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Research paper

A cross-continental analysis of tree-related microhabitats, stress symptoms, and biodiversity assessed through eDNA in *Acer platanoides* L. and *Quercus rubra* L. trees located in urban parks and on streets

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ABSTRACT

As urban forestry becomes increasingly vital as a nature-based solution to mitigate the risks of climate change and urbanization, a thorough understanding of the significance of tree-related microhabitats (TreMs) in trees growing in parks and along streets is essential. Previous studies highlight the importance of tree size, species origin, and management intensity in creating TreMs; however, the specific influence of species origin—whether native or introduced—on microhabitat provision and urban forest biodiversity remains unclear. To investigate this, we assessed 320 trees in the cities of New Haven (USA) and Karlsruhe (Germany) using TreM and stress-symptom evaluations, and conducted environmental DNA (eDNA) analysis on a subset of 24 trees. Comparing Red oak and Norway maple in two urban environments, we provide initial insights into how species origin, size, location, and tree vitality influence TreMs, and whether eDNA can reveal biodiversity patterns between New Haven and Karlsruhe. Generalized linear models that included both cities indicated that stress symptoms, species identity, and tree size were better predictors of TreM provision than site or species origin. Overall, Red oaks had lower TreM richness and abundance across our study cities than Norway maples, while our eDNA analysis revealed that Red oaks supported greater taxonomic diversity than Norway maples. The eDNA results demonstrated a distinct community of shared biodiversity across Germany and the USA, including vertebrates, invertebrates, fungi, and bacteria. These findings may be linked to Red oak's role as a long-lived, late-successional species in forest ecosystems and its larger size compared to Norway maple. We conclude that cities should retain larger trees to support habitat diversity and that additional research is needed to link biodiversity, TreMs, tree species identity, and arboricultural practices.

Introduction

The urban and peri-urban forest (UPF) comprises all trees and vegetation that have found homes in our cities. These include single-street trees to trees in backyards, community gardens, parks, and woodlands that are woven into the urban landscape (Salbitano, 2016). The UPF provides diverse ecosystem services to the urban populace and is seen as a nature-based solution to mitigate the risks of climate change and the impacts of urbanization in cities, thereby improving human well-being. Most notably, through services such as urban climate amelioration (Wang et al., 2018), carbon sequestration (Nowak et al., 2002), air pollution capture (Nowak et al., 2006), and stormwater management (Zölch et al., 2017). Restoration of ecosystems that provide ecosystem services has been shown to provide ecological, social, and economic benefits to cities (Elmqvist et al., 2015). Additionally, ecosystem services benefit more than just humans – UPFs can support biodiversity by creating habitats that provide homes for organisms.

Biodiversity indicates a healthy, functioning, and stable ecosystem (Cardinale et al., 2012). With the global biodiversity decline crisis, managing our growing urban landscapes for biodiversity preservation

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has become an ever-increasing topic (Ostoić & van den Bosch, 2015). In many ecosystems, large trees are considered important for habitat structures and wildlife (Tews et al., 2004; Stagoll et al., 2012). In urbanized environments, a single large tree can support an equivalent number of species and individuals as many smaller-sized trees (Le Roux et al., 2015). Still, as places for migration, habitat, and food, all trees in the UPF remain an important vector for many organisms living within or passing through our cities.

One way to evaluate a tree's capacity to support numerous and a variety of organisms is through the evaluation of tree-related microhabitats (TReMs). However, the link between TReMs and measured biodiversity remains speculative in UPF. TReMs are aboveground morphological structures or substrates on trees that provide habitat for an organism for at least one portion of their life cycle (Larrieu et al., 2018). Larrieu et al. developed a microhabitat typology and methodology for the assessment of TReMs across ecosystems. There are 47 distinct microhabitat types that can be categorized into 15 groups and further categorized into 7 forms (See Fig. A1 in Appendix based on Martin et al., 2022).

The diversity of TReM structures is matched by diverse drivers of TReM formation (Kõrkjas et al., 2021). They include biotic and abiotic factors such as environmental conditions, species-specific physiology, management preferences (Mölder et al., 2020), and temporality. However, many of these drivers can be attributed to metrices of tree health such as age (Ranius et al., 2009), resistance, predation, disease, and wounding (Jackson & Jackson, 2004). Tree health is important in influencing TReM formation and a tree's ability to be a long-standing structure in its ecosystem.

Despite TReMs usefulness as a survey method for habitat, a clearer understanding of their relationship to biodiversity measures is needed if they're to be used as a proxy. Biodiversity can be inventoried through various methods, but the use of environmental DNA (eDNA) based approaches has recently emerged as a powerful tool in conservation science. The eDNA method extracts and sequences DNA from the environment using soil, tissue, swabs, and water samples, providing a detailed and comprehensive breakdown of species present (Dickie et al., 2018). In this study, we utilize eDNA on a sub-selection of large trees to begin to bridge our understanding of these two methodologies.

The novelty of a well-understood and standardized assessment of TReMs means that the body of work on them is relatively small and has been applied only to a limited extent in urban ecosystems (Martin et al., 2022). Despite this, several studies have suggested that larger trees and native species tend to support higher TReM richness and abundance (Großmann et al., 2020; Laux et al., 2022). However, Species diversity and selection are at the forefront of urban forest programming as they are important determinants of tree and forest integrity and resilience in the high-stress conditions of the urban environment (Conway et al., 2019; Roloff et al., 2016).

Planting introduced (or post-Columbian) species in North American and European cities has become controversial due to the potential benefits and drawbacks of such plantings. It is argued that the inclusion of introduced species is essential, as many may be better adapted to the unique stressors of the urban environment than their native (or pre-Columbian in Europe and North America) counterparts, resulting in a long-lived, healthier urban forest (Sjöman et al., 2016). However, apprehension about introduced species stems from their capacity to displace native species and disrupt ecosystem processes, particularly through biological invasion (Delavaux et al., 2023). Still, it is well understood that planting native species has significant benefits for urban biodiversity, as their strong ecological relationships tend to support insect and bird populations, potentially creating a cascading effect within the trophic system (Tartaglia & Aronson, 2024).

Immense levels of human activity and trade mean cities are often the initial vector for invasive species (Lovett et al., 2016). It is well documented that the number of introduced species increases with proximity to urban cores, but the severity of biological invasion is more strongly influenced by local native phylogenetic and functional plant diversity (Kowarik et al., 1995; Delavaux et al., 2023). Regardless of species origin, the UPF will often be the first point of introduction for introduced species. This remains an untapped potential to use urban trees as a first line of defense by viewing them as 'sentinel trees' (Paap et al., 2017).

In this study, we aim to understand the impact of native origin status (native or pre-Columbian occurrence vs. introduced or post-Columbian occurrence in German and US cities) of single-standing trees along streets and within parks on tree stress and their ability to support biodiversity. To observe this phenomenon, we selected two contrasting yet common urban tree species: *Quercus rubra* L. – or Northern red oak – native to the northeastern United States, and *Acer platanoides* L. – or Norway maple – native to central Europe. These species were chosen for their abundance in the study cities of New Haven, USA, and Karlsruhe, DE, and for their demonstrated capacity to outcompete native counterparts within their family in introduced environments. Both species were declared invasive species in their introduced countries. Our research questions were:

1. How do tree-related microhabitats differ between Norway maple and red oak trees growing in contrasting habitats (i.e., park vs. street), as well as between New Haven, USA, and Karlsruhe, Germany? What factors most influence the richness and abundance of these microhabitats? (N=320)
2. How do vertebrates, invertebrates, bacteria, and fungi found on eDNA samples taken from leaves, bark, and sub-surface soil near trees differ between these two species from two different cities through metabarcoding analyses? (in a subsample of 24 largest trees from the 320 trees)

Methods and materials

Study areas

This study occurred in Karlsruhe, Baden-Württemberg, Germany, and New Haven, Connecticut, USA. Karlsruhe is located at 49.0069° N, 8.4037° E in southwestern Germany, covering approximately 173.4 km^2. New Haven is located at 41.3° N latitude and 72.9° W longitude in the northeast of the United States. Climatically, the two cities are similar with warm, wet, and temperate climates. The annual average temperature for both cities is approximately 11°C. However, New Haven receives more annual rainfall, with an average of 1355 mm compared to Karlsruhe's 871 mm . In Karlsruhe, the most common tree species belong to the genera maple, hornbeam, oak, lime, and cherry. New Haven's common tree species are oak, maple, beech, and honey locust (URI, 2011).

Species and tree selection

Karlsruhe and New Haven maintain databases on individual trees in their cities, including location, size, species, and various tree health characteristics. The Urban Tree Cadaster of Karlsruhe documents all trees growing on municipal-owned land, including street and park trees (Stadt Karlsruhe, 2022). Whereas New Haven's database, provided through the Urban Resources Initiative (URI), only details the city's street trees. As a result, New Haven's park trees were unable to be pre-selected. Instead, park trees were selected based on presence and size at the time of observation. If pre-selected trees were the wrong species, had been removed, or were dead, a new tree would be selected from the database or as a spontaneous availability.

Given that tree diameter was a known factor in microhabitat provision, only trees with a minimum DBH of 20 cm were selected, with an additional size class for trees with a DBH greater than 40 cm (Laux et al., 2022; Larrieu et al., 2018). We evaluated street and park trees separately as site location has been shown to influence TReM production (Laux et al., 2022). Based on the sampling population criteria, red oak and

Norway maple were identified as the most suitable species for this study (Table 1). We selected a total of 320 trees split evenly between species, city, and location (Fig. A2 in the Appendix). Of these 320 trees, the 24 largest were selected for a sub-study that quantified the number of species and taxa present using eDNA.

Red oak is a deciduous tree native to the eastern United States and southeastern Canada, extending as far west as Minnesota. Oaks, in general, are notable for their role as a keystone species in forested ecosystems, providing key support for a wide range of flora and fauna, including long-living and late-successional species, and supporting hundreds of species across North America. The oak tree's acorns are an important food source for wildlife, such as deer, squirrels, and birds (NRCS, n.d.). Red oak is a commonly planted species in the urban forest, given its tolerance for soil compaction, high light, and dry, hot conditions (CITREE, 2015). Previous studies have shown that red oak seedlings demonstrate enhanced growth under urban conditions (Searle et al., 2012). In European forests, the species is rapidly becoming more prominent as it is well adapted to conditions induced by climate change, but it is disrupting local ecology, forest dynamics, and seedling regeneration and recently declared as an invasive species in Germany (Major et al., 2013; Nicolescu et al., 2020 and see the website which was last accessed on 25.03.2026: https://www.bfn.de/gebietsfremde-baumarteninvasivitaet).

Norway maple is a deciduous tree native to western and central Europe. Norway maple is a shade-tolerant tree that typically grows in mixed stands; however, in North America, it is rapidly colonizing the seed bank and understory and is considered an invasive species. The species is gradually replacing historic oak stands in American forests. Norway maple is one of the most common urban trees in temperate region of Europe and America for its fast early growth rate and ability to grow in a wide range of conditions, particularly in urban areas, where it can withstand low light and harsh soil conditions (NRCS, n.d.).

Data collection

Survey data collection occurred between May and August of 2022. All surveys were completed by the first author, using standardized urban tree survey methods to minimize observer bias and error. For each survey, a single tree was observed using three different standardized assessments:

1. Site assessment

The site assessment documented relevant information about the tree condition and environment as per Nowak, 2024 and Roloff, 2016. This included identifying information, such as species (*Northern Red oak/Norway maple*) and location, site, and planting conditions (*street/park, strip/pit/NA, Impervious/shrub cover percentages*), tree measurements (*height, DBH, crown dimensions*), crown light availability, crown transparency (foliage density), and vitality class.

2. TReMs assessment

A standardized assessment and methodology for TReMs exists within the literature. This assessment is based upon a hierarchical typology that begins with 7 general forms that share physiognomy and functional characteristics. These forms are further subdivided into 15 groups, which share more specific morphological traits and can be further assigned into 47 specific microhabitat types. These 47 TReMs are defined by structure and size per the reference field guide (Kraus et al., 2016; Larrieu et al., 2018; See appendix – Table A1).

3. Tree health assessment

The tree health assessment was created using the methods recommended by the i-Tree pest detection field guide (USDA, 2010; See appendix – Table A2 and A3). This documented the different kinds of human and environmentally induced stress signs and their severity. Additionally, it documented pest and disease signs, as well as the percentage of the canopy affected.

eDNA sample collection and laboratory analyses

Sample collection was conducted concurrently in both cities in September 2023. Each team collected leaf, subsurface soil (5 to 10 cm deep), and bark surface samples of 12 survey trees per city, following the Global Biosciences Center's (https://www.sgs.com/en-es/sgs-global-biosciences-center) general sampling guidelines (Fig. 1). The SGS website presented here was last checked on 25.03.2026. The eDNA laboratory research and services from SGS can be found in this YouTube Video (SGS, 2026). In total, 72 biological samples were collected to create a biological profile of species diversity at each tree. To avoid cross-contamination, all field material was sterilized between sites and disposable items were discarded after single use. Samples were kept on ice and shipped at 4°C for extraction and sequencing. DNA quality and integrity were verified prior to PCR amplification using Qbit and Nanodrop analyses. Amplification of 5 distinct molecular targets enabled us to detect DNA from vertebrates, bacteria, fungi, insects, and other invertebrates.

Libraries were sequenced on an Illumina MiSeq equipment in paired-end 2 × 150 bp mode, resulting in 2 × 23,715,064 reads. Sequencing performance met platform specifications, and only high-quality reads were retained for downstream analyses. Sequenced reads were analysed using a custom metabarcoding analysis pipeline based on the QIIME 2 (Boylen et al., 2019) platform. Briefly, quality control of raw sequencing data was performed to verify sequencing accuracy and resulting non-satisfactory reads were discarded. The remaining were used to construct a set of representative sequences (ASVs) using DADA2 (Callahan et al., 2016) and filtered to discard amplicons of unexpected length. Taxonomic identification of the ASVs was done using Naïve Bayesian Classifiers trained on reference sequences expected to be amplified by each primer pair, with a minimum threshold of 95% confidence for taxonomic assignments.

The following reference sequence databases were used to construct the set of classifiers: MIDORI version GB245 (https://www.reference-midori.info/), SILVA version 138-NR99 (https://www.arb-silva.de/), and UNITE (https://unite.ut.ee/). The resulting count tables were corrected to mitigate the effect of tag-jumping. All website links presented here were last checked on 25.03.2026.

Statistical analyses

Accumulation curves were generated for each sampling group to assess sampling adequacy. Curves approaching asymptotes indicate a

Table 1
Breakdown of the number of trees surveyed by city, species, site, and size class.

City	Karlsruhe				New Haven			
Site	Park		Street		Park		Street	
Species	20 ≤ 39 cm	40 cm ≥	20 ≤ 39 cm	40 cm ≥	20 ≤ 39 cm	40 cm ≥	20 ≤ 39 cm	40 cm ≥
Quercus rubra	20	20	20	20	20	20	20	20
Acer platanoides	20	20	20	20	20	20	20	20

Fig. 1. Sample collection for leaves (a), sub-surface soil (b), and tree bark swabs in Karlsruhe. Leaves sample were collected using a telescopic pruning sheer whereas bark swabs were collected using paint brush dipped in a specific cell buffer solution and then mounted on a telescopic pole (Photo source: Ferdinand Betting).

representative sample size sufficient for TReMs analysis.

TReMs were quantified using different metrics depending on their structural characteristics: cavities were counted, bryophyte coverage was recorded as a percentage, and webs were noted as presence/absence. To standardize data for richness and abundance calculations, percentage-based and binary data were converted through a scoring system: 0% = 0, ≤50% = 1, and >50% = 2; presence/absence was scored as 1/0. TReMs richness is defined as the number of distinct TReMs per tree with a value greater than 1, while TReMs abundance was determined by summing the total number of TReMs present on each tree.

Normality of variables was determined using Shapiro-Wilk tests. Due to heteroscedasticity and right-skewed distribution, Box-Cox transformations were applied to normalize key variables (see Table A5 in the Appendix). We used the Mann-Whitney U test to assess the statistical significance of differences in the sum of microhabitat groups. To evaluate differences in mean TReMs abundance and richness between species and sites, Mann-Whitney U tests were conducted, and boxplots were used for visualization.

Generalized linear models (GLMs) were employed to examine the predictors of TReM richness and abundance across the entire dataset (New Haven and Karlsruhe combined). Box-Cox transformed variables were included to optimize model performance for variables DBH, TReM richness, and abundance. Models were evaluated for collinearity, and residual diagnostics confirmed assumptions of normality and homoscedasticity. The abundance and richness of TreMs were used as the target variables in two separate GLMs. The structures of the GLMs were as follows:

TreM richness/abundance ~ Tree species*Site + Location + DBH + Crown dieback + Defoliation + Wilted foliage

Tree species and site were included as an interaction term, assuming differences between species (*Quercus rubra* vs. *Acer platanoides*) and within sites (park vs. street). Location (New Haven vs. Karlsruhe) was included in the model to test whether two cities on different continents affect TReM richness and abundance. DBH, crown dieback, and wilted foliage were variables that were not autocorrelated and were therefore selected to test their impact on TReM richness and abundance.

We performed both descriptive and multivariate analyses of biodiversity assessment using eDNA data. Dimension reduction using Principal Component Analysis (PCoA) to determine whether organisms detected in the samples were distinct between New Haven and Karlsruhe, as well as among substrates (vegetation leaves, subsurface soil samples, and bark swabs), tree species, and sites.

All analyses and figures were conducted in R version 4.x (R Core Team, 2024), using the packages vegan (Oksanen et al., 2022), dplyr (Wickham et al., 2023), ggplot2 (Wickham, 2016), MASS (Venables & Ripley, 2002), car (Fox & Weisberg, 2019), and corrplot (Wei & Simko, 2021).

Results

Tree dimensions and site conditions

Table A4 in the Appendix summarizes the mean values for DBH, tree height, light availability, crown transparency, and percent impervious and shrub cover below the canopy for the 320 sampling trees. We found that Red oak and park trees were larger than Norway maple and street trees in both cities. Additionally, those results demonstrated the differences in planting conditions between Karlsruhe and New Haven: in New Haven, all trees exhibited higher crown light availability and impervious surface cover beneath their canopies; in Karlsruhe, they had more shrub cover and greater crown transparency. Differences in the built environment and planting regimes led to less competition and increased tree size. However, these results also indicated that sampled trees in New Haven's UPF perhaps faced harsher light and water conditions than Karlsruhe's.

Tree-related microhabitats (TReMs) by city

The accumulation curve (Fig. 2) revealed that the number of trees sampled for all groups in both cities was sufficient as a representative sample size in Karlsruhe, as they neared their asymptote with the number of trees. However, it also demonstrated that we would had needed more sample trees in New Haven, indicated by the rising asymptote. It also implied that the data in New Haven showed substantial heterogeneity, or a higher degree of variation, compared to the

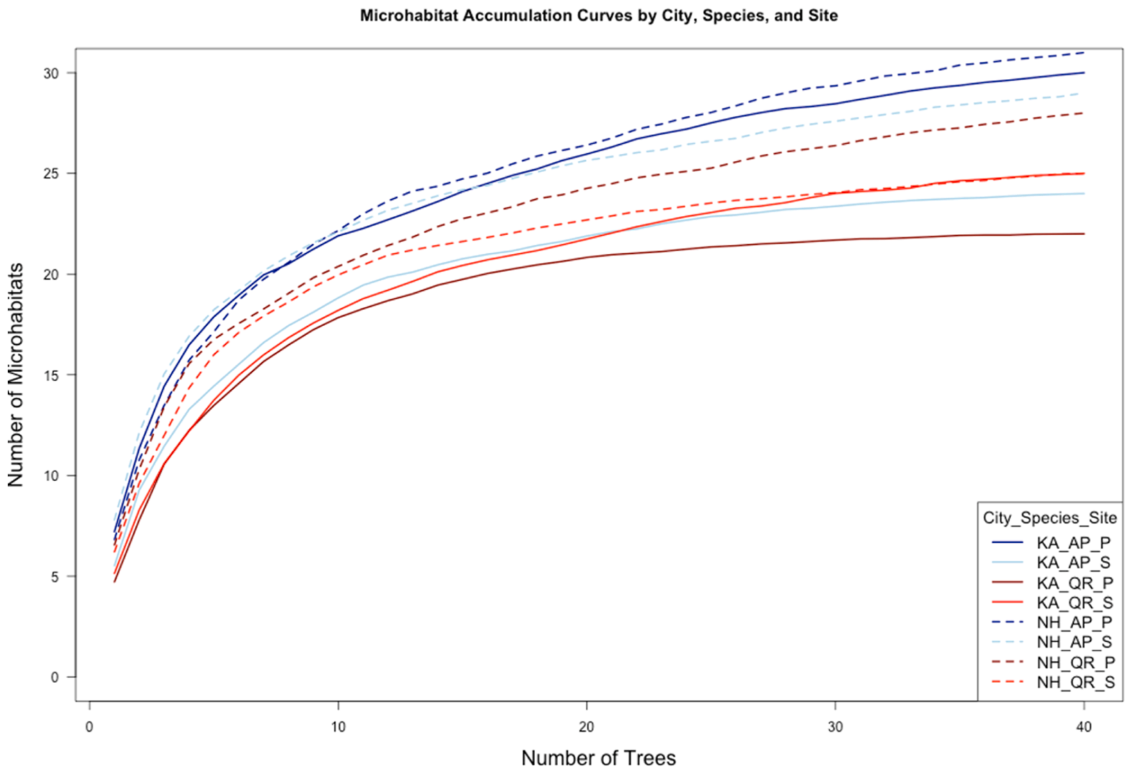

Fig. 2. Accumulation curve for TReMs in Karlsruhe and New Haven (KA_AP_P: Norway maple in parks in Karlsruhe; KA_AP_S: Norway maple near streets in Karlsruhe; KA_QR_P: Red oak in parks in Karlsruhe; KA_QR_S: Red oak near streets in Karlsruhe; NH_AP_P: Norway maple in parks in New Haven; NH_AP_S: Norway maple near streets in New Haven; NH_QR_P: Red oak in parks in New Haven; NH_QR_S: Red oak near streets in New Haven).

data from Karlsruhe.

Table 2 shows the sum of microhabitat groups in both cities, organized by tree species, along with the results of a Mann-Whitney U test. Table A6 (see the Appendix) presents a similar analysis but also incorporates site conditions (park vs. street). These tables illustrated how tree species identity contributed to differences in microhabitat group totals across locations. Key groups of microhabitats in which both species showed a strong response to the change in location are fungi and insect galleries. Both microhabitat groups showed a greater presence in New Haven than in Karlsruhe, suggesting differences in urban tree health concerns between the two cities.

Red oaks showed a strong positive response for nests in New Haven, consistent with its ecological role as a key habitat provider in the Northeastern United States. It also exhibited more concavities in New Haven, primarily in park trees (see in appendix Table A6), likely reflecting the presence of older and larger individuals in these settings. Norway maple showed a strong positive response for small cavities and parasitic structures (moss and lichen) in Karlsruhe, particularly in park trees, suggesting distinct ecological interactions in the park setting.

Comparison of TReM richness and abundance

We compared the total TReM richness and abundance calculated for each tree using a series of Mann-Whitney U tests (see Fig. 3 and Table A7 in the Appendix). We first tested whether Norway maple and Red oak trees grown at the same site (park vs. street) differed in their provision of TReM richness and abundance in their native and introduced locations (Karlsruhe vs. New Haven). The Red oak trees in the parks of New Haven had significantly higher TReM richness than those in the parks of Karlsruhe. But no significant difference in TReM richness was observed between cities when they were grown near streets. The TReM abundance was not significant when comparing individuals of two species across cities and among park and street trees. Second, we wanted to test whether TReM richness and abundance would vary within tree species growing under different site conditions in New Haven or Karlsruhe. Hence, we compared TReM richness and abundance within the same tree species grown in parks or near streets in each city. None of those comparisons was statistically significant. Third, we tested whether TReM richness and abundance would vary between tree species grown at the same site within each city. We found that Norway maple trees in the parks and near the streets of Karlsruhe had significantly higher TReM richness than Red oak trees in the parks and near the streets of Karlsruhe. The abundance of TReM was significantly higher in Norway maple park trees than in Red oak park trees in Karlsruhe, although a similar result was not observed for street trees. None of the comparisons of TReM richness and abundance between Norway maple and Red oak trees grown under similar site conditions were significant in New Haven city. Finally, we compared TReM richness and abundance between Norway maple and Red oak trees, irrespective of their site and location across the whole data set. We found that Norway maple, in general, had significantly higher TReM richness and abundance when the entire data set was used in the comparison.

Stress symptoms of trees between species, habitat, and parks in New Haven and Karlsruhe

In Karlsruhe, all stress symptoms differed significantly across species and site groups, whereas in New Haven, all symptoms but discoloration were significant (Table A9 in the Appendix). While the Kruskal-Wallis test did not detect a significant difference in mean discoloration between groups in New Haven, the difference between Red oak and Norway maple was among the largest observed, likely reflecting high within-group variability (Table A10 in the Appendix). Patterns in dieback varied by city. In Karlsruhe, street trees of both species exhibited more intense dieback than park trees. However, in New Haven species identity was the strongest determinant of dieback intensity, with

Table 2
Sum and result of Mann-Whitney U test for TReM groups by species in Karlsruhe and New Haven (n.s.: not significant).

Group	Species	Karlsruhe	New Haven	*p*-value
Burr and canker	*Acer platanoides*	66	80	n.s.
	Quercus rubra	80	77	n.s.
Concavities	*Acer platanoides*	226	206	n.s.
	Quercus rubra	173	229	$p < 0.05$
Crown deadwood	*Acer platanoides*	194	190	n.s.
	Quercus rubra	193	162	n.s.
Epiphytic and parasitic	Acer platanoides	214	202	$p < 0.05$
	Quercus rubra	170	178	n.s.
Exposed sap and heartwood	*Acer platanoides*	73	86	n.s.
	Quercus rubra	86	78	n.s.
Exposed sapwood	*Acer platanoides*	233	219	n.s.
	Quercus rubra	131	181	$p < 0.05$
Exudates	*Acer platanoides*	66	78	n.s.
	Quercus rubra	82	79	n.s.
Fungi	*Acer platanoides*	66	107	$p < 0.05$
	Quercus rubra	88	127	$p < 0.05$
Insect galleries	*Acer platanoides*	196	567	$p < 0.05$
	Quercus rubra	192	576	$p < 0.05$
Microsoils	*Acer platanoides*	99	115	n.s.
	Quercus rubra	105	109	n.s.
Nests	*Acer platanoides*	101	125	n.s.
	Quercus rubra	113	137	$p < 0.05$
Rot holes	*Acer platanoides*	73	91	n.s.
	Quercus rubra	81	85	n.s.
Small cavities	*Acer platanoides*	267	206	$p < 0.05$
	Quercus rubra	177	190	n.s.
Twig tangles	*Acer platanoides*	60	79	n.s.
	Quercus rubra	80	76	n.s.

Norway maple showing substantially higher die back than Red oak. While Norway maple consistently exhibited higher dieback in both cities, the difference was especially pronounced in New Haven. This suggests that surveyed Norway maples might had a physiological difference or might be more vulnerable to urban stressors compared to Red oak, an effect that was potentially amplified in its introduced range.

Defoliation followed a different trend: in both cities, park trees had higher mean defoliation than street trees. Notably, in New Haven, the native Red oak had significantly more defoliation (i.e., predation) compared to Norway maple. This implies a higher level of faunal interaction in the environment of parks than on streets, especially for native species in New Haven. Wilt was significantly higher in New Haven street trees but showed no clear species or site trend in Karlsruhe. The one stress symptom associated with a single species is discoloration, where Norway maple exhibited a consistently higher mean, particularly in New Haven. Field observations in New Haven indicated a high prevalence of Tar Spot Disease in the Norway Maple population; however, the disease-inducing fungus, *Rhystisma acerninum*, was detected in a similar number of trees in both species by eDNA analysis.

Impact of multiple factors on microhabitat richness and abundance

The results of the GLM analyses are presented in Table 3. Among the predictors, DBH had the largest and most significant positive effect in both models, reinforcing the role of tree size as a primary driver of TReM provision. Health-related variables, such as crown dieback and wilted foliage, had a significant positive impact on TReM richness and abundance, though with smaller effect sizes. Notably, defoliation had a stronger positive effect on TReM abundance, implying that pest and disease presence might correlate with the development of microhabitat structures (e.g., fungal growths, insect boreholes). This interpretation is supported by the relatively high proportions of detritivores and phytophagous species (see appendix – Fig. A18 (top)), which may capitalize on degraded or decaying tissue. The model revealed a significant difference between the two species, with red oak generally exhibiting lower TReM abundance and richness than Norway maple. However, red oak supported greater overall species diversity than Norway maple, as confirmed by the eDNA analysis described in the following section. The origin effect, defined as the assumed difference in TReM richness and abundance between two cities on two continents (New Haven vs. Karlsruhe) within the same species, was not significant. In the GLM analysis, street trees, in general and regardless of species or city, had lower TReM abundance than park trees. However, this effect was not significant for TReM richness.

Observed biodiversity through eDNA assessment

From the 390 species detected through eDNA analysis of 24 trees, we observed a difference in species richness between Karlsruhe and New Haven for vertebrates and invertebrates (Fig. 4). In both tree species, the richness of arthropods and vertebrates was higher in park samples than street samples when all three types of samples were combined. But that was only the case for Norway maple in New Haven, and for Red oaks, there were even opposite trends in park trees compared to street trees, compared to what was found in Karlsruhe. While 18 species of mammals, primarily small- to medium-sized rodents, were detected in both cities, Karlsruhe samples had much higher bird diversity, with 29 species compared to New Haven's 12. Furthermore, 3 reptile species were detected in Karlsruhe, whereas none were detected in New Haven. Overall, the Karlsruhe samples had much higher species richness among vertebrates and invertebrates than those from New Haven, regardless of species and site (Figures A3 through A13 for detailed taxonomic breakdown in the Appendix). 24 vertebrate and invertebrate species whose DNA was found in at least 8 or more samples are listed in Table A11 in the Appendix.

Furthermore, in the streets and parks of Karlsruhe and New Haven, the Red oak supported more species than the Norway maple (Figures A3 through A6 in the Appendix). Generally, the community groups between the two cities were distinct, except for several mammal and invertebrate species with a widespread distribution. While no community differences were detected between tree species, communities were associated with the sample substrate (e.g., soil, leaf, and surface). This is exemplified in Fig. 5 (Fig. A14 and A19 for other taxonomic groups in the Appendix), where we can see the overlap and separation of biological communities of arthropods by the surface rollers compared to the vegetative or leaf samples.

Similar to vertebrate species diversity, the Karlsruhe samples had much higher invertebrate species diversity. Not only were there a higher prevalence of species, but whole families of insects present plentifully in Karlsruhe were absent in New Haven. This could have implications for the ecological community. For example, families within the Diptera order that are present in Karlsruhe but have low or no presence in New Haven, such as Chironomidae, Muscidae, Dolichopodidae, and Drosophilidae, are essential as both predators of other insects and prey for birds. There was also much higher species diversity and sample detection frequency among herbivorous families in the Insecta order, such as Hymenoptera, Orthoptera, and Psocoptera, in Karlsruhe compared to New Haven. Beyond species diversity, functional diversity of species, which can be evaluated through eDNA, could impact inter-animal and animal-fauna interactions in these cities (Fig. A18 (top) in the Appendix). eDNA analysis further revealed several significant differences between New Haven and Karlsruhe. Firstly, soil bacterial diversity was much higher in the New Haven samples than in the Karlsruhe samples.

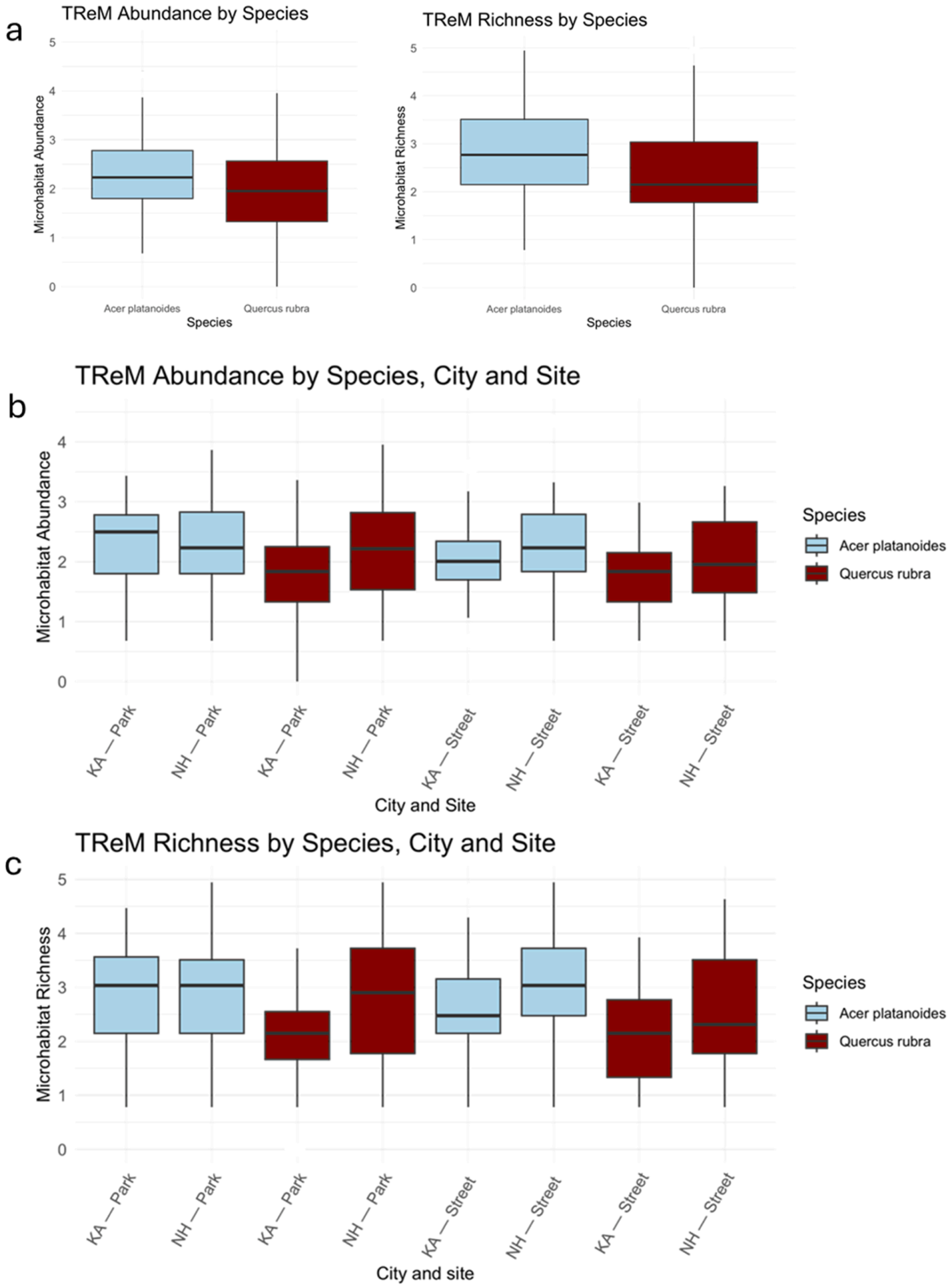

Fig. 3. Comparison through Box plots TReM richness between species, sites, and two cities. Statistical results of Mann-Whitney *U* tests for those comparisons are provided in Table A7.

About 300 different bacterial taxa were detected in New Haven compared to the 200 found in Karlsruhe (Fig. A20 through A22 in the Appendix). Additionally, many of the most abundant bacterial genera detected in our samples play a functional role in upkeeping soil health through the production of antibiotics, the degradation of pollutants, or the biocontrol of microorganisms (Table A12 in the Appendix).

The results from our eDNA analysis for fungal groups contrast with this. Overall, Karlsruhe's samples demonstrated much higher species diversity of soil fungi, with around 140 taxa detected, compared to New Haven, at 60 (see appendix – Figures A23-A28). Most of the taxa detected belonged to the phyla Ascomycota, Basidiomycota, and Mortierellomycota with varying levels of functions (Table A13 in the Appendix). Across both cities, Ascomycota typically had the highest prevalence and species diversity. Generally, the park trees had

Table 3

Generalized linear model (GLM) parameter estimates and results for models testing our hypotheses on TReM richness and abundance and other significant variables influencing them. The table includes the estimated effect size (Estimate), standard error (Std. Error), *t* value, and significance codes for each explanatory variable retained in the final model. Explanatory variables were selected based on our variables of interest and lack of multicollinearity (p value <0,001 = ***; p value <0.01 = **;p value <0.05 = *; n.s.= not significant).

Target variables	Coefficients/Explanatory variables	Parameters			
		Estimate	Standard error	*t* value	Significance codes
TreM richness	Intercept	-13.24	0.96	-13.88	***
	Species (*Quercus rubra* vs. *Acer platanoides*)	-0.72	0.11	-6.52	***
	Site (Street vs. Park)	-0.02	0.08	-0.2	n.s.
	Location (New Haven vs. Karlsruhe)	0.02	0.11	0.18	n.s.
	DBH	9.08	0.54	16.82	***
	Crown dieback	0.008	0.002	3.31	**
	Defoliation	0.003	0.002	1.38	n.s.
	Wilted foliage	0.004	0.001	2.40	*
	Species-*Quercus rubra*:Site-Street	0.25	0.16	1.55	n.s.
TreM abundance	Intercept	-8.10	0.79	-10.33	***
	Species (*Quercus rubra* vs. *Acer platanoides*)	-0.47	0.09	-4.95	***
	Site (Street vs. Park)	-0.16	0.09	-1.71	*
	Location (New Haven vs. Karlsruhe)	0.05	0.06	0.81	n.s.
	DBH	5.84	0.44	13.10	***
	Crown dieback	0.007	0.002	3.62	***
	Defoliation	0.005	0.002	2.98	**
	Wilted foliage	0.003	0.001	2.41	*
	Species-*Quercus rubra*:Site-Street	0.14	0.13	1.06	n.s.

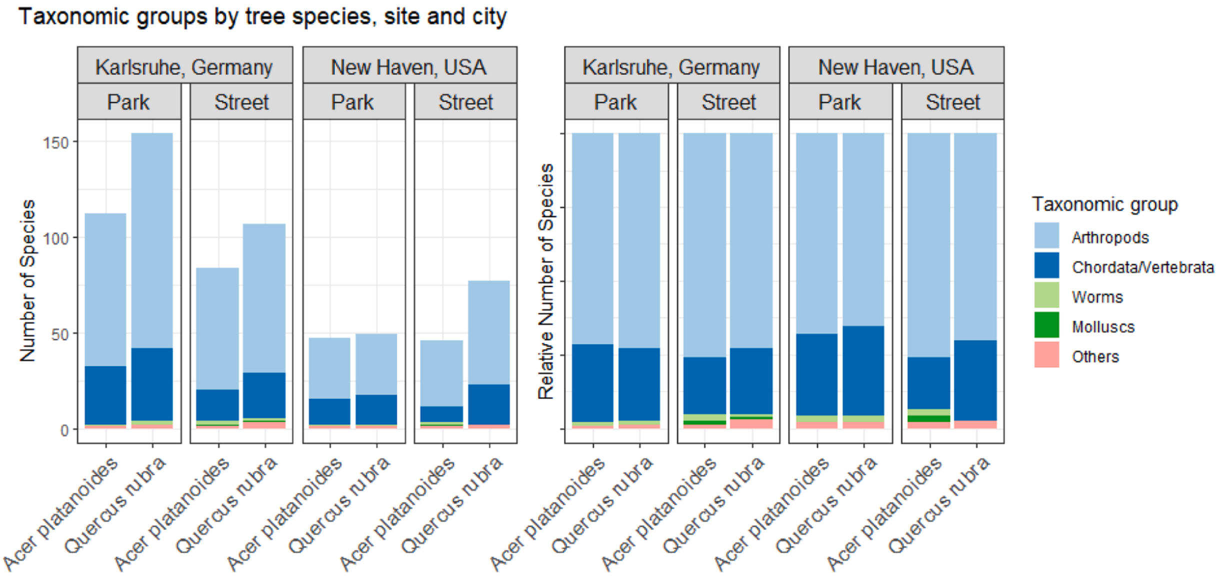

Fig. 4. Left) Number of species per taxonomic group; Right): Relative number of species per taxonomic group. Samples are organized by city, tree species, and sampling location (park vs. street).

significantly higher soil fungal diversity levels than street trees. This can be attributed to the physical differences between parks and streets that allow greater soil connectivity. Among these fungi, we captured disease-causing species, including those that cause tar spot, anthracnose, phyllosticta spot, and leaf blister. Additionally, two harmful pathogenic fungi, *Mycosphaerella tassiana* and *Sawadaea tulasnei*, were found only in Karlsruhe, with no comparable species in New Haven.

Discussion

Previous research demonstrates that planting native and introduced species in urban environments can yield diverse biodiversity outcomes. These responses have been linked to multiple factors, including species nativeness, species-specific association, physiological differences, and functional group traits (Berthon et al., 2021). Our results demonstrate that both species, site (park vs street) and origin (exotic or native), can differently influence biodiversity, TReMs richness and abundance. We demonstrated that Red oaks growing in parks within their native range in New Haven had higher TReM richness than Red oaks in their introduced park areas in Karlsruhe, but this effect was not observed for Red oak street trees or for Norway maple trees grown in parks or on streets in both cities. Size of trees (DBH) and their stress symptoms were also crucial factor impacting TReM richness and abundance. At the same time, our eDNA analysis revealed that Red oak supported more taxa and species than Norway maple across both cities and site types. We will discuss the results, highlighting local ecology, physiology, habitat characteristics, and biodiversity measures, within the context of urban planning. Finally, we will present the limitations encountered in this study, the future scope of research, and the conclusions.

Local ecology, species physiology, and tree habitat

Despite observing the same species, the microhabitat composition between the two cities differs significantly, highlighting broader

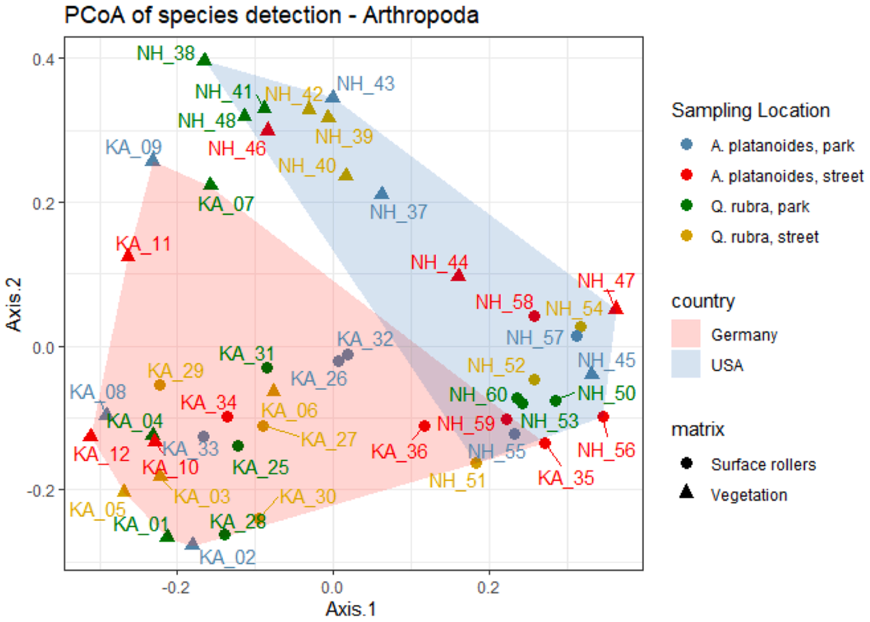

Fig. 5. Dimensional reduction (PCoA) of **Arthropod species** detection matrix separated by country (Germany vs. USA), sampling location (street vs. park), tree species (*Q. rubra* vs. *A. platanoides*), and matrix (vegetation, which was tree leaves samples vs. surface rollers).

ecological factors that shape microhabitat abundance and richness. In its native range, Norway maple supported significantly more moss and lichen than in its introduced range and than Red oak. Furthermore, both species experienced a significant increase in the number of boreholes in New Haven. While borer insects are plentiful globally, the forests of the northeastern United States are particularly vulnerable to a high proportion of introduced borer pests (Liebhold et al., 2013; Lovett et al. 2016). The major difference in TReMs composition between the two cities may reflect regional forest concerns in the northeastern United States. These contrasts underscore that species alone are not the primary determinant of microhabitat type and abundance. Instead, the distinct ecosystems in which these trees exist exert differential impacts, leading to varying TReMs compositions.

A species-specific stress association did appear with Norway maple and discoloration arising from the presence of tar spot disease. Beyond susceptibility to pests and pathogens, tree species also differ physiologically in ways that influence TReM richness and abundance. For instance, occlusion or wound-healing rates after pruning or mechanical damage vary by species, depending on their physiology, wood density, pruning quality, and the time of year when wounding occurs (Lund et al., 2023; Dujesiefken et al., 2005). As TReMs can develop from injury or declining health, a better understanding of how physiological differences between species interact with growth conditions and management practices may lead to better insight and management strategies.

The stress symptom assessment further revealed the impact of urban form on the health of trees existing in the UPF. Differences in crown dieback among cities, species, and sites revealed that street trees experience more prolonged stress than park trees. This is relatively intuitive, given that harsh urban conditions are likely to be more intense on a street than in a park. While not observed in Karlsruhe, wilt in New Haven is similarly higher among street trees than among park trees. This may be indicative of climate or management differences between the two cities, given that wilt fluctuates daily. Streets are not the only site with environmental pressures. We see this as park trees experience higher rates of herbivory-induced predation than street trees, resulting in greater defoliation.

Bivariate comparison of TReM richness showed higher richness in Red oak trees in parks in New Haven than in Karlsruhe, perhaps because some microhabitat groups, such as concavities, nests, fungi, and exposed sapwood, were more abundant in their native range than in Karlsruhe. Nevertheless such origin effect (i.e. native vs. exotic trees) were not significant in GLM analyses when multiple variables were included. For example, the GLM models identified other factors, such as stress symptoms, as significant predictors in TReM abundance and richness, suggesting that the conditions experienced by trees in the UPF play a stronger role in TReM development. For example, in New Haven, harsher growing conditions created by high crown light exposure and impervious surface color may have driven stress responses and TReM formation in Norway Maple.. The findings from Konarska et al. (2023) similarly highlight the dominant influence of built conditions on ecosystem provision, showing that the negative impact of paving outweighed any species effect. TReM abundance was higher in park trees than in street trees, possibly due to pruning and tree care associated with safety measures. Park trees often have large crowns and may occur more frequently in specific microhabitats, such as mosses, lichens, dead branches, and cavities. Additionally, the models reinforce the importance of large trees (Laux et al., 2022) in the UPF as habitat providers through more TReM abundance and richness. This highlights the importance of this study to preserve and care the large trees in cities for increasing biodiversity.

Biodiversity measures and biological security in the urban context

Our findings point to the need to refine the application of TReM assessments in the UPF. While TReMs are a valuable tool for identifying habitat structures and signs of past ecological interaction, our eDNA assessment revealed the limitations in using TReMs as a direct proxy for biodiversity. For example, on one hand, although eDNA detected higher bird, insect, and fungal diversity in Karlsruhe, more nests and fungal bodies were documented through the field TReM survey in New Haven than in Karlsruhe. On the other hand, eDNA assessment's ability to detect distinct community groups across different substrates and cities offers an opportunity to tailor biodiversity monitoring to specific conservation or management goals, depending on which part of the tree, species, site, or city is prioritized.

Given the small sample size, conclusions remain preliminary, underscoring the need for additional studies to refine and validate the use of eDNA for doing meaningful biodiversity in the UPF. Beyond its role in biodiversity assessment, eDNA may hold promise for biosecurity applications. In 2017, Paap et al. 2017 reframed the role of urban trees from potential vectors of pests to proactive sentinels by recognizing their capacity as early-warning systems. In this context, eDNA, combined with comprehensive monitoring, could support the early detection of pests, pathogens, and invasive species that threaten the ecological stability and resilience of our urban forests.

Limitations and scope of future research

This study was constrained in several ways. First, we could focus only on 2 tree species. The patterns of TReM richness and abundance could have differed with more sampling across different seasons and the inclusion of more tree species. Second, while sampling in Karlsruhe met the accumulation curve criteria, the sample size in New Haven may have been insufficient to assess patterns of richness and abundance. This may have limited the statistical power of the analysis, particularly within our eDNA sub-study, which had an even smaller sample size. Third, this study was limited to streets and parks, excluding other UPF site types, such as forests within cities or at their edges. Finally, only two cities with similar ecosystem types were observed, limiting the applicability to broader urban contexts. This study underscores the need for more comprehensive research to clarify the ecological role of species origin (exotic or native) in supporting urban biodiversity. Increasing the representation of species, sample sizes, geographic areas, and sites observed in UPFs will be critical to refining our understanding, but the human and financial resources required for fieldwork, data analyses, and laboratory costs (e.g., eDNA analysis) were limited in this study. Future research should clarify the objectives and practical applications of tools such as TReMs and eDNA in urban forest contexts. For example, more research is needed to connect specific types of microhabitats, like cavities and holes, to the abundance of particular beetle and bird groups, which warrants a larger sample size and wider spatial and temporal replication.

Furthermore, increasing understand around the influence of the built environment on tree health and capacity to provide ecosystem services is crucial to stewarding healthier UPFs. In particular, stress-tolerant exotic trees are often planted in cities as a climate change adaptation measure, but their impact on the biodiversity of native flora and fauna and on ecosystem function warrants more research in UPF. Integrating long-term monitoring and experimental approaches will be essential for developing adaptive management strategies that strengthen urban forests' resilience and ecological function amid intense climate and biodiversity crises.

Conclusion

In this study, we observed two popular urban tree species: one, a fast-growing, widespread generalist, and the other, a long-living and late-successional species. We found that species-level differences, tree stress symptoms, and tree size are crucial factors in explaining patterns in both biodiversity metrics.

Furthermore, our results highlight the importance of refining biodiversity monitoring efforts.

We call for further research to identify the most effective ways to support the habitat function of urban trees, and we conclude that retaining large trees should be a priority for biodiversity provision, as well as for other benefits related to climate regulation and human well-being.

CRediT authorship contribution statement

Anastasia Rubio: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sara Pardal:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis. **Daniel Pinto-Neves:** Writing – review & editing, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Ferdinand Betting:** Writing – review & editing, Project administration, Investigation, Data curation. **Zoe Petridis:** Writing – review & editing, Project administration, Methodology, Investigation, Data curation. **Tamalika Chakraborty:** Writing – review & editing, Methodology, Conceptualization. **Mario Köhler:** Writing – review & editing, Resources, Data curation. **Aaron Peter:** Writing – review & editing, Investigation, Data curation. **Somidh Saha:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflicts of interest. The Karlsruhe City Horticulture Office and the Urban Resources Initiative at Yale University granted permission for fieldwork in Karlsruhe and New Haven, respectively.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.baae.2026.06.001.

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