Received: 26 September 2022 | Accepted: 5 December 2022

DOI: 10.1002/ajb2.16120

REVIEW

American Journal of Botany

Environmental DNA as an emerging tool in botanical research

Mark D. Johnson[1,2] | Joanna R. Freeland[3] | Laura Parducci[4,5] | Darren M. Evans[6] | Rachel S. Meyer[7] | Brenda Molano-Flores[2] | Mark A. Davis[2]

[1]Engineering Research and Development Center, Construction Engineering Research Laboratory (CERL), Champaign, IL, USA

[2]Illinois Natural History Survey, Prairie Research Institute, University of Illinois Urbana-Champaign, Champaign, IL, USA

[3]Department of Biology, Trent University, 1600 West Bank Drive, Peterborough, ON, K9L 0G2, Canada

[4]Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy

[5]Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, Norbyvagen 18D, SE-75236, Uppsala, Sweden

[6]School of Natural and Environmental Sciences, Newcastle University, Newcastle Upon Tyne, UK

[7]Department of Ecology and Evolutionary Biology, University of California Santa Cruz, Santa Cruz, CA, USA

Correspondence

Mark A. Davis, Illinois Natural History Survey, Prairie Research Institute, University of Illinois Urbana-Champaign, Champaign, IL 61820 USA.
Email: davis63@illinois.edu

Abstract

Over the past quarter century, environmental DNA (eDNA) has been ascendant as a tool to detect, measure, and monitor biodiversity (species and communities), as a means of elucidating biological interaction networks, and as a window into understanding past patterns of biodiversity. However, only recently has the potential of eDNA been realized in the botanical world. Here we synthesize the state of eDNA applications in botanical systems with emphases on aquatic, ancient, contemporary sediment, and airborne systems, and focusing on both single-species approaches and multispecies community metabarcoding. Further, we describe how abiotic and biotic factors, taxonomic resolution, primer choice, spatiotemporal scales, and relative abundance influence the utilization and interpretation of airborne eDNA results. Lastly, we explore several areas and opportunities for further development of eDNA tools for plants, advancing our knowledge and understanding of the efficacy, utility, and cost-effectiveness, and ultimately facilitating increased adoption of eDNA analyses in botanical systems.

KEYWORDS

ancient botanical eDNA, aquatic botanical eDNA, botanical eDNA, eDNA, organism derived botanical eDNA, review

Extinction rates across the globe are increasing, large swaths of natural habitat are being altered by humans, and habitat degradation continues apace (Brondizio, 2019). Sala et al. (2000) identified land use and climate change as major drivers of biodiversity loss over the next century. These reports, and many others like them, highlight the urgent need for researchers to gather information on the ecology and biodiversity of natural habitats to conserve as many species as possible. The foundation upon which these ecological insights are built is biodiversity monitoring. While conventional biodiversity monitoring methods can provide detailed species information, they are also oftentimes destructive to the environment, require high levels of expertise, are geographically biased, require extensive time commitments, and the quality of results depends on the resources used throughout the process (Herrick et al., 2005; Garrard et al., 2008). Further, these challenges are often exacerbated by the so-called "taxonomic impediment," with increasingly fewer taxonomic professionals available to render accurate delimitation (Wheeler and Wilson, 2004;

BOX 1 Definitions of terms commonly used within the field of eDNA study

- **Environmental DNA (eDNA)** DNA from an organism and present in the environment and bulk environmental samples.
- **Botanical eDNA** eDNA from plants (e.g., pollen, leaf fragments, flower fragments) and collectible from bulk samples such as air, water, soil, feces, and from pollinators.
- **Aquatic botanical eDNA** Plant eDNA in freshwater (lotic and lentic) and saltwater.
- **Ancient eDNA** Degraded eDNA from ancient plant and animal communities.
- **Contemporary soil/sediment eDNA** eDNA from soils.
- **Airborne botanical eDNA** Plant eDNA in air and dust.

Agnarson and Kunter, 2007; Raposo et al., 2021). To help address these limitations and the need for rapid, cost-effective biodiversity information, researchers are increasingly looking to molecular genetic tools.

One ascendent molecular method is the collection and analysis of environmental DNA (eDNA). Historically, the definition of eDNA has taken either a sensu stricto (DNA of macrobial organisms in a bulk sample) or sensu lato (total pool of DNA in a bulk sample) approach (Pawlowski et al., 2020). In this context, we refer to eDNA as the DNA shed from a host organism into its environment (Ficetola et al., 2008; Thomsen and Willerslev, 2015; Barnes and Turner, 2016; Box 1). Species presence can be determined without a targeted organismal sample, thereby reducing environmental disturbance, sample bias, and sampling time (Taberlet et al., 2018). Furthermore, eDNA is collected via bulk environmental samples across an array of ecological contexts, including marine (Thomsen et al., 2012), freshwater (Jerde et al., 2011; Deiner et al., 2015), sediment/soil (Yoccoz et al., 2012; Parducci et al., 2017; Lin et al., 2021), and air (Johnson et al., 2019a). It can also be collected from various media including artifacts (Foley et al., 2012), animal legs (Arstingstall et al., 2021), feces (Wang et al., 2022), honey and resins (Chui et al., 2021), pitcher plant liquid (Littlefair et al., 2018) and flowers (Harper et al., 2022).

Early eDNA studies were largely reliant on single-species approaches via polymerase chain reaction (PCR) to assess presence/absence in a sample (Ficetola et al., 2008). However, eDNA technology has rapidly evolved, allowing researchers to track multiple species or even multiple population haplotypes (Adams et al., 2019) by means of multiplexed quantitative PCR, multiplexed metabarcoding, target capture (Foster et al., 2021) or metagenomics (Taberlet et al., 2018; Box 2). On the whole, these advances have ignited an explosion of research leveraging this genetic tool.

BOX 2 Common methods and terms used to describe the analysis of eDNA and interpret the results

- **Polymerase chain reaction (PCR)** A method to amplify a target DNA region and detect a species within an eDNA sample.
- **Quantitative PCR (qPCR)** PCR method that uses fluorescence to quantify target DNA in real-time for a species.
- **Multiplex qPCR** A qPCR method to simultaneously amplify two or more target DNA regions.
- **Digital droplet PCR (ddPCR)** The sample is partitioned into thousands of droplets, and amplification occurs in each individual droplet.
- **Target capture** DNA or RNA molecules are attached to a chip/bead designed to bind to target DNA regions and are then separated from nontarget sequences using a magnet.
- **Metagenomics** The study of total DNA or eDNA from all organisms in a bulk sample instead of on a specific DNA region.
- **Metabarcoding** The process of using DNA markers with high-throughput sequencing to identify numerous taxa within a sample and analyze and assess the total biodiversity. Species-specific genes or gene fragments (barcodes) are used to detect a target taxon.
- **Bioinformatics** Acquisition, storage, computation and analysis of biological data such as metabarcoding and metagenomic sequences to interpret and filter the results and assign taxa.
- **Primer** A short nucleic acid sequence that is complementary to a target DNA fragment from a target organism(s) and needed for amplification and sequencing.
- **Reference library** Known genetic sequence information that is used to match and identify unknown sequences, e.g., those generated from high-throughput sequencing of eDNA.

Environmental DNA has been deployed extensively and in multiple environments, but the focus has largely been on metazoans. However, botanical eDNA has emerged as the next frontier in eDNA research. Here we define botanical eDNA as DNA shed from plants (for this review, our scope is Streptophyta) into their environment and collected via nontargeted bulk sampling (Box 1). Such samples consist of pollen, leaf and flower fragments, and other plant tissues (e.g., roots) yielding freely available DNA, constituting trace DNA from plants in air, water, and sediment samples, directly on pollinators and other plant-visiting animals, or even present in

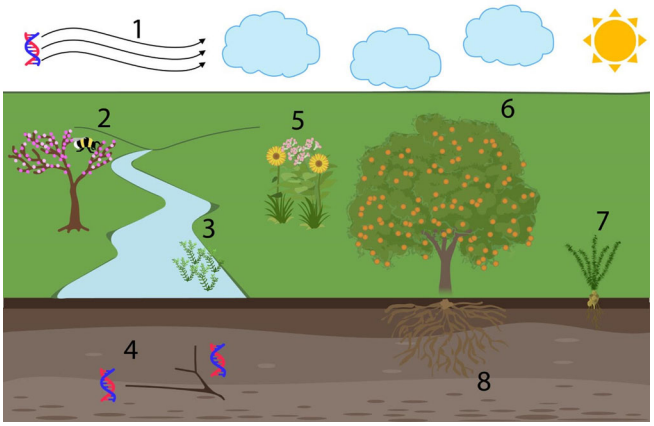

FIGURE 1 Sources of botanical eDNA and where samples can be collected in the environment. (1) Air. Airborne eDNA analysis can be used to assess plant biodiversity. (2) Organisms and their products (e.g., insects, honey, herbivores, feces). Samples can provide information on plant–animal interactions. (3) Water. (4) Ancient and contemporary soil. Samples may also be used for targeted rhizosphere analyses. (5) Flower fragments and pollen. (6) Fruit and seeds, released or carried into the environment. (7) Leaf fragments. (8) Root systems. Fragments can shed light on root–microbe interactions.

feces (Figure 1). While genetic detection and identification of pollen has been studied for years (Parducci et al., 2005; Kraaijeveld et al., 2015), eDNA approaches allow researchers to track plant species through pollen and other associated plant materials (seed, leaf, flower, free floating DNA, etc.) independent of pollination (Johnson et al., 2019b).

Characterizing botanical eDNA from aquatic, ancient, contemporary sediment, air, and biotic surface samples could revolutionize the ways researchers monitor invasive species, rare, threatened and/or endangered species, plant communities and even plant–animal interactions (Evans and Kitson, 2020; Johnson et al., 2023). Despite the vast potential for botanical eDNA, this method has been sparsely used across several botanical subdisciplines with little cohesion. And while Banerjee et al. (2022a) conducted a review introducing the concept of eDNA, and the methodology, and comparisons with conventional surveying in botanical systems, no comprehensive review has yet explored the current state of eDNA in botanical systems, methodological issues specific to botanical eDNA, and areas of need for future inquiry. Here, we review botanical eDNA applications with goals (1) to describe the current state of eDNA in botanical systems and (2) to delineate the challenges and opportunities for botanical eDNA into the future. Ultimately, our goal is to facilitate and increase the adoption of eDNA in botanical research using the best available information and methods.

AQUATIC eDNA IN BOTANICAL SYSTEMS

Early eDNA research focused primarily on aquatic systems, but studies investigating eDNA signatures of plant taxa are scant compared to metazoan aquatic eDNA studies (Beng and Corlett, 2020). The first botanical eDNA proof-of-concept study found that botanical eDNA could be isolated and characterized from water samples to detect invasive plants (Scriver et al., 2015). Since then, multiple studies have effectively isolated botanical eDNA from water samples in both laboratory and mesocosm settings (Fujiwara et al., 2016; Matsuhashi et al., 2016; Gantz et al., 2018; Coghlan et al., 2020; Kuehne et al., 2020). This foundational research paved the way for detecting plant species in lotic and lentic systems via species-specific assays (i.e., qPCR; Fujiwara et al., 2016; Gantz et al., 2018; Anglès d'Auriac et al., 2019; Chase et al., 2020; Kuehne et al., 2020; Miyazono et al., 2020; Doi et al., 2021) and community metabarcoding methods (Shackleton et al., 2019; Coghlan et al., 2020; Palacios Mejia et al., 2021; Tsukamoto et al., 2021). A particular area of emphasis is assessing the efficacy of eDNA approaches to detect invasive plants in aquatic systems. Fujiwara et al. (2016) developed primers for *Egeria densa*, a plant native to Brazil that has invaded areas of North America, Europe, and Asia. These markers have since been used to survey natural water bodies in Japan, revealing this approach to be more effective than conventional surveys (Miyazono et al., 2020). In another example, Gantz et al. (2018) used several assays to detect experimental and natural populations of invasive *Hydrilla verticillata* in the United States, recovering a correlation between plant observation and eDNA detection. However, Kuehne et al. (2020) used aquatic eDNA to screen invasive *Egeria densa* and *Myriophyllum spicatum* with species-specific ITS1 primers, revealing low detection rates, even where plants were highly visible, with weak relationships between eDNA concentration and plant abundance. Similarly, while species-specific primers detected invasive *Stratiotes aloides* eDNA from experimental systems, target eDNA from natural water bodies was detected infrequently, even when the invasive population was abundant (Marinich, 2017).

ANCIENT eDNA FOR BOTANICAL SYSTEMS

Ancient eDNA is perhaps the best-established source of botanical eDNA, taking advantage of DNA that has been preserved for impressively long periods of time (up to 2 million years in sediments and up to 800,000 years in basal ice; Kjaer et al., 2022; Willerslev et al., 2007) to examine past plant communities, compositional shifts over time, and potential impacts of climate change (Estrada et al., 2018). Oftentimes, studies on ancient eDNA have substantially different goals and foci because the material and temporal scale vary greatly from those of contemporary samples. Like in aquatic eDNA studies, the study of ancient botanical eDNA has lagged behind comparable investigations in vertebrate systems (Estrada et al., 2018). Nevertheless, groundbreaking work by Willerslev et al. (2003) found both plant and animal genetic information could be obtained from permafrost cores ranging from 10,000 to

400,000 years old, introducing a new method for accessing genetic records of past environments. Following these results, ancient plant DNA from a variety of media including basal ice (Willerslev et al., 2007; Gould et al., 2010), sediment (Kjaer et al., 2022), permafrost (Jørgensen et al., 2011; Pawlowska et al., 2014; Willerslev et al., 2014), lake cores (Matisoo-Smith et al., 2008; Anderson-Carpenter et al., 2011; Parducci et al., 2012; Parducci et al., 2013), herbivore middens (Murray et al., 2012), fecal material (Poinar et al., 2001; Hofreiter et al., 2003), dental calculus (Weyrich et al., 2015) and even caves (Haile et al., 2007; Haouchar et al., 2014; Pedersen et al., 2015; Slon et al., 2017; Stahlschmidt et al., 2019) have been assessed. Advances in ancient eDNA approaches have paved the way for assessing whole plant communities from sediment samples, facilitating novel comparisons of community change and biodiversity monitoring. For example, studies of lake sediments or mires have found temporal shifts in Holocene plant communities and their interactions (Gugerli et al., 2013; Pedersen et al., 2013; Parducci et al., 2019; Rijal et al., 2021), revealing how plant communities respond to a changing global climate.

Ancient eDNA studies continue to effectively leverage emergent technologies to understand how this genetic information can be harnessed to shape our understanding of history and inform our predictions for the future. Pedersen et al. (2015) highlighted the shift from metabarcoding to fully realized shotgun metagenomics. While metabarcoding uses specific barcoding genes to determine taxonomic composition of mixed samples, for shotgun metagenomics, the total eDNA from all the organisms in a sample is directly sequenced (Cowart et al., 2018). Ancient eDNA can be leveraged in this approach to detect species and estimate, in some cases, plant abundances (Pedersen et al., 2016; Parducci et al., 2019). Capo et al. (2021) noted that metagenomics would also allow for identification of the ultrashort DNA sequences that are common in ancient eDNA studies while removing taxonomic biases and blind spots linked to PCR-based approaches. To fully utilize this method in ancient eDNA, target capture approaches and more comprehensive genomic reference databases are essential (see below; Capo et al., 2021). Additionally, as the application of botanical ancient eDNA progresses, research could be used to shape climate change policy, track invasive species emergence, and inform conservation interventions (Ruppert et al., 2019). Plants have been present on the earth since the Paleozoic era, meaning they are valuable markers for large-scale, long-term climate change. Knowledge of past drivers, mechanisms, and responses could help predict future conditions (Estrada et al., 2018) because the study of ancient eDNA can help us elucidate how ancient plants responded to climate change, facilitating extrapolation to current communities and ultimately informing native species conservation. Wood et al. (2018) studied pack rat middens ranging from 200 to 49,600 years old to examine how plant pathogens changed over time and gain insights into how they may shift in the future. Additionally, Allaby et al. (2015) used ancient eDNA detections to model human and plant community coevolution, finding that oftentimes this evolution resulted in rapid adaptation of humans and/or plants at specific points in time.

CONTEMPORARY SOILS AND SEDIMENT eDNA FOR BOTANICAL SYSTEMS

While studies exploring ancient eDNA rely on sediment and core samples to determine the ancient record of plant species, some studies use soil to examine modern day botanical eDNA (Meyer et al., 2021). Buee et al. (2009) developed one of the first terrestrial soil metabarcoding studies examining fungal biodiversity in forest systems. Yoccoz et al. (2012) produced among the first metabarcoding surveys of plant biodiversity via soil samples, revealing that biodiversity detected via botanical eDNA from the soil matched aboveground biodiversity in boreal communities, tropical systems, and recovered decades-old crops. These groundbreaking studies have paved the way for studies documenting plant community biodiversity (Fahner et al., 2016; Edwards et al., 2018; Carvalho-Silva et al., 2021; Osathanunkul et al., 2021; Barnes et al., 2022; Ariza et al., 2022), rare plant conservation (Hartvig et al., 2021), sampling sand for meiofaunal communities (Castro et al., 2021), assessing community structure, including root associations (Blaalid et al., 2012; Martínez-García et al., 2014; Ruppert et al., 2019), eDNA ecology in the soil (Foscari et al., 2022), and tracking human land use (Foucher et al., 2020). Lastly, contemporary soil eDNA is beginning to be utilized for forensic ecology, by connecting soil ecological habitats and eDNA to the origin of soil and sediments (Flojgaard et al., 2019; Frankl et al., 2022).

AIRBORNE eDNA IN BOTANICAL SYSTEMS

Still in its infancy, the study of airborne eDNA represents an innovative frontier in assessing terrestrial plant biodiversity. Airborne eDNA was recently highlighted as a global conservation issue (Sutherland et al., 2022). Early airborne botanical genetic sampling focused specifically on pollen detection and its human health impacts (Folloni et al., 2012; Kraaijeveld et al., 2015; Korpelainen and Pietilainen 2017; Mohanty et al., 2017). Johnson et al. (2019a, 2019b) expanded on these early studies to explore airborne eDNA as a bulk environmental sample instead of focusing explicitly on pollen and human health. Johnson et al. (2019b) detected the presence of nonflowering insect-pollinated species via airborne eDNA, illustrating that this material expands past pollen and could be used to detect whole plant communities. Banchi et al. (2020) expanded on this research, assessing airborne plant and fungal diversity

over a 9-month period and tying it to human health impacts. Furthermore, Johnson et al. (2021a) revealed spatiotemporal variation in species composition, reflective of differences in phenology and of acute disturbances at a landscape scale. Both Banchi et al. (2020) and Johnson et al. (2021a) found airborne eDNA to be ideal for tracking changes in phenology over time. Expanding into applied uses of airborne eDNA, researchers have explored the impact airborne eDNA can have on determining the geographic origin of dust or examining eukaryotic communities within the global dust belt (Aalismail et al., 2021; Lennartz et al., 2021). Most recently, Johnson et al. (2021b) found that airborne eDNA metabarcoding was more sensitive and efficient than conventional transect-based plant community surveys, while also detecting more invasive plant species, thereby offering a potential large-scale, multifaceted biodiversity monitoring paradigm. Furthermore, Johnson et al. (2021b) explored the detection of species throughout the year. While they found that wind-pollinated species' eDNA was more abundant during the pollination season, they also found that they could detect species that were not flowering, considered dormant, or were insect-pollinated, further highlighting the lack of reliance on pollen for detecting plant species with airborne eDNA.

As the field advances, we need to understand more about how botanical airborne eDNA can be used for specific applications in ecology and conservation. Johnson et al. (2021a) found that airborne eDNA can be used to track acute, landscape-scale disturbance, indicating this method may be useful for monitoring long-term climate change and/or restoration. Lennartz et al. (2021) revealed that airborne eDNA can be used in forensic applications by tracking the source of dust depositions. This research has only scratched the surface of what may be possible. Studying invasive species detection, long-distance dispersal, and metapopulation dynamics are all viable applications to be addressed.

ORGANISM-DERIVED SAMPLES FOR BOTANICAL eDNA

There are myriad possible organism-derived sources of botanical eDNA, including honey, animal shedding on plants, gut contents, and frass and fecal samples. Botanical eDNA from a variety of sources has been leveraged to understand plant–animal interactions. Banerjee et al. (2022) assessed the applications of eDNA for elucidating plant–animal interactions, highlighting studies of mutualism (Rasmussen et al., 2021), pollination (Thomsen and Sigsgaard 2019; Evans and Kitson 2020), frugivory (Monge et al., 2020), parasitism (Thomsen and Sigsgaard, 2019; Miller et al., 2021), dispersal (Harrer and Levi 2018), and herbivory (de Sousa et al., 2019) for plant species through mammals, birds, and insects. Two of the most-studied aspects include pollinators and feces. Wilson et al. (2010) were among the first to study pollinator interactions by examining pollen on Hawaiian bees to document what flower species were regularly visited and preferred. Later, Keller et al. (2015) examined mixed-pollen samples via metabarcoding and conventional microscopy. Presently, multiple studies have examined pollinator preference via plant eDNA (Richardson et al., 2015; Sickel et al., 2015; Evans and Kitson 2020). Furthermore, eDNA has also been used to create network analyses based on the DNA collected from insects (Evans and Kitson 2016; Pornon et al., 2016; Galliot et al., 2017). Recently, Thomsen et al. (2019), Harper et al. (2022), and Gomez et al. (2022) detected residual insect eDNA on flower heads, highlighting a novel avenue for elucidating plant–insect interactions. Honey is another source of botanical eDNA representing plant–insect interactions, containing a wide array of DNA sources such as pollen, plant fragments, pathogens, and parasites (Ribani et al., 2020). Laube et al. (2010) designed primers and detected plant species linked to various commercial brands of honey. Valentini et al. (2010) then used honey and metabarcoding to distinguish and determine plant species origin for two commercial honey brands. Since then, additional studies have explored botanical eDNA in honey to elucidate the mutualistic roles of plants and bees and to produce accurate labels for specific commercial honey brands (Jain et al., 2013; Hawkins et al., 2015; de Vere et al., 2017).

Botanical eDNA has also been isolated from fecal samples to assess diets. Hoss et al. (1992) was the first to examine the genetic component of feces, exploring the diet of European brown bears in Italy. Bradley et al. (2007) revealed that both gorilla and monkey feces yielded eDNA from several local plants. Since then, fecal eDNA has been used to examine plants in mammal, bird, and fish diets (Harrer and Levi, 2018; de Sousa et al., 2019; Ruppert et al., 2019). As applications for botanical eDNA continue to expand, new sources of eDNA are constantly being discovered. For example, plant species themselves have come into focus as a viable source of eDNA, either through washing plant material and collecting the runoff (Valentin et al., 2020) or directly swabbing plants to detect mammals or insects (Kudoh et al., 2020; Lyman et al., 2022).

Whether collected from the water, sediment, soil, air, feces, or other source, botanical eDNA is an emerging and powerful tool to assess a diverse suite of factors (biodiversity, networks, interactions) and answer unique research questions within plant communities. However, despite this incredible and rapidly expanding area of inquiry, there remain foundational challenges to be addressed and unique opportunities to explore.

CHALLENGES WITH CENSUSING PLANT DIVERSITY FROM eDNA

As botanical eDNA is in its infancy, there remain several caveats and challenges to address to expand this method as a sensitive and reliable conservation and monitoring tool. To

advance eDNA research in botanical systems, lessons gleaned from the development of eDNA analysis in nonbotanical systems may be instructive. Here we identify several challenges to botanical systems that may influence the efficacy of eDNA methodologies. While many of these questions have been and continue to be addressed, our understanding of how they influence botanical eDNA efficacy and efficiency remains limited.

Influence of abiotic factors

How abiotic factors influence the transport, persistence, and fate of eDNA in botanical systems remains the most substantial challenge. In aquatic systems, numerous abiotic factors, including stream flow (Curtis et al., 2021), substrate type (Buxton et al., 2017), temperature (Jo et al., 2019), UV light (Kessler et al., 2020), and pH (Strickler et al., 2015; reviewed by Harrison et al., 2019) impact the availability and longevity of eDNA. In the recent exploration of airborne systems, the focus has been on understanding the basic ecology of eDNA (origin, state, transport, and fate; Barnes and Turner, 2016), including what influences the detection distance for airborne eDNA, the impact of height on collection, and methods of collection. Abiotic factors undoubtedly influence botanical eDNA across all sampling media. For example, rainfall has been found to limit the detection of airborne eDNA particles (Johnson et al., 2019b), while ancient botanical eDNA collection is subject to climatic and temporal limitations, often being most successful in frozen and arid environments (Rawlence et al., 2014; Ruppert et al., 2019). Furthermore, microbial activity can play a significant role in the degradation of eDNA, ostensibly impacting botanical eDNA from all media (Zulkefli et al., 2019). In short, while some studies have examined the abiotic impacts on the availability, longevity, and transport of botanical eDNA (Zhu, 2006; Pote et al., 2009; Yoccoz et al., 2012; Johnson et al., 2021a), considerable research is required on a per system basis to understand the full intricacies and interplay of abiotic factors. Furthermore, understanding how abiotic factors impact degradation may be of particular importance in botanical systems, as botanical eDNA includes a variety of material that may differentially degrade. For example, pollen has evolved strategies for long-term persistence, sometimes remaining viable for years (Yuan et al., 2018), ostensibly yielding contemporary botanical samples biased toward pollen detection because other materials degrade much faster. These intricacies are laid bare by ancient eDNA studies, which do not focus primarily on pollen but instead on DNA conservation since most samples come not from pollen, but from nonviable material such as macrofossils (seeds, leaves fruits, roots, etc.; Parducci et al., 2019).

Influence of biotic factors

Phenologies critically impact detections in nonbotanical systems (de Souza et al., 2016; Curtis et al., 2021) and in botanical systems as well. Some plants deposit substantial amounts DNA, while others do not, often depending on environmental conditions, developmental stage, and plant anatomy and physiology. Johnson et al. (2021b) revealed that while species could be detected throughout the year, detections and reads increased during a species' flowering season. In aquatic systems, species phenology heavily impacts the amount of botanical eDNA released into the environment. Matsuhashi et al. (2019) found aquatic botanical eDNA samples had higher concentrations of *Hydrilla verticillata* in the growing season versus the dormant season. This trend was reinforced in a variety of studies, collectively suggesting that aquatic botanical eDNA concentrations may increase later in the season when plant biomass has peaked and seasonal decay has begun, at least in temperate zones, although investigations across taxa and ecological contexts are required (Anglès d'Auriac et al., 2019; Kuehne et al., 2020; Kodama et al., 2022). Additionally, phytochemistry may influence DNA yield from plant tissue fragments. As plant cells lyse in senescence, vacuolar nucleases and other enzymes that degrade DNA are released. Furthermore, many plants are well known to yield little DNA when dry or to possess difficult tannins that are coextracted and are PCR inhibitors (Pyle and Adams, 1989). Overall, the abundance and detectability of botanical eDNA is heavily influenced by biotic factors including growth form, flower morphology, pollination syndrome, and flowering phenology. These relationships need to be examined on a case-by-case basis to ensure that detection is optimized, especially in cases of rare, threatened, endangered, and invasive species detection.

It is vital to ensure that biotic contamination is both understood and proper control procedures are in place. Across eDNA studies, the utilization of field, extraction, and amplification blanks, sterilized equipment, and bleach solutions to control for contamination control have been established as best practices to account for contamination at all stages of sampling and sample processing (Goldberg et al., 2016; Sepulveda et al., 2020; Hutchins et al., 2021). Most recently, airborne eDNA has been shown to have the potential to be a significant source of contamination. Klepke et al. (2022) found that both plant and animal airborne eDNA can accumulate over time in water samples. More research into the potential impact of airborne contamination needs to be explored, since within every eDNA project, samples are exposed to the air (Klepke et al., 2022). These results also highlight the need for rigorous field controls and replication across an experiment to control for potential contaminants.

Taxonomic resolution and primer choice

While reference library availability and use has been discussed extensively for eDNA (Ruppert et al., 2019; Stoeckle et al., 2020; Jerde et al., 2021), the availability of botanical reference library information has lagged behind vertebrate data (Prieto et al., 2021). This lack of botanical reference library information indicates that the continued bolstering of curated, online sequence repositories is essential to maximize the potential of eDNA approaches to document plant communities. Additionally, reference libraries have shown geographic inequality toward the global north and first-world countries (Schenekar, 2022), so improved equity in global coverage is imperative for successful eDNA metabarcoding. There are also challenges related to choosing botanical eDNA markers. The Consortium for the Barcode of Life (CBOL) Plant Working group has recommended the *rbcL* and *matK* chloroplast genes as standard DNA plant barcodes (CBOL Plant Working Group, 2009). Additionally, Chen et al. (2010) found that the ITS2 marker was effective for detecting plant species and focused on nuclear DNA instead of chloroplast DNA. Environmental DNA is often degraded, low quality, and low quantity, which may unsuitable for longer reads (e.g., *matK*). As a result, a smaller *trnL* chloroplast marker has been developed to identify plants via eDNA (Taberlet et al., 2007; Bell et al., 2017). For samples with degraded DNA, mini-barcodes (Little, 2013) are more likely to be amplified, but shorter barcodes contain fewer diagnostic sites. Although multiple plant markers are available, no consensus exists within botanical eDNA due to various primer biases (based on interactions with both target organism DNA and reference library information available), which can have substantial impacts on downstream results. Johnson et al. (2019a, 2021b) found that the c and h *trnL* primers detected more plant eDNA than ITS2 did, but also had more unassigned reads than ITS2, indicating *trnL* may be ideal for bulk eDNA studies and species-specific approaches, while ITS2 could detect more species in a metabarcoding analysis with the current state of genetic reference information. By contrast, *rbcL* and ITS2 had higher taxonomic resolution and reference data than *matK and trnL* did for terrestrial plant communities (Fahner et al., 2016), whereas for aquatic plants one study found that *matK* had higher species resolution than either *rbcL* or *trnL-psbA* (Scriver et al., 2015). Yet still another study revealed *rbcL* was more informative than either *matK* or ITS2 for identifying taxa (Coghlan et al., 2020). In terms of ancient eDNA, oftentimes choice is limited to *trnL*-based approaches because the fragment is short with high resolution and occasionally to the longer ITS fragment (Parducci et al., 2017). These conflicting results highlight the challenge associated with choosing the appropriate marker for botanical research and the need for better understanding of both primer and pipeline biases and their interactions. Study-by-study, region-by-region assessments of ideal markers and reference libraries are critical to ensure maximization of information gleaned from botanical eDNA research. Primer choice may also falsely lead to negative detections if the DNA is too fragmented compared to the marker size or if there are mismatches. Assays should be vetted with positive (e.g., mock communities) and negative controls, and models such as site occupancy modeling can help estimate absence. We still have much to learn, so we encourage researchers and editors to publish not only aquatic plant eDNA investigations that have generated positive results, but also those that have generated results that are largely and unexpectedly negative or are too erratic to allow for easy explanation.

Undefined spatiotemporal scales

Environmental DNA from plants does not necessarily align with other environmental plant evidence, and scientists are only beginning to describe the different spatiotemporal scales that botanical eDNA represents. Using pollen as an example, eDNA analysis may measure different spatiotemporal scales from pollen in the same sample. Nogueira et al. (2021) found that dust from across Africa regularly reaches the Amazon Basin, while Johnson et al. (2021b) found that species from several miles outside the study site were detectable. Thus, eDNA surveys can detect both large scale plant biodiversity patterns and smaller-scale plant communities. Researchers have been identifying plants through morphological study of pollen grains in sediment (Parducci et al., 2005) or ambient air (Kraaijeveld et al., 2015) for decades, but eDNA preserved in sediment is thought to largely come from non-pollen sources (Parducci et al., 2019). When pollen and eDNA from the same lake sediment have been compared, researchers observe that pollen signals are often more regional in scale—like that of surrounding hills—than eDNA, because sediment eDNA reveals local plant communities (Parducci et al., 2017). Furthermore, in aquatic and airborne systems, water flow and air streams have been shown to act as conveyor belts for downstream and downwind detection (Deiner et al., 2016; Johnson et al., 2021b). Considerable knowledge gaps also exist for all aquatic-based eDNA assays (i.e., not just plants) regarding patterns and processes associated with degradation, diffusion, and dilution of eDNA in water bodies (Harrison et al., 2019; Deiner, 2021; Lacoursière-Roussel and Deiner, 2021).

Relative abundance

Environmental DNA researchers often hope to estimate relative abundance or density of organismal biomass from environmental samples, but relative proportions of eDNA are unlike clip strip data or forest surveys of diameter at breast height. There has been no correlation established between plant eDNA concentrations and relative biomass or

abundance. Differences in pollen size and mass lead to major gradients in travel distance of pollen granules (Mohanty et al., 2017), and again, phytochemicals can degrade eDNA and inhibit PCR. However, laboratory assays introduce their own biases that distort observed relative abundances from actual abundances. Polymerases in PCR usually have some level of "GC bias" where some templates with a certain GC content compared to AT content will not amplify equivalently. Nichols et al. (2018) demonstrated this bias can have an outsized effect on the number of plants recovered in a metabarcoding assay with *trnL* primers from soil. PCR is also innately stochastic, and in most environmental substrates (e.g., water, soil), samples are dominated by microbes and nontarget taxa (Leese et al., 2020). Therefore, plant DNA is relatively rare, and thus, technical PCR replicates may exhibit major differences in presence and relative abundance of plants simply by random chance (Shirazi et al., 2021).

THE FUTURE PROMISE OF BOTANICAL eDNA

As research on botanical eDNA has evolved, several exciting frontiers have emerged. Global conservation efforts rely on our ability to monitor biodiversity, but also understand more about genetic lineages within species that are often not considered. While studies are already using botanical eDNA to determine the presence of invasive and endangered species (Johnson et al., 2021b; Tsukamoto et al., 2021), opportunities are emerging to gain population-level insights and estimate abundance from botanical eDNA. Sigsgaard et al. (2016) found that eDNA analysis of water samples can be used to detect long and abundant mtDNA, facilitating population genetic analyses. Additionally, Andres et al. (2021) used eDNA samples to detect intraspecific genetic diversity using microsatellite frequencies for an aquatic invasive species. As described by Sigsgaard et al. (2019), most studies examining population level data from eDNA samples have focused on vertebrates in aquatic environments, including marine (Sigsgaard et al., 2016; Stat et al., 2017; Baker et al., 2018; Parsons et al., 2018; Turon et al., 2019) and freshwater (Uchii et al., 2016; Goricki et al., 2017; Marshall and Stepien, 2019; Sigsgaard et al., 2019; Stepien et al., 2019) systems. In botanical systems, pollen has been used for decades to study plant population genetics (Ennos, 1994), and others such as Monge et al. (2020) found that avian saliva on fruit provided a source of eDNA from which several microsatellite markers could be amplified. However, no research exploring the potential of botanical eDNA for population level analyses has been published to date. Such studies need to examine the relationship between the preservation of varying botanical eDNA sources and allelic frequency in a target system. Exploring population genetics using botanical eDNA samples would contribute information on how populations are responding to changes in the environment and how genetic diversity shifts in imperiled taxa.

In addition to understanding more about specific populations, botanical eDNA also presents an opportunity for long-term monitoring of anthropogenic change and disturbance regimes. Like population genetics, most of the research into how eDNA can be used to monitor disturbance comes from water and sediment. Cowart et al. (2020) collected substratum samples to track the recolonization of benthic invertebrate communities around deep-sea vents after an induced disturbance. Other studies have tracked anthropogenic disturbance in marine (Bakker et al., 2017; Xie et al., 2018; Nichols and Marko, 2019; DiBattista et al., 2020) and freshwater systems (Klymus et al., 2017; Sun et al., 2019; Hempel et al., 2020). We also highlighted several studies that have examined how botanical eDNA changes due to abiotic (e.g., UV, wind, water conditions) and biotic factors (e.g., phenology, growing season), while fewer studies have examined the impact of anthropogenic change on botanical eDNA. Yoccoz et al. (2012) used sediment botanical eDNA to track which soils had been cultivated and with which crops. Giguet-Covex et al. (2014) tracked plants and domestic mammals using ancient DNA from lake sediments and revealed the effects of anthropogenic factors on landscape-scale changes since the Neolithic period. More recently, Johnson et al. (2021a) analyzed airborne eDNA to track differences in botanical eDNA concentration due to human disturbance in the form of honey mesquite removal. These preliminary results with modern botanical eDNA indicate that it may be a useful tool in tracking long-term ecological change (e.g., climate change, fire, weather events, invasive species) or assist with adaptive restoration projects where continued monitoring is vital to the process (Walters, 1986; Johnson et al., 2021a). Exploring how botanical eDNA can assist with monitoring anthropogenic disturbance will be vital as disturbances across the world continue to increase.

As work with botanical eDNA advances, there will be opportunities for increased use of metabarcoding, metagenomic, and targeted capture approaches as opposed to targeted (e.g., single-species) detection (Box 2). We are already seeing the impact of eDNA metabarcoding in botanical systems (Yoccoz et al., 2012; Giguet-Covex et al., 2014; Coghlan et al., 2020; Johnson et al., 2021b; Tsukamoto et al., 2021), but there are many more questions that can be addressed. By increasing the frequency and regularity of metabarcoding analyses within the field of botanical eDNA, subsequent results will be better suited for longer-term surveys. Furthermore, these long-term surveys with a multispecies focus will generate more information related to community composition, response to invasive plants, impact of abiotic change on species distribution and abundance, and much more. In aquatic systems, these applications are being thoroughly explored, providing a script of possible questions, challenges, and opportunities for using botanical eDNA more broadly (Ruppert et al., 2019). Target capture approaches in particular are showing promise in extending the inferential. Target capture approaches use RNA or DNA molecules designed

to bind to target DNA regions, which are then separated magnetically from nontarget sequences (Foster et al., 2021). Target capture approaches remove the need for global primers and eliminates the risk of primer and PCR bias (Foster et al., 2021). This method is increasingly used with ancient eDNA botanical systems. Murchie et al. (2020) used this method to detect Pleistocene–Holocene plants and animals, while Foster et al. (2021) used a target capture approach to detect botanical eDNA within a sediment core. Furthermore, in aquatic systems, eDNA metabarcoding has been shown to be a cost-effective alternative to large-scale, conventional sampling (Balint et al., 2018). Botanical eDNA metabarcoding, on the other hand, requires more research to assess its cost effectiveness compared to that of conventional surveying.

Lastly, as technology progresses, exploring aspects of population ecology, species presence/absence, and long-term monitoring based on environmental RNA (eRNA) will be critical. Because eDNA can persist in an environment, detections can raise questions of whether a species is contemporarily present, whether its DNA was transported from elsewhere, shed by a deceased individual, or stored long ago in subsequently released from sediment (Marshall et al., 2021). However, eRNA is a much less stable source of genetic material (Tsuri et al., 2020; Farrell et al., 2021). Nevertheless, it may be possible to determine the presence of metabolically active populations, conduct population and community diagnostics as eRNA signals change in response to health and stress, and examine the age of eDNA samples (Marshall et al., 2021; Yates et al., 2021). While promising, the use of eRNA as a tool remains in its infancy, and very few studies have examined botanical eRNA and its potential benefits (Marshall et al., 2021; Yates et al., 2021). Studying the applications of eRNA for detecting and assessing the health of plant species in water, sediment, and air represents a new frontier in environmental sampling.

CONCLUSIONS

As habitat degradation, invasive species introduction, climate change, and anthropogenic disturbance fuel the global biodiversity crisis, the primary way to combat these losses includes biodiversity monitoring (Thomsen and Willerslev, 2015). Tracking plant community change is vital, because these communities often represent the foundation of an ecosystem (Wilsey and Potvin, 2000). Currently, botanical eDNA analyses have been useful for detecting plant species in a variety of environments (i.e., aquatic, ancient, contemporary sediment, airborne) of different ages, while also shedding light on environmental conditions and plant–animal interactions (Banerjee et al., 2022b). While several challenges remain for the promise of botanical eDNA analysis to be fully realized, it has clearly emerged as a viable and important tool for plant ecologists. As we continue to solve the challenges of botanical eDNA and look to the future, we suggest that botanical eDNA will help to revolutionize the field of biological monitoring in global botanical systems.

AUTHOR CONTRIBUTIONS

M.D.J., B.M.F., and M.A.D. planned the manuscript. M.D.J. and M.A.D. wrote the manuscript. J.R.F., L.P., D.M.E., R.S.M., B.M.F., and M.A.D. provided editorial feedback.

ACKNOWLEDGMENTS

We thank the Botanical Society of America, who provided funding for the symposium Frontiers in Botany: Environmental DNA as an Emerging Tool for Measuring, Monitoring, and Managing Plant Biodiversity and for inviting this review. We also thank an anonymous reviewer and an *AJB* Associate Editor for providing thoughtful comments that substantively and substantially improved this manuscript. Finally, we thank Dylan Sarver for graphic design of Figure 1.

DATA AVAILABILITY STATEMENT

The authors have no data to report.

ORCID

Mark D. Johnson http://orcid.org/0000-0002-0460-945X
Joanna R. Freeland http://orcid.org/0000-0002-5251-7680
Laura Parducci http://orcid.org/0000-0003-1956-4757
Darren M. Evans http://orcid.org/0000-0003-4061-6726
Rachel S. Meyer http://orcid.org/0000-0002-4907-5797
Brenda Molano-Flores http://orcid.org/0000-0002-0899-3102
Mark A. Davis http://orcid.org/0000-0001-9034-9430

REFERENCES

Aalismail, N. A., R. Díaz-Rúa, N. Geraldi, M. Cusack, and C. M. Duarte. 2021. Diversity and sources of airborne eukaryotic communities (AEC) in the global dust belt over the red sea. *Earth Systems and Environment* 5: 459–471.

Adams, C. I. M., M. Knapp, N. J. Gemmell, G, Jeunen, M, Bunce, M. D. Lamare, and H. R. Taylor. 2019. Beyond biodiversity: Can environmental DNA (eDNA) cut it as a population genetics tool? *Genes* 10: 192.

Agnarson, I., and M. Kunter. 2007. Taxonomy in a changing world: seeking solutions for a science in crisis. *Systematic Biology* 56: 531–539.

Allaby, R. G., L. Kistler, R. M. Gutaker, R. Ware, J. L. Kitchen, O. Smith, and A. C. Clarke. 2015. Archaeogenomic insights into the adaptation of plants to the human environment: pushing plant–hominin co-evolution back to the Pliocene. *Journal of Human Evolution* 79: 150–157.

Anderson-Carpenter, L. L., J. S. McLachlan, S. T. Jackson, M. Kuch, C. Y. Lumibae, and H. N. Poinar. 2011. Ancient DNA from lake sediments: bridging the gap between paleoecology and genetics. *BMC Ecology and Evolution* 11: 30.

Andres, K. J., S. A. Sethi, D. M. Lodge, and J. Andres. 2021. Nuclear eDNA estimates population allele frequencies and abundance in experimental mesocosms and field samples. *Molecular Ecology* 30: 685–697.

Anglès d'Auriac, M. B., D. A. Strand, M. Mjelde, B. O. L. Demars, and J. Thaulow. 2019. Detection of an invasive aquatic plant in natural water bodies using environmental *DNA*. *PLoS One* 14: e0219700.

Ariza, M., B. Fouks, Q. Mauvisseau, R. Halvorsen, I. G. Alsos, and H. J. Boer. 2022. Plant biodiversity assessment through soil eDNA

reflects temporal and local diversity. *Methods in Ecology and Evolution* (in press). https://doi.org/10.1111/2041-210X.13865

Arstingstall, K. A., S. J. DeBano, X. Li, D. E. Wooster, M. M. Rowland, S. Burrows, and K. Frost. 2021. Capabilities and limitations of using DNA metabarcoding to study plant–pollinator interactions. *Molecular Ecology* 30: 5266–5297.

Baker, C. S., D. Steel, S. Nieukirk, and H. Klinck. 2018. Environmental DNA (eDNA) from the wake of the whales: Droplet digital PCR for detection and species identification. *Frontiers in Marine Science* 5: 133.

Bakker, J., O. S. Wangensteen, D. D. Champman, G. Boussarie, D. Buddo, T. L. Guttridge, H. Hertler, et al. 2017. Environmental DNA reveals tropical shark diversity in contrasting levels of anthropogenic impact. *Scientific Reports* 7: 16886.

Balint, M., C. Nowak, O. Marton, S. U. Pauls, C. Wittwer, J. L. Aramayo, A. Schulze, et al. 2018. Accuracy, limitations and cost efficiency of eDNA-based community survey in tropical frogs. *Molecular Ecology Resources* 18: 1415–1426.

Banchi, E., C. G. Ametrano, E. Tordoni, D. Stankovic, S. Ongaro, M. Tretiach, A. Pallavicini, et al. 2020. Environmental DNA assessment of airborne plant and fungal seasonal diversity. *Science of the Total Environment* 738: 140249.

Banerjee, P., K. A. Stewart, G. Dey, C. M. Antognazza, R. K. Sharma, J. P. Maity, S. Saha, et al. 2022a. Environmental DNA analysis as an emerging non-destructive method for plant biodiversity monitoring: a review. *AoB Plants* 14: 1–14.

Banerjee, P., K. A. Steward, C. M. Antognazza, I. V. Bunholi, K. Deiner, M. A. Barnes, and S. Saha. 2022b. Plant–animal interactions in the era of environmental DNA (eDNA)—a review. *Environmental DNA* 4: 987–999.

Barnes, C. J., I. B. Nielsen, A. Aagaard, R. Ejrnaes, A. J. Hansen, and T. G. Froslev. 2022. Metabarcoding of soil environmental DNA replicates plant community variation but not specificity. *Environmental DNA* 4: 732–746.

Barnes, M. A., and C. R. Turner. 2016. The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics* 17: 1–17.

Bell, K. L., L. V. Loeffler, and B. J. Brosi. 2017. An *rbcL* reference library to aid in the identification of plant species mixtures by DNA metabarcoding. *Applications in Plant Sciences* 5: 1600110.

Beng, K. C., and R. T. Corlett. 2020. Applications of environmental DNA (eDNA) in ecology and conservation: Opportunities, challenges and prospects. *Biodiversity and Conservation* 29: 2089–2121.

Blaalid, R., T. Carlsen, S. Kumar, R. Halvorsen, K. I. Ugland, G. Fontana, and H. Kauserud. 2012. Changes in the root-associated fungal communities along a primary succession gradient analysed by 454 pyrosequencing. *Molecular Ecology* 21: 1897–1908.

Bradley, B., M. Stiller, D. M. Doran-Sheehy, T. Harris, C. A. Chapman, L. Vigilant, and H. Poinar. 2007. Plant DNA sequences from feces: Potential means for assessing diets of wild primates. *American Journal of Primatology* 69: 699–705.

Brondizio, E. S., J. Settele, S. Diaz, and H. T., Ngo. 2019. Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES). *IPBES secretariat, Bonn, Germany*. 1148p.

Buee, M., M. Reich, C. Murat, E. Morin, R. H. Nilsson, S. Uroz, and F. Martin. 2009. 454 Pyrosequencing analysis of forest soils reveal an unexpectedly high fungal diversity. *New Phytologist* 184: 449–456.

Buxton, A. S., J. J. Groombridge, and R. A. Griffiths. 2017. Is the detection of aquatic environmental DNA influenced by substrate type? *PLoS One* 12: e0183371.

Capo, E., C. Gigue-Covex, A. Rouillard, K. Nota, P. D. Heintzman, A. Vuillemin, D. Ariztegui, et al. 2021. Lake sedimentary DNA research on past terrestrial and aquatic biodiversity: overview and recommendations. *Quaternary* 4: 6.

Carvalho-Silva, M., L. H. Rosa, O. H. B. Pinto, T. H. da Silva, D. K. Henriques, P. Convey, and P. E. A. S. Camara. 2021. Exploring the plant environmental DNA diversity in soil from two sites on Deception Island (Antarctica, South Shetland Islands) using metabarcoding. *Antarctic Science* 35: 469–478.

Castro, L. R., R. S. Meyer, B. Shapiro, S. Shirazi, S. Cutler, A. M. Lagos, and S. Y. Quiroga. 2021. Metabarcoding meiofauna biodiversity assessment in four beaches of Northern Colombia: Effects of sampling protocols and primer choice. *Hydrobiologia* 848: 3407–3426.

CBOL Plant Working Group. 2009. A DNA barcode for land plants. *Proceedings of the National Academy of Sciences, USA* 106: 12794-12797.

Chase, D. M., L. M. Kuehne, J. D. Olden, and C. O. Ostberg. 2020. Development of a quantitative PCR assay for detecting *Egeria densa* in environmental DNA samples. *Conservation Genetics Resources* 12: 545–548.

Chen, S., H. Yao, J. Han, C. Liu, J. Song, L. Shi, Y. Zhu, et al. 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS One* 5: e8613.

Chui, S. X., A. Keller, and S. D. Leonhardt. 2021. Functional resin use in solitary bees. *Ecological Entomology* 47: 115–136.

Coghlan, S. A., A. B. A Shafer, and J. R. Freeland. 2020. Development of an environmental DNA metabarcoding assay for aquatic vascular plant communities. *Environmental DNA* 3: 372–387.

Cowart, D. A., M. Matabos, M. I. Brandt, J. Marticorena, and J. Sarrazin. 2020. Exploring environmental DNA (eDNA) to assess biodiversity of hard substratum faunal communities on the Lucky Strike Vent Field (Mid-Atlantic Ridge) and investigate recolonization dynamics after an induced disturbance. *Frontiers in Marine Science* 6: 783.

Cowart, D. A., D. R. Murphy, and C. H. C. Cheng. 2018. Metagenomic sequencing of environmental DNA reveals marine faunal assemblages from the West Antarctic Peninsula. *Marine Genomics* 37: 148–160.

Curtis, A. N., J. S. Tiemann, S. A. Douglass, M. A. Davis, and E. R. Larson. 2021. High stream flows dilute environmental DNA (eDNA) concentrations and reduce detectability. *Diversity and Distributions* 27: 1918–1931.

de Sousa, L. L., S. M. Silva, and R. Xavier. 2019. DNA metabarcoding in diet studies: unveiling ecological aspects in aquatic and terrestrial ecosystems. *Environmental DNA* 1: 199–214.

de Souza, L. S., J. C. Godwin, M. A. Renshaw, and E. R. Larson. 2016. Environmental DNA (eDNA) detection probability is influenced by seasonal activity of organisms. *PLoS One* 11: e0165273.

de Vere, N., L. E. Jones, T. Gilmore, J. Moscrop, A. Lowe, D. Smith, M. J. Hegarty, et al. 2017. Using DNA metabarcoding to investigate honey bee foraging reveals limited flower use despite high floral availability. *Scientific Reports* 7: 42838.

Deiner, K., E. A. Fronhofer, E. Machler, J. Walser, and F. Altermatt. 2016. Environmental DNA reveals that rivers are conveyer belts of biodiversity information. Nature *Communications* 7: 12544.

Deiner, K., J. Walser, E. Machler, and F. Altermatt. 2015. Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. *Biological Conservation* 183: 53–63.

Deiner, K., H. Yamanaka, and L. Bernatchez. 2021. The future of biodiversity monitoring and conservation utilizing environmental DNA. *Environmental DNA* 3: 3–7.

DiBattista, J. D., J. D. Reimer, M. Stat, G. D. Masucci, P. Biondi, M. De Brauwer, and S. P. Wilkinson. 2020. Environmental DNA can act as a biodiversity barometer of anthropogenic pressures in coastal ecosystems. *Scientific Reports* 10: 8365.

Doi, H., Y. Akamatsu, M. Goto, R. Inui, T. Komuro, M. Nagano, and T. Minamoto. 2021. Broad-scale detection of environmental DNA for an invasive macrophyte and the relationship between DNA concentration and coverage in rivers. *Biological Invasions* 23: 507–520.

Edwards, M. E., I. Greve Alsos, N. Yoccoz, E. Coissac, T. Goslar, L. Gielly, J. Haile, et al. 2018. Metabarcoding of modern soil DNA give a highly local vegetation signal in Svalbard tundra. *Holocene* 28: 2006–2016.

Estrada, O., J. Breen, S. M. Richards, and A. Cooper. 2018. Ancient plant DNA in the genomic era. *Nature Plants* 4: 394–396.

Evans, D. M., and J. J. N. Kitson. 2020. Molecular ecology as a tool for understanding pollination and other plant–insect interactions. *Current Opinion in Insect Science* 38: 2020.

Evans, D. M., and J. J. N. Kitson. 2016. Merging DNA metabarcoding and ecological network analysis to understand and build resilient terrestrial ecosystems. *Functional Ecology* 30: 1904–1916.

Fahner, N., S. Shokralla, D. Baird, and M. Hajibabaei. 2016. Large-scale monitoring of plants through environmental DNA metabarcoding of soil: recovery, resolution, and annotation of four DNA markers. *PLoS One* 11: e015750.

Farrell, J. A., L. Whitmore, and D. J. Duffy. 2021. The promise and pitfalls of environmental DNA and RNA approaches for the monitoring of human and animal pathogens from aquatic sources. *BioScience* 71: 609–625.

Ficetola, G. F., C. Miaud, F. Pompanon, and P. Taberlet. 2008. Species detection using environmental DNA from water samples. *Biology Letters* 4: 423–425.

Folloni, S., D. Kagkli, B. Rajcevic, N. C. C. Guimaraes, B. Van Droogenbroeck, F. H. Valicente, G. V. Eede, and M. V. Bulcke. 2012. Detection of airborne genetically modified maize pollen by real-time PCR. *Molecular Ecology Resources* 12: 810–821.

Foscari, A., G. Alberti, M. Zotti, and G. Incerti. 2022. Species-specific DNA distribution in spruce–beech forest soil. *Environmental DNA* 4: 1120–1135.

Flojgaard, C., T. G. Froslev, A. K. Brunbjerg, H. H. Bruun, J. Moeslund, A. J. Hansen, and R. Ejenaes. 2019. Predicting provenance of forensic soil samples: linking soil to ecological habitats by metabarcoding and supervised classification. *PLoS One* 14: e0202844.

Foley, B. P., M. C. Hansson, D. P. Kourkoumelis, and T. A. Theodoulou. 2012. Aspects of ancient Greek trade re-evaulated with amphora DNA evidence. *Journal of Archaeological Science* 29: 389–398.

Foucher, A., O. Evrand, G. F. Ficetola, L. Gielly, J. Poulain, C. Giguet-Covex, J. P. Laceby, et al. 2020. Persistence of environmental DNA in cultivated soils: implication of this memory effect for reconstructing the dynamics of land use and cover changes. *Scientific Reports* 10: 10502.

Foster, N. R., K. V. Dijk, E. Biffin, J. M. Young, V. A. Thomson, B. M. Gillanders, A. R. Jones, and M. Waycott. 2021. A multi-gene region targeted capture approach to detect plant DNA in environmental samples: a case study from coastal environments. *Frontiers in Ecology and Evolution* 9: 735744.

Frankl, A., O. Evrard, E. Cammeraat, B. Tytgat, E. Verleyen, and A. Stokes. 2022. Tracing hotspots of soil erosion in high mountain environments: How forensic science based on plant eDNA can lead the way. An opinion. *Plant and Soil* 476: 729–742.

Fujiwara, A., S. Matsuhashi, H. Doi, S. Yamamoto, and T. Minamoto. 2016. Use of environmental DNA to survey the distribution of an invasive submerged plant in ponds. *Freshwater Science* 35: 748–754.

Galliot, J., D. Brunel, A. Berard, A. Chauveau, A. Blanchetete, L. Lanore, and A. Farruggia. 2017. Investigating a flower–insect forager network in a mountain grassland community using pollen DNA barcoding. *Journal of Insect Conservation* 21: 827–837.

Gantz, C. A., M. A. Renshaw, D. Erickson, D. M. Lodge, and S. P. Egan. 2018. Environmental DNA detection of aquatic invasive plants in lab mesocosm and natural field conditions. *Biological Invasions* 20: 2535–2552.

Garrard, G. E., S. A. Bekessy, M. A. McCarthym, and B. A. Wintle. 2008. When have we looked hard enough? A novel method for setting minimum survey effort protocols for flora surveys. *Austral Ecology* 33: 986–998.

Giguet-Covex, C., J. Pansu, F. Arnaud, P. Rey, C. Griggo, L. Gielly, and I. Domaizon. 2014. Long livestock farming history and human landscape shaping revealed by lake sediment DNA. *Nature Communications* 5: 3211.

Goldberg, C. S., C. R. Turner, K. Deiner, K. E. Klymus, P. F. Thomsen, M. A. Murphy, S. F. Spear, et al. 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution* 7: 1299–1307.

Gomez, N. G., D. H. Sørensen, P. Y. S. Chua, and L. Sigsgaard. 2022. Assessing flower-visiting arthropod diversity in apple orchards through metabarcoding of environmental DNA from flowers and visual census. *Environmental DNA* 5: 117–131.

Goricki, S., D. Stankovic, A. Snoj, M. Kuntner, W. R. Jeffery, P. Trontelij, M. Pavicevic, et al. 2017. Environmental DNA in subterranean biology: range extension and taxonomic implications for *Proteus*. *Scientific Reports* 7: 45054.

Gould, B. A., B. Leon, A. M. Buffen, L. G. Thompson. 2010. Evidence of a high-Andean, mid-Holocene plant community: An ancient DNA analysis of glacially preserved remains. *American Journal of Botany* 97: 1579–1584.

Gugerli, F., N. Alvarez, and W. Tinner. 2013. A deep dig—hindsight on Holocene vegetation composition from ancient environmental DNA. *Molecular Ecology* 22: 3433–3436.

Haile, J., R. Holdaway, K. Oliver, M. Bunce, M. T. P. Gilbert, R. Nielsen, K. Munch, et al. 2007. Ancient DNA chronology within sediment deposits: Are paleobiological reconstructions possible and is DNA leaching a factor? *Molecular Biology and Evolution* 24: 982–989.

Haouchar, D., J. Haile, M. C. McDowell, D. C. Murray, N. E. White, R. J. N. Allcock, M. J. Phillips, et al. 2014. Thorough assessment of DNA preservation from fossil bone and sediments excavated form a late Pleistocene–Holocene cave deposit on Kangaroo Island, South Australia. *Quaternary Science Reviews* 84: 56–64.

Harper, L. R., M. L. Niemiller, J. B. Benito, L. E. Paddock, E. Knittle, B. Molano-Flores, and M. A. Davis. 2022. BeeDNA: Microfluidic environmental DNA metabarcoding as a tool for connecting plant and pollinator communities. *Environmental DNA* 5: 191–211.

Harrer, L. E. F., and T. Levi. 2018. The primacy of bears as seed dispersers in salmon-bearing ecosystems. *Ecosphere* 9: e02076.

Harrison, J. B., J. M. Sunday, and S. M. Rogers. 2019. Predicting the fate of eDNA in the environment and implications for studying biodiversity. *Proceedings of the Royal Society, B, Biological Sciences* 286: 2019140.

Hartvig, I., C. Kosawang, E. D. Kjaer, and L. R. Nielsen. 2021. Detecting rare terrestrial orchids and associated plant communities from soil samples with eDNA methods. *Biodiversity and Conservation* 30: 3879–3901.

Hawkins, J., N. de Vere, A. Griffith, C. R. Ford, J. Allainguillaume, M. J. Hegarty, L. Baillie, and B. Adams-Groom. 2015. Using DNA metabarcoding to identify the floral composition of honey: A new tool for investigating honey bee foraging preferences. *PLoS ONE* 10: e0134735.

Hempel, C. A., B. Peinert, A. J. Beermann, V. Elbrecht, J. N. Macher, T. H. Macher, G. Jacobs, and F. Leese. 2020. Using environmental DNA to monitor the reintroduction success of the Rhine sculpin (*Cottus rhenanus*) in a restored stream. *Frontiers in Ecology and Evolution* 8: 81.

Herrick, J. E., J. W. Van Zee, S. E. McCord, E. M. Courtright, J. W. Karl, and L. M. Burkett. 2005. Monitoring manual for grassland, shrubland and savanna ecosystems, vol. I, Quick start. University of Arizona Press, Tucson, AZ, USA.

Hofreiter, M., J. I. Mead, P. Martin, H. N. Poinar. 2003. Molecular caving. *Current Biology* 16: 693–695.

Hoss, M., M. Kohn, S. Paabo, F. Knauer, and W. Schroder. 1992. Excrement analysis by PCR. *Nature* 259: 199.

Hutchins, P. R., L. N. Simantel, and A. J. Sepulveda. 2021. Time to get real with qPCR controls: the frequency of sample contamination and the informative power of negative controls in environmental DNA studies. *Molecular Ecology Resources* 22: 1319–1329.

Jain, S., F. Jesus, G. M. Marchioro, and D. de Araujo. 2013. Extraction of DNA from honey and its amplification by PCR for botanical identification. *Food Science and Technology* 33: 753–756.

Jerde, C. L., A. R. Mahon, W. L. Chadderton, and D. M. Lodge. 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters* 4: 150–157.

Jerde, C. L., A. R. Mahon, T. Campbell, M. E. McElroy, K. Pin, J. N. Childress, M. Armstrong, et al. 2021. Are genetic reference libraries sufficient for environmental DNA metabarcoding of Mekong River Basin fish? *Water* 13: 1767.

Jo, T., H. Murakami, S. Yamamoto, R. Masuda, T. Minamoto. 2019. Effect of water temperature and fish biomass on environmental DNA shedding, degradation, and size distribution. *Ecology and Evolution* 9: 1135–1146.

Johnson, M. D., R. D. Cox, and M. A. Barnes. 2019a. Analyzing airborne environmental DNA: A comparison of extraction methods, primer type, and trap type on the ability to detect airborne eDNA from terrestrial plant communities. *Environmental DNA* 1: 176–185.

Johnson, M. D., R. D. Cox, and M. A. Barnes. 2019b. The detection of a non-anemophilous plant species using airborne eDNA. *PLoS One* 14: e0225262.

Johnson, M. D., R. D. Cox, B. A. Grisham, D. Lucia, and M. A. Barnes. 2021a. Airborne eDNA reflects human activity and seasonal changes on a landscape scale. *Frontiers in Environmental Science* 8: 563431.

Johnson, M. D., M. Fokar, R. D. Cox, and M. A. Barnes. 2021b. Airborne environmental DNA metabarcoding detects, more diversity, with less sampling effort, than a traditional plant community survey. *BMC Ecology and Evolution* 21: 218.

Johnson, M. D., A. D. Katz, M. A. Davis, D. Edlund, S. Tomczyk, B. Molano-Flores, T. Wilder, and J. H. Sperry. 2023. Environmental DNA metabarcoding from flowers reveals arthropod pollinators, plant pests, parasites, and predator–prey interactions. *Environmental DNA* 5: in press.

Jørgensen, T., J. Haile, P. Möller, A. Andreev, S. Boessenkool, M. Rasmussen, F. Kienast, et al. 2011. A comparative study of ancient sedimentary DNA, pollen, and macrofossils from permafrost sediments of northern Siberia reveals long-term vegetational stability. *Molecular Ecology* 21: 1989–2003.

Keller, A., N. Danner, G. Grimmer, M. Ankenbran, K. van der Ohe, S. Rost, S. Hartel, and I. Steffan-Dewenter. 2015. Evaluating multiplexed next-generation sequencing as a method in palynology for mixed pollen samples. *Plant Biology* 17: 558–566.

Kessler, E. J., K. T. Ash, S. N. Barratt, E. R. Larson, and M. A. Davis. 2020. Radiotelemetry reveals effects of upstream biomass and UV exposure on environmental DNA occupancy and detection for a large freshwater turtle. *Environmental DNA* 2: 13–23.

Kjaer, K. H., M. W. Pedersen, B. De Sanctis, B. De Cashan, T. S. Korneliussen, C. S. Michelsen, K. K. Sand, et al. 2022. A 2-million-year-old ecosystem in Greenland uncovered by environmental DNA. *Nature* 612: 283–291.

Klepke, M. J., E. E. Sigsgaard, M. R. Jensen, K. Olsen, and P. F. Thomsen. 2022. Accumulation and diversity of airborne, eukaryotic environmental DNA. *Environmental DNA* 4: 1323–1339.

Klymus, K. E., C. A. Richter, N. Thompson, and J. E. Hinck. 2017. Metabarcoding of environmental DNA samples to explore the use of uranium mine containment ponds as a water source for wildlife. *Diversity* 9: 54.

Kodama, T., S. Miyazono, Y. Akamatsu, S. Tsuji, and R. Nakao. 2022. Abundance estimation of riverine macrophyte *Egeria densa* using environmental DNA: Effects of sampling season and location. *Limnology* 23: 299–308.

Korpelainen, H., and M. Pietilainen. 2017. Biodiversity of pollen in indoor air samples as revealed by DNA metabarcoding. *Nordic Journal of Botany* 35: 602–608.

Kraaijeveld, K., L. A. Weger, M. V. Garcia, H. Buermans, J. Frank, P. S. Hiemstra, and J. T. Dunnen. 2015. Efficient and sensitive identification and quantification of airborne pollen using next-generation DNA sequencing. *Molecular Ecology Resources* 15: 8–16.

Kudoh, A., T. Minamoto, and S. Yamamoto. 2020. Detection of herbivory: eDNA detection from feeding marks on leaves. *Environmental DNA* 2: 627–634.

Kuehne, L. M., C. O. Ostberg, D. M. Chase, J. J. Duda, and J. D. Olden. 2020. Use of environmental DNA to detect the invasive aquatic plants *Myriophyllum spicatum* and *Egeria densa* in lakes. *Freshwater Science* 39: 521–533.

Lacoursière-Roussel, A., and K. Deiner. 2021. Environmental DNA is not the tool by itself. *Journal of Fish Biology* 98: 383–386.

Laube, I., H. Hird, P. Brodmann, S. Ullmann, M. Schone-Michling, J. Chisholm, and H. Broll. 2010. Development of primer and probe sets for the detection of plant species in honey. *Food Chemistry* 118: 979–986.

Leese, F., M. Sander, D. Buchner, V. Elbrecht, P. Haase, and V. M. A. Zizka. 2020. Improved freshwater macroinvertebrate detection from environmental DNA through minimized nontarget amplification. *Environmental DNA* 3: 261–276.

Lennartz, C., J. Kurucar, S. Coppola, J. Crager, J., Bobrow, L. Bortolin, and J. Comolli. 2021. Geographic source estimation using airborne plant environmental DNA in dust. *Scientific Reports* 15: 16238.

Lin, M., A. L. Simons, R. J. Harrigan, E. E. Curd, F. D. Schneider, D. V. Ruiz-Ramos, Z. Gold, et al. 2021. Landscape analyses using eDNA metabarcoding and Earth observation predict community biodiversity in California. *Ecological Applications* 31: e02379.

Little, D. P. 2013. A DNA mini-barcode for land plants. *Molecular Ecology Resources* 14: 437–446.

Littlefair, J. E., A. Zander, C. D. S. Costa, and E. L. Clare. 2018. DNA metabarcoding reveals changes in the contents of carnivorous plants along an elevation gradient. *Molecular Ecology* 28: 281–292.

Lyman, J. A., D. E. Sanchez, S. N. Hershauer, C. J. Sobek, C. L. Chambers, J. Zahratka, F. M. Walker. 2022. Mammalian eDNA on herbaceous vegetation? Validating a qPCR assay for detection of an endangered rodent. *Environmental DNA* 4: 1187–1197.

Marinich, A. K. 2017. *Evaluating environmental DNA (eDNA) detection of invasive water soldier (Stratiotes aloides)* (Thesis). Peterborough, ON: Trent University.

Marshall, N. T., and C. A. Stepien. 2019. Invasion genetics from eDNA and thousands of larvae: a targeted metabarcoding assay that distinguishes species and population variation of zebra and quagga mussels. *Ecology and Evolution* 9: 3515–3538.

Marshall, N. T., H. A. Vanderploeg, and S. R. Chaganti. 2021. Environmental (e)RNA advances the reliability of eDNA by predicting its age. *Scientific Reports* 11: 2769.

Martínez-García, L. B., S. J. Richardson, J. M. Tylianakis, D. A. Peltzer, and I. A. Dickie. 2014. Host identity is a dominant driver of mycorrhizal fungal community composition during ecosystem development. *New Phytologist* 205: 1565–1576.

Matisoo-Smith, E., R. K. Welikala, G. Tannock, P. I. Chester, D. T. Feek, and J. R. Flenley. 2008. Recovery of DNA and pollen from New Zealand lake sediments. *Quaternary International* 184: 139–149.

Matsuhashi, S., H. Doi, A. Fujiwara, S. Watanabe, and T. Minamoto. 2016. Evaluation of the environmental DNA method for estimating distribution and biomass of submerged aquatic plants. *PLoS One* 11: e0156217.

Matsuhashi, S., T. Minamoto, and H. Doi. 2019. Seasonal change in environmental DNA concentration of a submerged aquatic plant species. *Freshwater Science* 38: 654–660.

Meyer, R. S., M. M. Ramos, M. Lin, T. M. Schweizer, Z. Gold, D. R. Ramos, S. Shirazi, et al. 2021. The CALeDNA program: Citizen scientists and researchers inventory California's biodiversity. *California Agriculture* 75: 20–32.

Miller, K. E., A. Polaszek, and D. M. Evans. 2021. A dearth of data: fitting parasitoids into ecological networks. *Trends in Parasitology* 37: 863–874.

Miyazono, S., T. Kodama, Y. Akamatsu, R. Nakao, and M. Saito. 2020. Application of environmental DNA methods for the detection and abundance estimation of invasive aquatic plant *Egeria densa* in lotic habitats. *Limnology* 22: 81–87.

Mohanty, R., M. Buchheim, and E. Levetin. 2017. Molecular approaches for the analysis of airborne pollen a case study of *Juniperus* pollen. *Annals of Allergy, Asthma, and Immunology* 118: 204–211.

Monge, O., D. Dumas, and I. Baus. 2020. Environmental DNA from avian residual saliva in fruits and its potential uses in population genetics. *Conservation Genetics Resources* 12: 131–139.

Murchie, T. J., M. Kuch, A. T. Duggan, M. L. Ledger, K. Roche, J. Klunk, E. Karpinski, et al. 2020. Optimizing extraction and targeted capture of ancient environmental DNA for reconstruction past environments

using the PalaeoChip Arctic-1.0 bait-set. *Quaternary Research* 99: 305–328.

Nichols, P. K., and P. B. Marko. 2019. Rapid assessment of coral cover from environmental DNA in Hawaii. *Environmental DNA* 1: 40–53.

Nichols, R. V., C. Vollmers, L. A. Newsom, Y. Wang, P. D. Heintzman, M. Leighton, R. E. Green, and B. Shapiro. 2018. Minimizing polymerase biases in metabarcoding. *Molecular Ecology Resources* 18: 927–939.

Nogueira, J., H. Evangelista, C. M. Valeriano, A. Sifeddine, C. Neto, G. Vaz, L. S. Moreira, et al. 2021. Dust arriving in the Amazon basin over the past 7,500 years came from diverse sources. *Communications Earth and Environment* 2: 5.

Osathanunkul, M., N. Sawongta, W. Pheera, N. Pechlivanis, F. Psomopoulos, and P. Madesis. 2021. Exploring plant diversity through soil DNA in Thai national parks for influencing land reform and agriculture planning. *PeerJ* 9: e11753.

Palacios Mejia, M., E. Curd, K. Edalati, M. A. Renshaw, R. Dunn, D. Potter, N. Fraga, et al. 2021. The utility of environmental DNA from sediment and water samples for recovery of observed plant and animal species from four Mojave Desert springs. *Environmental DNA* 3: 214–230.

Parducci, L., K. D. Bennett, G. F. Ficetola, I. G. Alsos, Y. Suyama, J. R. Wood, and M. W. Pedersen. 2017. Ancient plant DNA in lake sediments. *New Phytologist* 214: 924–942.

Parducci, L., I. Greve Alsos, P. Unneberg, M. W. Pedersen, L. Han, Y. Lammers, J. Sakari Salonen, et al. 2019. Shotgun environmental DNA, pollen, and macrofossil analysis of late glacial lake sediments from southern Sweden. *Frontiers in Ecology and Evolution* 7: 189.

Parducci, L., T. Jørgensen, M. M. Tollefsrud, E. Elverland, T. Alm, S. L. Fontana, K. D. Bennett, et al. 2012. Glacial survival of boreal trees in northern Scandinavia. *Science* 335: 1083–1086.

Parducci, L., I. Matetovici, S. L. Fontana, K. D. Bennett, Y. Suyama, J. Haile, K. H. Kjaer, et al. 2013. Molecular- and pollen-based vegetation analysis in lake sediments from central Scandinavia. *Molecular Ecology* 22: 3511–3524.

Parducci, L., Y. Suyama, M. Lascoux, and K. D. Bennett. 2005. Ancient DNA from pollen: a genetic record of population history in Scots pine. *Molecular Ecology* 14: 2873–2882.

Parsons, K. M., M. Everett, M. Dahlheim, and L. Park. 2018. Water, water everywhere: Environmental DNA can unlock population structure in elusive marine species. *Royal Society Open Science* 5: 180537.

Pawlowska, J., F. Lejzerowicz, P. Esling, W. Szczucinski, M. Zajaczkowski, and J. Pawlowski. 2014. Ancient DNA sheds new light on the Svalbard foraminiferal fossil record of the last millennium. *Gebiology* 12: 277–288.

Pawlowski, J., L. Apotheloz-Perret-Gentil, and F. Altermatt. 2020. Environmental DNA: What's behind the term? Clarifying the terminology and recommendations for its future use in biomonitoring. *Molecular Ecology* 29: 4258–4264.

Pedersen, M. W., A. Ginolhacc, L. Orlando, J. Olsen, K. Andersen, J. Holm, S. Funder, et al. 2013. A comparative study of ancient environmental DNA to pollen and macrofossils from lake sediments reveals taxonomic overlap and additional plant taxa. *Quaternary Science Reviews* 75: 161–168.

Pedersen, M. W., S. Overballe-Petersen, L. Ermini, C. D. Sarkissian, J. Haile, M. Hellstrom, J. Spens, et al. 2015. Ancient and modern environmental DNA. *Philosophical Transactions of the Royal Society, B, Biological Sciences* 370: 20130383.

Pedersen, M. W., A. Ruter, C. Schweger, H. Friebe, R. A. Staff, K. K. Kjeldsen, M. L. Z. Mendoza, et al. 2016. Postglacial viability and colonization in North America's ice-free corridor. *Nature* 537: 45–49.

Poinar, H. N., M. Kuch, K. D. Sobolik, I. Barnes, A. B. Stankiewicz, T. Kuder, W. G. Spaulding, et al. 2001. A molecular analysis of dietary diversity for three archaic Native Americans. *Anthropology* 98: 4317–4322.

Pornon, A., N. Escaravage, M. Burrus, H. Holota, A. Khimoun, J. Mariette, C. Pellizzari, et al. 2016. Using metabarcoding to reveal and quantify plant-pollinator interactions. *Scientific Reports* 6: 27282.

Pote, J., R. Ackermann, and W. Wildi. 2009. Plant leaf mass loss and DNA release in freshwater sediments. *Ecotoxicology and Environmental Safety* 72: 1378–1383.

Prieto, A. E., L. Hardion, and J. Beisel. 2021. Designing robust DNA barcode libraries for metabarcoding of freshwater plants by integrating herbarium collections and contemporary floristic inventories. *ARPHA Conference Abstracts* 4: e64713.

Pyle, M. M., and R. P. Adams. 1989. *In situ* preservation of DNA in plant specimens. *Taxon* 38: 576–581.

Raposo M. A., G. M. Kirwan, A. C. Calijorne Lourenco, G. Sobral, F. A. Bockmann, and R. Stopiglia. 2021. On the notions of taxonomic 'impediment', 'gap', 'inflation', and 'anarchy', and their effects on the field of conservation. *Systematics and Biodiversity* 19: 296–311.

Rasmussen, A. J., M. Nielsen, S. S. Mak, J. Doring, F. Klincke, S. Gopalakrishnan, R. Dunn, et al. 2021. eDNA-based biomonitoring at an experimental German vineyard to characterize how management regimes shape ecosystem diversity. *Environmental DNA* 3: 70–82.

Rawlence, N. J., D. J. Lowe, J. R. Wood, J. M. Young, G. J. Churchman, Y. Huang, and A. Cooper. 2014. Using palaeoenvironmental DNA to reconstruct past environments: progress and prospects. *Journal of Quaternary Science* 29: 610–626.

Ribani, A., V. J. Utzeri, V. Taurisano, and L. Fontanesi. 2020. Honey as a source of environmental DNA for the detection and monitoring of honey bee pathogens and parasites. *Veterinary Sciences* 7: 113.

Richardson, R. T., C. Lin, J. Quijia, N. S. Riusech, K. Goodell, and R. M. Johnson. 2015. Rank-based characterization of pollen assemblages collected by honey bees using a multi-locus metabarcoding approach. *Applications in Plant Sciences* 3: 1500043.

Rijal, D. P., P. D. Heintzman, Y. Lammers, N. G. Yoccoz, K. E. Lorberau, I. Pitelkova, T. Goslar, et al. 2021. Sedimentary ancient DNA shows terrestrial plant richness continuously increased over the Holocene in northern Fennoscandia. *Science Advances* 7: eabf9557.

Ruppert, K. M., R. J. Kline, and M. S. Rahman. 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: a systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation* 17: e00547.

Sala, O. E., F. S. Chapin, J. J. Armesto, E. Berlowm, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, et al. 2000. Global biodiversity scenarios for the year 2100. *Science* 287: 1770–1774.

Schenekar, T. 2022. The current state of eDNA research in freshwater ecosystems: Are we shifting from the developmental phase to standard application in biomonitoring? *Hydrobiologia* https://doi.org/10.1007/s10750-022-04891-z

Scriver, M., A. Marinich, C. Wilson, and J. Freeland. 2015. Development of species-specific environmental DNA markers for invasive aquatic plants. *Aquatic Botany* 122: 27–31.

Sepulveda, A. J., H. R. Patrick, M. Forstchen, M. McKeefry, and A. M. Swigris. 2020. The elephant in the lab (and field): contamination in aquatic environmental DNA studies. *Frontiers in Ecology and Evolution* 8: 609973.

Shackleton, M. E., G. N. Rees, G. Watson, C. Campbell, and D. Nielsen. 2019. Environmental DNA reveals landscape mosaic of wetland plant communities. *Global Ecology and Conservation* 19: e00689.

Shirazi, S., R. S. Meyer, and B. Shapiro. 2021. Revisiting the effect of PCR replication and sequencing depth on biodiversity metrics in environmental DNA metabarcoding. *Ecology and Evolution* 11: 15766–15779.

Sickel, W., M. J. Ankenbrand, G. Grimmer, A. Holzschuh, S. Hartel, J. Lanzen I. Steffan-Dewenter, and A. Keller. 2015. Increased efficiency in identifying mixed pollen samples by metabarcoding with a dual-indexing approach. *BMC Ecology* 15: 20.

Sigsgaard, E. E., M. D. Jensen, I. E. Winkelmann, P. R. Moller, M. M. Hansen, and P. F. Thomsen. 2019. Population-level inferences from environmental DNA—current status and future perspectives. *Evolutionary Applications* 13: 245–262.

Sigsgaard, E. E., I. B. Nielsen, S. S. Bach, E. D. Lorenzen, D. P. Robinson, S. W. Knudsen, M. W. Pedersen, et al. 2016. Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA. *Nature Ecology and Evolution* 1: 0004.

Slon, V., C. Hopfe, C. L. Weib, F. Mafessoni, M. Rasilla, C. Lalueza-Fox, A. Rosas, et al. 2017. Neandertal and Denisovan DNA from Pleistocene sediments. *Science* 356: 605–608.

Stahlschmidt, M. C., T. C. Collin, D. M. Fernandes, G. Bar-Oz, A. Belfer-Cohen, Z. Gao, N. Jakeli, et al. 2019. Ancient mammalian and plant DNA from late Quaternary stalagmite layers at Solkota Cave, Georgia. *Scientific Reports* 9: 6628.

Stat, M., M. J. Huggett, R. Bernasconi, J. D. BiBattista, T. E. Berry, S. J. Newman, E. S. Harvey, and M. Bunce. 2017. Ecosystem biomonitoring with eDNA: Metabarcoding across the tree of life in a tropical marine environment. *Scientific Reports* 7: 12240.

Stepien, C. A., M. R. Snyder, and A. E. Elz. 2019. Invasion genetics of the silver carp *Hypophthalmichthys molitrix* across North America: Differentiation of fronts, introgression, and eDNA metabarcode detection. *PLoS One* 14: e0203012.

Stoeckle, M. Y., M. D. Mishum, and Z. Charlop-Powers. 2020. Improved Environmental DNA reference library detects overlooked marine fishes in New Jersey, United States. *Frontiers in Marine Science* 7: 226.

Strickler, K. M., A. K. Fremier, and C. S. Goldberg. 2015. Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation* 183: 85–92.

Sun, Z., M. Majaneva, E. Sokolova, S. Rauch, S. Meland, and T. Ekrem. 2019. DNA metabarcoding adds valuable information for management of biodiversity in roadside stormwater ponds. *Ecology and Evolution* 9: 9712–9722.

Sutherland, W. J., P. W. Atkinson, S. H. M. Butchart, M. Capaja, L. V. Dicks, E. Fleishman, K. J. Gaston, et al. 2022. A horizon scan of global biological conservation issues for 2022. *Trends in Ecology and Evolution* 37: 95–104.

Taberlet, P., A. Bonin, L. Zinger, and E. Coissac. 2018. Environmental DNA for biodiversity research and monitoring. Oxford University Press, Oxford, UK.

Taberlet, P., E. Coissac, F. Pompanon, L. Gielly, C. Miquel, A. Valentini, T. Vermat, et al. 2007. Power and limitations of the chloroplast *trnL* (UAA) intron for plant DNA barcoding. *Nucleic Acids Research* 35: e14.

Thomsen, P. F., J. Kielgast, L. L. Iverson, P. R. Moller, M. Rasmussen, and E. Willerslev. 2012. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS One* 7: e41732.

Thomsen, P. F., and E. E. Sigsgaard. 2019. Environmental DNA metabarcoding of wild flowers reveals diverse communities of terrestrial arthropods. *Ecology and Evolution* 9: 1665–1679.

Thomsen, P. F., and E. Willerslev. 2015. Environmental DNA—an emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation* 183: 4–18.

Tsukamoto, Y., S. Yonezawa, N. Katayama, and Y. Isagi. 2021. Detection of endangered aquatic plants in rapid streams using environmental DNA. *Frontiers in Ecology and Evolution* 8: 622291.

Tsuri, K., S. Ikeda, T. Hirohara, Y. Shimada, T. Minamoto, and H. Yamanaka. 2020. Messenger RNA typing of environmental RNA (eRNA): A case study on zebrafish tank water with perspectives for the future development of eRNA analysis on aquatic vertebrates. *Environmental DNA* 3: 14–21.

Turon, X., A. Antich, C. Palacin, K. Praebel, and O. S. Wangensteen. 2019. From metabarcoding to metaphylogeography: Separating the wheat from the chaff. *Ecological Application* 30: e02036.

Uchii, K., H. Doi, and T. Minamoto. 2016. A novel environmental DNA approach to quantify the cryptic invasion of non-native genotypes. *Molecular Ecology Resources* 16: 415–422.

Valentin, R. E., D. M. Fonseca S. Gable K. E. Kyle, G. C. Hamilton, A. L. Nielsen, and J. L. Lockwood. 2020. Moving eDNA surveys onto land: Strategies for active eDNA aggregation to detect invasive forest insects. *Molecular Ecology Resources* 20: 746–755.

Valentini, A., C. Miquel, and P. Taberlet. 2010. DNA barcoding for honey biodiversity. *Diversity* 2: 610–617.,

Walters, C. J. 1986. Adaptive management of renewable resources. McMillan, New York, New York, USA.

Wang, B., Y. Li, G. Zhang, J. Yang, C. Deng, H. Hu, L. Zhang, X. Xu, and C. Zhou. 2022. Seasonal variations in the plant diet of the Chinese Monal revealed by fecal DNA metabarcoding analysis. *Avian Research* 13: 100034.

Weyrich, L. S., K. Dobney, and A. Copper. 2015. Ancient DNA analysis of dental calculus. *Journal of Human Evolution* 79: 119–124.

Wheeler, Q. D., and E. O. Wilson. 2004. Taxonomy: Impediment or expedient? *Science* 303: 285.

Willerslev, E., E. Cappellni, W. Boomsma, R. Nielsen, M. B. Hebsgaard, T. B. Brand, M. Hofreiter, et al. 2007. Ancient biomolecules from deep ice cores reveal a forested southern Greenland. *Science* 317: 111–114.

Willerslev, E., J. Davison, M. Moora, M Zobel, E. Coissac, M. E. Edwards, E. D. Lorenzen, et al. 2014. Fifty thousand years of Arctic vegetation and megafaunal diet. *Nature* 506: 47–51.

Willerslev, E., A. Hansen, J. Binladen, T. B. Brand, M. Thomas, P. Gilbert, B. Shapiro, et al. 2003. Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* 300: 791–795.

Wilsey, B. J., and C. Potvin. 2000. Biodiversity and ecosystem functioning: importance of species evenness in an old field. *Ecology* 81: 2000.

Xie, Y., X. Zhang, J. Yang, S. Kim, S. Hong, J. P. Giesy, U. H. Yim, et al. 2018. eDNA-based bioassessment of coastal sediments impacts by an oil spill. *Environmental Pollution* 238: 739–748.

Yates, M. C., A. M. Derry, and M. E. Cristescu. 2021. Environmental RNA: A revolution in ecological resolution? *Trends in Ecology and Evolution* 36: 601–609.

Yoccoz, N. G., K. A. Brathen, L. Gielly, J. Haile, M. E. Edwards, T. Goslar, H. V. Stedingk, et al. 2012. DNA from soil mirrors plant taxonomic and growth form diversity. *Molecular Ecology* 21: 3647–3655.

Yuan, S., S. Chin, C. Lee, and F. Chen. 2018. Phalaenopsis pollinia storage at sub-zero temperature and its pollen viability assessment. *Botanical Studies* 59: 1.

Zhu, B. 2006. Degradation of plasmid and plant DNA in water microcosms monitored by natural transformation and real-time polymerase chain reaction (PCR). *Water Research* 40: 3231–3238.

Zulkefli, N. S., K. H. Kim, and S. Hwang. 2019. Effects of microbial activity and environmental parameters on the degradation of extracellular environmental DNA from a eutrophic lake. *International Journal of Environmental Research and Public Health* 16: 3339.

How to cite this article: Johnson, M. D., J. R. Freeland, L. Parducci, D. M. Evans, R. S. Meyer, B. Molano-Flores, and M. A. Davis. 2023. Environmental DNA as an emerging tool in botanical research. *American Journal of Botany* e16120. https://doi.org/10.1002/ajb2.16120