

Doi: 10.46793/MAK2026.029U

POSSIBILITIES OF USING eDNA TECHNIQUES FOR DETECTING WEEDS AND OTHER PLANTS FROM THE SOIL SEED BANK

Ahmet Uludağ^{1*}, Muhamad Shakirin Mispan², Sze-Looi Song³

¹Çanakkale Onsekiz Mart University, Faculty of Agriculture, Plant Protection Department, Çanakkale, Türkiye,

²Institute of Biological Sciences, Faculty of Science, Universiti Malaya, Kuala Lumpur, Malaysia,

³Institute for Advanced Studies, Universiti Malaya, Kuala Lumpur, Malaysia,

***Corresponding author: ahuludag@yahoo.com**

Abstract: Environmental DNA (eDNA) is short DNA fragments left behind by organisms into the nonliving elements of environment and have become a foremost tool of life and environmental sciences to develop models for future, detect current level of ecosystems, get info from past situations since 1980's. The early use of eDNA to detect microorganisms in water, sediment and soil followed by macroorganisms detection in seas and freshwater in the beginning of the 21st century. Today it is possible to detect non-living pollutants in various environments. eDNA barcoding and metabarcoding has opened new horizons. Combining traditional methods or newest ones such as environmental RNA and using apps on site, modelling approach, and new generation sequencing and bioinformatics enabled getting more precise results. eDNA has been used in plant sciences and agriculture but its use in weed science is not common. Introducing eDNA with other related innovations into weed science and invasive alien plants strategies to detect alien introductions and current soil seed bank can make ecological approach more applicable.

Keywords: Weeds, eDNA, Soil seed bank

INTRODUCTION

Humanity faces unprecedented challenges in protecting the environment, sustaining ecosystems, and preserving life amid climate change, biodiversity loss, population growth, and resource exploitation issues largely stemming from the industrialization era. Agriculture both contributes to and suffers from these problems: it must produce sufficient food and resources at affordable costs while ensuring safety for producers and consumers. Consequently, agronomists and farmers should prioritize reducing synthetic agrochemicals, fossil fuel use, soil disturbance, and irrigation, alongside conserving biodiversity. However, weed control has become increasingly reliant on chemicals.

Frequent use of herbicides, especially in developed countries from mid-1990's led to side effects on the environment and non-target organisms, followed by herbicide resistance issues toward 20th century's end. This shift prompted a focus on ecological approaches and integrated weed management (Forcella et al., 1993; Jones and Medd, 2000). Understanding weed seed banks, including seed longevity, has been central to ecological research mainly on annual weeds (Uremiş et al., 2003; Schwartz-Lazaro and Copes, 2019; Šikuljak et al.,

2024; Asav et al., 2025). Seed bank studies extend beyond agricultural weeds to forest weeds, pasture weeds, invasive alien plants, wildlife relationships, and habitat dynamics (Mahé et al., 2021; Warriar and Kunhikannan, 2022; Fabšičová et al., 2024; Kushbokov et al., 2025). Extending seed bank analysis to natural and semi-natural areas reveals methodological challenges, such as time consumption and the need for deep botanical knowledge, necessitating novel methods like environmental DNA (eDNA) (Mahé et al., 2021; Kestel et al., 2022). This review summarizes eDNA applications for detecting weeds and plants across environments and highlights recent advances beyond eDNA.

Brief History of eDNA

eDNA refers to short DNA fragments left behind by organisms into the nonliving elements of environment detectable in soil, air, freshwater, seawater, mud, silt, ice, feces, and permafrost. It comprises DNA from diverse organisms in a given area. However, DNA extracted from isolated microorganisms from environment does not qualify as eDNA (Bohmann et al., 2014).

Although the term "environmental DNA" was first used by Lakay et al. (2007), nucleic acid studies in organic soils date back to 1913 (Ogram, 1988; Banerjee et al., 2022). Early research focused on microbial DNA extraction methods (Catlin, 1956; Torsvik, 1980; Ogram et al., 1987). Detecting *Escherichia coli* in water samples marked as one of the early eDNA applications followed by soil and estuarine studies (Echeverria et al., 1982; Nannipieri et al., 1986; Paul et al., 1988). In the early 1990s, eDNA identified microbes in marine sediments and phytoplankton in saline water, often termed "DNA particles" (Rishan et al., 2023).

DNA-DNA colony hybridization advanced quantification of anabolic and catabolic plasmids, and monitoring of recombinant DNA in environments (Sayler et al., 1985). Detection of aquatic invasive species by using eDNA by Ficetola et al. (2008) was a pioneering work for macroorganism detection (Banerjee et al., 2022). So far, eDNA has been used in many different environments from sea to air, from soil to freshwater to detect macroorganisms for varying aims from species detection to trophic relations, to find out current situation to get information about past, which can be seen in several reviews (Pedersen et al., 2015; Banerjee et al., 2022; Rishan et al., 2023). Species-specific eDNA assays have detected invasive aquatic plants like *Limnobium laevigatum* (Zhu et al., 2024).

eDNA applications include wildlife forensics, low-density population detection, invasive species monitoring, biodiversity assessment, population dynamics, community structures, ecosystem health, trophic interactions, and historical distributions (Willerslev et al., 2003; Diaz-Ferguson and Moyer, 2014). Beyond these environmental uses of eDNA, it has been used in agriculture sector because agriculture has very large diversity at microorganism and macroorganism levels which many mutualistic and antagonistic species are interconnected with the cultivation of crops and livestock (Kestel et al., 2022). eDNA can be used to identify pests (insects, pathogens other pests), to get information on ecosystem health (soil, microbes and pollinator diversity), and to decide management practices such as fertilizer and pesticide applications (Kestel et al., 2022).

Despite studies on crop pests and diseases, weed research (including environmental weeds) remains limited. Plant eDNA from mud, soil, or sediment approximates seed bank studies, though samples may include remains beyond seeds, organic matter, or extracellular DNA bound to particles (Foucher et al., 2020). Crop DNA persisted up to eight years post-cultivation, peaking in the first three (Yoccoz et al., 2012; Foucher et al., 2020). Grapevine eDNA lingered 65 years post-cultivation, which shows woody plant parts stay longer. Some species (e.g., mustard, rape) are indistinguishable, while dominant weeds like bindweed, knotweed, and ryegrass were prevalent (Foucher et al., 2020). These findings suggest eDNA's potential for weed science: early warning, decision support, herbicide resistance detection so on.

Pros and Cons of eDNA

eDNA's power is evident across life and environmental sciences, yet it is underutilized in agriculture, especially weed science possibly due to protocol gaps or methodological issues. For instance, post-*Cabomba* eradication, eDNA could not distinguish viable from dead plants, indicating persistence years after death (Collins et al., 2022).

Strengths (adapted from Matsushashi, 2016; Kestel et al., 2022; Vasar et al., 2023; Rishan et al., 2024; Martins et al., 2025):

- Detects specific organisms and assemblages in substrates from soil to air.
- Monitors beneficial/harmful organisms in food systems.
- *In silico, in vitro, in vivo* approaches mitigate limitations.
- Complements traditional methods for improved agricultural monitoring.
- Metabarcoding is simple, cost-effective, sensitive, scalable, avoiding microscopy.
- Minimally destructive (small environmental samples).

Weaknesses (from Banerjee et al., 2022, 2024; Hernández Martínez de la Riva et al., 2025):

- Poor understanding of eDNA ecology/interactions.
- Degradation in environment and false positives/negatives.
- Quantification challenges.
- Lack of standardized protocols (especially for plants), practitioner adoption.
- Need for reference databases, group-specific primers.
- Bioinformatics pipeline improvements.
- High-throughput instrument availability.

Beyond eDNA

DNA barcoding/metabarcoding uses degraded environmental DNA for high-throughput taxa identification via short fragments, enabling large-scale biodiversity studies (Yoccoz et al., 2012; Espinosa Prieto et al., 2023). Universal markers (chloroplast *rbcL*, *trnH-psbA*, *matK*; nuclear ITS etc.) standardize approaches and ITS within the nucleus led to universal barcoding system, which made use of DNA barcoding and metabarcoding (Kress, 2017). Sequencing the chloroplast *trnL* P6 loop from 325 global soil samples advanced vegetation metabarcoding (Vasar et al., 2023). Consistent markers/primers will build libraries for comparable results and ITS within the nucleus led to universal barcoding system, which made use of DNA barcoding and metabarcoding (Espinosa Prieto et al., 2024, 2025).

Not only improving barcoding and metabarcoding methods, but also some other studies have been conducted to use eDNA efficiently. For instance, different PCR methods have been developed to provide rapid precise on-site eDNA detection for fish and suggested to be used in varying taxa and environments including terrestrial ones (Doi et al., 2021; Wei et al., 2024). As mentioned above, eDNA has some drawbacks compared to classical methods as well as viceversa is true as it has been mentioned in the literature related to plant biomonitoring (Banerjee et al., 2022). Coupling eDNA use with classical monitoring methods improved mostly plant biomonitoring as well as other taxa (Johnson et al., 2021; Pont et al., 2022; Martins et al., 2025).

Environmental RNA (eRNA) has gained attraction, building on early microbial RNA work that had focused on microbial RNA and use of RNA in evolution studies (Nannipieri et al., 1986; Olsen et al., 1986; Kagzi et al., 2023). eRNA outperforms eDNA for pollution/ecotoxicology (e.g., microplastics, metals) studies (Greco et al., 2022; Giroux et al., 2023). eRNA suits vertebrates; eDNA, invertebrates although the research question might determine methods selection (Macher et al., 2024). In ponds, eDNA detected higher biodiversity (plants, algae, fungi) than eRNA (Janik-Superson et al., 2025), though results vary (Miyata et al., 2022). Plant eRNA lags other taxa. eRNA degrades faster, originates from living organisms (unlike eDNA from live/dead) (Deiner et al., 2021; Ahi and Schenekar, 2025), but it can be better to detect the recent elements of soil seed bank.

Concluding Remarks

Seed bank analysis is vital for weed management and modeling, but traditional methods are labor-intensive. eDNA offers new avenues for seed bank estimation and weed management. Its use in life/environmental sciences has grown since the 1980s, though bryophytes, pteridophytes, and gymnosperms need more studies (Banerjee et al., 2022). Applications now include pollution assessment, especially estimation of nonliving things. In addition, eDNA techniques have been used in plant science and crop production but not much studies related to weed science and invasive alien plants.

Integrating eDNA with traditional methods and/or eRNA methods improves accuracy. Regional biases (e.g., understudied tropics) must be addressed (Banerjee et al., 2022). Technique/gadget advances are needed (Vasar et al., 2023). Metabarcoding and bioinformatic strategies require focus (Abdelfattah et al., 2018).

Future priorities: global plant eDNA libraries, genomic sequencing, hybrid techniques, big data. Complementary technologies (sequencing, apps, modeling) enable precision, automation, scalability (Clement et al., 2025; Javed et al., 2025).

REFERENCES

- Abdelfattah, A., Malacrinò, A., Wisniewski, M., Cacciola, S.O., Schena, L. (2018). Metabarcoding: A powerful tool to investigate microbial communities and shape future plant protection strategies. *Biological Control*, 120, 1-10.
- Ahi, E.P., Schenekar, T. (2025). The promise of environmental RNA research beyond mRNA. *Molecular Ecology*, 34(12), e17787.
- Asav, Ü., Serim, A.T., Kaya, Y., Başaran, B. (2025). Herbicide Strategies for Weed Control and Soil Seed Bank Dynamics in Winter Wheat. *Journal of Crop Health*, 77(4), 129.

- Banerjee, P., Stewart, K.A., Dey, G., Antognazza, C.M., Sharma, R.K., Maity, J.P. *et al.* (2022). Environmental DNA analysis as an emerging non-destructive method for plant biodiversity monitoring: a review. *AoB Plants*, 14(4), plac031.
- Catlin, B.W. (1956). Extracellular deoxyribonucleic acid of bacteria and a deoxyribonuclease inhibitor. *Science*, 124(3219), 441-442.
- Clement, R.A., Lee, H., Manoukis, N.C., Pacheco, Y.M., Ross, F., Sisterson, M.S., Owen, C.L. (2025). Addressing Biological Invasions in Agriculture with Big Data in an Informatics Age. *Agriculture*, 15(11), 1157.
- Collins, C., Price, T., Dugdale, T. (2022). *Cabomba caroliniana* eradication-integrated weed control success in the NT.
- Deiner, K., Yamanaka, H., Bernatchez, L. (2021). The future of biodiversity monitoring and conservation utilizing environmental DNA. *Environmental DNA*, 3(1), 3-7.
- Díaz-Ferguson, E.E., Moyer, G.R. (2014). History, applications, methodological issues and perspectives for the use environmental DNA (eDNA) in marine and freshwater environments. *Revista de biología tropical*, 62(4), 1273-1284.
- Doi, H., Watanabe, T., Nishizawa, N., Saito, T., Nagata, H., Kameda, Y. *et al.* (2021). On-site environmental DNA detection of species using ultrarapid mobile PCR. *Molecular Ecology Resources*, 21(7), 2364-2368.
- Echeverria, P., Seriwatana, J., Chityothin, O., Chaicumpa, W., Tirapat, C. (1982). Detection of enterotoxigenic *Escherichia coli* in water by filter hybridization with three enterotoxin gene probes. *Journal of Clinical Microbiology*, 16(6), 1086-1090.
- Espinosa Prieto, A.E., Hardion, L., Debortoli, N., Bournonville, T., Marescaux, J., van der Zon, K.A.E., Beisel, J.N. (2025). Environmental DNA metabarcoding for catchment-scale detection of aquatic plants, invasive species, and land-use indicators in a large river. *Ecological Indicators*, 178, 113943.
- Espinosa Prieto, A., Beisel, J.N., Verschuren, P., Hardion, L. (2023). Toward freshwater plant diversity surveys with eDNA barcoding and metabarcoding. *Environmental DNA*, 5(4), 648-670.
- Espinosa Prieto, A., Hardion, L., Debortoli, N., Beisel, J. N. (2024). Finding the perfect pairs: A matchmaking of plant markers and primers for multi-marker eDNA metabarcoding. *Molecular ecology resources*, 24(4), e13937.
- Fabšičová, M., Vymyslický, T., Frei, I., Zdražilková, M., Smetanová, S., Winkler, J., Jiroušek, M. (2024). The importance of soil seed banks for biodiversity restoration in degraded grasslands. *Folia Geobotanica*, 59(1), 17-37.
- Forcella, F., Eradat-Oskoui, K., Wagner, S.W. (1993). Application of weed seedbank ecology to low-input crop management. *Ecological Applications*, 3(1), 74-83.
- Foucher, A., Evrard, O., Ficetola, G.F., Gielly, L., Poulain, J., Giguet-Covex, C. *et al.* (2020). Persistence of environmental DNA in cultivated soils: implication of this memory effect for reconstructing the dynamics of land use and cover changes. *Scientific Reports*, 10(1), 10502.
- Giroux, M.S., Reichman, J.R., Langknecht, T., Burgess, R.M., Ho, K.T. (2023). Using eRNA/eDNA metabarcoding to detect community-level impacts of nanoplastic exposure to benthic estuarine ecosystems. *Environmental Pollution*, 338, 122650.
- Greco, M., Lejzerowicz, F., Reo, E., Caruso, A., Maccotta, A., Coccioni, R. *et al.* (2022). Environmental RNA outperforms eDNA metabarcoding in assessing impact of marine pollution: A chromium-spiked mesocosm test. *Chemosphere*, 298, 134239.
- Hernández Martínez de la Riva, A., Rytwinski, T., Spetka, M., Bennett, J.R. (2025). An evidence map and guide for using community science, remote sensing, and environmental DNA for rare plant detection. *Conservation Science and Practice*, 7(10), e70156.
- Janik-Superson, K., Krawczyk, D., Baranowska, M., Królikowska, K., Seweryn, M., Lach, J. *et al.* (2025). Comparing eDNA and eRNA Sampling Methodologies From Pond Environments. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 35(2), e70083.

- Javed, Q., Bouhadi, M., Ban, S.G., Ban, D., Heath, D., Iqbal, B. *et al.* (2025). Smart Chip Technology for the Control and Management of Invasive Plant Species: A Review. *Plants*, 14(10), 1510.
- Johnson, M.D., Fokar, M., Cox, R.D., Barnes, M.A. (2021). Airborne environmental DNA metabarcoding detects more diversity, with less sampling effort, than a traditional plant community survey. *BMC Ecology and Evolution*, 21(1), 218.
- Jones, R.E., Medd, R.W. (2000). Economic thresholds and the case for longer term approaches to population management of weeds. *Weed Technology*, 14(2), 337-350.
- Kagzi, K., Millette, K.L., Littlefair, J.E., Pochon, X., Wood, S.A., Fussmann, G.F., Cristescu, M.E. (2023). Assessing the degradation of environmental DNA and RNA based on genomic origin in a metabarcoding context. *Environmental DNA*, 5(5), 1016-1031.
- Kestel, J.H., Field, D.L., Bateman, P.W., White, N.E., Allentoft, M.E., Hopkins, A.J. *et al.* (2022). Applications of environmental DNA (eDNA) in agricultural systems: Current uses, limitations and future prospects. *Science of the Total Environment*, 847, 157556.
- Kushbokov, A., Deák, B., Valkó, O. (2025). Characteristics of soil seed bank in global drylands - A review. *Arid Land Research and Management*, 1-22.
- Lakay, F.M., Botha, A., Prior, B.A. (2007). Comparative analysis of environmental DNA extraction and purification methods from different humic acid-rich soils. *Journal of applied microbiology*, 102(1), 265-273.
- Longhi, S., Cristofori, A., Gatto, P., Cristofolini, F., Grando, M.S. *et al.* (2009). Biomolecular identification of allergenic pollen: a new perspective for aerobiological monitoring? *Annals of allergy, asthma and immunology*, 103(6), 508-514.
- Macher, T.H., Arle, J., Beermann, A.J., Frank, L., Hupało, K., Koschorreck, J. *et al.* (2024). Is it worth the extra mile? Comparing environmental DNA and RNA metabarcoding for vertebrate and invertebrate biodiversity surveys in a lowland stream. *PeerJ*, 12, e18016.
- Mahé, I., Cordeau, S., Bohan, D.A., Derrouch, D., Dessaint, F., Millot, D., Chauvel, B. (2021). Soil seedbank: Old methods for new challenges in agroecology?. *Annals of Applied Biology*, 178(1), 23-38.
- Martins, V.C., Nunes, G.L., Oliveira, R.R., Gastauer, M., Oliveira, G., Vasconcelos, S. (2025). DNA Metabarcoding as a Complementary Approach to Traditional Surveys for Monitoring the Plant Diversity in the Amazon canga. *Environmental DNA*, 7(4), e70155.
- Matsushashi, S., Doi, H., Fujiwara, A., Watanabe, S., Minamoto, T. (2016). Evaluation of the environmental DNA method for estimating distribution and biomass of submerged aquatic plants. *PLoS One*, 11(6), e0156217.
- Miyata, K., Inoue, Y., Amano, Y., Nishioka, T., Nagaike, T., Kawaguchi, T. *et al.* (2022). Comparative environmental RNA and DNA metabarcoding analysis of river algae and arthropods for ecological surveys and water quality assessment. *Scientific reports*, 12(1), 19828.
- Nannipieri, P., Ciardi, C., Badalucco, L., Casella, S. (1986). A method to determine soil DNA and RNA. *Soil Biology and Biochemistry*, 18(3), 275-281.
- Ogram, A., Saylor, G.S., Barkay, T. (1987). The extraction and purification of microbial DNA from sediments. *Journal of microbiological methods*, 7(2-3), 57-66.
- Ogram, A.V. (1988). The extraction and purification of microbial DNA from sediments. PhD diss., University of Tennessee.
- Olsen, G.J., Lane, D.J., Giovanoni, S.J., Pace, N.R., Stahl, D.A. (1986). Molecular ecology and evolution: a ribosomal RNA approach. *Annu. Rev. Microbiol.*, 40, 337-355.
- Paul, J.H., DeFlaun, M.F., Jeffrey, W.H. (1988). Mechanisms of DNA utilization by estuarine microbial populations. *Applied and environmental microbiology*, 54(7), 1682-1688.
- Pedersen, M.W., Overballe-Petersen, S., Ermini, L., Sarkissian, C.D., Haile, J., Hellstrom, M. *et al.* (2015). Ancient and modern environmental DNA. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370(1660), 20130383.
- Pont, D., Meulenbroek, P., Bammer, V., Dejean, T., Erős, T., Jean, P. *et al.* (2023). Quantitative monitoring of diverse fish communities on a large scale combining eDNA metabarcoding and qPCR. *Molecular Ecology Resources*, 23(2), 396-409.

- Rishan, S.T., Kline, R.J., Rahman, M.S. (2023). Applications of environmental DNA (eDNA) to detect subterranean and aquatic invasive species: a critical review on the challenges and limitations of eDNA metabarcoding. *Environmental Advances*, 12, 100370.
- Rishan, S.T., Kline, R.J., Rahman, M.S. (2024). Exploitation of environmental DNA (eDNA) for ecotoxicological research: A critical review on eDNA metabarcoding in assessing marine pollution. *Chemosphere*, 141238.
- Sayler, G. S., Shields, M. S., Tedford, E. T., Breen, A., Hooper, S. W., Sirotkin, K., & Davis, J. W. (1985). Application of DNA-DNA colony hybridization to the detection of catabolic genotypes in environmental samples. *Applied and Environmental Microbiology*, 49(5), 1295-1303.
- Schwartz-Lazaro, L.M., Copes, J.T. (2019). A review of the soil seedbank from a weed scientists perspective. *Agronomy*, 9(7), 369.
- Šikuljak, D., Uludag, A., Anđelković, A., Trkulja, N., Božić, D., Vrbničani, S. (2024). Evaluation of the viability of old seeds of several important agricultural weeds. *Pesticides and Phytomedicine*, 39(1), 13-26.
- Torsvik, V.L. (1980). Isolation of bacterial DNA from soil. *Soil Biology and Biochemistry*, 12(1), 15-21.
- Uremis, I., Uludag, A., Aksoy, E.O., Gonen, O., Kadioglu, I. (2003). Relations between seedbank and weed flora in cotton areas. *Aspects of Applied Biology*, 69, 113-118.
- Vasar, M., Davison, J., Moora, M., Sepp, S.K., Anslan, S., Al-Quraishy, S. *et al.* (2023). Metabarcoding of soil environmental DNA to estimate plant diversity globally. *Frontiers in Plant Science*, 14, 1106617.
- Warrier, R.R., Kunhikannan, C. (2022). Significance of soil seed bank in forest vegetation - a review. *Seeds*, 1(3), 181-197.
- Wei, Z., Zhang, X., Chen, Y., Liu, H., Wang, S., Zhang, M. *et al.* (2024). A new strategy based on a cascade amplification strategy biosensor for on-site eDNA detection and outbreak warning of crown-of-thorns starfish. *Science of The Total Environment*, 927, 172258.
- Willerslev, E., Hansen, A.J., Binladen, J., Brand, T.B., Gilbert, M.T.P., Shapiro, B. *et al.* (2003). Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science*, 300(5620), 791-795.
- Yoccoz, N.G., Bråthen, K.A., Gielly, L., Haile, J., Edwards, M.E., Goslar, T. *et al.* (2012). DNA from soil mirrors plant taxonomic and growth form diversity. *Molecular ecology*, 21(15), 3647-3655.
- Zhu, X., Bell, K.L., Wu, H., Gopurenko, D. (2024). Development of an Environmental DNA Assay for Prohibited Matter Weed Amazon Frogbit (*Limnobium laevigatum*). *Environments*, 11(4), 66.