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 negative relationships simultaneously contribute to ecosystem functioning. Extinction of a single plant species may have deleterious effects on associated animals and vice-versa, and loss of interactions may occur prior to species-extinction. Monitoring species-interactions is therefore directly related to environmental health and functioning, and studying complex interactions through accurate, cost-effective sampling can aid in the management of detrimental anthropogenic impacts. Conventional PAI monitoring methods (e.g., camera, malaise, and pitfall traps) are potentially invasive, time-consuming, and often unable to achieve species-specific detection. While DNA barcoding of gut contents or bulk samples provides species-specific detection, saves time, and enables simultaneous detection of many taxa, these methods remain potentially

invasive and may require the sacrifice of study organisms. Alternatively, non-invasive environmental DNA (eDNA)-based monitoring provides accessible collection of biological signatures from the environment (air, water, soil) that can elucidate PAI. Environmental DNA methods have accurately detected plant-pollinator, plant-herbivore, and even some mutualistic relationships, from single-interacting species to the whole interacting community. In addition, a time-series of ecological interactions can be facilitated with eDNA methods. Although PAI studies using eDNA methods remain in their infancy, to date they have identified higher numbers of taxa in several direct comparisons to DNA-based gut/bulk sampling and conventional survey methods. Therefore, research into the influencing factors of eDNA detection involved in PAI (e.g., sources and types, methodological standardization, database limitations, validation with conventional surveys, and existing ecological models) will benefit the growth of this application. Involvement of environmental RNA (eRNA) can further strengthen eDNA-based methods, provide a better understanding of complex species-interactions, and help to avoid false positive results. Thus, implementation of eDNA methods to study PAI can particularly benefit environmental biomonitoring surveys that are imperative for biodiversity health assessments.

Keywords

Plant-animal interactions (PAI), environmental DNA (eDNA), molecular ecology, biodiversity loss, non-invasive biodiversity sampling, biodiversity monitoring, conservation management, ecosystem functioning

Introduction

Biodiversity arguably plays a prominent role in ecosystem stability (McNeely et al. 1990). However, rampant exploitation of natural resources have increased extinction rates (Myers 1990, Arneth et al., 2020), and altered land-use patterns (Daily, 1995), which would adversely affect ecosystem functioning (Arneth et al., 2020). According to the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), more than one million species are at risk of becoming threatened with extinction (IPBES, 2019), heralding the Anthropocene as the sixth mass extinction (Myers, 1990; Román-Palacios & Wiens, 2020). Yet 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 rate (Simmons et al., 2020). This is especially pertinent for specialist species, which have developed mechanisms of interaction on specific taxa (Colles et al., 2009). In fact, given that the loss of successive interactions is an important early warning system for the deterioration of ecosystem health (Valiente-Banuet et al., 2015), documenting and conserving such complex interactions is critical to retain ecosystem functioning.

One of the principle 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 negative relationships (e.g. predation; frugivory and herbivory, parasitism, and mutualism). For example, predation via frugivory contributes to propagation and thus facilitates plant restoration (Chama et al., 2013; Monge et al., 2020) and gene flow (Robledo-Arnuncio & Garcia, 2007). Herbivory leads to defoliation or root removal, which can regulate or diminish overall phytomass, but can also increase species diversity and influence plant distribution (Milchunas & Lauenroth, 1993; Castagneyrol et al., 2017), thereby regulating ecosystem stability (Wirth et al., 2008; Schallhart et al., 2012; Castagneyrol et al., 2017). In pollinator-plant mutualisms, the former acquires food 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 has already shed light on the complex network of species-specificity, habitat preference, and co-evolution between plants and their pollinators (Sargent & Ackerly, 2008). Mutualisms also assist with growth and offer protection from pathogens (e.g., plant-microbiome associations; Schirawski & Perlin, 2018). In contrast, negative interactions (e.g., parasites, parasitoids) can also affect the growth of plants and result in economical and ecological loss (Derocles et al., 2015). Thus, PAI underpin 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., camera, malaise, and pitfall traps, and gut-content analysis), are 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') may enable researchers and managers to scale up the documentation and monitoring of such relationships and do so at increased temporal and spatial frequencies with more cost effectiveness (see Figure 1).

Here we review the use of eDNA-based methods to study PAI. We discuss the advantages and current limitations of such methods, and propose research directions that are important next steps to enable eDNA-based methods for PAI analysis of ecosystems and their functions. Within this context, our goal is to highlight, for both researchers and managers, the potential utility of non-invasive eDNA-based methods, but we also aim to identify and clarify uncertainties and next steps needed to advance the methods for broad application.

Why use eDNA-based methods for studying PAI?

Species interactions are dynamic processes and their subsequent observation is difficult using discrete means of data collection (i.e. the conventional methods which are difficult to scale up in space and time). Studying species interactions would therefore require sampling methods that provide broad spatial and temporal inference. DNA-based methods offer a broad output with the capability of identifying multiple PAI simultaneously, and the ease at which DNA is collected and analysed also affords multiple sampling events for an integrative approach. The conventional applications of DNA-based methods (e.g., metabarcoding of gut contents and bulk samples) have already proved useful in elucidating complex species and trophic interactions (Garcia-Robledo et al., 2013). For instance, conventional DNA analysis from gut content or bulk samples have identified different nodes across various food webs, and reconstructed the trophic links in terrestrial (Wirta et al. 2014, 2015a, 2015b, 2016; Gogarten et al., 2020), 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 conventional DNA-based methods across multiple fields (Symondson, 2002; Valentini et al., 2009; Pompanon et al., 2012; Clare, 2014; Kress et al., 2015; Evans et al., 2016).

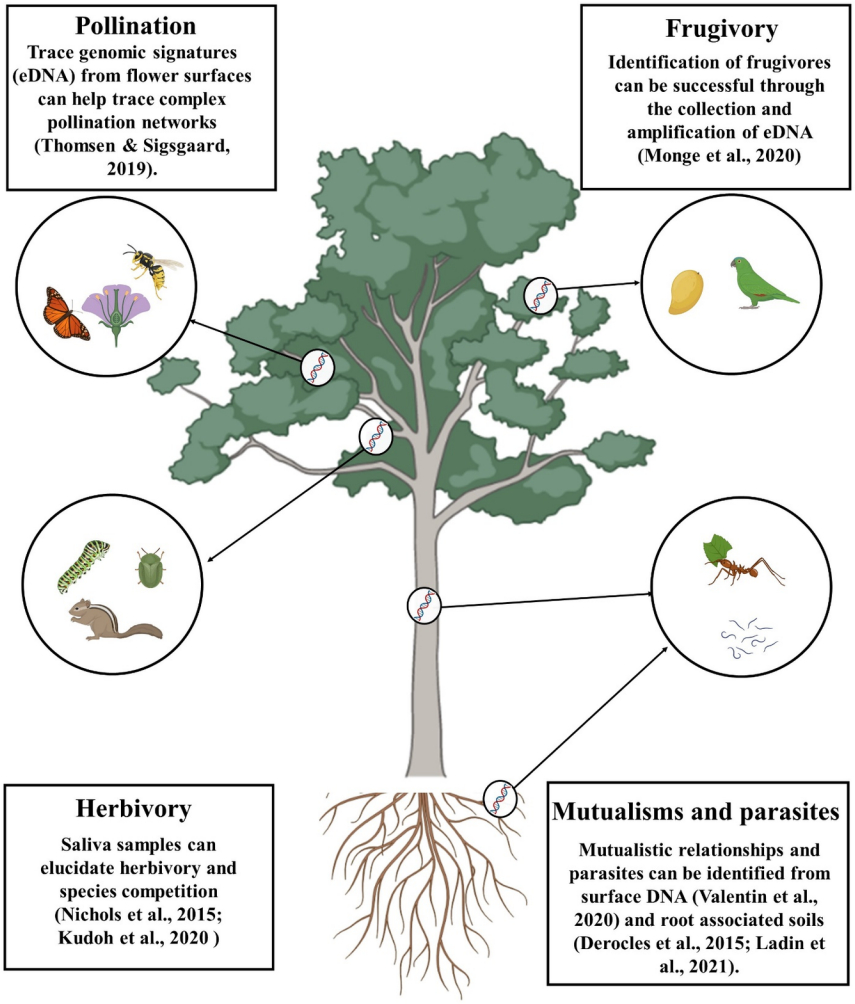


Figure 1: Biological signatures in the form of eDNA or eRNA can be detected from plants non-invasively to trace out complex interactions. Illustration presents hypothetical examples of PAI (e.g., pollination, herbivory, frugivory, and mutualism) including representative examples in the literature.

However, conventional DNA-based methods with tissue, bulk, and gut content samples can face practical limitations such as (i) its highly invasive nature that sometimes leads to the sacrifice of organisms; (ii) in gut content analysis - secondary consumption can manipulate or destroy possible interactive links (Guenay et al., 2021); and (iii) plant specimens in diet/dung can contain indigestible secondary metabolites that bind with DNA, which may hinder the isolation and amplification of DNA from samples (Echevarría-Machado et al. 2005).

In eDNA-based barcoding or metabarcoding, sample collections are not limited to direct sources (e.g., fecal, urine, or fur samples), but rather from the extra-organismal DNA, extracted from samples such as air, water and soil (Ficetola et al., 2008; Taberlet et al., 2012; Deiner et al., 2017). Novel developments, such as the collection of DNA from the surface of organisms (e.g. DNA from leaf surfaces; Valentin et al., 2020) have further highlighted eDNA's non-invasive advantages. While eDNA can be detected through non-target organisms such as insect derived DNA (iDNA) to target mammals (Gogarten et al., 2020), or DNA from flowers to target arthropods (Thomsen & Sigsgaard, 2019), these are still potentially invasive to non-target

taxa. Given the need for conservation-friendly sampling without harming any organism (including non-target taxa), collecting eDNA samples from soil, water, air, and even from the surface of an organism offers a completely non-invasive method (Valentin et al., 2020), preventing the scarification or sacrifice of organisms.

These non-invasive eDNA samples have cetainly advanced our ability to accurately detect the presence/absence of species (Deiner et al., 2021; Rodriguez-Ezpeleta 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 (McElroy et al. 2020; Fediajevaite et al. 2021). Additionally, eDNA-based methods have been shown to capture increased taxonomic diversity compared to conventional methods, which can be applicable for large scale monitoring (Macher et al., 2018). Thus, eDNA-based methods have gradually overcome limitations associated with the need for taxonomic expertise (currently a dwindling skill) and morphological identification. The latter being time consuming, laborious, and unable to detect phenotypic plasticity. Perhaps most importantly for the assessment of ecological integrity and functionality, eDNA has the ability to detect entire communities in a very short period of time.

Methodological development for the sampling and sequencing of eDNA has rapidly evolved from presence/absence detection of organisms (Ficetola et al., 2008) and the abundance and quantification data of eDNA signals (Taberlet et al., 2012), to the detection of the whole communities (Deiner et al., 2021), and even their trophic interactions (Thomsen & Sigsgaard, 2019; D'Alessandro & Mariani 2021). Indeed, eDNA-based methods have seen a sharp increase in adoption (Veilleux et al., 2021) and development into 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 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 (plant insects, plant-animal), predation (e.g., herbivory, frugivory), and mutualism (plant-nematodes, plant-ants, plant-animals) (Thomsen & Sigsgaard, 2019; Van Beeck Calkoen et al., 2019; Rasmussen et al., 2021) 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.

Current advancements in eDNA for the study of PAI

Although still in its infancy, species-specific assays as well as metabarcoding of eDNA have demonstrated great application for understanding PAI in nature (see Table 1). 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 myriad central ecological and evolutionary principles and theories. The loss of even a singular plant species can trigger rapid extinction of pollinators (e.g., honey bee), which is also of serious ecological and economical concern (Klein et al., 2007; NEA, 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 are analysed rapidly and cost-effectively (Hawkins et al., 2015; De Vere et al., 2017). 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 shows 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 (Thomsen & Sigsgaard., 2019; Kudoh et al., 2020). For example, Thomsen & 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 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 molecules to detect specific interactions of fruit eaters have now made this prospect more convenient. For example, Monge et al. (2020) successfully amplified salivary eDNA of frugivorous birds (*Ara macao*) from tropical almond (*Terminalia catappa*) fruit remains. Albeit with limited succes, this study further provided proof-of-concept for the use of eDNA in non-invasive 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 number of plant taxa eaten by particular herbivores and also the number of herbivores visiting particular plants. Importantly, eDNA-based methods have been shown to detect large numbers of taxa more efficiently than other sampling methodologies (e.g., microscopic analysis of fecal sample, bulk DNA metabarcoding; Tournayre et al., 2021). Environmental 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 of fecal matter may be a restricted application to large organisms, yet the collection of eDNA can be acquired through the rinsing of water from leaf surfaces to identify smaller taxa (Valentin et al., 2020). To overcome this limitation, saliva samples can be collected to identify herbivores that have fed upon specific plants (e.g., from browsed twigs, Valentin et al., 2020; or leaves, Nichols et al., 2012). In fact, Nichols et al. (2015) applied eDNA analysis across a large forest landscape, proving the utility of this method for studying cryptic browsing behaviour. 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, suggesting eDNA signatures might be able to quantitatively delineate the amount of herbivory.

Detecting negative PAI interactions through eDNA has also become possible recently. Derocles et al. (2015) for example, successfully amplified trace DNA from plants- leaf miners-parasitoid interactions and Thomsen & Sigsgaard (2019) detected large numbers of phytophagous species, parasitoids, gall inducers, and predator insects through the metabarcoding of flowers. Cumulatively, these studies provide a foundation for detecting negative 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 were 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 occured over one hundred years ago via ancient honey samples, highlighting eDNA's potential to reveal a time series of species interaction.

Current limitations

There remains a need to understand eDNA's current limitations, especially when it pertains to PAI detection and interpretation. Limitations are spread out among each step of the collection-analysis-interpretation process (Figure 2). It is therefore imperative to identify the necessary strategies before establishment of eDNA as one newer branch of PAI analysis. The existing limitations of this method are:

(I) The complex, and often idiosyncratic, ecology of eDNA. In effect, practitioners may sample different

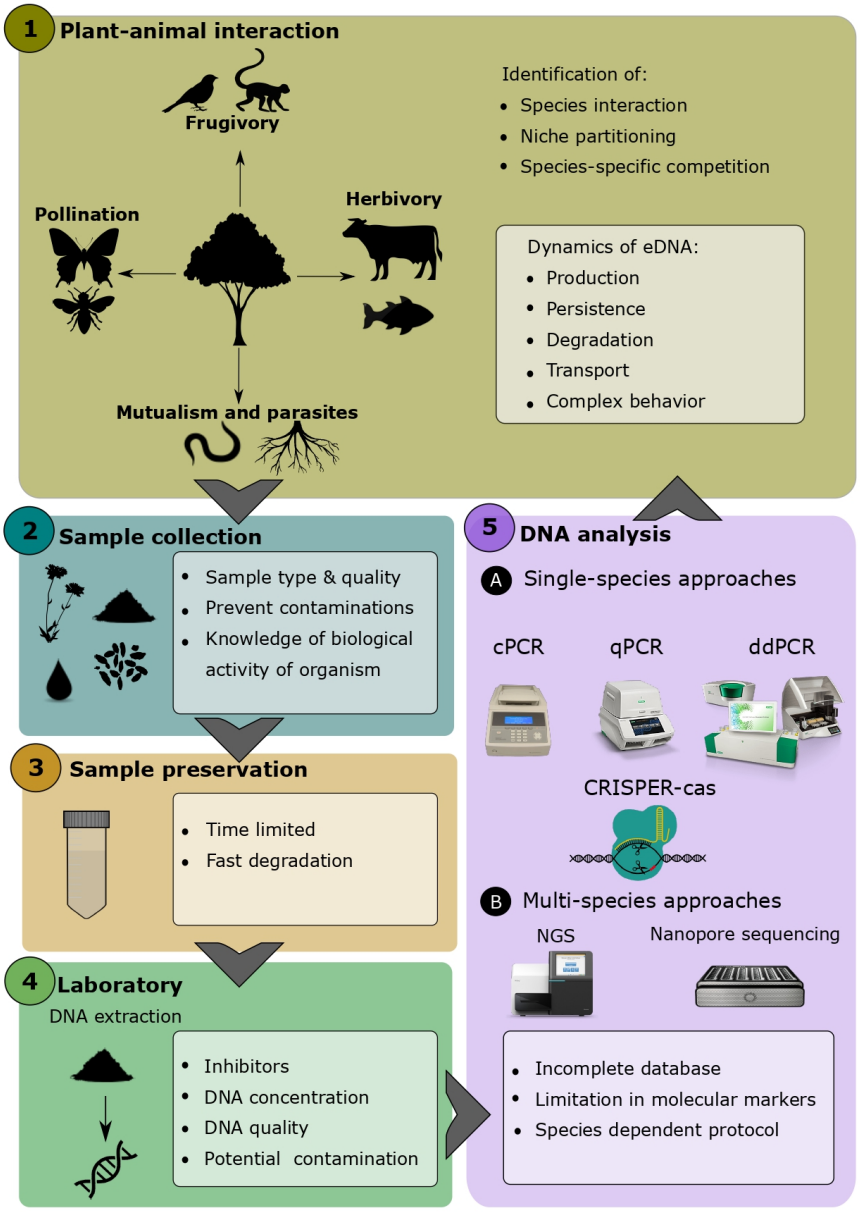
sources of eDNA (cellular, extracellular, extra-organismal, etc.) (Stewart, 2019; Rodriguez-Ezpeleta et al., 2021), which may lead to different PAI interpretations. For example, pollen and spores (extra organismal DNA) are more or less ubiquitous in the atmosphere, travel long distances (through wind or water), and contain adaptations to remain in dormant stages for long periods of time. These, when settled on non-targeted and non-interacting organisms, lead to misinterpretation. 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 their behavior may help to draw more precise conclusions. 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, production rate of eDNA is most likely influenced by species interactions themselves (e.g., competition between/among species) (Stewart, 2019). In fact, mixed-species fish populations have been shown to increase eDNA production rates when housed together compared to single species populations (Sassoubre et al., 2016). Beside 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 mediums (air, water, soil) should also be considered (Barnes & Turner, 2016; Lacoursiere-Roussel & Deiner, 2021), especially given these parameters have yet to be standardized for many taxa (Barnes & Turner, 2016).

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

(III) 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 also lead to the failure in decrypting accurate PAI (Sheppard et al., 2005; Roslin & Majaneva, 2016).

(IV) Comparative validations between the detection efficiency of eDNA to that of conventional surveys (e.g., camera, malaise traps), are necessary to justify the consistency of eDNA methods.

(V) 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 behaviour on the same plant) is difficult with current eDNA analysis.



(VI) 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). Laboratory protocols, including the method of standardization, is 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 group-specific primer development, protocol standardization, and removing the barrier of inhibitors (Burian et al., 2021), collection and analysis optimization may still be required.

Figure 2: Workflow including potential limitations (inserted box) in each step for eDNA analysis in Plant-animal interactions (PAI) detection (cPCR = conventional PCR, qPCR = quantitative PCR, ddPCR = droplet digital PCR, CRISPR-cas = Clustered Regularly Interspaced Short Palindromic Repeats- CRISPR-associated protein, NGS= next generation sequencing).

Table 1: Plant-animal interaction (PAI) studies using eDNA methods between 2009 and 2021. 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".

Types of interactions	Organisms involved	Applications
Mutualism /Symbiosis	Arthropods; grapevines and plants	Sustainable agricultural methods
Parasite/parasitoid	Parasitoid and plants	Environmental integrity and pest manageme
	Parasitoid and plants	Identification of plant -leaf miner parasitoids
Pollination	Arthropods and wild flowers	Environmental integrity and pest manageme
	Honey bees and plants	Pollinator -plant preference
	Honey bees; other plant sucking insect and plants	Ecosystem monitoring
	Plant, arthropods, bacteria, fungi, viruses	Describe trophic interactions of honey bees.
	Smith bees and plants	Historic plant-pollinator interactions
Predation	Birds and plants	Species interaction and potential use in popu
	Deers and plants	Forest management
	Gazelle and plants	Dietary assessment for conservation purpose
	Gorillas and plants	Dietary assessment for conservation purpose
	Grouse and plants	Dietary assessment for conservation purpose
	Herbivorous insects and plants	Verify insect-plant interactions
	Idaho ground squirrel and plants	Dietary assessment for conservation purpose
	Italian hare and plants	Dietary assessment for conservation purpose
	Lambs and plants	Dietary assessment and feeding selectivity
	Large mammalian herbivores and plants	Dietary assessment and niche partitioning
	Lemur and plants	Dietary assessment for conservation purpose
	Lotus roots (plants) and turtles	Dietary assessment and feeding activity
	Mammals and plants	Trophic interactions and key species for ecos
	Mammals and plants	Dietary assessment
	Pacific pocket mouse and plants	Dietary assessment for conservation purpose
	Tapir and plants	Dietary assessment and trophic interactions
	Ungulates and plants	Dietary assessment and foraging behaviours
	Ungulates and plants	Predict ecological interactions
	Woodland caribou and plants	Dietary assessment for conservation purpose

Future perspectives

The advent of eDNA-based methods 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; DiBattistaet 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 could envision research into the congruence or discordance of plant flowering time and their pollinators, or whether adaptive plasticity to changing environments marches in concert among tightly linked taxa. Certainly, the sampling ease of eDNA collections 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 lends itself to the monitoring of invasive species, and here too eDNA methodology may illuminate how invasive species change complex species interactions on an

ecosystem scale. While it is true invasive species, at least initially, add to the gross 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 to well-established species interactions, may be circumvented.

Recent methodological developments to collect and extract environmental RNA (eRNA) might also be leveraged to detect gene expression (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). In fact, eRNA's high concentration and short persistence time within the environment may help to avoid false positive results (Marshall et al., 2021). However, to date, detection methods for eRNA are not yet well established.

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, and in a non-invasive manner. Threats to species 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 (Valiente-Banuet et al., 2015; Simmons et al., 2020) and studying a single species may limit our full understanding of the changes and threats to an entire ecosystem as species interaction involves multiple species together (Roslin & Majaneva, 2016). In fact, positive and negative interactions synergically work to maintain the stability, health, and function of an ecosystem. This thus demands a fast, reliable and non-invasive 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.

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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 analysed during the current study.

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.

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