Review

Environmental DNA analysis as an emerging non-destructive method for plant biodiversity monitoring: a review

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Abstract

Environmental DNA (eDNA) analysis has recently transformed and modernized biodiversity monitoring. The accurate detection, and to some extent quantification, of organisms (individuals/populations/communities) in environmental samples is galvanizing eDNA as a successful cost and time-efficient biomonitoring technique. Currently, eDNA’s application to plants remains more limited in implementation and scope compared to animals and microorganisms. This review evaluates the development of eDNA-based methods for (vascular) plants, comparing its performance and power of detection with that of traditional methods, to critically evaluate and advise best-practices needed to innovate plant biomonitoring. Recent advancements, standardization and field applications of eDNA-based methods have provided enough scope to utilize it in conservation biology for numerous organisms. Despite our review demonstrating only 13% of all eDNA studies focus on plant taxa to date, eDNA has considerable environmental DNA has considerable potential for plants, where successful detection of invasive, endangered and rare species, and community-level interpretations have provided proof-of-concept. Monitoring methods using eDNA were found to be equal or more effective than traditional methods; however, species detection increased when both methods were coupled. Additionally, eDNA methods were found to be effective in studying species interactions, community dynamics and even effects of anthropogenic pressure. Currently, elimination of potential obstacles (e.g. lack of relevant DNA reference libraries for plants) and the development of user-friendly protocols would greatly contribute to comprehensive eDNA-based plant monitoring programs. This is particularly needed in the data-depauperate tropics and for some plant groups (e.g., Bryophytes and Pteridophytes). We further advocate to coupling traditional methods with eDNA approaches, as the former is often cheaper and methodologically more straightforward, while the latter offers non-destructive approaches with increased discrimination ability. Furthermore, to make a global platform for eDNA, governmental and academic-industrial collaborations are essential to make eDNA surveys a broadly adopted and implemented, rapid, cost-effective and non-invasive plant monitoring approach.

Keywords: DNA barcoding; DNA metabarcoding; environmental DNA (eDNA); molecular ecology; non-destructive biodiversity monitoring; plant conservation, population management.

Introduction

The deterioration of biodiversity is accelerating at an unprecedented rate (Arneth et al. 2020), with 25 % of all monitored populations (Bongaarts 2019), and a staggering 39 % of vascular plants in particular (Antonelli et al. 2020; Nic Lughadha et al. 2020) currently threatened with extinction, forewarning a phase of global mass extinction (Myers 1990). In fact, plant diversity underpins all ecosystem functioning, suggesting that...
plant community loss will likely accelerate other biodiversity declines (Cardinale et al. 2012; Wang et al. 2020), and further impact the various ecosystem services that humans rely upon (Turnbull et al. 2016). Without strong conservation strategies and implementation, biodiversity integrity could reach a limit of destabilization, thereby reducing the Earth’s ability to resist abrupt change (viz. anthropogenic perturbations; Arnett et al. 2020). However, conservation efforts directed towards plant diversity can be hampered by a lack of monitoring data required for prioritizing conservation action, representing often diffuse, difficult to access, or outdated information, ultimately resulting in poorly designed management schemes (Corlett et al. 2020). Thus, to prevent further loss of biodiversity, we need to innovate, modernize and prioritize plant conservation and management monitoring programs.

In traditional monitoring systems across taxa, organisms are detected by visual and/or acoustic identification, or through manual collection methods. All of these require the help of taxonomic experts; a commodity in rapid decline (Jorgensen et al. 2020). Assuming that experts can be utilized, there still remains high sampling/analysis costs (Qu and Stewart 2019), the risk of misidentification, incorrect detection due to phenotypic plasticity, failure to identify cryptic species and potentially incorrect differentiation of individuals in juvenile stages (Eiler et al. 2018). It is also nearly impossible to detect all the members of a particular community simultaneously, thus making ecosystem-level inferences difficult or reliant on taxonomic proxies (Eiler et al. 2018). Additionally, collection methods further risk injury to both organisms and researchers—an important consideration especially for rare organisms at low density, or places where sampling is difficult. Perhaps, most importantly, individuals of threatened taxa are often discouraged or even banned from collection regimes. In conclusion, relying solely on traditional monitoring methods can be more time-consuming, costly, potentially invasive/destructive and inaccurate, making conservation efforts unsuccessful even for species of ecological concern (Thomsen and Willerslev 2015; Piggott et al. 2021). Therefore, alternative methods (coupled or stand-alone) need to be considered for fast, cost-effective and large-scale plant biodiversity monitoring (Deiner et al. 2021): an especially pressing ecological and political issue.

Sampling methods and molecular techniques using DNA-based monitoring either from direct or bulk samples, have caught the attention of ecologists and conservation managers and have been critically evaluated in several recent reviews (Krishnamurthy and Francis 2012; Taylor and Harris 2012; Sheth and Thaker 2017; DeSalle and Goldstein 2019). The implementation of DNA barcoding (focusing on single species) and metabarcoding (barcoding coupled with high-throughout sequencing methods to detect multiple species or whole communities) in biodiversity monitoring has proved to be effective in terms of detecting rare (Hosein et al. 2017), endangered (Lee et al. 2011; Xu et al. 2018), understanding community composition (Matesanz et al. 2019), plant–animal interactions (e.g. DNA from honey samples, diet analysis) (Pornon et al. 2017) and reconstructing past flora (Jorgensen et al. 2012; Alsos et al. 2018). DNA-based methods provide powerful tools for quick identification and discrimination of taxa. Furthermore, implementation of eDNA-based methods, where the collection and detection of species through DNA from air, water and soil represents a novel non-destructive approach that could revolutionize species monitoring programs (Minamoto et al. 2012; Miya et al. 2015; Deiner et al. 2017; Yamamoto et al. 2017; Cristescu and Hebert 2018; Taberlet et al. 2018; Ruppert et al. 2019; Calderón-Sanou et al. 2020; Banerjee et al. 2021). Environmental DNA is shed by organisms into their surroundings and thus lends itself to easy collection procedures. Indeed, these molecules represent remnant signatures of species, and are not only restricted to cellular DNA or extra-organismal DNA (e.g. epidermal cells, pollens, spores and other traces) but also include naked DNA (extracellular DNA) (Fig. 1) (Pawlowski et al. 2020b; Rodriguez-Ezpeleta et al. 2021).

Research employing such non-destructive eDNA-based methods in both aquatic (freshwater and marine systems) and terrestrial environments (soil and air) has provided valuable findings (Minamoto et al. 2012; Deiner et al. 2016; Berry et al. 2019; Ritter et al. 2020; Valentin et al. 2020). In recent decades, eDNA-based methods have been successfully employed to understand many critical concepts of ecology (e.g. habitat preference, migration, species interaction; Wu et al. 2019), including the detection and monitoring of focal or rare organisms.

![Figure 1. Different types of (plant) eDNA that can be collected and extracted from the environment.](image_url)
where the collection of samples is critical for conservation initiatives (Stewart et al. 2017). The early detection of invasive species at low density (Muha et al. 2019), or entire communities from virgin areas (Ritter et al. 2020) has also been carried out for numerous taxa. But while eDNA-based methods have been successfully used for detecting a diversity of taxa, from microorganisms (Abdel fattah et al. 2018) to macro-organisms (Deiner et al. 2021), less research has focused on the development of eDNA-based methods in higher plants.

The relative paucity of eDNA applications using plants may, in part, be reflective of their (apparent) ease in traditional sampling methods, where the focal taxa are static and also potentially because of their less charismatic standing for conservation awareness in comparison to their animal counterparts (Clucas et al. 2008). But cross-taxon congruence between plants and animal groups is known across monitored sites and biodiversity metrics (e.g. Radford and Odé 2009), suggesting a clear and urgent need to not only identify plant conservation priorities but also increase plant-specific monitoring on a systematic and global scale for maximum impact on environmental decision-making. Here, we argue that eDNA methods could spearhead plant monitoring programs, filling up large knowledge gaps in plant biodiversity data; particularly for species of urgent conservation needs.

The slower methodological development of eDNA analysis for plants may reflect the many hurdles associated with using DNA methods for plant taxa in general (e.g. incomplete reference libraries and development of universal primers) (Kress 2017). In fact, the implementation of DNA-based tools for plant species identification was initially questioned due to the shortfall of a ‘universal’ barcode. However, barcoding regions rbcL, trnH-psbA, matK (on the chloroplast genome) and ITS within the nucleus have now been identified and validated for such uses, making barcoding and metabarcoding options a reality (Kress 2017).

In order to systematically review the literature, comparing studies that use eDNA for plant biomonitoring to all other eDNA studies performed to date, we searched the online database PubMed with the criteria ‘((environmental DNA[Title/Abstract]) OR (eDNA[Title/Abstract]) OR (metabarcoding[Title/Abstract]))’ for all eDNA (e.g. barcoding or otherwise) or related metabarcoding studies, including those focused on animals or microscopic taxa. We then searched the literature using the terms ‘((environmental DNA[Title/Abstract]) OR (eDNA[Title/Abstract]) OR (metabarcoding[Title/Abstract])) AND (plants[Title/Abstract])’ for studies specifically targeting plants, including diet (faecal) and pollinator (e.g. pollen, honey) analysis, across all plant taxa (Fig. 2). Subsequently, we then refined our search by selecting only those studies dealing with eDNA-based methods (focused on air, water, soil excluding ancient eDNA samples) and on vascular plants (pteridophytes, gymnosperms and angiosperms) (Table 1; see Supporting Information—Data 1). The endeavour was made to draw the attention of practitioners and scientists who may otherwise be unfamiliar with the achievements of the eDNA-based methods and its application in plant ecology and conservation, specifically highlighting case studies in vascular plants.

**Emergence of eDNA in Macro-organism Community Studies**

The concept of eDNA-based species detections originally emerged from microbiological studies (Ogram et al. 1987). In these studies, DNA-based methods focused on extracellular DNA (which plays a crucial role in biofilm development) for monitoring of phytoplankton and bacterial communities. Here, researchers mostly targeted particulate, extracellular and dissolved DNA to detect DNA outside of the cell (Ogram et al. 1987; Rondon et al. 2000; Levy-Booth et al. 2007). In the early 2000s, the term ‘environmental DNA’ was introduced in microbial community analysis (Lakay et al. 2007), but implementation of eDNA to detect macroorganisms non-invasively and non-destructively did not come to the forefront until 2008, with the detection of aquatic invasive species (Ficetola et al. 2008). Later on, the methodology was updated by pioneer studies to detect rare aquatic animals (Darling and Mahon 2011; Jerde et al. 2011). Further, successive studies on eDNA persistence and transport (Dejean et al. 2011; Goldberg et al. 2011; Pilioud et al. 2013), release rates (Maruyama et al. 2014; Andruszkiewicz Allan et al. 2021), changes in concentration in relation to organismal abundance and seasonal activities were validated (Dejean et al. 2012; Takahara et al. 2012; Thomsen et al. 2012; Spear et al. 2015). The eDNA-based method thrived rapidly and became a multidisciplinary branch of science (Deiner et al. 2021). In fact, methodological optimization has remained a primary focus (Deiner et al. 2015; Miya et al. 2015; Banerjee et al. 2021; Bruce et al. 2021b), wherein, researchers have successfully utilized eDNA for species detection to reveal many ecological questions (Minamoto et al. 2012), such as organism presence/absence (Ficetola et al. 2008), abundance and habitat preference (Wu et al. 2019), detection of rare, threatened (Qu and Stewart 2019) and invasive species (Muha et al. 2019), monitoring whole biodiversity (Ritter et al. 2020; Yamamoto et al. 2017), study of species interactions (Banerjee et al. 2022), population ecology (Sigsgaard et al. 2020), behavioural biology (Dunn et al. 2017), anthropogenic effects (Zhang et al. 2020), ecosystem health (Fossay et al. 2020) and even disease monitoring (Barnes et al. 2020) across numerous taxa.

For plants specifically, eDNA biomonitoring has been deployed using air (Longhi et al. 2009), soil (Yoccoz et al. 2012) as well as water (Matsuhashi et al. 2016) samples. Our literature review quantified a total of 4114 eDNA studies across all organisms, illustrating a precipitous increase in recent years. Out of these, only 558 (13 % of total) of all cumulative studies conducted to date have used eDNA-based
methods to detect plant species or communities (species-specific or metabarcoding). Although, more studies incorporated eDNA-based biomonitoring on plant communities in 2020 and 2021, this number still remained low at approximately 15 % of all studies within those years (Fig. 2; see Supporting Information—Data 1). However, these studies also include past biodiversity monitoring through sediment DNA/ancient DNA (Zobel et al. 2018b; Stoof-Leichsenring et al. 2020), other indirect sampling approaches, e.g. DNA from honey samples (Khansaritoreh et al. 2020), diet analysis (Bhattacharyya et al. 2019), species identification from herbal products (Raclariu et al. 2018), as well as DNA from the environmental samples (eDNA). Interestingly, present-day studies using eDNA-based methods (focused on air, water, soil) on vascular plants represent only 4 % of studies on plants, and <1 % of all eDNA or related metabarcoding studies that could demonstrate great utility for community- or ecosystem-level quantification and monitoring [see Supporting Information—Data 1].

Workflow and Recent Advances in eDNA-Based Methods

Traces of eDNA in general, and of plants in particular, can be detected from different environments, where the sampling approaches and extracting protocols may be modified and adapted according to the type of sample and specific aim of the study (Deiner et al. 2015, 2021; Bruce et al. 2021a). Like animals, detection of plant eDNA can be possible across large zones due to the ejection of reproductive propagules and transportation of eDNA in and between the mediums (Bell et al. 2016) (Fig. 3). Thus, before application of eDNA methods for plant species, methodological standardization and understanding of the

| eDNA target | Environment | Plant taxon | Country           | Reference                                      |
|-------------|-------------|-------------|-------------------|-----------------------------------------------|
| Species-specific | Aquatic     | *Egeria densa* | Japan, USA        | (Fujisawa et al. 2016; Matsuhashi et al. 2016; Chase et al. 2020; Doi et al. 2021a; Miyazono et al. 2021) |
|              |             | *Elodea canadensis* | USA               | (Gantz et al. 2018; Angles d’Auriac et al. 2019) |
|              |             | *Hydrilla verticillata* | Japan, USA       | (Matsuhashi et al. 2016; Gantz et al. 2018) |
|              |             | *Potamogeton crispus, Stuckenia pectinata, P. foliosus, S. filiformis and Zannichellia palustris* | USA               | (Kuzmina et al. 2018) |
| Community   | Aquatic     | *Sapria bimalayana* | Thailand          | (Osathanunkul 2019) |
|              |             | *Angiosperm* | Canada            | (Coghlan et al. 2021) |
|              |             |              | China             | (Ji et al. 2021b) |
|              |             |              | Japan             | (Tsukamoto et al. 2021) |
|              |             | *Podostemaceae* | The Netherlands   | (Krajeveld et al. 2015) |
|              |             |              | Finland           | (Korpelainen and Pietilainen 2017) |
|              |             |              | Italy             | (Banchi et al. 2020b) |
|              |             |              | USA               | (Johnson et al. 2019, 2021) |
|              |             | *Gymnosperm, angiosperm* | Italy           | (Leontidou et al. 2021) |
|              |             |              | Japan             | (Uetake et al. 2021) |
|              |             |              | USA               | (Lennartz et al. 2021) |
|              |             | *Poaceae (grass family)* | UK               | (Brennan et al. 2019b) |
| Terrestrial (air) |             |              | Japan             | (Ohta et al. 2018) |
| Terrestrial (petal surface) |             | *Angiosperm* | Australia          | (van der Heyde et al. 2020) |
|              |             |              | Canada            | (Fahner et al. 2016) |
| Terrestrial (soil) |             | *Pteridophyte, gymnosperm, angiosperm* | Norway, France, French Guiana | (Yoccoz et al. 2012) |

Table 1. Vascular plant eDNA-based monitoring studies focused on air, water and soil environments between 2008 and 2021.

Adapted from the original document with necessary edits for formatting and coherence.
habitats of target taxa are essential. Here, we do not attempt to furnish a complete guide to the methodology (see Taberlet et al. 2018; Tsuji et al. 2019; Kumar et al. 2020b; Bruce et al. 2021a; Minamoto et al. 2021 for further details), but summarized the total workflow in a few steps as described below.

**Sampling approaches and environmental influences (Step I)**

In aquatic environments, typically a well-cleaned DNA-free bottle or one-time use sampler is suitable for collecting water from the surface (e.g. for surface plants), whereas a sampler...
equipped with pole/rope-like structure (e.g. Van Dorn sampler) is used for submerged water (Berry et al. 2019; Doi et al. 2021). However, as technology is progressing to simplify sample collection and improving efficiency, replicability and sterility of water sampling, a fully integrated sampling system can also be utilized (Thomas et al. 2018). Furthermore, for sampling ease, mobile polymerase chain reaction (PCR) and field preparation for eDNA amplifications have also been developed to provide rapid on-site eDNA detection (Doi et al. 2021b), thereby rapidly scaling-up biomonitoring speed and breadth. As any strategy of eDNA sample collection may not be suitable for all organisms, an objective-based sampling strategy (e.g. sample quantity, volume, locations) should be designed prior to fieldwork (Bruce et al. 2021a).

In terrestrial environments, specific collection protocols for soil samples include using a sterile digger, auger or debris metal screens (Ritter et al. 2020), and for sediments, sterile tubes, modified plastic syringes or drilling cores. Importantly, depth of sampling may vary depending on the target taxa. For air samples, individuals can use a volumetric sampler equipped with filter paper, adhesive tape or sterile collection tubes (Brennan et al. 2019; Banchi et al. 2020b; Rowney et al. 2021; Tordoni et al. 2021). But eDNA collection is not restricted to these three habitats only and has radically advanced towards innovative point-sampling. For example, eDNA can also be sampled from non-target organisms such as insect-derived DNA to study plant diversity (Gogarten et al. 2017; Jones et al. 2019) in aquatic environments (e.g. Van Dorn sampler, 2022). For soil samples, field preparation for eDNA amplifications have also been developed to provide rapid on-site eDNA detection (Doi et al. 2021b), thereby rapidly scaling-up biomonitoring speed and breadth. As any strategy of eDNA sample collection may not be suitable for all organisms, an objective-based sampling strategy (e.g. sample quantity, volume, locations) should be designed prior to fieldwork (Bruce et al. 2021a).

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Preservation (Step II)

Post-collection, samples are generally preserved by storing on ice or 4 °C temperature, frozen at −20 or −80 °C, dry preservation with absorbents (e.g. silica gel) (Kumar et al. 2020), or liquid preservation with pure preservative (e.g. ethanol, benzalkonium chloride (0.01 %)) (Jo et al. 2021) or lysis agents (e.g. Longmire’s buffers) (Kumar et al. 2020; Bruce et al. 2021a).

Capture and extraction (Step III and IV)

Samples may be further processed through filtration, centrifugation, ultracentrifugation or precipitation methods to accumulate eDNA (Tsui et al. 2019) but samples that are not subjected to an accumulation step can undergo direct extractions (Fig. 3). Filtration method uses fine porous membrane (e.g. 0.22 l, 0.45 l) to capture DNA; precipitation method uses ethanol and salt to precipitate DNA, whereas in centrifugation and ultracentrifugation method, DNA can be accumulated without adding any chemical (Bruce et al. 2021a). Filtration method are more common in use because the process larger volume of water (generally 0.5–2 μm; Tsui et al. 2019); however, other methods (e.g. precipitation) can be used where collection of samples is difficult (Tsui et al. 2019). Nowadays, both on-site and off-site eDNA filtration equipment are also available commercially (e.g. EnvirodNA; https://www.envirodna.com/). Moreover, implementation of these capture methods depends on volume of sample needed, which further depends on species abundance. Furthermore, there are many DNA extraction approaches and the method used can affect the quality of the resulting DNA template. It is important to test the DNA extraction method to ensure that it is suitable for the downstream DNA application (Deiner et al. 2017).

Amplification and sequencing (Step V)

Target species detection focuses on a particular species (one or few) and uses species-specific primers to amplify particular targets with conventional PCR (cPCR) for ‘presence and absence’, or quantitative PCR (qPCR) for DNA copy number quantification or used for more sensitive/accurate detection when DNA molecules are scarce (Wineland et al. 2019). Specific primers need to be designed for the target species and validation carried out to ensure that they do not cross-amplify related taxa (Rowney et al. 2021). Another kind of PCR, the droplet digital PCR (ddPCR), has also demonstrated very high sensitivity (Nathan et al. 2014), and species detection with the CRISPR-Cas method has also been used (Williams et al. 2019).

On the other hand, DNA metabarcoding approaches use universal primers coupled with high-throughput sequencing to analyse many samples in parallel and can identify multiple species in each sample (Bush et al. 2019). Target species detection is used to monitor, quantify, as well as study the behaviour (e.g. seasonal influence) of one or few species; whilst metabarcoding is used to detect whole plant communities, study complex interactions and give equal emphasis on a large number of target taxa (Bylemans et al. 2019; Blackman et al. 2020). However, in all of the above methods, choice of markers is extremely important to detect and discriminate the target taxa. In the case of animals, universal or species-specific primers are often based on mitochondrial cytochrome c oxidase I (CO1), 12s, 16s rRNA (Hall 1999;
Che et al. (2012), but no single barcode region has been found to be perfect in resolving all plant taxa adequately (Jones et al. 2021b). The low mutation rate of the mitochondrial CO1 region in higher plants makes it unsuitable, leading instead to the use of chloroplast (cpDNA) and nuclear DNA (nDNA) regions (Lee et al. 2016). The two core plastid DNA barcodes, cpDNA maturase K (∼matK) and ribulose-bisphosphate carboxylase (rbcL) gene, in combination are found to be effective for plants and especially for angiosperms (Kreft and Jetz 2007). Furthermore, cpDNA psb–trnH intergenic spacer and nuclear ribosomal internal transcribe spacer- ITS1 or ITS2 are also effective in species-level discrimination (Kress and Erickson 2007; Chen et al. 2010; Group et al. 2011). These barcode regions are typically used in plant barcoding and metabarcoding, but the longer length of ∼matK makes its use in metabarcoding more difficult. A combination of rbcL and ITS2 is recommended for plant metabarcoding studies (Jones et al. 2021b). DNA mini-barcodes are more preferable for eDNA, due to degradation of longer fragment in environment (Hajibabaei and McKenna 2012; Little 2014), however, this may reduce taxonomic resolution.

Following amplification, most studies currently use the Illumina MiSeq platform with v3 that can provide sequence read lengths of 300–550 base pair reads. New long-read sequencing technologies (e.g. PacBio HiFi long-read sequencing) have the potential to increase sequence length, which could provide increased taxonomic resolution. Meanwhile, short-read sequencing technologies, such as Illumina NovaSeq, have the potential to increase throughput making sample processing faster and cheaper. Portable sequencing devices, like the Oxford Nanopore MinION, can allow fast analysis within the field. Thus, whole or reduced genome approaches are increasingly being used within ecological studies and have significant potential for plant monitoring.

Bioinformatics (Step VI)

The quantity of data produced from eDNA and metabarcoding studies requires automated processes for the curation of sequences and assigning taxonomy. Various off-the-shelf as well as custom pipelines exist and the settings used within these pipelines must be thoroughly validated (Demer et al. 2017). The choice of the perfect bioinformatic pipelines is important to obtain accurate results. Newly developed pipelines (Mathon et al. 2021) as well as existing ones (e.g. Barque, QiIME 2) can be applied according to study. Furthermore, choice between use of OTU (operational taxonomic units) and ASV (amplicon sequence variant) can also influence taxonomic assignment. OTUs overcoming PCR and sequencing error are generally clustered sequences based on a threshold similarity, whereas ASVs identify unique sequence variations also filter out, PCR and sequencing errors, providing more precise and accurate measurements of single nucleotide variations. The use of ASV is growing due to its precision, reproducibility and comprehensiveness, thus may possibly replace OTU (Callahan et al. 2017). Overall, the choice of these parameters will depend on the reference database, marker used and aim of study.

Precautions

Limitations and precautions do exist with the use of eDNA methods for plants, for example, ensuring suitable primers for the questions being addressed, the requirement for standardized methodologies and the creation of suitable and complete reference libraries (Echevarria-Machado et al. 2005). To reduce false-positive and -negative error (including PCR inhibition) and eliminate chances of contamination during all the described steps in Fig. 3, positive controls (PC) (e.g. IPC: internal positive control, IAC: internal amplification control) and negative controls (NC) (e.g. collection blank, preservation blank, extraction blank) should be used (Jorgensen et al. 2020; Pawlowski et al. 2020a), and all possible types of error should be considered (Darling and Mahon 2011). The use of 10–50 % bleach solution followed by 75 % ethanol, DNA Away, Decon 90, DNA-exitusPlus is recommended for sterilization purposes. Furthermore, a major consideration for PCR-based approaches is how quantitative can they be considered. Quantification is affected by the combination of marker and primer used, DNA template, mixture characteristics and PCR conditions (Lamb et al. 2019). However, eDNA methods using metabarcoding and other amplicon-based approaches should be considered as semi-quantitative with the abundance of DNA reads treated as estimates of relative abundance (Deagle et al. 2019).

**eDNA in Relation to Traditional Plant Biodiversity Monitoring**

**eDNA compared to traditional monitoring**

Aquatic environment. Environmental DNA-based monitoring has been directly compared to traditional monitoring across several studies. For example, Kuzmina and colleagues (2018) detected three rare plant species (Potamogeton foliosus, Stuckenilia filiformis and Zannichellia palustris) that had been overlooked using traditional methods during their field visit but amplified through eDNA. Coghlan et al. (2021) similarly reported additional biodiversity information with eDNA-based metabarcoding, where nine alien taxa were identified, and out of them five did not have any previous records. Shackleton et al. (2019) compared eDNA-based metabarcoding with previous traditional monitoring data for wetland plants and found more information about endemic species. Tsukamoto et al. (2021) applied eDNA-based metabarcoding to detect endangered species of Podostemaceae in Japan where traditional methods were not fruitful due to low abundance and the submerged nature of these species. In this study, Tsukamoto and colleagues (2021) detected four species that showed similarity with previous records, although they found eDNA-based monitoring to be more effective in detecting rare species than simultaneous field surveys. For information about changes in plant diversity in relation to landscape or season, Banchi et al. (2020b) and Uetake et al. (2021) have further found eDNA to be as effective as traditional methods, especially over very short periods of time. Together, these studies suggest eDNA methods for plant biomonitoring may represent a more accurate and sensitive means compared to traditional monitoring approaches.

Terrestrial environment. Air eDNA includes bulk DNA (e.g. plant parts), and even naked DNA, which can be utilized in understanding the abundance, distribution and interactions of plants (Lennartz et al. 2021). Kraaijveld et al. (2015), for example, reported that detection and identification of plants from air–eDNA metabarcoding were found to be more effective than microscopic analysis. Brennan et al. (2019) showed a strong relationship between air-borne pollen and
the phytology of vegetation, whilst Rowney et al. (2021) showed a link between the abundance and composition of airborne pollen measured using eDNA and respiratory health in humans. In fact, for plant monitoring through air samples, most traditional surveys (microscopic analysis of pollen) and even some (air) eDNA-based surveys have focused primarily on pollen samples. Interestingly, Johnson et al. (2019) reported that detection of plant diversity is not necessarily based on pollen nor limited to anemophilous/entomophilous species. Rather, collections may represent a broad category of biological signatures detected from air through eDNA.

Environmental DNA methods using soil have been very popular to uncover ancient DNA from sediment samples (Zobel et al. 2018a; Evard et al. 2019; Lentz et al. 2021) and have even been implemented to detect large numbers of local vegetation from surface soil (Yoccoz et al. 2012; Fahner et al. 2016; Edwards et al. 2018). Interestingly, soil eDNA analysis helps in detecting plants with occasional appearance (e.g. where most of the body parts are present underground and only appear during flowering), where traditional surveys have historically faced difficulties in tracing them. For example, Osathanunkul (2019) developed eDNA-based methods to detect the occasionally visible endangered parasitic plant (Sapria himalayana) to increase its conservation success. Here, traditional surveys depended solely on flowering time but eDNA unearthed presence throughout the year. In fact, detecting a large number of taxa from soil eDNA has recently revolutionized plant biomonitoring (van der Heyde et al. 2020), where traditional sampling methods have been limited to above-ground visualization. Detection of plants and their interactions has also been studied with eDNA from rhizosphere samples (Montagna et al. 2018). Thus, eDNA has the ability to provide additional biodiversity data over traditional methods.

eDNA coupled with traditional monitoring

Although eDNA-based methods have provided successful results in recent studies compared to traditional methods (Banerjee et al. 2021), both have drawbacks. Thus, combining them may reduce the chance of error for final plant biomonitoring data (Roussel et al. 2015; Zaiko et al. 2018; Banerjee et al. 2022). In a comparison with traditional survey (e.g. line-point interrupt survey), Johnson et al. (2021) found that detection rate may vary with the type of species, where as eDNA recorded more grass where as traditional survey identified more showy flowers and both of them identified equal portion of forb species. This suggests both methods have their potential limitations. In order to understand the combined effects of eDNA-based methods and traditional surveys, Ji et al. (2021) noted that eDNA revealed more plant taxa per sampling site, but the combination of both methods was found to be more useful. Matushashi et al. (2016) found the equal effectiveness of eDNA-based methods and visual observation in submerged aquatic plant (H. verticillata); however, eDNA detection was more frequent. In another aquatic invasive plant E. densa, eDNA was also found to be equally effective or more beneficial than traditional surveys (Fujiwara et al. 2016; Gantz et al. 2018; Chase et al. 2020; Doi et al. 2021a; Miyazono et al. 2021).

However, it is evident that in its early stage of implementation, collecting eDNA for plant biomonitoring is fruitful and impressive, although the presence of potential limitations needs to be considered for its further progress, such as (i) literature understanding about ecology and interactions of eDNA, (ii) degradation of eDNA in environment and false-positive and -negative concerns, (iii) improvements in quantification, (iv) lack of standardized protocols, especially for plants (but see Minamoto et al. 2021) and practitioners adaption, (v) urgent need of reference database and group-specific primers, (vi) improvements to bioinformatics pipelines, and (vii) availability of high-throughput instrument. (Zaiko et al. 2018; Harper et al. 2019; Banerjee et al. 2022).

Conclusions and Future Perspectives

Environmental DNA methods have proven to be highly successful for surveying species, populations, communities and monitoring overall biodiversity. Despite eDNA's potential valuable role in plant biomonitoring however, many aspects to date remain unexplored. For example, we are currently experiencing worldwide degradation of forests, particularly in the tropics (40–50 % loss in forest cover; Barlow et al. 2016; Corlett 2016; Giam 2017; Roe 2019). We thus are in dire need of fast and effective monitoring methods, especially for these highly biodiverse regions. However, our search detected most studies incorporating eDNA methods do not occur in the tropics where species extinction is rapidly accelerating. What's more, while eDNA metabarcoding in animals has now specific focus on particular taxonomic groups (e.g. fish, bird, insect) more focused conservation initiatives are required for particular plant groups, e.g. bryophytes, pteridophytes (but see also Brennan et al. 2019; Tsukamoto et al. 2021; Table 1). In fact, it is worthwhile to note that our literature search revealed no scientific publications pertaining to eDNA-based monitoring involving bryophytes, which happen to be the second largest plant group, next only to flowering plants. The bryophytes are often ‘pioneer species’ and have significant roles in ecosystem functioning such as soil development, nutrient cycling, hydrology and carbon budgets (O’Neill 2000; DeLucia et al. 2003). Furthermore, pteridophytes and gymnosperms are also equally important plant taxa that need urgent monitoring and management.

The importance of these groups therefore cannot be underestimated and this calls for immediate attention. However, as biomonitoring technology keeps updating and procedures optimized, eDNA-based approaches are likely to become an extremely versatile and an essential method for plant science, despite some limitations. Biomonitoring based on eDNA will allow researchers to understand the molecular basis of plant ecological functioning, such as (i) distribution, (ii) abundance, (iii) coexistence, (iv) interactions and (v) coevolution. Recent development of environmental RNA (eRNA) and potentially in future, environmental protein (eProtein) may further lead to the molecular basis of many biological questions (e.g. health of an organism, stress response, gene expression) (Marshall et al. 2021; Yates et al. 2021). Still, elimination of potential obstacles (e.g. reference database, barcode gap) and the development of user-friendly interfaces (e.g. standardize methodology, proper bioinformatic pipelines) would contribute to improving the wide-spread implementation of these methods for plant biodiversity monitoring and conservation implementation. Sampling methodology is rapidly developing but it still may be important at this stage to couple traditional and molecular methods together as we have noticed the increase of species detection rate when both methods are employed (Ji et al. 2021). The latter method would provide a (i)
cost-effective, (ii) accurate, (iii) versatile, (iv) safe and perhaps most importantly (v) non-destructive (Berry et al. 2019) approach. In this way, the scientific community could reach a more comprehensive plant monitoring program for a variety of taxa and environments, allowing scientists, managers and policymakers to provide a global framework for actionable plant biodiversity conservation.

Supporting Information
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None declared.

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Literature Cited
Abdelfattah A, Malacrinò A, Wisniewski M, Cacciola SO, Schena L. 2018. Metabarcoding: a powerful tool to investigate microbial communities and shape future plant protection strategies. Biological Control 120:1–10.

Alsos IG, Lammers Y, Yoccoz NG, Jørgensen T, Sjögren P, Gielly L, Edwards ME. 2018. Plant DNA metabarcoding of lake sediments: how does it represent the contemporary vegetation. PLoS One 13:e0193403.

Andruszkiewicz Allan E, Zhang WG, Lavery AC, Govindarajan AF. 2021. Environmental DNA shedding and decay rates from diverse animal forms and thermal regimes. Environmental DNA 3:492–514.

Angles d’Auriac MB, Strand DA, Mjelde M, Demars BO, Thaujov L. 2019. Detection of an invasive aquatic plant in natural water bodies using environmental DNA. PLoS One 14:e0219700.

Antonelli A, Smith R, Fry C, Simmons MS, Kersey PJ, Pritchard H, Abbé M, Acedo C, Adams J, Ainsworth A. 2020. State of the world’s plants and fungi. Royal Botanic Gardens (Kew); Sfumato Foundation, 13–17. https://hal.archives-ouvertes.fr/hal-02957519 (15 April 2022).

Arneth A, Shin YJ, Leadley P, Rondinini C, Bukvareva E, Kolb M, Midgley GF, Oberdorff T, Palomo I, Saito O. 2020. Post-2020 biodiversity targets need to embrace climate change. Proceedings of the National Academy of Sciences of the United States of America 117:30882–30891.

Banchi E, Amertrano CG, Greco S, Stankovic D, Muggia L, Pallavicini A. 2020a. PLANI Ts: a curated sequence reference dataset for plant ITS DNA metabarcoding. Database. doi: 10.1093/database/baz155.

Banchi E, Amertrano CG, Tordoni E, Stankovic D, Ongaro S, Trettiach M, Pallavicini A, Muggia L, Group AW. 2020b. Environmental DNA assessment of airborne plant and fungal seasonal diversity. Science of the Total Environment 738:140249.

Banerjee P, Dey G, Antognazza CM, Sharma RK, Maity JP, Chan MW, Huang Y-H, Lin P-Y, Chao H-C, Lu C-M. 2021. Reinforcement of environmental DNA based methods (sensu stricto) in biodiversity monitoring and conservation: a review. Biology 10:1223.

Banerjee P, Stewart KA, Antognazza CM, Banchi E, Barnes MA, Saha S, Verdiel H, Doi H, Maity JP. 2022. Plant–animal interactions in the era of environmental DNA (eDNA)—a review. Environmental DNA. doi:10.1002/edn3.308.

Barlow J, Lennox GD, Ferreira J, Berenguer E, Lees AC, Mac Nally R, Thomson JR, de Barros Ferraz SF, Louzada J, Oliveira VH. 2016. Anthropogenic disturbance in tropical forests can double biodiversity loss from deforestation. Nature 533:144–147.

Barnes MA, Brown AD, Daum MN, de la Garza KA, Driskill J, Garrett BJ. 2016. Pollen DNA barcoding: current applications and future prospects. Genome 59:629–640.

Berry TE, Saunders BJ, Coghlan ML, Stat M, Jarman S, Richardson AJ, Davies CH, Berry O, Harvey ES, Bunce M. 2019. Marine environmental DNA biomonitoring reveals seasonal patterns in biodiversity and identifies ecosystem responses to anomalous climatic events. PLoS Genetics 15:e1007943.

Bhattacharyya S, Dawson DA, Hipserson H, Ishtiaq F. 2019. A diet rich in C3 plants reveals the sensitivity of an alpine mammal to climate change. Molecular Ecology 28:250–265.

Blackman RC, Ling KKS, Harper LR, Shum P, Hanfling B, Lawson-Handley L. 2020. Targeted and passive environmental DNA approaches outperform established methods for detection of quagga mussels, Dres sena rostroformis bugensis in flowing water. Ecology and Evolution 10:13248–13259.

Bongaarts J. 2019. Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. Population and Development Review 45:680–681.

Brennan GL, Potter C, de Vere N, Griffith GW, Skjoth CA, Osborne NJ, Wheeler BW, McNness RN, Clewlow Y, Barber A, Hanion HM, Hegarty M, Jones L, Kurganskiy A, Rowney FM, Armitage
C, Adams-Groom B, Ford CR, Petch GM, Creer S, Elliot A, Frisk CA, Neilson R, Potter S, Rafiq AM, Roy DB, Selby K, Steinberg N, Consortium P. 2019. Temperate airborne grass pollen defined by spatio-temporal shifts in community composition. *Nature Ecology & Evolution* 3:750–754.

Bruce K, Blackman R, Bourlat SJ, Hellstrom AM, Bakker J, Bista I, Bohmann K, Bouchez A, Brys R, Clark K. 2022a. A practical guide to DNA-based methods for biodiversity assessment. *Advanced Books* 1:68634.

Bruce K, Blackman RC, Bourlat SJ, Hellstrom M, Bakker J, Bista I, Bohmann K, Bouchez A, Brys R, Clark K. 2022b. A practical guide to DNA-based methods for biodiversity assessment. doi:10.3897/ab.68634.

Bush A, Compson ZG, Monk WA, Porter TM, Steeves R, Emilson E, Gagne N, Hajibabaei M, Roy M, Baird DJ. 2019. Studying ecosystems with DNA metabarcoding: lessons from biomonotoring of aquatic macroinvertebrates. *Frontiers in Ecology and Evolution* 7:434.

Bylemans J, Gleeson DM, Duncan RP, Hardy CM, Furlan EM. 2019. A performance evaluation of targeted eDNA and eDNA metabarcoding analyses for freshwater fishes. *Environmental DNA* 1:402–414.

Calderón-Sanou I, Müenkemüller T, Boyer F, Zinger L, Thuiller W. 2020. Development of an environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. *Journal of Applied Ecology* 49:953–959.

Chase DM, Kuehne LM, Olden JD, Ostberg CO. 2020. Development of eDNA metabarcoding analyses for freshwater fishes. *Environmental DNA* 1:402–414.

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

Chen S, Tao Y, Han J, Liu C, Song J, Shi L, Zhu Y, Ma X, Gao T, Pang X. 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS One* 5:e8613.

Chen S, Tao Y, Han J, Liu C, Song J, Shi L, Zhu Y, Ma X, Gao T, Pang X. 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS One* 5:e8613.

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

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

Dejene T, Valentin A, Duparc A, Pellerin-Cuit S, Pompanon F, Taberlet P, Miaud C. 2011. Persistence of environmental DNA in freshwater ecosystems. *PLoS One* 6:e23398.

Dejean T, Valentin A, Miquel C, Taberlet P, Bellemain E, Miaud C. 2012. Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. *Journal of Applied Ecology* 49:953–959.

DeLucia EH, Turnbull MH, Walcroft AS, Griffin KL, Tissue DT, Glenn D, McSevney TM, Whitehead D. 2003. The contribution of bryophytes to the carbon exchange for a temperate forest. *Global Change Biology* 9:1158–1170.

DeSalle R, Goldstein P. 2019. Review and interpretation of trends in DNA barcoding. *Frontiers in Ecology and Evolution* 7. doi:10.3389/fevo.2019.00302.

Doi H, Akamatsu Y, Goto M, Inui R, Komuro T, Nagano M, Minamoto T. 2021a. 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.

Doi H, Watanabe T, Nishizawa N, Saito T; Nagata H, Kameda Y, Maki N, Ikeda K, Fukuzawa T. 2021b. On-site environmental DNA detection of species using ultrarapid mobile PCR. *Molecular Ecology Resources* 21:2364–2368.

Dunn N, Priesvly V, Heraza A, Arnold R, Savolainen V. 2017. Behavior and season affect crayfish detection and density inference using environmental DNA. *Ecology and Evolution* 7:7777–7785.

Echevarria-Machado I, Sanchez-Cach LA, Hernandez-Zepeda C, Rivera-Madrid R, Moreno-Valenzuela OA. 2005. A simple and efficient method for isolation of DNA in high mucilaginous plant tissues. *Molecular Biotechnology* 31:129–135.

Edward ME, Alsos IG, Yoccoz N, Coissac E, Goslar T, Gielly L, Haile J, Langdon CT, Tribisch A, Binney HA, von Stedingh H, Taberlet P. 2018. Metabarcoding of modern soil DNA gives a highly local vegetation signal in Svalbard tundra. *Holocene* 28:2006–2016.

Eiler A, Lofgren A, Hernøe O, Norden S, Saetre P. 2018. Environmental DNA (eDNA) detects the pool frog (*Pelophylax lessonae*) at times when traditional monitoring methods are insensitive. *Scientific Reports* 8:5452.

Evrard NA, Mace GM, Tilman D, Wardle DA. 2012. Biodiversity loss and its impact on humanity. *Nature* 486:59–67.

Eltermann F, Creer S, Bista I, Lodge DM, De Vere N. 2017. Environmetal DNA metabarcoding: the example of the American bullfrog *Lithobates catesbeianus*. *Journal of Applied Ecology* 49:953–959.

Fahner NA, Shokralla S, Baird DJ, Hajibabaei M. 2016. Large-scale DNA metabarcoding analyses for freshwater fishes. *Environmental DNA* 486:59–67.

Ficetola GF, Miaud C, Pompanon F, Taberlet P, Bellemain E, Miaud C. 2012. Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. *Journal of Applied Ecology* 49:953–959.

Fogus S, Brandsegg H, Sivertsgård R, Pettersen O, Sandercock BK, Solem O, Hindar K, Mo TA. 2020. Monitoring presence and abundance of two gyrodactylyid ectoparasites and their salmonid hosts using environmental DNA. *Environmental DNA* 2:53–62.

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

Ganz CA, Renshaw MA, Erickson D, Lodge DM, Egan SF. 2018. Environmental DNA detection of aquatic invasive plants in lab mesocosm and natural field conditions. *Biological Invasions* 20:2555–2552.

Giam X. 2017. Global biodiversity loss from tropical deforestation. *Proceedings of the National Academy of Sciences of the United States of America* 114:5775–5777.

Gogarten JF, Hoffmann C, Arangjelovic M, Sachtse A, Merkel K, Dieguez P, Agbor A, Angedakin S, Brazzola G, Jones S. 2020.
Fly-derived DNA and camera traps are complementary tools for assessing mammalian biodiversity. *Environmental DNA* 2:63–76.

Goldberg CS, Pilliod DS, Arkle RS, Waits LP. 2011. Molecular detection of vertebrates in stream water: a demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. *PLoS One* 6:e22746.

Group CPB, LI D-Z, Gao L-M, LI H-T, Wang H, Ge X-J, Liu J-Q, Chen Z-D, Zhou S-L, Chen S-L. 2011. Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. *Proceedings of the National Academy of Sciences of the United States of America* 108:19643–19646.

Hajibabaei M, McKenna C. 2012. DNA mini-barcodes. In: DNA barcodes. Totowa, NJ: Humana Press, 339–353. doi:10.1007/978-1-61779-591-6_15.

Hall T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95–98.

Harper LR, Buxton AS, Rees HC, Bruce K, Brys R, Halfaerden D, Read DS, Watson HV, Sayer CD, Jones EP, Priestley V, Machler E, Murria C, Garces-Pastor S, Medupin C, Burgess K, Benson G, Boonham N, Griffiths RA, Handley LL, Hanfling B. 2019. Prospects and challenges of environmental DNA (eDNA) monitoring in freshwater ponds. *Hydrobiologia* 826:25–41.

Hosein FN, Austin N, Maharaj S, Johnson W, Rostant L, Ramdass AC, Rampersad SN. 2017. Utility of DNA barcoding to identify rare endemic vascular plant species in Trinidad. *Ecology and Evolution* 7:7311–7333.

Jerde CL, Mahon AR, Chadderton WL, Lodge DM. 2011. “Sight-unseen” detection of rare aquatic species using environmental DNA. *Conservation Letters* 4:150–157.

Ji F, Yan L, Yan S, Qin T, Shen J, Zha J. 2021. Estimating aquatic plant diversity and distribution in rivers from Jingjinji region, China, using environmental DNA metabarcoding and a traditional survey method. *Environmental Research* 199:111348.

Jo T, Sakata MK, Murakami H, Masuda R, Minamoto T. 2021. Universal performance of benzalkonium chloride for the preservation of environmental DNA in seawater samples. *Liniology and Oceanography: Methods* 19:758–768.

Johnson MD, Cox RD, Barnes MA. 2019. The detection of a non-anemophilous plant species using airborne eDNA. *PLoS One* 14:e0225262.

Johnson MD, Fokar M, Cox RD, Barnes MA. 2021. Airborne environmental DNA metabarcoding detects more diversity, with less sampling effort, than a traditional plant community survey. *BMC Ecology and Evolution* 21:1–15.

Jones L, Brennan GL, Lowe A, Creer S, Ford CR, De Vere N. 2021a. Shifts in honeybee foraging reveal historical changes in floral resources. *Communications Biology* 4:1–10.

Jones L, Twyford AD, Ford CR, Rich TC, Davies H, Forrest LL, Hart ML, McHaffie H, Brown MR, Hollingsworth PM. 2021b. Barcode UK: a complete DNA barcoding resource for the flowering plants and conifers of the United Kingdom. *Molecular Ecology Resources*. doi:10.1111/1755-0998.13388.

Jørgensen LVG, Nielsen JW, Villadsen MK, Vissman B, Dalvin S, Mathiessen H, Madsen L, Kania PW, Buchmann K. 2020. A non-lethal method for detection of *Bonamia ostreae* in flat oyster (*Ostrea edulis*) using environmental DNA. *Scientific Reports* 10:16143.

Jørgensen T, Kjær KH, Haile J, Rasmussen M, Boesenskool S, Andersen K, Coisac T, Taberlet P, Brochmann C, Orlando L. 2012. Islands in the ice: detecting past vegetation on Greenlandic nunataks using historical records and sedimentary ancient DNA meta-barcoding. *Molecular Ecology* 21:1980–1988.

Khasansitroche F, Salmaki Y, Ramezani E, Azirani TA, Keller A, Neumann K, Alizadeh K, Zarrre S, Beckh G, Behling H. 2020. Employing DNA metabarcoding to determine the geographical origin of honey. *Helthyon* 6:e05596.

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

Kraaijeveld K, De Weger LA, Garcia MV, Buermans H, Frank J, Hiemstra PS, Den Dunnen JT. 2015. Efficient and sensitive identification and quantification of airborne pollen using next-generation DNA sequencing. *Molecular Ecology Resources* 15:8–16.

Kreft H, Jetz W. 2007. Global patterns and determinants of vascular plant diversity. *Proceedings of the National Academy of Sciences of the United States of America* 104:5925–5930.

Kress WJ. 2017. Plant DNA barcodes: applications today and in the future. *Journal of Systematics and Evolution* 55:291–307.

Kress WJ, Erickson DL. 2007. A two-locus global DNA barcode for land plants: the coding rblc gene complements the non-coding trnH-psba spacer region. *PLoS One* 2:e508.

Krishnamurthy PK, Francis RA. 2012. A critical review on the utility of DNA barcoding in biodiversity conservation. *Biodiversity and Conservation* 21:1901–1919.

Kuehne LM, Ostberg CO, Chase DM, Duda J. 2020. Use of environmental DNA to detect the invasive aquatic plants *Myriophyllum spicatum* and *Egeria densa* lakes. *Freshwater Science* 39:521–533.

Kumar G, Eble JE, Gaither MR. 2020. A practical guide to sample preservation and pre-PCR processing of aquatic environmental DNA. *Molecular Ecology Resources* 20:29–39.

Kuzmina ML, Braukmann TWA, Zakharov EV. 2018. Finding the pond through the weeds: eDNA reveals underestimated diversity of pondweeds. *Applications in Plant Sciences* 6:e01155.

Lakay FM, Botha A, Prior BA. 2007. Comparative analysis of environmental DNA extraction and purification methods from different humic acid-rich soils. *Journal of Applied Microbiology* 102:265–273.

Lamb PD, Hunter E, Pinnekaj JK, Creer S, Davies RG, Taylor MI. 2019. How quantitative is metabarcoding: a meta-analytical approach. *Molecular Ecology* 28:420–430.

Lee SY, Ng WL, Mahat MN, Nazre M, Mohamed R. 2016. DNA barcoding of the endangered *Aquilaria* (*Thymelaeaceae*) and its application in species authentication of agarwood products traded in the market. *PLoS One* 11:e0154631.

Lennartz C, Kurucar J, Coppola S, Craiger J, Bobrow J, Borvoll I, Comolli J. 2021. Geographic source estimation using airborne plant environmental DNA in dust. *Scientific Reports* 11. doi:10.1038/s41598-021-95702-3.

Lentz DL, Hamilton TL, Dunning NP, Tepe EJ, Scarborough VL, Meyers SA, Grazioso L, Weiss AA. 2021. Environmental DNA reveals arboreal cityscapes at the Ancient Maya Center of Tikal. *Scientific Reports* 11:12725.

Leonidou K, Yokou D, Sandioniagi A, Bruno A, Lazarina M, De Groeve J, Li M, Varotto C, Girardi M, Casiraghi M. 2021. Plant biodiversity assessment through pollen DNA metabarcoding in Natura 2000 habitats (Italian Alps). *Scientific Reports* 11:1–12.

Levy-Booth DJ, Campbell RG, Gulden RH, Hart MM, Powell JR, Klironomos JN, Pauls KP, Swanton CJ, Trevor JS, Dunfield KE. 2007. Cycling of extracellular DNA in the soil environment. *Soil Biology and Biochemistry* 39:2977–2991.

Little DP. 2014. A DNA mini-barcode for land plants. *Molecular Ecology Resources* 14:437–446.

Liu J, Moeller M, Gao LM, Zhang DQ, Li DZ. 2011. DNA barcoding for the discrimination of Eurasian yews (*Taxus* L., *Taxaceae*) and the discovery of cryptic species. *Molecular Ecology Resources* 11:89–100.

Longhi S, Cristofori A, Gatto P, Cristofolini F, Grando MS, Gottardini E. 2009. Biomolecular identification of allergenic pollen: a new perspective for aerobiological monitoring? *Annals of Allergy Asthma & Immunology* 103:508–514.

Lucas A, Bodger Q, Brovi BJ, Ford CR, Forman DW, Greig C, Hegarty M, Jones I, Neyland PJ, De Vere N. 2018a. Floral resource partitioning by individuals within generalised hoverfly pollination
networks revealed by DNA metabarcoding. *Scientific Reports* 8:1–11.

Lucas A, Bodger O, Brogi BJ, Ford CR, Forman DW, Greig C, Hegarty M, Neyland P, De Vere N. 2018b. Generalisation and specialisation in hoverfly (Syrphidae) grassland pollen transport networks revealed by DNA metabarcoding. *Journal of Animal Ecology* 87:1008–1021.

Marshall NT, Vanderploeg HA, Chaganti SR. 2021. Environmental (e) RNA advances the reliability of eDNA by predicting its age. *Scientific Reports* 11. doi:10.1038/s41598-021-92205-4.

Maruyama A, Nakamura K, Yamanaka H, Kondoh M, Minamoto T. 2014. The release rate of environmental DNA from juvenile and adult fish. *PLoS One* 9:e114639.

Matesanz S, Pascador DS, Pias B, Sánchez AM, Chacón-Labella J, Ilumaini A, de la Cueva M, López-Angulo J, Mari-Mena N, Vizcaíno A. 2019. Estimating belowground plant abundance with DNA metabarcoding. *Molecular Ecology Resources* 19:1265–1277.

Mathon L, Valentin A, Guérin PE, Normandene E, Noel C, Lionnet C, Boulanger E, Thuiller W, Bernatchez L, Mouillot D. 2021. Benchmarking bioinformatic tools for fast and accurate eDNA metabarcoding species identification. *Molecular Ecology Resources* 21:2563–2579.

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

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

Minamoto T, Miya M, Sado T, Seino S, Doi H, Kondoh M, Nakamura K, Takahara T, Yamamoto S, Yamanaka H. 2021. An illustrated manual for environmental DNA research: water sampling guidelines and experimental protocols. *Environmental DNA* 3:8–13.

Minamoto T, Yamamoto H, Takahara T, Honjo MN, Kawabata Z. 2012. Surveillance of fish species composition using environmental DNA. *Limnology* 13:193–197.

Miya M, Sato Y, Fukunaga T, Sado T, Poulsen J, Sato K, Minamoto T, Yamamoto S, Yamanaka H, Araki H. 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. *Royal Society Open Science* 2:150088.

Miyazono S, Kodama T, Akamatsu Y, Nakao R, Saito M. 2021. Application of environmental DNA methods for the detection and abundance estimation of invasive aquatic plant *Egeria densa* in lotic habitats. *Limnology* 22:81–87.

Montagna M, Berruti A, Bianciotti V, Cremonesi P, Giannico R, Guzmérolfo L, Lumini E, Pierse S, Pizzi F, Turri E, Gandini G. 2018. Differential biodiversity responses between kingdoms (plants, fungi, bacteria and metazoa) along an Alpine succession gradient. *Molecular Ecology* 27:3671–3685.

Muha TR, Skukan R, Borrell YJ, Rico JM, de Leaniz CG, García-Vazquez E, Consuegra S. 2019. Contrasting seasonal and spatial distribution of native and invasive *Codium* seaweed revealed by targeting species-specific eDNA. *Ecology and Evolution* 9:8567–8579.

Myers N. 1990. Mass extinctions: what can the past tell us about the present and the future? *Palaeogeography, Palaeoclimatology, Palaeoecology* 82:175–185.

Nathan LM, Simmons M, Wegleitner BJ, Jerde CL, Mahon AR. 2014. Quantifying environmental DNA signals for aquatic invasive species across multiple detection platforms. *Environmental Science & Technology* 48:12800–12806.

Nic Lughadha E, Bachman SP, Leão TC, Forest F, Halley JM, Moat J, Acero C, Bacon KL, Brewer RF, Gätäble G. 2020. Extinction risk and threats to plants and fungi. *Plants, People, Planet*, 2:389–408.

O’Neill K. 2000. Role of bryophyte-dominated ecosystems in the global carbon budget. In: *Bryophyte biology*. Cambridge: Cambridge University Press, 344–368.

Ogram A, Sayler GS, Barkay T. 1987. The extraction and purification of microbial DNA from sediments. *Journal of Microbiological Methods* 7:57–66.

Ohta T, Kawashima T, Shinozaki NO, Dobashi A, Hiraoka S, Hoshino T, Kanno K, Kataoka T, Kawashima S, Matsui M, Nemoto W, Nishijima S, Suganuma N, Suzuki H, Taguchi YH, Takenaka Y, Tanigawa Y, Tsuneyoshi M, Yoshitake K, Sato Y, Yamashita R, Arakawa K, Iwasaki W. 2018. Collaborative environmental DNA sampling from petal surfaces of flowering cherry *Cerasus × yedoensis* *Somei-yoshino* across the Japanese archipelago. *Journal of Plant Research* 131:709–717.

Ortega A, Geraldi NR, Diaz-Rua R, Orberg SB, Wessellmann M, Krause-Jensen D, Duarte CM. 2021. A DNA mini-barcode for marine macrophytes (vol 20, pg 920, 2020). *Molecular Ecology Resources* 21:1000–1000.

Otsahnukel M. 2019. eDNA-based monitoring of parasitic plant (*Sapria himalayana*). *Scientific Reports* 9. doi:10.1038/s41598-019-45647-5.

Pawloski J, Apotheo-Perrt-Gentil L, Mächler E, Alterfrett M. 2020a. Environmental DNA applications for biomonitoring and bioassessment in aquatic ecosystems. *Environmental Studies*. doi:10.5167/uzh-187800.

Pawloski J, Apotheo-Perrt-Gentil L, Alterfrett M. 2020b. Environmental DNA: what's behind the term? Clarifying the terminology and recommendations for its future use in biomonitoring. *Molecular Ecology* 29:4258–4264.

Piggott MR, Banks SC, Broadhurst BT, Fulton CJ, Lintermans M. 2021. Comparison of traditional and environmental DNA survey methods for detecting rare and abundant freshwater fish. *Aquatic Conservation: Marine and Freshwater Ecosystems* 31:173–184.

Pilliod DS, Goldberg CS, Arkle RS, Waits LP. 2013. Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. *Canadian Journal of Fisheries and Aquatic Sciences* 70:1123–1130.

Pornon A, Andalo C, Burrus M, Escaravage N. 2017. DNA metabarcoding data unveils invisible pollination networks. *Scientific Reports* 7:1–11.

Potter C, De Vere N, Jones LE, Ford CR, Hegarty MJ, Hodder KH, Diaz A, Franklin EL. 2019. Pollen metabarcoding reveals broad and species-specific resource use by urban bees. *PeerJ* 7:e5999.

Qu C, Stewart KA. 2019. Evaluating monitoring options for conservation: comparing traditional and environmental DNA tools for a critically endangered mammal. *The Science of Nature* 106:1–9.

Racal, Luci, Devereux CE, Ichim MC, Cipercă OT, Bryston AK, de Boer H. 2018. What’s in the box? Authentication of *Echinacea* herbal products using DNA metabarcoding and HPTLC. *Phytochemistry* 44:32–38.

Radford EA, Odé B (Eds). 2009. *Conserving important plant areas: investing in the Green Gold of South East Europe*. Salisbury: Plantlife International.

Ritter CD, Dunthorn M, Ansland S, de Lima VX, Tedesco L, Nilsson RH, Antonelli A. 2020. Advancing biodiversity assessments with environmental DNA: long-read technologies help reveal the drivers of Amazonian fungal diversity. *Ecology and Evolution* 10:7509–7524.

Rodriguez-Ezpeleta N, Morisette O, Bean CW, Manu S, Banerjee P, Lacoursière-Roussel A, Beng KC, Alter SE, Roger F, Holman LE, Stewart KA, Monaghan MT, Mauvisseau Q, Mirimin L, Wangensteen OS, Helyar SJ, de Boer H, Monchamp ME, Nijland R, Abbott CL, Doi H, Barnes MA, Leray M, Hablutzel PL, Deiner K. 2021. Trade-offs between reducing complex terminology and producing accurate interpretations from environmental DNA: comment on “Environmental DNA: what’s behind the term?” by Pawloski et al., (2020). *Molecular Ecology* 30:4601–4603.

Roe D. 2019. Biodiversity loss is a development issue: a rapid review of evidence. *IIED Issue Paper* 2019 e019-45647-5.

Rondon MR, August PR, Bettermann AD, Brady SF, Grossman TH, Liles MR, Loiacano KA, Lynch BA, MacNeil IA, Minor C, Tiong CL, Gilman M, Osborne MS, Clardy J, Handelsman J, Goodman RM. 2000. Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. *Applied and Environmental Microbiology* 66:2541–2547.
Zhang S, Lu Q, Wang Y, Wang X, Zhao J, Yao M. 2020. Assessment of fish communities using environmental DNA: effect of spatial sampling design in lentic systems of different sizes. *Molecular Ecology Resources* 20:242–255.

Zobel M, Davison J, Edwards ME, Brochmann C, Coissac E, Taberlet P, Willerslev E, Moora M. 2018a. Ancient environmental DNA reveals shifts in dominant mutualisms during the late Quaternary. *Nature Communications* 9:1–9.

Zobel M, Davison J, Edwards ME, Brochmann C, Coissac E, Taberlet P, Willerslev E, Moora M. 2018b. Ancient environmental DNA reveals shifts in dominant mutualisms during the late Quaternary. *Nature Communications* 9:1–9.