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. Thus, 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 for innovating 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. eDNA also 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 the 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 less-concern plant groups. We further advocate it may be valuable to couple traditional methods with eDNA approaches, as the former is often cheaper and methodologically more straightforward, while the latter offers a non-destructive approach with the ability to identify plants in situations where morphological identification is difficult or impossible. Furthermore, in order to make a global platform for eDNA, governmental and

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academic-industrial collaborations are essential to make eDNA surveys a broadly adopted and implemented, rapid, cost-effective, and non-invasive plant monitoring approach.

Keywords

Environmental DNA (eDNA), Plant conservation, Non-destructive biodiversity monitoring, Population management, Molecular ecology, DNA barcoding, DNA metabarcoding

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1. 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 (Wang et al., 2020, Cardinale et al., 2012), 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; (Arneth 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, 2016). 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 the taxon, 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

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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. 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 (Piggott et al., 2021, Thomsen and Willerslev, 2015). 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 (Taylor and Harris, 2012, Krishnamurthy and Francis, 2012, Sheth and Thaker, 2017, DeSalle and Goldstein, 2019). The implementation of DNA barcoding (focusing on single species) and metabarcoding (barcoding coupled with high-throughput sequencing methods to detect multiple species or whole communities) in biodiversity monitoring has proved to be effective in term of detecting rare (Hosein et al., 2017), endangered (Lee et al., 2016), cryptic and invasive species (Liu 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 (Jørgensen et al., 2012, Alsos et al., 2018). DNA-based methods provide powerful tools for quick identification and discrimination of taxa. Furthermore, the use of environmental DNA (eDNA) based sampling, 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

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programs (Deiner et al., 2017, Ruppert et al., 2019, Taberlet et al., 2018, Calderón- Sanou et al., 2020, Cristescu and Hebert, 2018, Miya et al., 2015, Yamamoto et al., 2017, Minamoto et al., 2012, Banerjee et al., 2021). eDNA 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) (Figure 1) (Rodriguez-Ezpeleta et al., 2021, Pawlowski et al., 2020b).

Research employing such non-destructive eDNA-based methods in both aquatic (freshwater and marine systems) and terrestrial environments (soil and air) have provided valuable findings (Deiner et al., 2016, Berry et al., 2019, Valentin et al., 2020, Ritter et al., 2020b, Minamoto et al., 2012) . 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 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., 2020a) have also been carried out for numerous taxa. But while eDNA-based methods have been successfully used for detecting a diversity of taxa, from microorganisms (Abdelfattah et al., 2018) to macroorganisms (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

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between plants and animal groups are 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, particularly in 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., the development of universal primers and incomplete DNA reference libraries) (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 [fecal] and pollinator [e.g., pollen, honey] analysis, across all plant taxa (Figure 2). Subsequently, we

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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; Supplementary data 1). The endeavor 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.

2. Emergence of eDNA in macroorganism 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 (Rondon et al., 2000, Levy-Booth et al., 2007, Ogram et al., 1987). In the early 2000's, 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 (Jerde et al., 2011, Darling and Mahon, 2011). Further, successive studies on eDNA persistence and transport (Dejean et al., 2011, Pilliod et al., 2013, Goldberg et al., 2011), 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, Thomsen et al., 2012, Takahara et al., 2012, Spear et al., 2015). The eDNA-based method thrived rapidly and became a multidisciplinary branch of science

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(Deiner et al., 2021). Predominantly in the last few years, methodological upgradation was one of the main attentions (Miya et al., 2015, Deiner et al., 2015, Bruce et al., 2021b, Banerjee et al., 2021). Across all organisms, 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., 2020a, Yamamoto et al., 2017), study of species interactions (Banerjee et al., 2022), population ecology (Sigsgaard et al., 2020), behavioral biology (Dunn et al., 2017), anthropogenic effects (Zhang et al., 2020), ecosystem health (Fossøy et al., 2020) and even disease monitoring (Barnes et al., 2020).

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. The 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 (Figure 2, Supplementary 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

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metabarcoding studies that could demonstrate great utility for community or ecosystem-level quantification and monitoring (Supplementary data 1).

Of the available research that has utilized eDNA methods (air, water, soil) for plant detection and/or quantification, studies have successfully detected invasive, rare, and endangered plants (Matsuhashi et al., 2016, Osathanunkul, 2019) as well as entire communities (Banchi et al., 2020b) and their interactions (Banerjee et al., 2022). In fact, monitoring plant biodiversity with eDNA has been validated in both terrestrial (Fahner et al., 2016, Banchi et al., 2020b, Lentz et al., 2021) and aquatic (Kuzmina et al., 2018, Doi et al., 2021a) environments (Table 1). Indeed, gradually progressing towards greater methodological standardization, including development of specific primers for single-species detection and universal primers for community analysis (Scriver et al., 2015, Ortega et al., 2021, Jones et al., 2021c), assay validation (Matsuhashi et al., 2016), building up reference databases (Banchi et al., 2020a), and comparison to traditional surveys (Gantz et al., 2018, Kuehne et al., 2020, Johnson et al., 2021), have all demonstrated efficient and effective application of eDNA collections.

3. 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, Deiner et al., 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/between the mediums (Bell et al., 2016) (Figure 3). Thus, before application of eDNA methods for plant species, methodological standardization and understanding of the

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habitat of target taxa is essential. Here, we do not attempt to furnish a complete guide to the methodology (see Kumar et al. (2020b), Tsuji et al. (2019), Taberlet et al. (2018), Bruce et al. (2021a) and (Minamoto et al., 2021) for further details), but summarized the total workflow in a few steps as described below.

3.1 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 (Doi et al., 2021a, Berry et al., 2019). 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 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., 2020a), 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 (Banchi et al., 2020b, Tordoni et al., 2021, Rowney et al., 2021, Brennan et al., 2019a). But eDNA collection is not restricted to these three habitats only and has radically advanced toward innovative point-sampling. For example, eDNA can also be sampled from non-target organisms such as insect-

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derived DNA to study plant diversity (Gogarten et al., 2020), as well as from flower surfaces to study plant-pollinators-interactions (Ohta et al., 2018, Thomsen and Sigsgaard, 2019, Ushio et al., 2015). Plant-pollinator interactions and pollinator floral preferences can be also monitored by sampling pollen from the bodies of pollinators (Lucas et al., 2018b, Lucas et al., 2018a, Potter et al., 2019) or from honey (Jones et al., 2021a, De Vere et al., 2017), however non-destructive monitoring approaches should be implemented if working with taxa of ecological concern.

Interpretation of species identification data with eDNA may depend upon plant's life history, phenotype, abundance, seasonal and reproductive activity of the taxon (Berry et al., 2019, Stewart, 2019, Wacker et al., 2019, Wood et al., 2020). Moreover, the persistence of eDNA may depend upon the physicochemical characteristics of the environment (temperature, pH, oxygen, conductivity, moisture content, light (visible/UV) exposure, transportation and mobilization) and biotic factors (nuclease activity, microbial activity) (Stewart, 2019, Wood et al., 2020). These factors strongly effect the final outcome, thus understanding their role is important. eDNA copy number is often related with the abundance and activity of plant species (Gantz et al., 2018), however sampling seasons also influence the eDNA concentration. For example, Matsuhashi et al. (2019) noted eDNA concentration in aquatic plants (*Hydrilla verticillata*) significantly differed between seasons, with eDNA concentration highest during the growth period (spring to autumn) compared to dormant period (winter). Similar findings have also been reported by Doi et al. (2021a) in *Egeria densa* and Anglès d'Auriac et al. (2019) in *Elodea canadensis*. Although, the effect of these above-mentioned biotic and abiotic factors on eDNA detection have been observed in animals (Stewart, 2019), they have not fully been evaluated in plants (but see also, Gantz et al. (2018), Matsuhashi et al. (2019), Doi et al. (2021a)).

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3.2 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., 2020a), or liquid preservation with pure preservative (e.g., ethanol, benzalkondium chloride (0.01%)) (Jo et al., 2021) or lysis agents (e.g., Longmire's buffers) (Kumar et al., 2020b, Bruce et al., 2021a).

3.3 Capture and extraction (Step III and IV): Samples may be further processed through filtration, centrifugation, ultracentrifugation or precipitation methods to accumulate eDNA (Tsuji et al., 2019) but samples that are not subjected to an accumulation step can undergo direct extractions (Figure 3). Filtration method uses fine porous membrane (e.g., 0.22µm, 0.45µm) 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 is more common in use because it processes larger volume of water (generally 0.5-2 µm; (Tsuji et al., 2019)), however, other methods (e.g., precipitation) can be used where collection of samples is difficult (Tsuji et al., 2019). Nowadays both onsite 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).

3.4 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 Polymerase Chain Reaction (cPCR)

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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, metabarcoding approaches use universal primers coupled with high-throughput sequencing to analyze 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 behavior (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 (Che et al., 2012, Hall, 1999), 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

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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 chemistry that can provide sequence read lengths of 300-550 base pair reads. New long-read sequencing technologies (for example 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.

3.5 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 (Deiner 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

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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 data base, marker used, and aim of study.

3.6 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 Figure 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 are 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).

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4. Environmental DNA in relation to traditional plant biodiversity monitoring

4.1 Environmental DNA 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 (Kuzmina et al., 2018) detected three rare plant species (*Potamogeton foliosus*, *Stuckenia 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 be fruitful due to low abundance and the submerged nature of these species. In this study, Tsukamoto and colleagues (Tsukamoto et al., 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). Kraaijeveld et al. (2015) for example, reported that detection

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and identification of plants from air-eDNA metabarcoding was found to be more effective than microscopic analysis. Brennan et al. (2019a) showed a strong relationship between air-borne pollen and the phenology of below-ground vegetation, whilst Rowney et al. (2021) showed a link between the abundance and composition of air-borne 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. (2019a) 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, Evrard 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 have 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.

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4.2 Environmental DNA 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 (Zaiko et al., 2018b, Roussel et al., 2015, 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 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 of the methods have their potential limitations. In order to understand the combined effects of eDNA-based methods and traditional surveys, Ji et al. (2021b) noted that eDNA revealed more plant taxa per sampling site, but the combination of both methods was found to be more useful. Matsuhashi et al. (2016) found the equal effectiveness of eDNA-based methods and visual observation in submerged aquatic plant (*Hydrilla verticillata*), however eDNA detection was more frequent. In another aquatic invasive plant *Egeria 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, Miyazono et al., 2021, Doi et al., 2021a).

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) little 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, and (vi) improvements to bioinformatic pipelines, (vi) availability of high-trough put instrument etc. (Zaiko et al., 2018a, Harper et al., 2019, Banerjee et al., 2022).

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5. 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, Giam, 2017, Roe, 2019, Corlett, 2016)). 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., 2019b, 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 2nd 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 essential method for plant science, despite some limitations. Biomonitoring based on eDNA will allow researchers to

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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 a 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., 2021a). 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 comprehensive plant monitoring program for a variety of taxa and environments, allowing scientists, managers and policy-makers to provide a global framework for actionable plant biodiversity conservation.

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Figure Captions

Figure 1. Different types of (plant) eDNA that can be collected and extracted from the environment.

Figure 2. Cumulative total number of eDNA or related metabarcoding studies (blue) and those studies focusing on plants (red). Data collected from 2008- the date of search (September 2021) from PUBMED.

Figure 3. Detailed workflow of eDNA-based methods (air, water, or soil). NC = negative control; PC = Positive Control; IPC = Internal Positive Control; IAC = Internal Amplification Control; PCI= Phenol/Chloroform/Isoamyl alcohol; CTAB = cetyl-tri-methyl-ammonium bromide; DNeasy B&T = DNeasy blood & tissue kit; PowerWater = DNeasy powerwater kit; cfPure= cell free DNA extraction Kit; MagMAX = MagMAX viral/pathogen nucleic acid isolation kit.

Table Caption

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

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Authors' contributions

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

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

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eDNA Target	Environment	Plant Taxon	Country	Reference
Species-specific	Aquatic	*Egeria densa*	Japan, USA	(Fujiwara et al., 2016, Miyazono et al., 2021, Doi et al., 2021, Chase et al., 2020, Matsuhashi et al., 2016)
		Elodea canadensis	USA	(Anglès d'Auriac et al., 2019, Gantz et al., 2018)
		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)
	Terrestrial (Soil)	*Sapria himalayana*	Thailand	(Osathanunkul, 2019)
Community	Aquatic	Angiosperm	Canada	(Coghlan et al., 2021)
			China	(Ji et al., 2021)
		Podostemaceae	Japan	(Tsukamoto et al., 2021)
	Terrestrial (Air)	Angiosperm	The Netherlands	(Kraaijeveld et al., 2015)
			Finland	(Korpelainen and Pietilainen, 2017)
			Italy	(Banchi et al., 2020)
			USA	(Johnson et al., 2019, Johnson et al., 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., 2019)
	Terrestrial (Petal surface)	Angiosperm	Japan	(Ohta et al., 2018)
	Terrestrial (Soil)	Pteridophyte, Gymnosperm, Angiosperm	Australia	(van der Heyde et al., 2020)
			Canada	(Fahner et al., 2016)

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	Pteridophytes, Angiosperm	Norway, France, French Guiana	(Yoccoz et al., 2012)

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Figure 1

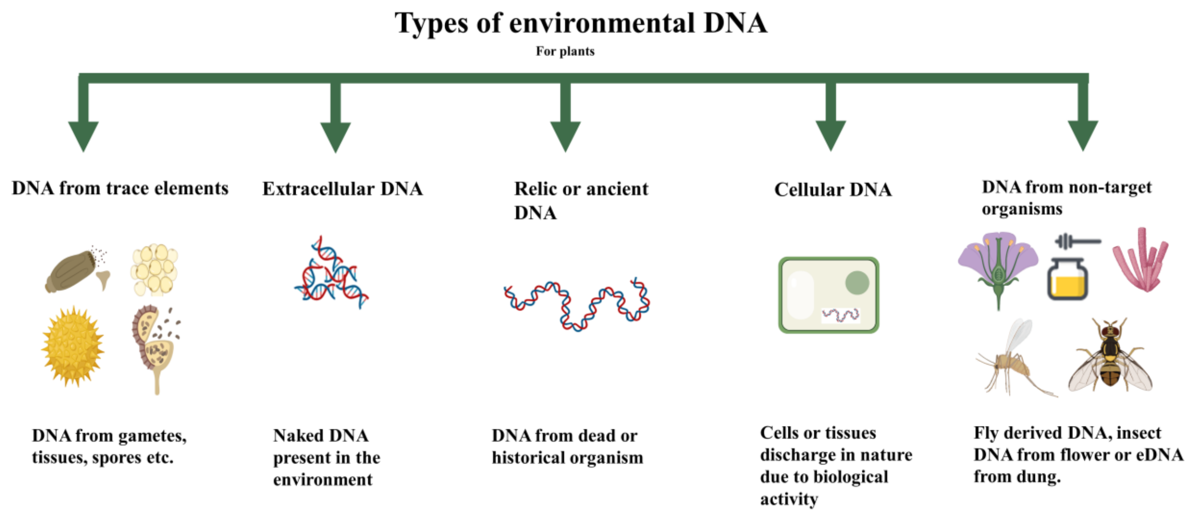

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Figure 2

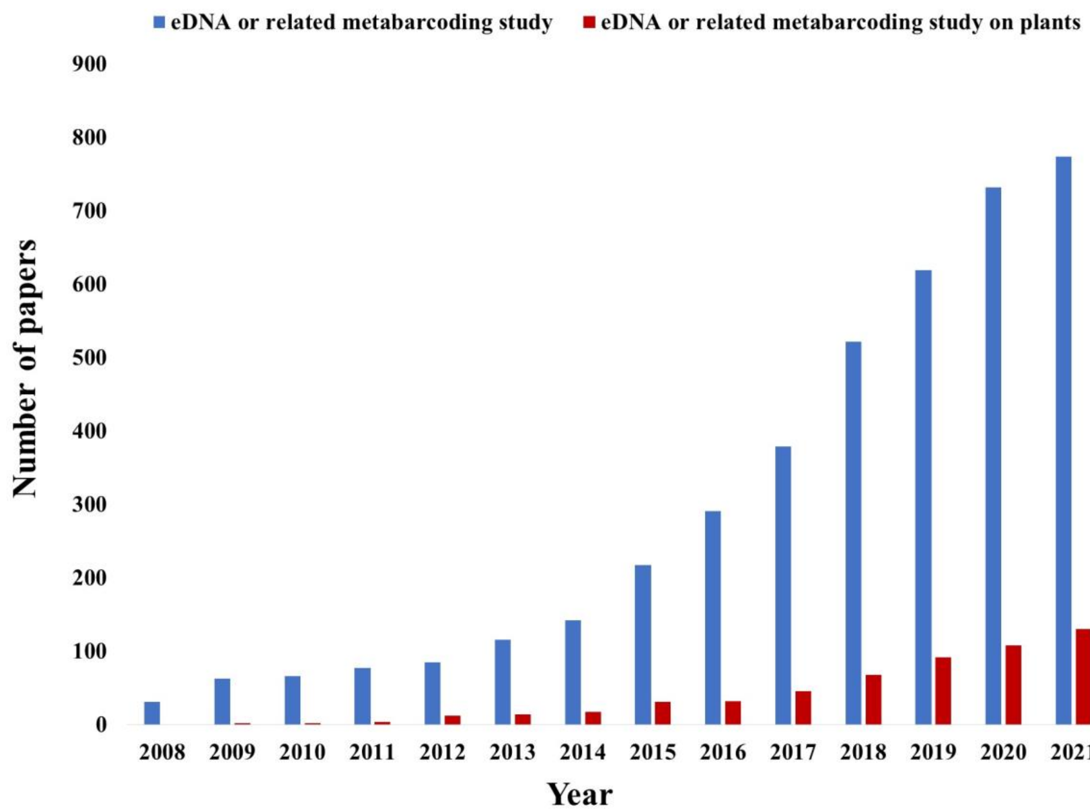

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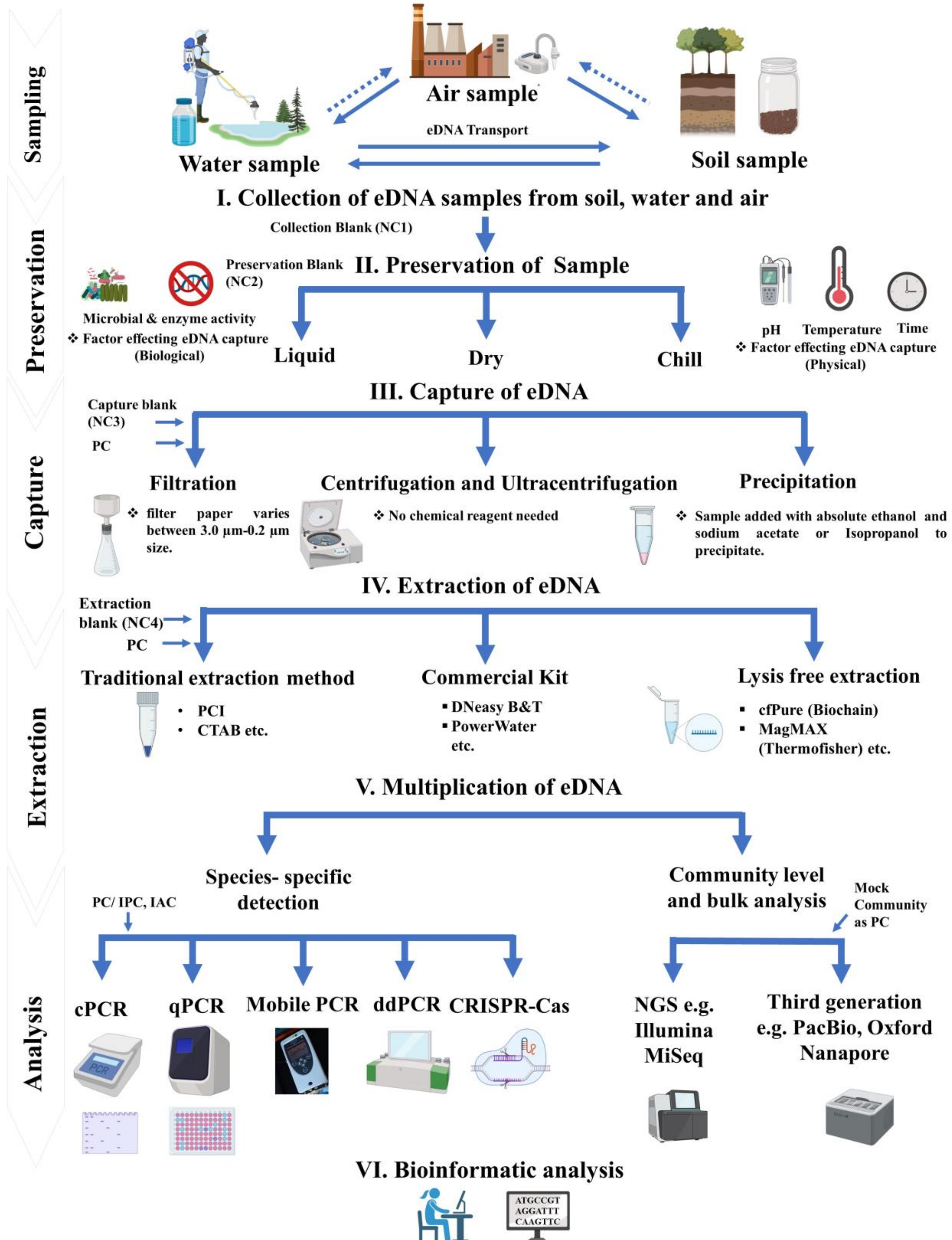
Figure 3



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