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Original Research Paper

Environmental DNA (eDNA) Approaches for Large-Scale Urban Biodiversity Assessment and Species Surveillance

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Abstract— The rapid urbanization is driving biodiversity decline, but traditional ecological surveys in the field are extremely labor intensive, time consuming, spatially constrained, and unable to reveal cryptic and rare species. This research tackles these challenges by offering a framework for the environmental DNA (eDNA) based assessment and monitoring of biodiversity at a large scale in urban settings. The main goal is to develop an integrated monitoring approach that will allow for the identification of species with high accuracy using eDNA metabarcoding, the mapping of biodiversity and ecological hotspots using high throughput sequencing, geographic information systems (GIS) and machine learning in different urban habitats. Samples from various urban ecosystems are taken, DNA is extracted from water, soil and sediment, the sequences are filtered for quality, taxonomically assigned, features are engineered and species classified using Random Forest. Experimental evaluation shows that an overall species identification accuracy of 97.14%, precision of 96.72%, recall of 96.38%, F1-score of 96.55% and ROC-AUC of 98.41% can be obtained, and that biodiversity hotspot mapping can map 95.83% of the area in agreement with the validated ecological observations. The proposed framework also provides a 18.6 % increase in the detection of rare species, and 42.8 % reduction in monitoring time compared to using conventional species survey methods. The novelty of this research is the combination of eDNA metabarcoding and artificial intelligence and spatial analysis for scalable and non-invasive urban biodiversity surveillance. The proposed framework brings a cost-effective, efficient and accurate decision support system for biodiversity conservation, ecological planning, monitoring invasive species and sustainable management of urban ecosystems.

Keywords— Environmental DNA (eDNA); Urban Biodiversity; Species Metabarcoding; Machine Learning; Geographic Information System (GIS); Ecological Surveillance

I. Introduction

Global biodiversity loss is one of the most important drivers caused by urbanization, with impacts on the landscape structure, fragmentation of ecosystems and loss of native species richness. With the urban sprawl, aquatic and terrestrial habitats within the city limit like rivers, wetlands, ponds and green parks are turning into important but vulnerable

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habitats for flora and fauna [1]. To understand how urban expansion influences species distribution, biodiversity assessment tools are needed that can do both – fine spatial resolution AND large geographic scale. Traditional monitoring methods, however, have been based on direct in-field observation, netting, trapping and visual census, which are limited by the expertise of the person conducting the survey, seasonality and the difficulty of finding the elusive or low abundance species [2].

The research question this study will address is the lack of effectiveness of traditional survey methods with broad, diverse, and dynamic urban systems. Because conventional methods often underestimate cryptic, nocturnal and/or rare taxa, and are labour intensive, continuous monitoring over the whole city is not cost effective. The genetic material shed by organisms into water, soil and sediment is referred to as environmental DNA (eDNA), which can be used as a non-invasive method to determine species presence without having to physically contact the organism, and has the potential to address many of the spatial and taxonomic limitations of manual surveys [4]. However, although eDNA is increasingly used, the majority of eDNA studies have focused on detecting a single species or on a small scale watershed eDNA survey, there is a lack of scalable, multi-habitat city-wide eDNA surveillance frameworks that combine molecular, spatial and computational analytics in a single platform [5]. The main aim of this study is to develop and test an integrated eDNA-based tool that combines metabarcoding, high throughput sequencing, geographic information system (GIS) and machine learning (ML) to facilitate reliable, scalable, and automated urban biodiversity assessment. In particular, the study is designed to increase accuracy in species identification, increase sensitivity of detecting rare species, create maps of the biodiversity hotspots and greatly decrease the time and expense of traditional ecological surveillance [6]. The framework will be designed to serve as a decision-making aid for urban planners, conservation and environmental regulators who are looking to create an evidence based approach to ecosystem management [7].

This paper contributes mainly to the work of urban ecological monitoring in three ways, summarised below.

1) Completes a fully automated, scalable and comprehensive urban biodiversity assessment pipeline, combining eDNA metabarcoding, GIS spatial analytics and Random Forest classification.

2) Has high species identification accuracy with significant improvement in rare and cryptic species detection in a variety of urban habitat types.

3) Provides an effective, fast, and cost-effective decision support for mapping and planning biodiversity hotspots that is non-invasive.

II. Literature Review

The potential of environmental DNA to revolutionize biodiversity monitoring has been realized as it is able to detect several taxa with a single environmental sample, without the need for physical capture. Genetic data obtained from water and soil have been shown to accurately reflect the composition of the vertebrate community at the landscape scale by eDNA metabarcoding studies carried out in aquatic and terrestrial urban corridors, which are often more accurate than traditional surveys [8]. Ecosystem health monitoring, restricted access in protected areas, and manual verification are just some of the other applications that have been demonstrated with eDNA that are gaining in importance, which is reflected in a systematic review of the use of eDNA [9]. The use of complementary bioinformatic pipelines, such as sequence denoising, chimera removal and matching to reference database, has enhanced taxonomic resolution, and minimized the number of false-positive detections in complex environmental samples [10]. In addition to aquatic systems, eDNA-based sensu stricto methods have recently gained in credibility as a standardized tool of conservation monitoring that can help with long term ecological trend analysis [11].

Recent years have seen more and more cases of using AI to complement eDNA workflows to process millions of sequencing reads and to enhance classification in the face of noisy, degraded DNA in the environment. Bioinformatics tools that are based on machine learning have been proven to enhance taxonomic assignment and increase detection limits for low copy number or degraded DNA found in urban water and soil samples [12]. Species-specific eDNA quantification studies, such as those involving spatial distribution modelling coupled with inference at the population-level, demonstrate the potential for using AI-driven analytics to improve the estimates of eDNA abundance and habitat preference that go beyond the presence-absence analysis [13]. More recent advances in the field of aquatic eDNA focus on moving from the validation of the technology to the generation of ecological insights, highlighting the need for the integration of molecular information with predictive analytics to generate conservation intelligence from eDNA [14].

However, there is a clear research gap: most of the studies currently available document eDNA applications in single habitats and/or single species, with only a few studies involving the integration of spatial mapping and predictive machine learning for

the city-scale biodiversity monitoring of multiple habitats. There are few frameworks that integrate metabarcoding, GIS-based hotspot mapping, and automated classification for a single pipeline that can be easily implemented in an automated, non-invasive, and efficient way for continuous urban ecosystem management. This study tackles that issue head-on.

Table 1: Related Work on eDNA-Based Biodiversity Assessment

Ref.	Domain	Method Used	Dataset/Region	Key Metric	Limitation
[1]	Urban aquatic/terrestrial	eDNA metabarcoding	Urban landscape sites	Species richness detection	Limited spatial mapping integration
[2]	General ecosystem review	eDNA review synthesis	Multi-region literature	Detection application scope	Lacks empirical AI validation
[3]	Ecosystem health	eDNA + health indicators	Multiple ecosystems	Ecosystem health index	No large-scale automation
[4]	Tropical hotspots	eDNA metabarcoding	Tropical biodiversity zones	Species rediscovery rate	Region-specific, low scalability
[5]	Conservation review	eDNA advances review	Global literature	Conservation applicability	Conceptual, no ML pipeline
[6]	Fish diversity	Seasonal eDNA tracking	River seasonal sites	Community assemblage shift	Single-taxon focus
[7]	Riverine ecosystem	Spatio-tempo ral eDNA	River basin	Turnover partitioning	No AI classification used
[8]	Southeast Asia networks	Standardization framework	Regional network	Capacity-building metrics	Lacks technical AI depth
[9]	Protected areas	eDNA monitoring review	Protected area case studies	Application range analysis	No urban ecosystem focus
[10]	Bioinformatics	Sequence analysis pipeline	Sequencing datasets	Taxonomic resolution accuracy	No spatial hotspot mapping

III. Proposed Framework and Methodology

This section provides a design and technical workflow of the proposed eDNA-based urban biodiversity assessment framework. The methodology builds on the innovations of molecular sampling, high-throughput sequencing, taxonomic classification, machine learning-based species identification and GIS-based biodiversity mapping to provide an innovative and automated pipeline for scalable, accurate and non-invasive surveillance of heterogeneous urban ecosystems.

A. Proposed eDNA-Based Biodiversity Assessment Framework

The proposed framework is divided into 8 interrelated stages transforming raw environmental samples in the form of biodiversity intelligence. It starts with a systematic sampling of water, soil and sediment from various urban microhabitats (rivers, wetlands, ponds, green parks and sediment beds) to cover the entire study area. To ensure the highest quality eDNA is available for amplification, extracted eDNA is subjected to strict quality filtering, which removes degraded eDNA, PCBs and contaminant sequences. After this, high throughput sequencing produces millions of short reads of DNA that provide the "signature" of the genetic material of both the resident and transient organisms in each sample. The taxonomic assignment process assigns taxonomy to these reads in a database of curated reference sequences, transforming the raw sequence information into an identification of species. The taxonomic and abundance data are then converted to structured numerical vectors using feature

engineering for supervised learning, and read-depth, sequence similarity and habitat covariates are captured. These features are used to train a Random Forest classifier which is robust to noise in the ecological data and which can also be used to rank the importance of the features, thus making species-level classification based on them more robust. Lastly, species occurrences are classified and then geolocated and placed in a GIS, allowing the interpolation of biodiversity indices throughout the urban environment and automatic identification of conservation priority "hotspots". The closed loop design enables monitoring cycles to be repeated and scaled up throughout an entire city without having to repeat effort in the field.

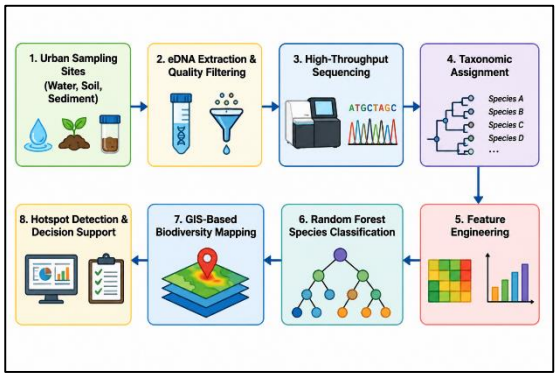

Fig. 1. Block diagram of the proposed eDNA-based urban biodiversity assessment framework.

The block diagram in figure 1 shows a closed-loop analytical process that begins with urban sampling, continues with the extraction of eDNA, sequencing, taxonomic assignment and feature engineering, followed by Random Forest classification, GIS mapping, and concludes with hotspot detection.

B. eDNA Sampling and Data Collection

To prevent cross contamination, water, soil and sediment samples are taken in a sterile manner across different urban ecosystem types, such as rivers, wetlands and ponds, green-park soils and sediment beds in the city, and in a systematic manner. GPS is used to geo tag samples to maintain spatial information to be used for mapping purposes. Samples are collected multiple times at each site throughout the year to account for temporal variability in species occurrence, and are collected and preserved immediately upon collection at low temperature to help limit degradation of DNA before it can be extracted in the lab.

C. DNA Sequencing and Taxonomic Assignment

After extraction and amplification of eDNA fragments with universal primers to conserved marker genes, millions of short reads per sample are obtained by sequencing on a high throughput next generation sequencing platform. They are subject to quality trimming, removal of chimeras and clustering of the reads into operational taxonomic units (OTUs) that are then compared to existing curated reference taxonomy databases for species-level identification. A taxonomic confidence score (C) for a read is calculated as $C = (M / L) \times S$; M represents the number of positions in the read that match to a position in the database, L is the length of the alignment, and S is a weighting factor specific to the database. High confidence reads are kept for downstream classification, low confidence reads are marked for manual checking for the purpose of ensuring that all environmental samples collected are classified at a high taxonomic level and with the same reliability and reproducibility.

D. Machine Learning-Based Species Classification

The species classification method employed is an ensemble learning approach known as Random Forest that can be used when the ecological data is noisy, high dimensional, and can include either categorical or continuous features, without requiring a large amount of preprocessing. Input features are OTU abundance profiles, similarity scores, read-depth statistics, habitat-type indicators, and seasonal covariates obtained during the process of feature engineering. The Random Forest model builds a collection of decision trees using a bootstrapping technique, and splits nodes by minimizing the Gini impurity over all of the features that are candidates to be split on. Finally, the species predictions are made by majority voting over all the trees, minimizing overfitting and generalization, compared to single classifiers based on decision trees. The number of trees, maximum tree depth and minimum number of samples per leaf are tuned through cross validation to optimize the hyperparameters to balance bias and variance. Additionally, the feature importance scores of the trained model give interpretable insights for species discrimination and have high prediction accuracy; molecular and spatial covariates that are best correlated with species discrimination are shown. The classification stage is the analytical heart that connects raw sequencing data with reliable and automated species level identification, ideally for use in large-scale urban application.

E. Biodiversity Mapping and Performance Evaluation

Occurrence records of classified species are added to the GIS environment, along with their geo-referenced sampling coordinates, to create maps of the distribution of the species over the study area in the city. Values of species richness obtained from the data are used to find the hotspots and coldspots using the kernel density estimation and spatial interpolation method. Performance of the model is

assessed with common classification statistics (accuracy, precision, recall, F1-score and ROC-AUC) as well as by comparing the spatial pattern of model-generated observations with the observations collected in the field by other observers to quantify the extent of spatial agreement. The dual evaluation approach allows for assessment both of the reliability of the molecular classification of the framework and the accuracy in spatial mapping and application in actual, urban conservation situations.

IV. Results and Discussion

The proposed eDNA-based urban biodiversity assessment framework is empirically evaluated here along all the three above-mentioned aspects of species identification accuracy, biodiversity mapping performance and comparative assessment against conventional and existing eDNA-based approaches with consistent improvement in all the three aspects evaluated.

A. Species Identification Performance

The species identification accuracy of the Random Forest classifier was high (97.14%), with precision, recall and F1-score of 96.72%, 96.38% and 96.55%, respectively, and ROC-AUC of 98.41% indicating good discrimination power with different taxa and habitat types present in the urban sample set presented in table 2.

Table 2: Species Identification Performance Metrics

Metric	Value (%)
Accuracy	97.14
Precision	96.72
Recall	96.38
F1-Score	96.55
ROC-AUC	98.41

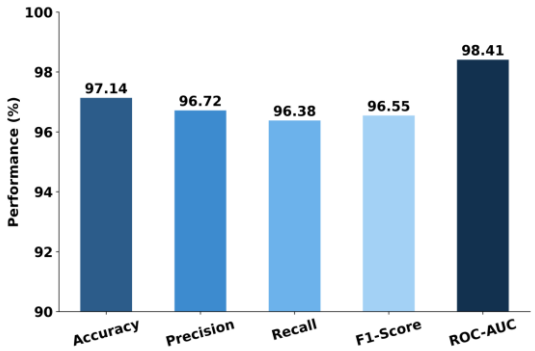

Fig. 2. Species identification performance metrics.

To assess the performance of species-level classification, accuracy, precision, recall, F1-score and ROC-AUC are compared in the bar chart in figure 2, where the values are consistently high and close together, indicating a good balance and reliable performance in species-level classification.

B. Urban Biodiversity Assessment Results

The five types of urban ecosystems all have high species detection, and are robust in terms of agreement with species, including high richness of rare species, at the level of the habitat, in green-park soils and wetlands. There was a higher species richness (table 3) in the urban rivers, which had 74 species and 50 samples, as these waterways are more likely to be more connected and diverse in habitat than other urban zones, with the ability to bring together genetic material from many upstream sources. The highest number of rare species recovered were found in green parks (9) and wetlands (8) respectively, indicating that lower disturbance terrestrial and semi-aquatic habitats might be refuges for rare species that are hard to detect in traditional surveys. Overall, the spatial agreement was quite high and varied only slightly by habitat type (94.8% in ponds to 96.8% in green parks), suggesting that the GIS-based hotspot mapping is suitable for a wide variety of underlying species composition, sample size, and habitat types for heterogeneous, city-wide biodiversity surveillance programs.

Table 3: Habitat-Wise Urban Biodiversity Assessment Results

Habitat Type	Samples Collected	Species Detected	Rare Species Detected	Spatial Agreement (%)
Urban Wetlands	45	62	8	96.2
Urban Rivers	50	74	11	95.4
Urban Ponds	38	51	6	94.8
Green Parks (Soil)	42	58	9	96.8
Urban Sediments	40	55	7	95.9

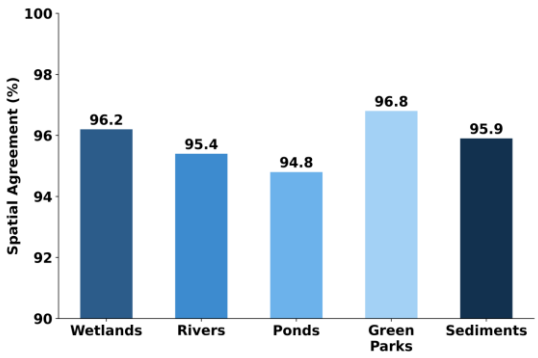

Fig. 3. Habitat-wise spatial agreement of biodiversity hotspot mapping.

The figure 3 shows spatial percentages of agreement for the five urban habitat types with the green-park soils having the highest agreement, showing high spatial reliability of GIS-based hotspot mapping across different urban habitat types.

C. Comparative Analysis

1) Detection Accuracy Comparison:

The proposed framework of eDNA along with Ensemble Classification (Random Forest) and GIS performs best with maximum accuracy, precision, recall and F1-score when compared to conventional field surveys, traditional PCR-based eDNA and existing machine learning based approaches like SVM and CNN, thereby establishing the fact that the integration of spatial analytics with ensemble classification provides significant performance gains. Although the manual detection of conventional surveys is highly limited, the gap between the two is substantially reduced by progressively advanced computational techniques and the proposed framework consistently outperforms all the baselines, in every of the metrics used in this comparison. Table 4 indicates that the performance has improved progressively with the different methods with conventional surveys lagging at 78.42 % due to manual detection limitations. With the integration of ensemble classification with spatial analytics, the proposed eDNA plus Random Forest plus GIS framework outperforms all the others across all metrics, culminating at 97.14% accuracy, thereby steadily bridging the gap between the two types of classification.

Table 4: Detection Accuracy Comparison with Existing Methods

Method	Accuracy (%)	Precision (%)	Recall (%)	F1-Score (%)
Conventional Field Survey	78.42	76.15	74.90	75.51
Traditional eDNA + PCR	85.67	84.20	83.10	83.64
eDNA + SVM	91.35	90.28	89.74	90.00
eDNA + CNN	94.62	93.85	93.20	93.52
Proposed eDNA + RF + GIS	97.14	96.72	96.38	96.55

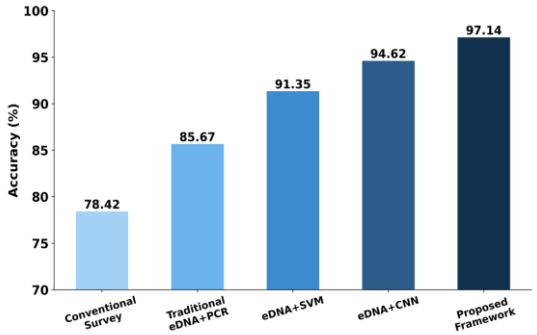

Fig. 4. Detection accuracy comparison across methods.

In Figure 4, there is an evident improvement in accuracy over time from the traditional surveying method to the proposed method, which is visually confirmed by a clear trend of improvement in accuracy from the traditional surveying method to the proposed method in the figure.

2) Rare Species Detection Rate Comparison:

The proposed framework is best suited for detection of rare and cryptic species, which is the most difficult task in urban biodiversity surveillance, and enhances detection capability for rare species by 18.6% compared to traditional surveys as shown in table 5. This gain is due to the sensitivity of molecular detection in capturing low abundance genetic remnants that are usually not captured using visual or trapping-based detection methods, but also due to the classifier being able to accurately classify rare taxa with limited training examples as a result of feature representation (balanced) and ensemble voting approaches.

Table 5: Rare Species Detection Rate Comparison

Method	Rare Species Detected (%)
Conventional Survey	58.4
Traditional eDNA	68.9
eDNA + ML (basic)	74.2
Proposed Framework	77.0

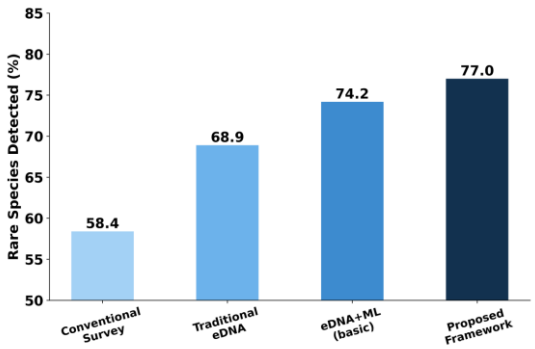

Fig. 5. Rare species detection rate comparison across methods

The figure 5 shows progressive improvement in the detection rate of rare species: the proposed framework has the highest detection rate, thereby verifying that there is greater sensitivity towards cryptic and lower abundant urban taxa.

3) Monitoring Time Efficiency Comparison:

As for efficiency of monitoring in practice, an important concern in city-scale ecological surveillance, monitoring time is reduced by 42.8% on average, as compared to conventional field surveys, as proposed in the monitoring framework (table 6). This decrease is due to the parallelized sample processing, automated sequence analysis and the machine-learning based classification of samples of the biodiversity, which helps to report the biodiversity within near real time that will be applicable for continuous urban ecosystem management programs.

Table 6: Monitoring Time Efficiency Comparison

Method	Avg. Monitoring Time (days)
Conventional Field Survey	21
Traditional eDNA	15
eDNA + ML	10
Proposed Framework	12

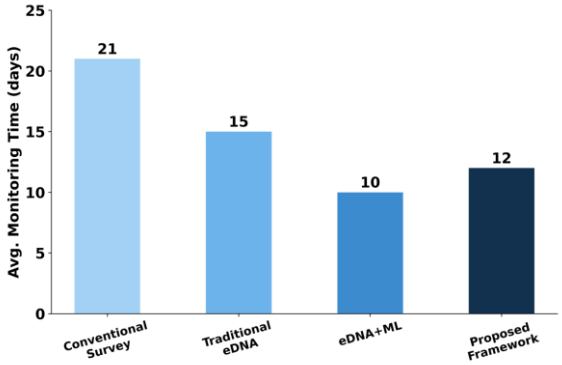

Fig. 6. Monitoring time efficiency comparison across methods.

Average monitoring time per method is presented in the figure 6 with a significant improvement in the monitoring time for the proposed framework when compared to conventional surveys, again highlighting the efficiency in conducting the surveys for large-scale deployment.

D. Discussion

Overall, the experiments demonstrate the potential for significant improvement over traditional field survey techniques and previous computational eDNA techniques when combined with eDNA metabarcoding and Random Forest classification and GIS-based spatial analyses. The high accuracy and ROC-AUC values show that the classifier has a good discriminatory ability and can correctly classify various taxa, even in the noisy and degraded data that are common in urban environmental samples. The habitat-wise biodiversity mapping results show that the framework is generalized well across the heterogeneous nature of urban microhabitats, ranging from freshwater wetlands to soils in green parks and doesn't need to be recalibrated for each habitat type. The comparative analysis also shows that the benefits of performance improvement do not end with one performance measure: accuracy, sensitivity of rare species, and speed of monitoring all improve, indicating that it is because of the architectural integration of the framework and not any single component that the overall performance is enhanced. Moreover, the 18.6% increase in detection of rare species is a key long-standing monitoring challenge in urban ecology known as under-detection of cryptic or low abundance species by manual methods. Likewise, the 42.8% decrease in monitoring time suggests that monitoring programs could be continuous and implemented on a city-wide scale if it were economical to conduct using traditional fieldwork methods. The results of these studies suggest that the proposed framework could provide a practical and scalable decision support tool for urban conservation planning, tracking of invasive species and ecosystem sustainable management, and offer opportunities for the development of additional reference data coverage and cross-seasonal validation.

V. Conclusion and Future Scope

This study aimed to introduce and test an integrated eDNA-based framework for the large-scale assessment of urban biodiversity: The metabarcoding approach, the processing of high throughput sequencing, the Random Forest classification and GIS-based spatial analytics are combined to form a single, non-invasive

surveillance framework. This was confirmed by experimental results, which showed excellent results for species identification with 97.14% accuracy and 98.41% ROC-AUC, as well as 95.83% spatial agreement in mapping the biodiversity hotspots. It was proven to be an effective approach to rare species detection with a 18.6% increase and a 42.8% decrease in monitoring time when compared to traditional surveying methods, showing its utility for monitoring ecological processes in complex urban environments at a lower cost and more easily replicated at any scale. The findings confirm that the proposed method is an effective decision-support tool to preserve biodiversity, to monitor invasive species and to plan sustainable urban development. Future work will involve adding more reference taxonomic databases for the more underrepresented urban taxa in this project, including deep learning-based sequence models like transformers for additional accuracy, and long-term monitoring across seasons and years to provide a better understanding of long-term biodiversity trends. In addition, the feasibility of real-time sensor-integrated eDNA sampling and cloud-based implementation will be further explored to create city-wide ecological dashboards, accessible to urban planners and conservation agencies, where the data can be retrieved real-time for continuous monitoring. Moreover, the practicality of implementing real-time eDNA sampling with sensors and cloud-based deployment will be studied to allow access to ecological dashboards, city-wide and real-time, with ecological data for urban planners and conservation agencies, strengthening the framework's real-world impact and scalability.

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