species and PAI quantification, such as understanding life histories, ages, metabolic activities, physiological conditions, and health of interacting organisms. Functional information of a species, population, or community and their functional genes can be detected from mRNA profiling or miRNA for studying the health of organisms. Furthermore, the short persistence time of eRNA and resulting low concentration within the environment may help to avoid false-positive results and even potentially provide an estimate for the relative time of eDNA deposition and thus organismal origin (Marshall et al., 2021). However, to date, detection methods for eRNA are not yet well-established, lack broad validation in the field, and insufficient reference data may raise concerns.

3 | CONCLUSIONS

In the context of global biodiversity decline where ecosystems are under heavy stress and subjected to rapid changes, it is critical to increase our knowledge of species interactions to support the restoration and conservation of ecosystems effectively. Threats to species and ecosystem integrity are often assessed in terms of habitat loss, overharvesting, or over-predation (Kerr & Deguise, 2004). Yet, populations may also decline through successive loss of species interactions (Simmons et al., 2020; Valiente-Banuet et al., 2015), and studying species in isolation may limit our full understanding of the changes and threats to the entire ecosystem of interacting species (Roslin & Majaneva, 2016). In fact, positive and antagonistic interactions synergically work to maintain the stability, health, and function of an ecosystem, demanding a fast, reliable, and noninvasive/destructive approach. Currently, eDNA-based methods exhibit accurate information about species-specificity, community dynamics, and ecological networks. Although to date there remains a limited number of investigations using eDNA to critically assess and identify PAI, we propose eDNA methods to herald a revolutionary era for studying complex and cryptic ecological links in nature.

AUTHOR CONTRIBUTION

P.B. conceived of the review; P.B., K.A.S., C.M.A, I.V.B., C.Y.C., H.V., and S.S. prepared the first draft and revised the manuscript. All authors gave extensive edits and revised the manuscript, from conception to final draft. P.B. and H.V. prepared the figure with input from all authors; I.V.B. prepared the table with input from all authors.

ACKNOWLEDGMENTS

P.B. has been supported by Overseas Research Scholarships (ORS) from National Chung Cheng University as well as Ministry of Education (MOE)- Industry-Academia project (Taiwan). The authors would like to thank the Ministry of Science and Technology (Taiwan) for financial support (MOST 109-2811-M-194-502; MOST 108-2811-M-194-510).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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How to cite this article: Banerjee, P., Stewart, K. A., Antognazza, C. M., Bunholi, I. V., Deiner, K., Barnes, M. A., Saha, S., Verdier, H., Doi, H., Maity, J. P., Chan, M. W., Chen, C. Y. (2022). Plant–animal interactions in the era of environmental DNA (eDNA)—A review. *Environmental DNA*, *4*, 987–999. https://doi.org/10.1002/edn3.308