Exertion of Environmental DNA in Terrestrial Ecosystems

Subjects: Plant Sciences

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The dearth of cardinal data on species presence, dispersion, abundance, and habitat prerequisites, besides the threats impeded by escalating human pressure has enormously affected biodiversity conservation. The innovative concept of eDNA, has been introduced as a way of overcoming many of the difficulties of rigorous conventional investigations, and is hence becoming a prominent and novel method for assessing biodiversity. The demand for eDNA in ecology and conservation has expanded exceedingly, despite the lack of coordinated development in appreciation of its strengths and limitations. Therefore it is pertinent and indispensable to evaluate the extent and significance of eDNA-based investigations in terrestrial habitats and to classify and recognize the critical considerations that need to be accounted before using such an approach.

Keywords: environmental DNA ; soil eDNA ; community characterization

1. Introduction

Assessing classical and extant biodiversity is conventionally anticipated by morphological and behavioral data obtained utilizing direct surveys, microscopes, binoculars, traps, and, most recently, bioacoustics [1]. These methods are often biased, intrusive, and/or predisposed by plummeting pool of taxonomic specialists for recognizing specimens [2]. Moreover, traditional surveys are mostly labor demanding and tedious and can be inefficacious at describing the accurate biodiversity in attendance [3]. The emergence of expeditious and moderately affordable DNA sequencing techniques has notably inflated biodiversity exploration and analysis by getting the better of labor-exhaustive long-established assessments and increased the latitude and scope to coherently distinguish biodiversity on a real-time basis utilizing systematized approaches [4].

Among the different techniques for biodiversity assessment, environmental deoxyribonucleic acid (eDNA), the complex mixture of genomic DNA obtained from an environmental sample, is becoming a key component of the ecologists' and environmental managers' toolbox, alluring worldwide attention [5][6]. The science of eDNA provides the opportunity to scrutinize the dynamics of species, populations and communities and map their geographical distribution over large scales as well as over long periods. It has the potential to revolutionize conservation science [7]. Environmental DNA uses standard, reproducible and auditable criteria that accurately identify target organisms in different environments [3][8], offering broad taxonomic extensiveness and real-time biodiversity assessment for a multitude of species [9].

The first investigation of environmental DNA based on the microbial diversity from the lake sediments was reported by Ogram et al. (1987) [10]. Within a short duration of time, the concept of environmental DNA witnessed tremendous growth. In the year 1990, an investigation disseminated and analyzed the diversity of 16S rRNA gene in bacterioplankton sampled from the Sargasso Sea using PCR amid cloning [11]. To discern the new pathways, metagenomics was used in uncultured microorganisms where cloning and sequencing of soil eDNA fragments were done [12]. Notable work on DNA metabarcoding published in 2003 described the extraction of megafaunal (mammoth, bison horse), ancient plant, and extinct ratite moa DNA from permafrost [13]. Next-generation sequencing (NGS) development after 2005 rendered the costly and time-consuming cloning phase unnecessary [14]. By 2010, DNA barcoding was stretched out to macroorganisms for diet analysis [15] and then for soil eDNA studies [16]. Over the years, different ways have been applied for eDNA analysis for targeting single species, standard or quantitative PCR for detecting all taxa from a given taxonomic group. PCR based assays are vital such as for bacteria [17], fungi [18], plants [19], eukaryotes [20], fishes [21] and so on.

Although eDNA-centered analysis has rapidly gained momentum in freshwater ecology, its success has been underestimated among terrestrial ecosystems [22]. Several variables affect how easily organisms may be detected [23]. When making management choices, it is important to understand the rate of eDNA degradation, the low end of detection, and the variables affecting eDNA detectability for a target species [24]. Terrestrial eDNA is often used as a metric to

quantify population distributions. Understanding the detection and variation of terrestrial eDNA across various species or taxonomic groups will be necessary for making these decisions [25]. The understanding of and possibility of using terrestrial eDNA as a tool in biodiversity conservation are limited by the deposition and degradation of eDNA in both historic and modern terrestrial ecosystems [26].

2. Plant Community Characterization

There is a considerable corpus of knowledge on plant communities gathered by the conventional above-ground botanical inventories that can be constructed with patterns inferred from plant eDNA found in soil [27]. Environmental DNA is a promising tool for identifying vigorous and quiescent seeds, pollen and detritus of species, thereby providing an extensive perspective of plant diversity [28]. This plant community structure can act as a noteworthy aid in outlining the ecological status of the soils [29]. A study carried out by Yacooz et al. [19], indicated that boreal plant communities can be reconstructed by using a short fragment of the P6 loop of the chloroplast trnL (UAA) intron amplified from soil DNA [30]. The results showed high consistency with the data obtained using classical botanical surveys. Yet, for plants, soil DNA is more representative of biomass turnover than actual biomass [31]. This highlights that DNA read frequencies can be difficult to relate to taxon abundances without a proper calibration [32]. This finding is significant as it recognized the limitation of using short DNA barcoding regions for absolute taxonomic resolve [33].

Likewise, there is comparatively a smaller number of investigations carried out on terrestrial eDNA as a contrivance for identifying, examining and/or analyzing plant pathogens. But eDNA techniques can be a potential tool for plant pathogen investigations dealing with identifying and analyzing pathogens [34][35]. For example, if a particular plant is showing symptoms, but no directed examination is possible at that stage, eDNA can come as a rescue for appropriate disease diagnosis [36]. Environmental DNA can also be used for monitoring infectious propagules. It could be an important caveat tool that will allow well-timed and absolute treatment of affected plants before the symptoms become noticeable. In another case, fungal pathogens were recognized in cities and agricultural fields to elucidate the potential for primary warning systems by sampling air [37]. However, one of the biggest challenges of using eDNA methods for identifying plant pathogens is that the genetic resolution of marker genes, in many cases, should be able to distinguish the strains (pathogenic and non-pathogenic). Such challenges have prompted the researchers to use markers that have high resolutions; for example, in *Fusarium*, to increase the resolution, an elongation factor-based marker has been used [38].

3. Earthworm Community Characterization

Because of their burrowing and casting activities, earthworms are among soil's most important ecosystem engineers [39]. They play a significant role in nutrient cycling, water retention, and soil fertility [40]. Due to their presence in a substantial proportion of soil animal biomass, the earthworm is sensitive to factors like land use and contamination and hence are considered good indicators of soil health [41].

Earthworm inventories traditionally rely on either passive or physical separation from soil or behavioral methods where earthworms are forced to the surface through physical or chemical stimulus [42]. But these traditional methods are invasive and time-consuming, requiring strong taxonomic skills. The results can be skewed by factors like soil properties, earthworm life stage and species characteristics [43].

The ultimate solution to such queries is to confront the earthworm community studies directly on soil samples using eDNA metabarcoding. Bienert et al. [42], conducted a pioneer study when they designed two metabarcodes regions in the mitochondrial 16sRNA gene specific to earthworms and tested them on French Alps soils detecting endogeic species efficiently. However, as eDNA metabarcoding samples did not contain a leaf litter layer, they missed several epigeic species. Pansu et al. [44], improved the sampling protocol vertically, including the leaf litter in soil samples they collected and increasing the sample scheme horizontal representativeness covering the entire surface of the studied area. This spatial heterogeneity allowed more detection of species in French Alps soils. This also proved that earthworm community composition is significantly affected by land use, a pattern that was not brought to light by classical survey methods [44]. In another study, it has been revealed that environmental DNA can be applied to monitor earthworms in agroecosystems, where it was able to detect more species per sample when compared with hand-sorting [45]. Similarly, a nested PCR method in Canada's boreal forest allowed strong detection of earthworms in archival soil samples stored for up to 30 years [46].

Though there is a tremendous increase in sequencing throughputs, the incompleteness of the reference databases of mitochondrial 16S or RNA gene remains a major impediment to the precise taxonomic identification of earthworms.

4. Bacterial Community Characterization

Soil microbiologists are probably the most receptive audience for the opportunities offered by the eDNA approaches relying on DNA sequencing to characterize microbial [47] and functional biodiversity [48] for the benefit of the planet and humankind [49].

Brian et al. [50], examined bacterial taxonomic biodiversity at different spatial scales to habitat scale (>10 m) up to global scale. Despite extreme variability, it was found that higher alpha diversity existed in fertilized plots. From the study, it was observed that 20% of the molecular taxonomic units overlap with other EMP samples collected around the world, and the figure reached 40% considerably only in EMP grassland soils, highlighting the existence of a core set of the cosmopolitan bacterial groups [50][51].

Fierer et al. [52], carried out a study to characterize the soil functional diversity and bacterial and archeal taxonomic composition for 16 sites in a wide range of biomass involving shotgun sequencing and 16srDNA sequencing. The results obtained from the study indicated that the desert biome (hot as well as cold) clearly showed apart from other biomes for both metagenome and bacterial community composition, indicating that in a desert environment, bacterial community structure is mainly determined by abiotic conditions instead of microbe-microbe competition [52]. Investigations have revealed that eDNA has the potential to measure microbial communities and forest biodiversity. Therefore leveraging these methods will enhance ability to detect extant species, describe new species and improve the understanding of ecological and community dynamics in forest ecosystems [53]. In another study, it has been demonstrated that eDNA underpins the great promise that could represent soil microbial eDNA metabarcoding for monitoring restoration progress and success [54].

5. Multi-Taxa Diversity Surveys

One of the fascinating opportunities offered by eDNA metabarcoding is the possibility of carrying out multi-taxa diversity surveys using the same sampling scheme and eDNA extracts [55]. Soil eDNA metabarcoding can be employed to detect eukaryotic diversities in retort to environmental fluctuations [56].

To analyze how fungal populations in soil and leaf litter responded to a bark beetle-caused tree dieback, Stursova et al. [57] employed metabarcoding. According to their study, the composition of fungal communities altered due to the loss of root-assist fungi and the rise in saprotrophic species, resulting in a drop in the biomass of these communities. [57].

Ramirez et al. [58], investigated biodiversity and biographic patterns from 600 soil cores collected in Central Park, New York City. They studied all three domains of life (bacteria, archaea and eukaryotes) utilizing 16sRNA gene amplification and sequencing for bacterial and archeal diversities and 18srRNA gene for eukaryotic diversity. The results obtained from the study showed that Central Park, an urban and managed ecosystem harbor, had an unscripted level of below-ground biodiversity for all three domains of life, much of which had never been described in public databases [58]. However, these results should be considered with caution as it is difficult to assess how raw data were filtered to discard PCR and sequencing artifacts from the study. These two parameters can greatly inflate biodiversity estimates [59]. Furthermore, it is unclear from the study whether the taxonomic and phylogenetic resolution of the metabarcodes (i.e., 90-bp long for bacteria) was appropriate to allow significant biographical patterns.

Multi-taxa eDNA surveys allow the comprehension of the factors governing soil community assembly and diversity [60]. For example, Zinger et al. [61], examined the fine-grained special distribution of soil bacteria, archaea and eukaryotes in a tropical rainforest plot using eDNA metabarcoding. The study found that soil community composition was highly variable, poorly explained by collected data, and suggested an overall random distribution of soil organisms. The study indicated a differential role of body size on soil community assembly across the tree of life and explains how the integration of diversity census across multiple taxonomic groups can help to test previous hypotheses on complex patterns of biodiversity and community structure [62].

6. Endangered Species

Only a few studies have been able to trace animals using the eDNA technique from soil [63] in areas where the animals were previously present under controlled conditions like Safari Parks or Zoos or from natural zones where the species are reported [64]. Similarly, an investigation revealed that the endangered New Mexico meadow jumping mouse (*Zapus hudsonius luteus*) is a prime candidate for creating a terrestrial eDNA detection tool because it is restricted to herbaceous riparian zones [65]. More study is necessary to create a reliable survey technique employing this eDNA detection

methodology. The research showed that mammalian eDNA might stay on nest vegetation for a very long time even after the animal has left, underscoring the potential of utilizing eDNA from plants to identify uncommon or threatened terrestrial species. Although it has been shown that eDNA can be a valuable tool for identifying invasive, cryptic, and/or decreasing species, this method is nevertheless constrained by the same limitations that apply to the interpretation of data from conventional survey approaches (e.g., imperfect detection). A quick and efficient way to track distribution and abundance is needed for the wood turtle, a cryptic semi-aquatic species in decline over much of its range [66].

Drammund et al. [67] studied eukaryotic species disparity above and below ground utilizing eDNA from the soil but were unable to identify endangered species [67]. Other investigations used iDNA (invertebrate-derived DNA) from insects like Carrion flies or leeches to monitor terrestrial mammal biodiversity [68]. Schnell et al. [69] succeeded in discovering two species described recently, the Truong Son muntjac (*Muntiacus truongsonensis*) and Annamite stripped rabbit (*Nesolagus timminsi*) [69].

Mammals and potentially endangered species can also be detected using samples from natural saltlicks utilizing eDNA metabarcoding [70]. Using eDNA from the soil to trace mammals [71] is not only environment dependent but also dependent on mammal abundance and size [72]. Hence, knowledge of the ecological behavior of the mammal is essential for the sampling design [65]. Researchers anticipate that eDNA technology will be crucial in delivering quick and widespread insights into the population genetics of endangered and challenging-to-sample species worldwide [73].

7. Bulk Specimens

Traditional ecosystem evaluations based on morphology or barcodes have been employed for terrestrial areas. But as these techniques are expensive, laborious, and less suitable for bulky samples, they allow bulk specimen eDNA metabarcoding to be executed in such ecosystems [74]. Yu et al. [75], provided techniques for classifying bulk arthropod samples using metabarcoding by creating seven arthropod communities and evaluating the richness and composition of the two datasets. They discovered that, although taxonomic information was affected to some extent, eDNA metabarcoding can accurately quantify community differences and diversities of bulk samples [75]. Ji et al. [76] verified bulk arthropod metabarcoding by comparing it to three high-quality reference datasets from Malaysia, China, and the United Kingdom. Metabarcoding produced equivalent statistical models in the same taxon, identified treatments and responses, and connected estimates of species richness for all sites [76]. In another related work, Gibson et al. [77], tested the potential of metabarcoding to characterize diversity by using numerous universal primers on bulk arthropod samples. They discovered that 91 percent of the arthropods could be recognized using metabarcoding, which could also identify microorganisms connected to the arthropods. They also discovered that eDNA metabarcoding was superior to previous approaches and significantly decreased the period and expense of biodiversity research, building a perfect tool for a range of other ecological applications, such as macro and micro-biome interactions [77]. Evaluating host-parasite and community interactions within biodiversity studies, a field of research crucial to biodiversity monitoring but challenging to identify using conventional techniques, may also benefit from the metabarcoding of bulk specimens. Sigut et al. [78] tested the feasibility of metabarcoding for identification using mock samples of insect larvae and parasitoids. They evaluated the completeness of the barcode database by comparing it to a known host-parasitoid database for the research region. Metabarcoding could reliably identify taxa et all taxonomic levels and correctly identify 92.8 percent of all species present in mock samples. Furthermore, they discovered that 39.4% of parasitoid and 90.74% of host taxa could be recognized using the reference database, demonstrating the clear necessity for expanding the parasitoid database. This metabarcoding data indicates more parasitoid diversity than that found in conventional surveys, demonstrating the potential of metabarcoding to discern between the variety of species and to get further accurate identifications via dependable archives [78]. To evaluate the effectiveness of the methodology aimed at vertebrate findings, Rodgers et al. [79], investigated vertebrate-specific metabarcoding of carrion flies in a region with a distinguished mammal diversity. They linked the metabarcoding results to diurnal transect counts and camera trapping. Metabarcoding was able to identify more species than other techniques used concurrently during the same period in each survey (including fly collection), but overall the number of species found was lower when the data from the previous seven years of surveys were taken into account. [79]. The findings indicated that metabarcoding is a useful and competent method for investigating biodiversity, particularly when combined with current monitoring approaches [80]. On the other hand, comprehensive reference databases, improved PCR processes, numerous markers, more extensive sampling efforts, method validation, and accurate sequencing methods are all necessary [81]. Similarly, further research must be done on the terrestrial ecosystem before it can be copiously included in programs for monitoring terrestrial biodiversity [82].

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