

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/385391202>

# REVOLUTIONIZING PEST CONTROL: HARNESSING EDNA TECHNOLOGY FOR PRECISION INSECT PEST MANAGEMENT

Article in Tropical Agroecosystems · October 2024  
DOI: 10.26480/taec.02.2024.54.62

CITATIONS  
0

READS  
125

6 authors, including:



**Aroni Preya Biswas**  
Bangabandhu Sheikh Mujibur Rahman Agricultural University  
2 PUBLICATIONS 0 CITATIONS

SEE PROFILE



**Disha Mallick**  
Bangabandhu Sheikh Mujibur Rahman Agricultural University  
2 PUBLICATIONS 6 CITATIONS

SEE PROFILE



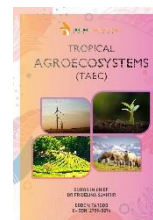
**Md. Motaheer Hossain**  
Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Dhaka  
109 PUBLICATIONS 2,302 CITATIONS

SEE PROFILE



**Md. Mamunur Rahman**  
Bangabandhu Sheikh Mujibur Rahman Agricultural University  
74 PUBLICATIONS 302 CITATIONS

SEE PROFILE



## RESEARCH ARTICLE

## REVOLUTIONIZING PEST CONTROL: HARNESSING EDNA TECHNOLOGY FOR PRECISION INSECT PEST MANAGEMENT

Aroni Preya Biswas<sup>a</sup>, Farhatun Nisa<sup>a</sup>, Disha Mallick<sup>a</sup>, Pratik Raj Karki<sup>b</sup>, Md. Motaher Hossain<sup>c</sup>, Md. Mamunur Rahman<sup>a\*</sup><sup>a</sup> Department of Entomology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.<sup>b</sup> Faculty of Agriculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.<sup>c</sup> Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.\*Corresponding author Email: [mamun@bsmrau.edu.bd](mailto:mamun@bsmrau.edu.bd)

This is an open access article distributed under the Creative Commons Attribution License CC BY 4.0, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## ARTICLE DETAILS

## Article History:

Received 23 August 2024  
Revised 18 September 2024  
Accepted 11 October 2024  
Available online 14 October 2024

## ABSTRACT

Global food production, supply chains, and food security are increasingly threatened by the burgeoning human population and the dwindling availability of arable land, exacerbating their vulnerability to both natural disasters and anthropogenic disturbances. Crop production hinges on a myriad of species interactions, encompassing both beneficial and detrimental organisms. The large-scale identification of these species within food production systems presents a formidable challenge, yet precise identification is paramount for accurately cataloging biodiversity and monitoring ecological changes. Enhancing our capabilities in detecting emergent pests and diseases, assessing soil and pollinator diversity, and collecting data to inform innovative management strategies such as targeted pesticide and fertilizer applications are critical components of this endeavor. Environmental DNA (eDNA) has emerged as a potent tool for the rapid and precise identification of individual organisms and species assemblages across various matrices, including air and soil. This paper explores the application of eDNA for the surveillance of agricultural environments and pest management. The scope of this review encompasses the utilization of eDNA technology in agricultural systems, focusing on its application in pest control and biodiversity monitoring. Despite the promising capabilities of eDNA, its implementation in pest management within agricultural systems remains underutilized, particularly in regions where food security is most at risk. A significant gap exists in the application of eDNA-based monitoring studies in food production systems globally, with a marked deficiency in developing nations. The objectives of this review are to evaluate the current use of eDNA in pest control and agricultural biodiversity monitoring, identify existing limitations and propose potential solutions to enhance eDNA applications, and highlight the need for increased adoption of eDNA technologies in underrepresented regions to improve global food security. Our comprehensive analysis underscores the efficacy of eDNA-based monitoring in pest control, delivering precise taxonomic identifications. Notably, 60% of eDNA research is concentrated on soil and plant substrates, predominantly focusing on bacterial and insect identification, with European studies accounting for a significant proportion (42%). There is a notable paucity of eDNA-based monitoring studies in numerous global food production systems, particularly within developing nations where food security is most precarious.

## KEYWORDS

Environmental DNA, metabarcoding, monitoring, surveillance, invasive species.

## 1. INTRODUCTION

Environmental DNA, also known as eDNA, is the genetic material in environmental substances such as sand, water, and air. The substance may consist of intact cells, extracellular DNA, and potentially whole organisms (Ficetola et al., 2008; Barnes and Turner, 2016). Environmental samples may contain environmental DNA (eDNA) that can be gathered, preserved, amplified, sequenced, and categorized according to its genetic sequence (Deiner et al., 2015). With this understanding, it is feasible to recognize and categorize several species. There are many different places from which eDNA can be obtained, such as the skin, mucous, saliva, sperm, secretions, eggs, feces, urine, blood, roots, leaves, fruit, pollen, and decaying carcasses of larger species. Microbes can be collected in their totality (Taberlet et al., 2012a; Bohmann et al., 2014; Barnes and Turner, 2016). The creation of eDNA is influenced by various factors, including the organism's biomass, age, eating habits, physiology, life history, and spatial use (Barnes and Turner, 2016; Goldberg et al., 2016; Hering et al., 2018).

Despite being a relatively new technique, eDNA surveys have already demonstrated significant potential for biological monitoring. Conventional methods of measuring species diversity are limited by the need for taxonomic classification, the possibility of habitat disturbance or loss, and the challenge of accurately assessing the diversity and abundance of entire ecological communities due to the complexities involved in identifying small or hard-to-find species. eDNA can improve existing procedures by specifically targeting individual species, collecting samples from a wider variety of taxonomic groups, and enhancing the precision of taxonomic identification (Deiner et al., 2017).

Moreover, eDNA is well-suited for augmenting traditional research due to its ability to detect rare species. Nevertheless, it does not possess the capability to provide data on demographic characteristics such as sex ratios and physical qualities (Goldberg et al., 2016; Deiner et al., 2017). However, it can be beneficial in detecting the early occurrences of native species previously thought to be extinct or endangered, invasive species,

## Quick Response Code



## Access this article online

Website:  
[www.taec.com.my](http://www.taec.com.my)

DOI:  
10.26480/taec.02.2024.54.62

and other rare species that are difficult to find using traditional approaches. The scope of eDNA research is limited by the deterioration of nearby eDNA, which often results in the conservation of minimal quantities of genetic material, particularly in hot, tropical settings. Furthermore, particular spatial and temporal patterns of species and communities can be understood differently depending on the environment and the ability of DNA to spread through various materials, such as water, and the varying rates at which it breaks down due to environmental factors (Coissac et al., 2012; Taberlet et al., 2012a; Eichmiller et al., 2016; Goldberg and Associates, 2016; Deiner and Associates, 2017; Hering and Associates, 2018).

Due to the inherent changes in environmental materials, measuring eDNA can be challenging. However, multiple studies have shown that environmental DNA (eDNA) can accurately predict the relative or rank abundance of species and is in agreement with biomass. While eDNA has various applications in monitoring, conservation, ecosystem evaluation, and other unexplored domains, it is crucial to continually improve the technology due to the fluctuating amounts of eDNA and the potential differences within the water body. This emphasis on improvement is intended to motivate and engage the audience in the field of research. Before implementing a new application, it is recommended to conduct a pilot study to ensure that the sampling design is appropriate for identifying the required species.

The study investigates the application of environmental DNA (eDNA) in the management of soil health and plant protection techniques, recognizing its potential to provide innovative solutions. Furthermore, the research explores the use of eDNA techniques for obtaining genetic markers, which are crucial for the detection of invasive species in biodiversity. The report aims to elucidate the application of eDNA for environmental monitoring and, importantly, for promoting agricultural sustainability, providing reassurance about the future of farming.

## 2. MATERIALS AND METHOD

This review paper explores current knowledge on collecting and integrating existing studies on using environmental DNA (eDNA) in pest management. The writers undertook an exhaustive examination of pertinent literature sourced from journals, conference proceedings, research papers, online sources, etc. The inclusion requirements specifically target research that provides comprehensive information on the advancement, authentication, and utilization of eDNA technologies to identify and monitor insect pest populations. The data retrieved focuses on eDNA sample methodologies, detection accuracy, and practical applications in integrated pest management (IPM). In addition, the review analyzes case studies and pilot projects to assess the practical effectiveness and difficulties encountered in real-world scenarios. The analysis of the findings aims to identify patterns, knowledge gaps, and

future research paths to offer a thorough perspective of how eDNA technology can enhance precision pest management tactics.

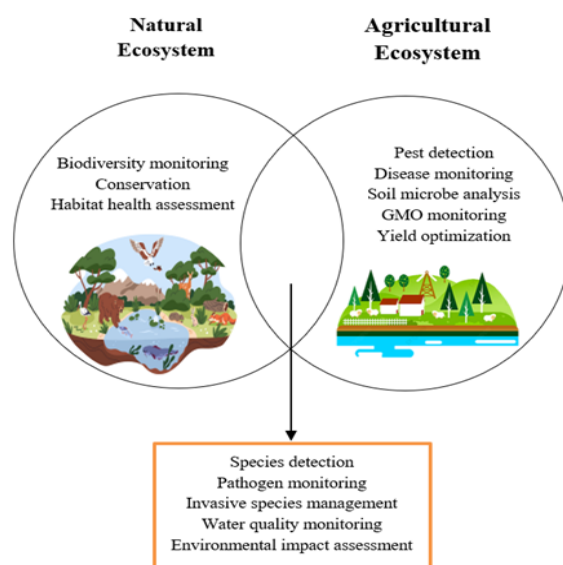
## 3. RESULTS AND DISCUSSION

### 3.1 Utilization of eDNA in Agricultural and Natural systems for management

Conventional agricultural monitoring techniques have encountered obstacles in their growth and are frequently unfeasible as most organisms cannot be cultivated or raised using them (Kudoh et al., 2020; Rappé and Giovannoni, 2003). Using small amounts of DNA or a single combined environmental sample provides a practical, consistent, and cost-efficient option for identifying species (Kudoh et al., 2020; Valentin et al., 2018; Littlefair et al., 2016). Performing individual assessments for pests and diseases on each plant or animal within a significant population is typically impractical due to time limitations and substantial costs (Brunner, 2020; Ceresini et al., 2019). In eDNA analysis, a composite sample containing multiple sub-samples can determine whether a specific organism is present or absent in an entire cargo.

This allows for a quick and thorough assessment, such as detecting the Khapra beetle (*Trogoderma granarium*) in shipping containers using a test specifically designed for this species (Brunner, 2020; Valentin et al., 2018). eDNA-based detections can be customized to target economically significant species or entire populations in situations where traditional identification methods based on morphology have been challenging (Aloo et al., 2020; Macgregor et al., 2019). This technique is beneficial for detecting microorganisms that are challenging to produce using specialized media, as studies indicate that more than 99% of bacteria cannot be grown in a laboratory setting (Rappé and Giovannoni, 2003; Sternhagen et al., 2020).

Moreover, the sophisticated automation in contemporary agriculture enables the incorporation of eDNA-based sample techniques with current technology and infrastructure to detect these specific species precisely. Acquiring information about these typically imperceptible organisms would enhance surveillance endeavors and potentially lead to more knowledgeable approaches for managing these species, contingent upon their association with the cultivated animal or plant of concern (such as targeted pesticide utilization or decreased fertilizer application) (Menta and Remelli, 2020; Willcox et al., 2019). The capacity to customize eDNA samples and specificity based on the desired species, community, or system has facilitated non-invasive surveys in various habitats and situations. However, eDNA surveys have yet to be extensively employed in the agricultural field despite their potential. The utilization of eDNA surveys has primarily been limited to natural environments (Bohmann et al., 2014; Evans and Kitson, 2020; Ruppert et al., 2019; Taberlet et al., 2012a).



**Figure 1:** Utilizations of environmental DNA (eDNA) in both natural and agricultural ecosystem

The use of eDNA in agricultural systems has been limited; however, there is a progressive movement towards increased utilization. As far as we know, there have been no thorough assessments of the application of eDNA barcoding and metabarcoding in both natural and agricultural systems (Fig 1). It is important to note that precise taxonomic

identifications are essential for monitoring in both natural and human-altered ecosystems. This oversight is particularly significant, as highlighted by studies conducted (Memmott et al., 2004; Van Elsas et al., 2002; Yue et al., 2020).

**Table 1:** Fourteen research were discovered from SCOPUS that aimed to identify the many applications of environmental DNA (eDNA) in agricultural and natural systems

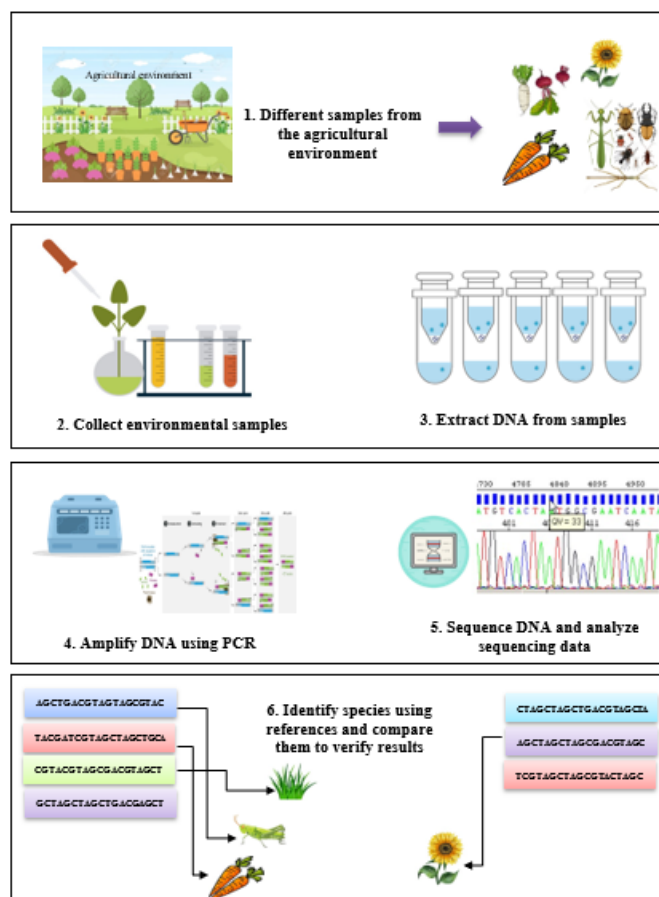
System	Substrate	Barcoding	Target taxa	Reference
Vineyard	Bulk-insect samples	Metabarcoding	Insects	Rasmussen et al., 2021
Agricultural fields	Bulk-insect samples	Metabarcoding	Insects	Zenker et al., 2020
Farmland	Bulk-insect samples	Metabarcoding	Insects	Song and Huang, 2016
Flowering fields and calcareous grasslands	Bulk-insect samples	Metabarcoding	Insects	Boetzel et al., 2021
Oil palm plantations	Bulk-insect samples	Metabarcoding	Insects	Edwards et al., 2014
Perennial cropland	Bulk-insect samples	Metabarcoding	Insects	Dopheide et al., 2020
Island habitat for long-distance	Moths	Metabarcoding	Plants	Chang et al., 2018
Caves within agricultural landscape	Faecal	Metabarcoding	Insects	Tournayre et al., 2021
Caves within agricultural landscape	Faecal	Metabarcoding	Insects	Aizpurua et al., 2017
Macadamia orchards	Faecal	Metabarcoding	Insects	Crisol-Martínez et al., 2016
Various orchards	Honey	Metabarcoding	Hemiptera species	Utzeri et al., 2018
Greenhouse and field trial	Leaf, root, seed,	Metabarcoding	Fungi	Latz et al., 2021
Agricultural fields	Soil	Metabarcoding	Bacteria, fungi and eukaryotes	Froslev et al., 2021
Agricultural landscapes	Pollen	Metabarcoding	Plants	Danner et al., 2017

### 3.2 Surveillance of pests and pathogens in agricultural systems

Plant pests and diseases possess an enhanced ability to undergo evolutionary changes, adapt to new settings, and spread within cultivated habitats (Brown and Hovmoll, 2002; Smith and Guegan, 2010). The presence of a susceptible host and favorable environmental circumstances has the potential to jeopardize agricultural productivity by promoting the spread of diseases and attracting pests. If not treated appropriately, worldwide food productivity is estimated to decrease by 20-40% per year (Flood, 2010). To attain food security by 2050, it is imperative to double crop yields, which will inevitably lead to a rise in the occurrence of crop diseases. There is an expected increase in the number of pathogenic bacteria that harm crops (Amari et al., 2021; Chaloner et al., 2021).

Hence, the emergence of novel plant pests and diseases, along with alterations in the intensity and geographical distribution of existing pests and diseases, can jeopardize the sustained viability of present agricultural

systems. This risk becomes more prominent when cutting-edge advancements and technologies are not employed to identify and monitor their emergence (Jones, 2009; Osunkoya et al., 2021; Wintermantel and Hladky, 2010). Two significant occurrences include the worldwide dissemination of the wheat blast fungus (*Magnaporthe oryzae*) and the existence of Ramularia leaf spot in barley (*Ramularia* sp.). Both pathogenic fungi provide challenges in terms of identification and cultivation, and they have quickly spread across international borders. A group researcher found that certain farms have seen a significant decline in their yearly production, with reductions of up to 70%, as a result of a low level of infection caused by *Ramularia* sp (Havis et al., 2015). In a similar vein, discovered that farms might experience a total cessation of productivity, reaching 100%, when the infection is attributed to *M. oryzae* (Ceresini et al., 2019). The use of eDNA-based methods for identifying pathogens in agricultural substrates, such as soil, leaf litter, or air, offers a strong and efficient approach (Ceresini et al., 2019).

**Figure 2:** Workflow for eDNA-based monitoring to identify the species

Research by Tordoni et al., 2021 revealed that eDNA metabarcoding, a cutting-edge technique, identified three times more fungal species from the air than manual identification. This breakthrough not only aids in the early detection of plant illnesses but also helps in acknowledging diseases that might otherwise go unnoticed in agricultural areas (Michael et al., 2020). A group researcher further demonstrated the potential of eDNA metabarcoding by using it in conjunction with passive and active air samplers to study the spatial and temporal variations of airborne fungal spores in landscapes with a mix of woods and agricultural regions (Redondo et al., 2020).

The results indicated that the communities were consistently controlled by the presence of fungal spores *Alternaria spp.* and *Ustilago spp.*, which possess the capacity to cause agricultural illnesses. Agricultural practitioners can strategically administer fungicides by focusing on specific areas where diseases have been verified by conducting similar observations on more minor geographical scales. This strategy maximizes the ROI (return on investment). Moreover, the potential for environmental damage can be diminished by minimizing and precisely targeting the utilization of fungicides instead of using more extensive application techniques (Sowunmi et al., 2019). Implementing a comparable surveillance technique using environmental DNA (eDNA) can provide advantages for biosecurity monitoring. One possible use is the integration of eDNA sampling, which involves collecting samples of air, water-wash, and crop surfaces into border control stations to improve existing approaches. This would enhance the identification of plant pests and pathogens and aid in preventing transnational epidemics.

Although still a relatively new method, using eDNA techniques in agricultural contexts can enhance current methods for detecting plant infections, such as *M. oryzae* and *Ramularia sp.* This can enhance the creation of flexible management strategies. Personalized eDNA surveys can rapidly and accurately detect new pests and pathogens, allowing for targeted sampling and the implementation of suitable management strategies. Herbivorous pest insects usually rely on a particular set of host species or specialized groups of plants as their primary food source (Imms, 1947). Previously, many methods, including direct observation, microscope examination, rearing of pest insects, and feeding trials, have been utilized to identify these potentially dangerous species (Hamilton et al., 2005; Symondson, 2002; Vu et al., 2018). These traditional methods require significant time investments and rely on extensive taxonomic expertise (Kudoh et al., 2020; Symondson, 2002).

Feeding trials can last up to 20 days, depending on the animal being studied. This period does not include the time needed for data processing (Clay et al., 1985; Dunse et al., 2010). Based on the studies conducted extended durations could lead to significant infestations and epidemics (Kudoh et al., 2020; Simberloff et al., 2013; Valentin et al., 2018). Delays can arise in both the identification of the problem and the implementation of targeted pesticide treatment, resulting in this consequence. In addition, the rapid rates of reproduction displayed by specific pest insects, such as aphids, along with the extensive areas that need to be monitored in agricultural systems, render traditional methods like direct observation by taxonomic experts impractical (Edwards et al., 2014; Rouland-Lefevre, 2010; Simberloff et al., 2013).

As a result, practitioners frequently have limited options and must depend on preventive pesticide treatments. These treatments are costly, harmful to the environment, and can contribute to developing pesticide resistance (Leskey et al., 2012; Morales, 2006; Rouland-Lefevre, 2010). Therefore, it is crucial to promptly and precisely identify recently appearing plant pests in food production systems. Barcoding and metabarcoding are highly effective techniques for rapidly identifying beneficial and pest insects throughout large agricultural areas. This involves the analysis of the DNA of herbivorous insects found on plants or collected in traps. Two kinds of traps are vane traps and funnel traps. This methodology has been scrutinized in studies undertaken (Thomsen and Valentin, 2018; Young et al., 2021).

Scientists have successfully identified the genetic makeup of insects that visit flowers, feed on plants, and prey on other insects by analyzing the DNA traces left on plant tissue after the insects eat or excrete waste (Bittleston et al., 2016; Derocles et al., 2015; Kudoh et al., 2020). Furthermore, these eDNA techniques have demonstrated potential in accurately identifying pest species from plant samples collected in orchards, vineyards, and croplands. A study showed that the use of environmental DNA (eDNA) is a more effective method for detecting the highly invasive and destructive brown marmorated stink bug (*Halymorpha halys*) compared to traditional approaches such as pheromone traps and black lights. Moreover, this technology is not only more effective but also economically efficient, utilizing a species-specific assay to selectively analyze rinse water collected after apples have been cleaned. Valentin et al. reported their findings in 2016 and 2018.

Moreover, eDNA-based surveys can be employed to detect the presence of co-occurring beneficial insects, such as native bees. This data can be utilized to assess the efficacy of the pesticide treatment in recovering from the impact of pests across a wide range of species. Moreover, it may be employed to predict the duration required for the reappearance of harmful and beneficial organisms. This information would be beneficial in formulating future schedules and strategies for applying pesticides. Bug traps are an alternative method for assessing the presence of pests. These technologies can improve molecular authentication and prevention strategies when used alongside traditional identifying procedures. eDNA detections can assist in promptly and accurately diagnosing emerging zoonotic pests and diseases.

This would facilitate the quick execution of preventive actions that improve herd production and animal welfare. DNA studies have been the preferred technique for identifying soil microorganisms in agricultural monitoring programs for about two decades (Hugenholtz and Pace, 1996; Rolf, 2005). The difficulty in identifying and growing soil microorganisms using traditional methods is mainly due to the low rate of success (0.1-1%) in culturing bacteria using conventional cultivation techniques (Rolf, 2005). The terminology used in soil literature may vary, but the DNA techniques employed to identify soil microorganisms are comparable to those utilized in eDNA and metagenomic studies (Taberlet et al., 2018). In soil studies in food production systems, the term "eDNA" is employed to measure the taxonomic diversity of the soil microbiome. This microbiome comprises bacteria, fungi, archaea, and eukaryotes, as specified in Table 2.

**Table 2:** Overview of key discoveries about the longevity of environmental DNA (eDNA) in monitoring the spread of pests and pathogens

Study site/ Sample habitat/ Substrate	Taxon Studied	Uses	Reference
Leaf and stem surfaces	Insects	Detecting an invasive pest insect	Allen et al., 2023
Fruit and leaf surfaces	Insects	State, transport, and fate of eDNA	Valentin et al., 2021
Palm plantations	Insects	Quantify the biological impacts of plantations	Edwards et al., 2014
Orchards	Insects	Information gathers from plant-sucking insects	Utzeri et al., 2018
Island habitat	Insects	Pollen grain analysis	Chang et al., 2018
Agricultural fields	Insects	Prey detection	Aizpurua et al., 2017
Agricultural fields	Bugs	Detection of invasive exotic insect	Valentin et al., 2018
Agricultural field and forest	Insects	Assessing insect biodiversity	Zenker et al., 2020
Farmland	Plants and moths	Construction, validation, and application of nocturnal pollen transport networks	Macgregor et al., 2019
Rice field	Bacteria	Transmission and biogeography of bacteria	Zhou et al., 2020
Agricultural field	Leptospira sp. and bacteria	Understanding leptospirosis co-epidemiology	Gamage et al., 2020
Soil	Earthworms	Comparing earthworms' biodiversity	Lilja et al., 2023



### 3.3 Management of the soil microbiome, macrofauna, mesofauna, and rhizobium

Plant productivity and overall health are inherently connected to the biological and functional diversity of the soil microbiome. Soils with diverse biological organisms can effectively control soil-borne pests and diseases through predation, competition, and parasitism. These tactics ultimately promote the widespread growth of crops. Intensifying agricultural activities, such as altering soil preparation methods, implementing grazing of animals, and limiting weed growth, can decrease the complexity of soil food webs. Consequently, this results in a reduction in the soil's capacity to regulate pests and disease-causing microorganisms. Incorporating strategies focused on preserving and improving soil biodiversity has been recognized as essential to sustainable agriculture and global food security. The application of eDNA in this study has facilitated categorizing the primary biological components of soil microbiomes in agricultural systems, including archaea, bacteria, fungi, and eukaryotes (Froslev et al., 2021).

Microorganisms constitute a constituent of the overall biodiversity found in soil. However, larger invertebrates, such as soil mesofauna (measuring more than 40 µm) and macrofauna (measuring more than 1 cm), also significantly maintain soil health. Notably, these species have not undergone comprehensive scrutiny in agricultural eDNA monitoring, accounting for only 7% of all studies conducted in this domain. The current method for monitoring soil meso- and macrofauna mostly depends on taxonomic keys to identify them based on their physical characteristics. Nevertheless, using eDNA-based detections provides a dependable and efficient alternative for categorizing the vast array of soil invertebrates (Taberlet et al., 2012a). An illustrative instance entails utilizing assays tailored to distinct taxa to detect extracellular DNA from earthworms in the soil, hence facilitating the categorization of species groupings. Unlike the labor-intensive process of physically identifying earthworms based on their physical characteristics, eDNA surveys provide a comprehensive examination of earthworm populations by studying the genetic material present in the environment. This technique also enables the differentiation of mesofauna populations linked to different crops. This study compared universal and species-specific testing and routine monitoring approaches in kiwifruit (*Actinidia sp.*) and apple (*Malus domestica*) orchards.

A group researcher identifies mesofauna by employing species-specific assays and examining their physical characteristics (Todd et al., 2020). The data unambiguously demonstrate that assays tailored explicitly for each species exhibit a 100% accuracy rate in identifying mesofaunal species. The range of the morphological study results varies from 40% to 100%. These statistics indicate that solely concentrating on morphological analysis may lead to incorrect species identification and decrease detection accuracy. Ecological investigations can enhance the identification of mesofauna by utilizing molecular techniques such as species-specific testing. The intricate symbiotic relationships between plants and rhizobium, a microorganism found in the soil, are crucial for plants' overall well-being and vigor (Dessaux et al., 2016). Recent research has shown that eDNA may be used to identify rhizospheres in agricultural environments, which is essential for developing new management strategies. For example, eDNA metabarcoding can demonstrate the variety of rhizosphere fungi connected to coffee plants (*Coffea sp.*).

### 3.4 Quantification of organism population size for the purpose of management

After numerous successful examples of species identification using eDNA, a promising question emerges: What additional data can be gleaned beyond mere species confirmation? A fascinating aspect of eDNA research is exploring the potential correlation between the quantity or abundance of eDNA in environmental samples and the population size of each species. The measurement of environmental DNA (eDNA) holds the promise of providing researchers and managers with valuable insights into endangered and threatened species, as well as guidance on data collection and interpretation. By utilizing eDNA quantification, we can uncover indications of habitat utilization and species preference that are otherwise difficult to discern. This information is invaluable for identifying the geographical regions that should be prioritized in conservation efforts. For instance, it enables us to pinpoint the habitats where animals reside, as well as their movement and dispersal patterns.

Environmental DNA (eDNA) measurement has been rigorously studied as a reliable method for collecting data on biomass or population size. A study conducted in a Japanese lagoon revealed a direct correlation between the

population of common carp fish and the concentration of environmental DNA (eDNA) discovered in the common carp (Takahara et al., 2012). The study conducted demonstrated a strong correlation between the fish population and the rate of detection and concentration of environmental DNA (eDNA) in a lake in Minnesota, United States of America (Eichmiller et al., 2014). The present investigation yielded results that were comparable to those obtained in the prior study. Researchers have identified new connections between different amphibian species in European ponds and Idaho streams (Thomsen et al., 2012a; Pilliod et al., 2013). The presence of these linkages was seen in both of these locations. In 2012, Andersen et al. did a study where they analyzed the genetic composition of soils in zoos and farms. The study focused on species that had previously been found in those locations. The research findings indicate a clear correlation between the composition of the local vertebrate community and the biomass of the native vertebrate species, as well as the soil samples. Despite the inherent challenge of measuring eDNA signals and understanding their geographical and temporal patterns, the efficiency of water and air in transporting eDNA, and the rapid degradation of eDNA in the environment, do not compromise the reliability of eDNA analysis. By integrating the processes of eDNA creation, transit, and degradation into our models, we can enhance the precision of our calculations regarding the abundance of organisms, which are dependent on the quantity of eDNA.

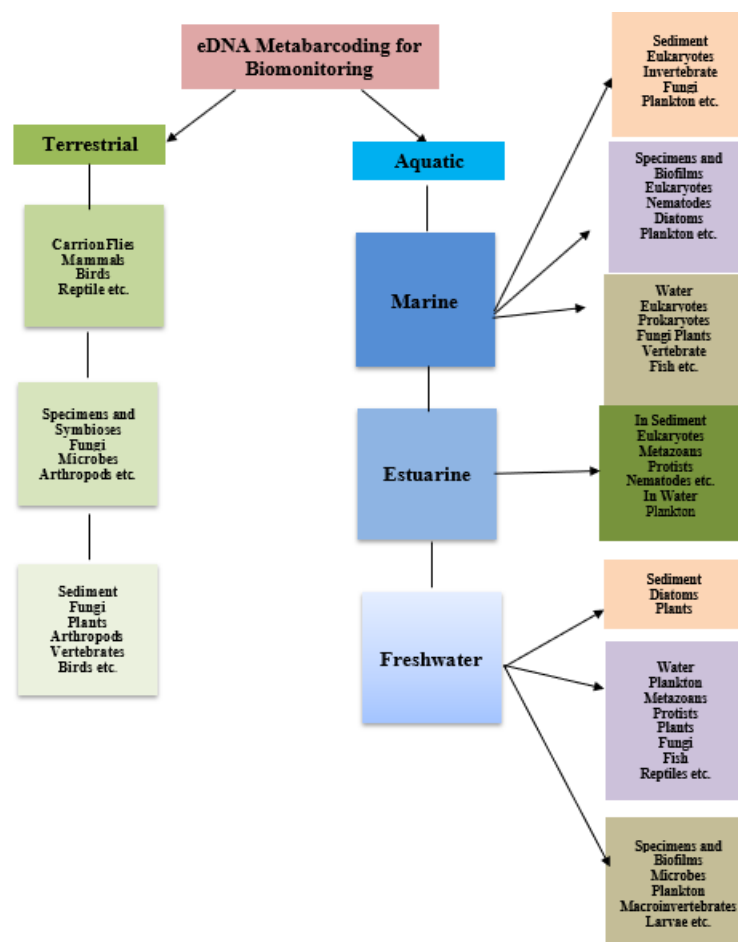
A study conducted in a freshwater lake ecosystem found that using eDNA analysis identified 25 distinct fish species, while conventional sampling methods only detected 17 species (Valentini et al., 2016). A study conducted revealed the presence of 37 different fish species in a maritime environment using environmental DNA (eDNA) compared to the 19 species detected by visual surveys (Kelly et al., 2017). Thomsen and Willerslev (2015) thoroughly reviewed environmental DNA (eDNA) investigations in different habitats. They discovered that the rate of species identification using eDNA was, on average, 32 percent greater than traditional survey methods. A total of 87 percent of the documented animal species inhabiting a tropical rainforest were accurately recognized by the use of eDNA surveys conducted in that specific area. Hassan and colleagues showed that the detection rate surpasses what can be accomplished using video trapping and acoustic monitoring.

A group researcher found that when studying aquatic invertebrates, eDNA analysis had a 92% accuracy in identifying known species, while traditional sampling methods only had a detection rate of 74% (Deiner et al., 2017). This finding was made by comparing it to the outcomes of the research. In a study, scientists performed eDNA analysis in a coral reef environment and identified 25 distinct coral species (Ardura et al., 2015). By comparison, the conventional ocular surveys only detected signs of 18 coral species. Compared to conventional monitoring of amphibian diversity, eDNA was found to have a much greater species detection rate, discovering seventy percent more species than visual encounter surveys (Thomsen et al., 2012). This was illustrated by an initial experiment that compared environmental DNA (eDNA) to traditional approaches. Bohmann and colleagues conducted a study that found that applying environmental DNA (eDNA) analysis successfully identified eighty percent of the targeted terrestrial animal species.

Conversely, conventional techniques could only detect sixty percent of the targeted species. An investigation of environmental DNA (eDNA) in a temperate climate woodland revealed the existence of 28 unique plant species. Conversely, conventional botanical research could only confirm the presence of 19 distinct species (Kartzinel et al., 2015). According to a meta-analysis of eDNA research conducted in freshwater settings, eDNA surveys discovered, on average, 1.4 times more fish species compared to traditional methods (Jerde et al., 2013). This was determined by comparing their findings to those of conventional techniques.

### 3.5 Using eDNA methodologies to identify invasive species in biodiversity

The ability to capture the "genetic signature" those organisms leave behind as a result of shedding, excreting, decomposing, and other processes is the foundation upon which eDNA-based biodiversity assessment is built (Seymour et al., 2019). Traditional techniques of measuring biodiversity, on the other hand, entail collecting or recording individuals to determine a species's presence or abundance. This is in contrast to the approaches that are currently being used. Research based on environmental DNA is therefore dependent on the capability of adequately matching the genetic signature left behind to the species relevant to the study.



**Figure 3:** Diagram of eDNA metabarcoding for global ecosystem and biodiversity monitoring

#### 4. CONCLUSION

The eDNA-based technology can significantly enhance our capacity to conduct scientific investigations and safeguard biodiversity and conservation efforts. Using environmental DNA (eDNA) in biodiversity monitoring offers a standardized, straightforward, and cost-effective approach for gathering crucial data on invasive species' abundance and spatial distribution in aquatic and subterranean ecosystems. Furthermore, it enhances the evaluation of species abundance and pest control, enhancing the distribution of limited conservation money and taxonomic understanding. An analysis of environmental DNA (eDNA) will yield valuable information for scientific investigations that seek to identify changes in biodiversity, identify areas with high species concentrations, and detect the presence of invasive species.

Moreover, research initiatives to direct conservation efforts and reveal ecological processes will significantly benefit from this unique knowledge. To maximize its effectiveness, eDNA-based biomonitoring should be used alongside well-established evaluation methodologies that have undergone significant and ongoing enhancements over a substantial duration. Using eDNA analysis is causing a fundamental change in monitoring biodiversity and implementing conservation efforts. Furthermore, it has created possibilities for prospects. While this approach shows promise for biodiversity monitoring and understanding environmental DNA (eDNA) in both land and water environments, it encounters specific problems that are distinct to these ecosystems and the usual challenges seen in other habitats. Using eDNA for monitoring should not be a substitute for the field observation techniques employed by knowledgeable taxonomic specialists and environmental scientists.

These folks can gather and safeguard data that surpasses the abilities of quantitative and qualitative observations. Therefore, it is crucial to establish established protocols and consistently demonstrate outcomes. Moreover, additional investigation is required to ascertain the ecological and physical limitations of using eDNA. Conducting a global evaluation of biodiversity using eDNA-based approaches is not feasible. The need for collaborative efforts to compare different approaches and combine established techniques like taxonomic and ecological data with emerging technologies at every implementation stage and ongoing improvements presents an additional significant challenge.

#### COMPETING INTEREST

We have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### REFERENCES

- Agerbo, R.J., Nielsen, M., Mak, S.S., Döring, J., Klincke, F., Gopalakrishnan, S., and Gilbert, M.T.P., 2021. eDNA-based biomonitoring at an experimental German vineyard to characterize how management regimes shape ecosystem diversity. *Environmental DNA*, 3 (1), Pp. 70-82.
- Aizpurua, O., Budinski, I., Georgiakakis, P., Gopalakrishnan, S., Ibañez, C., Mata, V., and Alberdi, A., 2018. Agriculture shapes the trophic niche of a bat preying on multiple pest arthropods across Europe: Evidence from DNA metabarcoding. *Molecular ecology*, 27 (3), 815-825.
- Allen, M.C., Kwait, R., Vastano, A., Kisurin, A., Zoccolo, I., Jaffe, B.D., and Lockwood, J.L., 2023. Sampling environmental DNA from trees and soil to detect cryptic arboreal mammals. *Scientific reports*, 13 (1), Pp. 180.
- Aloo, B.N., Mbega, E.R., and Makumba, B.A., 2020. Rhizobacteria-based technology for sustainable cropping of potato (*Solanum tuberosum* L.). *Potato Research*, 63, Pp. 157-177.
- Amari, K., Huang, C., and Heinlein, M., 2021. Potential impact of global warming on virus propagation in infected plants and agricultural productivity. *Frontiers in plant science*, 12, Pp. 649768.
- Andersen, K., Bird, K.L., Rasmussen, M., Haile, J., Breuning-Madsen, H.E.N.R.I.K., Kjaer, K.H., and Willerslev, E., 2012. Meta-barcoding of 'dirt' DNA from soil reflects vertebrate biodiversity. *Molecular ecology*, 21 (8), Pp. 1966-1979.
- Ardura, A., Zaiko, A., Martinez, J.L., Samuiloviene, A., Borrell, Y., and Garcia-Vazquez, E., 2015. Environmental DNA evidence of transfer of North Sea molluscs across tropical waters through ballast water. *Journal of Molluscan Studies*, 81 (4), Pp. 495-501.

- Barnes, M.A., and Turner, C.R., 2016. The ecology of environmental DNA and implications for conservation genetics. *Conservation genetics*, 17 (1), Pp. 1-17.
- Barnes, M.A., Turner, C.R., Jerde, C.L., Renshaw, M.A., Chadderton, W.L., and Lodge, D.M., 2014. Environmental conditions influence eDNA persistence in aquatic systems. *Environmental science & technology*, 48 (3), Pp. 1819-1827.
- Bittleston, L.S., Baker, C.C., Strominger, L.B., Pringle, A., and Pierce, N.E., 2016. Metabarcoding as a tool for investigating arthropod diversity in N ephenes pitcher plants. *Austral Ecology*, 41 (2), Pp. 120-132.
- Boetzel, F.A., Krauss, J., Heinze, J., Hoffmann, H., Juffa, J., König, S., Steffan-Dewenter, I., 2021. A multitaxa assessment of the effectiveness of agri-environmental schemes for biodiversity management. *Proceedings of the National Academy of Sciences*, 118 (10), Pp. e2016038118.
- Bohmann, K., Evans, A., Gilbert, M.T.P., Carvalho, G.R., Creer, S., Knapp, M., and De Bruyn, M., 2014. Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in ecology and evolution*, 29 (6), Pp. 358-367.
- Bohmann, K., Evans, A., Gilbert, M.T.P., Carvalho, G.R., Creer, S., Knapp, M., and De Bruyn, M., 2014. Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in ecology & evolution*, 29 (6), Pp. 358-367.
- Brown, J.K., and Hovmøller, M.S., 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science*, 297 (5581), Pp. 537-541.
- Carew, M.E., Pettigrove, V.J., Metzeling, L., and Hoffmann, A.A., 2013. Environmental monitoring using next generation sequencing: rapid identification of macroinvertebrate bioindicator species. *Frontiers in zoology*, 10, Pp. 1-15.
- Ceresini, P.C., Castroagudín, V.L., Rodrigues, F.Á., Rios, J.A., Aucique-Pérez, C.E., Moreira, S.I., and McDonald, B.A., 2019. Wheat blast: from its origins in South America to its emergence as a global threat. *Molecular Plant Pathology*, 20 (2), Pp. 155-172.
- Chaloner, T.M., Gurr, S.J., and Bebber, D.P., 2021. Plant pathogen infection risk tracks global crop yields under climate change. *Nature Climate Change*, 11 (8), Pp. 710-715.
- Chang, H., Guo, J., Fu, X., Liu, Y., Wyckhuys, K.A., Hou, Y., and Wu, K., 2018. Molecular-assisted pollen grain analysis reveals spatiotemporal origin of long-distance migrants of a noctuid moth. *International Journal of Molecular Sciences*, 19 (2), Pp. 567.
- Clay, K., Hardy, T.N., and Hammond, A.M., 1985. Fungal endophytes of grasses and their effects on an insect herbivore. *Oecologia*, 66, Pp. 1-5.
- Coissac, E., Riaz, T., and Puillandre, N., 2012. Bioinformatic challenges for DNA metabarcoding of plants and animals. *Molecular ecology*, 21 (8), Pp. 1834-1847.
- Creer, S., Deiner, K., Frey, S., Porazinska, D., Taberlet, P., Thomas, W.K., and Bik, H.M., 2016. The ecologist's field guide to sequence-based identification of biodiversity. *Methods in ecology and evolution*, 7 (9), Pp. 1008-1018.
- Crisol-Martínez, E., Moreno-Moyano, L.T., Wormington, K.R., Brown, P.H., and Stanley, D., 2016. Using next-generation sequencing to contrast the diet and explore pest-reduction services of sympatric bird species in macadamia orchards in Australia. *PLoS One*, 11 (3), Pp. e0150159.
- Cristescu, M.E., 2014. From barcoding single individuals to metabarcoding biological communities: towards an integrative approach to the study of global biodiversity. *Trends in ecology & evolution*, 29 (10), Pp. 566-571.
- Daniel, R., 2005. The metagenomics of soil. *Nature Reviews Microbiology*, 3 (6), Pp. 470-478.
- Danner, N., Keller, A., Härtel, S., and Steffan-Dewenter, I., 2017. Honey bee foraging ecology: Season but not landscape diversity shapes the amount and diversity of collected pollen. *PLoS one*, 12 (8), Pp. e0183716.
- Deiner, K., Bik, H.M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., and Bernatchez, L., 2017. Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular ecology*, 26 (21), Pp. 5872-5895.
- Deiner, K., Bik, H.M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., and Bernatchez, L., 2017. Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular ecology*, 26 (21), Pp. 5872-5895.
- Derocles, S.A., Evans, D.M., Nichols, P.C., Evans, S.A., and Lunt, D.H., 2015. Determining plant-leaf miner-parasitoid interactions: a DNA barcoding approach. *PloS one*, 10 (2), Pp. e0117872.
- Dessaux, Y., Grandclément, C., and Faure, D., 2016. Engineering the rhizosphere. *Trends in plant science*, 21 (3), Pp. 266-278.
- Dopheide, A., Makiola, A., Orwin, K.H., Holdaway, R.J., Wood, J.R., and Dickie, I.A., 2020. Rarity is a more reliable indicator of land-use impacts on soil invertebrate communities than other diversity metrics. *Elife*, 9, Pp. e52787.
- Dunse, K.M., Stevens, J.A., Lay, F.T., Gaspar, Y.M., Heath, R.L., and Anderson, M.A., 2010. Coexpression of potato type I and II proteinase inhibitors gives cotton plants protection against insect damage in the field. *Proceedings of the National Academy of Sciences*, 107 (34), Pp. 15011-15015.
- Edwards, D.P., Magrach, A., Woodcock, P., Ji, Y., Lim, N.T.L., Edwards, F.A., and Yu, D.W., 2014. Selective-logging and oil palm: Multitaxon impacts, biodiversity indicators, and trade-offs for conservation planning. *Ecological Applications*, 24 (8), Pp. 2029-2049.
- Eichmiller, J.J., Bajer, P.G., and Sorensen, P.W., 2014. The relationship between the distribution of common carp and their environmental DNA in a small lake. *PloS one*, 9 (11), Pp. e112611.
- Eichmiller, J.J., Best, S.E., and Sorensen, P.W., 2016. Effects of temperature and trophic state on degradation of environmental DNA in lake water. *Environmental science & technology*, 50 (4), Pp. 1859-1867.
- Evans, D.M., and Kitson, J.J., 2020. Molecular ecology as a tool for understanding pollination and other plant-insect interactions. *Current opinion in insect science*, 38, Pp. 26-33.
- Ficetola, G.F., Miaud, C., Pompanon, F., and Taberlet, P., 2008. Species detection using environmental DNA from water samples. *Biology letters*, 4 (4), Pp. 423-425.
- Flood, J., 2010. The importance of plant health to food security. *Food security*, 2 (3), Pp. 215-231.
- Frøslev, T.G., Nielsen, I.B., Santos, S.S., Barnes, C.J., Bruun, H.H., and Ejrnæs, R., 2022. The biodiversity effect of reduced tillage on soil microbiota. *Ambio*, 51 (4), Pp. 1022-1033.
- Gamage, C.D., Sato, Y., Kimura, R., Yamashiro, T., and Toma, C., 2020. Understanding leptospirosis eco-epidemiology by environmental DNA metabarcoding of irrigation water from two agro-ecological regions of Sri Lanka. *PLoS neglected tropical diseases*, 14 (7), Pp. e0008437.
- Gibson, J.F., Shokralla, S., Curry, C., Baird, D.J., Monk, W.A., King, I., and Hajibabaei, M., 2015. Large-scale biomonitoring of remote and threatened ecosystems via high-throughput sequencing. *PloS one*, 10 (10), Pp. e0138432.
- Goldberg, C.S., Turner, C.R., Deiner, K., Klymus, K.E., Thomsen, P.F., Murphy, M.A., and Taberlet, P., 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in ecology and evolution*, 7 (11), Pp. 1299-1307.
- Hamilton, J.G., Dermody, O., Aldea, M., Zangerl, A.R., Rogers, A., Berenbaum, M.R., and Delucia, E.H., 2005. Anthropogenic changes in tropospheric composition increase susceptibility of soybean to insect herbivory. *Environmental entomology*, 34 (2), Pp. 479-485.
- Hassan, S., Sabreena, Pocza, P., Ganai, B.A., Almalki, W.H., Gafur, A., and Sayyed, R.Z., 2022. Environmental DNA metabarcoding: a novel contrivance for documenting terrestrial biodiversity. *Biology*, 11 (9), Pp. 1297.
- Havis, N.D., Brown, J.K., Clemente, G., Frei, P., Jedryczka, M., Kaczmarek, J., and Hess, M., 2015. *Ramularia collo-cygni*—an emerging pathogen of barley crops. *Phytopathology*, 105 (7), Pp. 895-904.



- Hering, D., Borja, A., Jones, J.I., Pont, D., Boets, P., Bouchez, A., and Kelly, M., 2018. Implementation options for DNA-based identification into ecological status assessment under the European Water Framework Directive. *Water research*, 138, Pp. 192-205.
- Hugenholtz, P., and Pace, N.R., 1996. Identifying microbial diversity in the natural environment: a molecular phylogenetic approach. *Trends in biotechnology*, 14 (6), Pp. 190-197.
- Jerde, C.L., Chadderton, W.L., Mahon, A.R., Renshaw, M.A., Corush, J., Budny, M.L., and Lodge, D.M., 2013. Detection of Asian carp DNA as part of a Great Lakes basin-wide surveillance program. *Canadian Journal of Fisheries and Aquatic Sciences*, 70 (4), Pp. 522-526.
- Jones, R.A., 2009. Plant virus emergence and evolution: origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. *Virus research*, 141 (2), Pp. 113-130.
- Kartzinel, T.R., Chen, P.A., Coverdale, T.C., Erickson, D.L., Kress, W.J., Kuzmina, M.L., and Pringle, R.M., 2015. DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proceedings of the National Academy of Sciences*, 112 (26), Pp. 8019-8024.
- Kelly, R.P., Closek, C.J., O'Donnell, J.L., Kralj, J.E., Shelton, A.O., and Samhouri, J.F., 2017. Genetic and manual survey methods yield different and complementary views of an ecosystem. *Frontiers in Marine Science*, 3, 283.
- Kraaijeveld, K., De Weger, L.A., Ventayol García, M., Buermans, H., Frank, J., Hiemstra, P. S., and Den Dunnen, J.T., 2015. Efficient and sensitive identification and quantification of airborne pollen using next-generation DNA sequencing. *Molecular ecology resources*, 15 (1), Pp. 8-16.
- Kudoh, A., Minamoto, T., and Yamamoto, S., 2020. Detection of herbivory: eDNA detection from feeding marks on leaves. *Environmental DNA*, 2 (4), Pp. 627-634.
- Latz, M.A., Kerrn, M.H., Sørensen, H., Collinge, D.B., Jensen, B., Brown, J.K., and Jørgensen, H.J.L., 2021. Succession of the fungal endophytic microbiome of wheat is dependent on tissue-specific interactions between host genotype and environment. *Science of The Total Environment*, 759, Pp. 143804.
- Leskey, T.C., Lee, D.H., Short, B.D., and Wright, S.E., 2012. Impact of insecticides on the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae): analysis of insecticide lethality. *Journal of Economic Entomology*, 105 (5), Pp. 1726-1735.
- Lilja, M.A., Buivydyaitė, Ž., Zervas, A., Krogh, P.H., Hansen, B.W., Winding, A., and Sapkota, R., 2023. Comparing earthworm biodiversity estimated by DNA metabarcoding and morphology-based approaches. *Applied Soil Ecology*, 185, Pp. 104798.
- Littlefair, J.E., and Clare, E.L., 2016. Barcoding the food chain: from Sanger to high-throughput sequencing. *Genome*, 59 (11), Pp. 946-958.
- Macgregor, C.J., Kitson, J.J., Fox, R., Hahn, C., Lunt, D.H., Pocock, M.J., and Evans, D.M., 2019. Construction, validation, and application of nocturnal pollen transport networks in an agro-ecosystem: a comparison using light microscopy and DNA metabarcoding. *Ecological Entomology*, 44 (1), Pp. 17-29.
- Macgregor, C.J., Kitson, J.J., Fox, R., Hahn, C., Lunt, D.H., Pocock, M.J., and Evans, D.M., 2019. Construction, validation, and application of nocturnal pollen transport networks in an agro-ecosystem: a comparison using light microscopy and DNA metabarcoding. *Ecological Entomology*, 44 (1), Pp. 17-29.
- Memmott, J., Waser, N.M., and Price, M.V., 2004. Tolerance of pollination networks to species extinctions. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271 (1557), Pp. 2605-2611.
- Menta, C., and Remelli, S., 2020. Soil health and arthropods: From complex system to worthwhile investigation. *Insects*, 11 (1), Pp. 54.
- Michael, P.J., Jones, D., White, N., Hane, J.K., Bunce, M., and Gibberd, M., 2020. Crop-zone weed mycobiomes of the south-western Australian grain belt. *Frontiers in Microbiology*, 11, Pp. 581592.
- Morales, F.J., 2006. History and current distribution of begomoviruses in Latin America. *Advances in virus research*, 67, Pp. 127-162.
- Osunkoya, O.O., Lock, C.B., Dhileepan, K., and Buru, J.C., 2021. Lag times and invasion dynamics of established and emerging weeds: insights from herbarium records of Queensland, Australia. *Biological Invasions*, 23, Pp. 3383-3408.
- Pilliod, D.S., Goldberg, C.S., Arkle, R.S., and Waits, L.P., 2013. Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. *Canadian Journal of Fisheries and Aquatic Sciences*, 70 (8), Pp. 1123-1130.
- Rappé, M.S., and Giovannoni, S.J., 2003. The uncultured microbial majority. *Annual Reviews in Microbiology*, 57 (1), Pp. 369-394.
- Redondo, M.A., Berlin, A., Boberg, J., and Oliva, J., 2020. Vegetation type determines spore deposition within a forest-agricultural mosaic landscape. *FEMS Microbiology Ecology*, 96 (6), Pp. 082.
- Rouland-Lefèvre, C., 2011. Termites as pests of agriculture. *Biology of termites: a modern synthesis*, Pp. 499-517.
- Ruppert, K.M., Kline, R.J., and Rahman, M.S., 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, 17, Pp. e00547.
- Simberloff, D., Martin, J.L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J., and Vilà, M., 2013. Impacts of biological invasions: what's what and the way forward. *Trends in ecology & evolution*, 28 (1), Pp. 58-66.
- Smith, K.F., and Guégan, J.F., 2010. Changing geographic distributions of human pathogens. *Annual review of ecology, evolution, and systematics*, 41, Pp. 231-250.
- Song, Y., and Huang, Y., 2016. The application of DNA metabarcoding in the study of soil animal diversity in Taibai Mountain. *Acta Ecol. Sin*, 36 (9).
- Sowunmi, F.A., Famuyiwa, G.T., Oluyole, K.A., Aroyeun, S.O., and Obasoro, O.A., 2019. Environmental burden of fungicide application among cocoa farmers in Ondo state, Nigeria. *Scientific African*, 6, Pp. e00207.
- Sternhagen, E.C., Black, K.L., Hartmann, E.D., Shivega, W.G., Johnson, P.G., McGlynn, R.D., and Aldrich-Wolfe, L., 2020. Contrasting patterns of functional diversity in coffee root fungal communities associated with organic and conventionally managed fields. *Applied and environmental microbiology*, 86 (11), Pp. e00052-20.
- Symondson, W.O.C., 2002. Molecular identification of prey in predator diets. *Molecular ecology*, 11 (4), Pp. 627-641.
- Taberlet, P., Coissac, E., Hajibabaei, M., and Rieseberg, L.H., 2012. Environmental DNA. *Molecular ecology*, 21 (8).
- Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., and Kawabata, Z.I., 2012. Estimation of fish biomass using environmental DNA. *PloS one*, 7 (4), Pp. e35868.
- Thomsen, P.F., and Willerslev, E., 2015. Environmental DNA—An emerging tool in conservation for monitoring past and present biodiversity. *Biological conservation*, 183, Pp. 4-18.
- Thomsen, P.F., Kielgast, J., Iversen, L.L., Møller, P.R., Rasmussen, M., and Willerslev, E., 2012. Detection of a diverse marine fish fauna using environmental DNA from seawater samples.
- Thomsen, P.F., Kielgast, J.O.S., Iversen, L.L., Wiuf, C., Rasmussen, M., Gilbert, M.T.P., and Willerslev, E., 2012. Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular ecology*, 21 (11), Pp. 2565-2573.
- Todd, J.H., Simpson, R.M., Poulton, J., Barraclough, E.I., Villsen, K., Brooks, A., and Jones, D., 2020. Detecting invertebrate ecosystem service providers in orchards: traditional methods versus barcoding of environmental DNA in soil. *Agricultural and Forest Entomology*, 22 (3), Pp. 212-223.
- Tordoni, E., Ametrano, C.G., Banchi, E., Ongaro, S., Pallavicini, A., Bacaro, G., and Muggia, L., 2021. Integrated eDNA metabarcoding and morphological analyses assess spatio-temporal patterns of airborne fungal spores. *Ecological Indicators*, 121, Pp. 107032.

- Utzeri, V.J., Schiavo, G., Ribani, A., Tinarelli, S., Bertolini, F., Bovo, S., and Fontanesi, L., 2018. Entomological signatures in honey: An environmental DNA metabarcoding approach can disclose information on plant-sucking insects in agricultural and forest landscapes. *Scientific reports*, 8 (1), Pp. 9996.
- Valentin, R.E., Fonseca, D.M., Nielsen, A.L., Leskey, T.C., and Lockwood, J.L., 2018. Early detection of invasive exotic insect infestations using eDNA from crop surfaces. *Frontiers in Ecology and the Environment*, 16 (5), Pp. 265-270.
- Valentin, R.E., Kyle, K.E., Allen, M.C., Welbourne, D.J., and Lockwood, J.L., 2021. The state, transport, and fate of aboveground terrestrial arthropod eDNA. *Environmental DNA*, 3 (6), Pp. 1081-1092.
- Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P.F., and Dejean, T., 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular ecology*, 25 (4), Pp. 929-942.
- van Elsas, J.D., Garbeva, P., and Salles, J., 2002. Effects of agronomical measures on the microbial diversity of soils as related to the suppression of soil-borne plant pathogens. *Biodegradation*, 13 (1), Pp. 29-40.
- Vu, Q., Ramal, A.F., Villegas, J.M., Jamorain, A., Bernal, C.C., Pasang, J.M., Horgan, F.G., 2018. Enhancing the parasitism of insect herbivores through diversification of habitat in Philippine rice fields. *Paddy and water environment*, 16, Pp. 379-390.
- Willcox, B.K., Howlett, B.G., Robson, A.J., Cutting, B., Evans, L., Jesson, L., and Rader, R., 2019. Evaluating the taxa that provide shared pollination services across multiple crops and regions. *Scientific reports*, 9 (1), 13538.
- Wintermantel, W.M., and Hladky, L.L., 2010. Methods for detection and differentiation of existing and new crinivirus species through multiplex and degenerate primer RT-PCR. *Journal of Virological Methods*, 170 (1-2), Pp. 106-114.
- Young, R.G., Milián-García, Y., Yu, J., Bullas-Appleton, E., and Hanner, R.H., 2021. Biosurveillance for invasive insect pest species using an environmental DNA metabarcoding approach and a high salt trap collection fluid. *Ecology and Evolution*, 11 (4), Pp. 1558-1569.
- Yue, S., Munir, I.U., Hyder, S., Nassani, A.A., Abro, M.M.Q., and Zaman, K., 2020. Sustainable food production, forest biodiversity and mineral pricing: Interconnected global issues. *Resources Policy*, 65, Pp. 101583.
- Zenker, M.M., Specht, A., and Fonseca, V.G., 2020. Assessing insect biodiversity with automatic light traps in Brazil: Pearls and pitfalls of metabarcoding samples in preservative ethanol. *Ecology and Evolution*, 10 (5), 2352-2366.
- Zhou, X., Wang, J. T., Zhang, Z. F., Li, W., Chen, W., and Cai, L., 2020. Microbiota in the rhizosphere and seed of rice from China, with reference to their transmission and biogeography. *Frontiers in Microbiology*, 11, Pp. 529571.

