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Urban airborne allergenic pollen shows highly local patterns correlated with public green spaces

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ABSTRACT

Ongoing climate change is increasing global temperatures, with cities experiencing amplified warming due to the urban heat island effect. Urban trees, forbs, and grasses are widely planted to mitigate heating. However, many plant species produce allergenic pollen, causing hay fever symptoms that can discourage people from using green spaces. This avoidance may increase heat exposure and reduce the physical and mental benefits of urban greenery. Despite this, optimal urban green design that minimizes allergy risks remains unclear, partly due to limited knowledge of local airborne pollen dispersion in urban heat islands. To address this gap, we investigated whether (1) local vegetation influences urban pollen concentrations and composition, (2) urban trees, forbs, or grasses contribute most to allergenic pollen loads, and (3) public or private urban spaces are the main source of airborne allergenic pollen. We conducted spatial and temporal street-level pollen monitoring at 10 sites across two neighborhoods in Leiden, the Netherlands, over 12 months. Samples were analyzed using DNA metabarcoding and microscopy. We identified 366 plant species across 105 families. Results show that public green spaces are the primary source of allergenic pollen in cities. Grasses dominated pollen loads in summer, whereas trees contributed most in winter, spring, and autumn. Notably, allergenic tree pollen was detected well beyond peak flowering periods. These findings highlight the importance for critically selecting urban tree species and the use of more non-allergenic forbs and less grasses when designing healthy urban green infrastructure.

1. Introduction

Climate change is driving a rise in average air temperatures and frequency, intensity and duration of heatwaves [1]. These effects are amplified in cities, which are typically warmer than surrounding rural areas due to the urban heat island effect [2–4]. Impermeable surfaces such as concrete, asphalt and bare stone absorb heat and release it slowly at night, leading to persistently elevated urban temperatures [5]. As a result, urban heat poses serious health risks, particularly for vulnerable populations, including elderly people and individuals with pre-existing health conditions [6,7].

Urban greening is widely promoted as a mitigation strategy, as trees, shrubs and grasses provide cooling through shade, evapotranspiration, and reflection, thereby improving the urban microclimate [8–11]. When

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flowering, wind-pollinated plant species release pollen into the air, and exposure to certain species of pollen can trigger allergic rhinitis, commonly known as hay fever, in sensitized individuals.

Urban climatic conditions can directly influence pollen dynamics by advancing flowering phenology, increasing pollen production, and prolonging pollen seasons [12]. In the Netherlands, the prevalence of self-reported allergies increases with the degree of urbanization of one's living environment [13], highlighting the potential impact of urban pollen exposure on health and allergy prevalence.

Allergy symptoms can be so severe that sensitized individuals avoid urban green spaces during pollen seasons, thereby increasing their heat exposure [14,15], or remain indoors, reducing the physical and mental benefits of urban green areas [16–18]. This trade-off underscores the need for strategically designed, hypoallergenic urban green infrastructure. While avoiding highly allergenic, wind-pollinated species is a fundamental principle, effective mitigation strategies remain limited by poor understanding of pollen dispersal and composition within cities. A recent survey in the Netherlands revealed that only 7% of municipalities systematically consider hay fever when selecting tree species for urban greening [19]. Multiple municipalities indicated that they assume pollen exposure is not strongly localized, and therefore believe that limiting allergenic trees in high-traffic areas would have little impact on hay fever symptoms.

Recent studies have shown that a high abundance of allergenic trees in urban areas may lead to elevated concentrations of airborne allergenic pollen [20,21]. Moreover, global warming and the increase in carbon dioxide concentrations are driving earlier onset of flowering [22,23], and an extension and intensification of flowering periods, which increases allergenic pollen concentrations in urban heat islands [21,22]. It is generally recommended that urban green space planning should consider both planting more non-allergenic trees and improving public pollen information and warning systems [24,25]. However, determining which species can reliably be classified as non-allergenic is not straightforward, as allergenicity assessments vary regionally.

Sousa-Silva et al. [26] showed that the classification of pollen allergenicity varies across different regions of the world. To accurately assess the allergenicity of local plant species, it is essential to follow regional guidelines. However, many of these guidelines are based on expert opinion rather than robust empirical data, and often lack support from scientific evidence. They should instead be based on local parameters such as regional pollen production and population sensitization patterns, which can vary across different climates [27]. Improving allergy risk assessments requires not only better allergenicity classification, but also more accurate and spatially resolved data on actual pollen exposure.

Current prediction models for airborne pollen exposure risk are primarily based on regional monitoring data collected using Hirst-type pollen and spore traps [28]. However, reliance on a single pollen monitoring station is insufficient to characterize daily local pollen exposure [29], as pollen concentrations are distributed unevenly at the street level within urban environments [14,30]. Furthermore, pollen is detected weeks earlier at street- compared to rooftop level [30]. As a result, regional or rooftop may not capture all spatial and temporal human exposure risk. In contrast, street-level monitoring can provide a more accurate representation of local exposure dynamics. Beyond improving exposure estimates, street-level monitoring can also help identify the primary sources of allergenic pollen by distinguishing contributions from public spaces such as streets and parks and from private gardens, and by differentiating ornamental plants from wild flora [31]. This information is critical for determining which urban domains should be prioritized in redesign efforts to achieve the greatest reduction in allergenic pollen exposure risk. Accurate monitoring at street level, however, also depends on reliable and high-resolution pollen identification methods.

Traditionally, pollen is identified and quantified based on morphology using light microscopy. However, manual counting of pollen is time-consuming, labor-intensive, and requires expertise in palynology. In addition, morphological identification often limits taxonomic resolution up to the genus or family level. As an alternative, DNA metabarcoding offers higher taxonomic resolution and enables simultaneous analysis of many samples [32]. This approach has been successfully applied for the identification and semi-quantification of airborne pollen [33–36]. DNA metabarcoding reads can be processed using two common bioinformatic approaches: zero-radius Operational Taxonomic Units (ZOTUs) and Amplicon Sequence Variants (ASVs).

To address the current limitations, we conducted year-round, biweekly street-level monitoring of airborne pollen in the Dutch city of Leiden. By combining traditional light microscopy with DNA metabarcoding, and by processing sequence data using both ZOTU- and ASV-based bioinformatic pipelines, we investigate (i) how airborne pollen disperses through urban areas with different levels of greening throughout the year, (ii) whether species composition and quantities are predominantly local, (iii) which plant groups contribute most to allergenic airborne pollen pressure, and (iv) whether the private or public domain contributes most to local pollen composition in the air in urban areas. This study forms part of a broader project aimed at supporting the design of healthier urban green infrastructure. The insights generated here will serve as input for the development of a decision support system to guide urban planning strategies that balance heat mitigation and allergenic pollen exposure.

2. Material and methods

2.1. Sample collection

For this study we selected two urban localities divided over ten urban sites in Leiden, the Netherlands (Fig. 1). One sampling locality was Jan van Houtkade (52.15368, 4.49162), a relatively green street with many ornamental trees, shrubs, and grasses, hereafter referred to as the 'green street'. Vegetation inventories showed that the sampled street segment contained 39 trees. The other locality was sampled at nine sites (streets) in the 'Noorderkwartier' neighborhood (52.16493, 4.50284) (Fig. S1), a highly urbanized area with limited green infrastructure, hereafter referred to as the 'grey neighborhood'. The sampled streets in this neighborhood contained on average 11 trees despite being of similar length. Photos of the green street and three streets in the grey neighborhood are included in Fig. S2–5. The average perceived temperature on hot summer days was 2 °C higher in the grey neighborhood than in the green street. [37]. Local trees, forbs, and grasses in the two localities were identified using data available from the 'Open bomen kaart' web interface (Bomen Leiden [38]), curated through on-site visits where all flora was identified using the latest edition of the Heukels' Flora [31], and mapped using QGIS Desktop v3.44.1 [39]. Allergenicity of trees in the Netherlands was determined using a regional guide [27]. The number of plant individuals present at 0, 50 and 100 m around the sampling localities was calculated using buffers in QGIS. Spatial data from the Dutch national topographic database were used to distinguish between built-up areas, public spaces, and private gardens.

Between March 2023 and March 2024, airborne pollen samples were collected twice a week. Sampling was carried out at street level using two handheld pollen samplers in parallel, informally referred to as *pollensniffers* [30]. The pollensniffers feature a conical inlet and a sample cartridge holder. A battery-powered ventilator generates an airstream of 8.3–9.2 L min^{-1} into the inlet and past the sample holder. One pollensniffer captured pollen on glass microscope slide coverslips for microscopic analysis, and the other on plastic acetate sheets for DNA metabarcoding analysis, which were both covered with a thin layer of vaseline. At each site, the pollensniffers were operated continuously for 30 min while walking the entire length of the street. A total of 155 coverslips were analyzed microscopically, and 164 acetate sheets were processed for DNA metabarcoding. Although more samples were collected in the field, these subsets were selected for analysis.

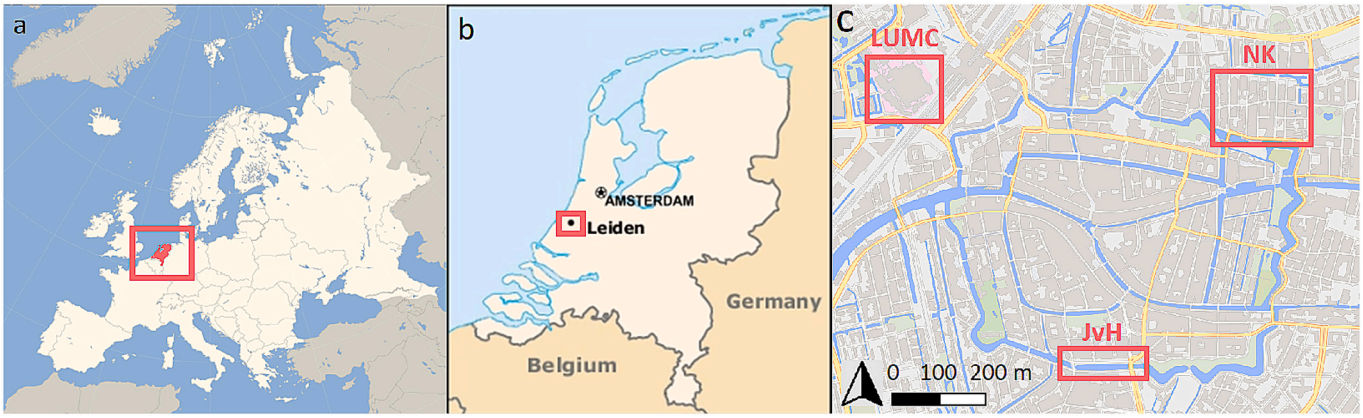

Fig. 1. A. Location of the Netherlands in Europe. B. Location of Leiden in the Netherlands. C. Sampling localities in Leiden: regional monitoring site (LUMC), Noorderkwartier (NK; grey neighborhood) and Jan van Houtkade (JvH; green street). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Microscopy samples with broken cover slips, and DNA samples that failed PCR amplification or with contaminated extraction blanks were excluded from further analysis. Daily regional pollen concentrations were measured at the Leiden University Medical Center using a Hirst-type 7-day volumetric spore trap [28] placed on a rooftop at 20 m height above ground level [22].

Additional targeted sampling was conducted beneath trees of several *Acer* and *Betula* species in Leiden between September and December 2025 to investigate potential late-season pollen presence suggested by initial data exploration. Airborne pollen was collected using the same pollensniffer setup and sampling protocol as described above. This additional campaign resulted in 48 microscopy slides, in which *Acer* and *Betula* pollen were quantified. Detailed sampling locations and dates are provided in Table S1.

2.2. Microscopy

Pollen on the 155 20x20mm glass coverslips collected with the pollensniffers was stained with a 7:6:1 glycerin-water-gelatin mixture containing 2% phenol and 0.002% *w/v* safranin. Coverslips were sealed on glass slides with a clear top coat of long lasting nail polish. A brightfield microscope with a micro raster at 200× magnification was used for the identification and quantification of pollen on the whole surface area of the coverslips. Pollen grains were identified based on morphological characteristics following [40,41].

2.3. DNA metabarcoding

2.3.1. DNA extraction, amplification, and sequencing

DNA was extracted from pollen collected on acetate sheets in a dedicated clean room using strict contamination-prevention measures. Pollen exines were disrupted by bead beating, followed by SDS incubation and DNA extraction with the QIAamp DNA Mini Kit. Extraction blanks were included and pooled during PCR. A two-step PCR protocol was used to amplify the nrITS2 region with plant-specific primers, generating dual-indexed Illumina libraries. Both negative and positive controls were included in a Latin square design. PCR products were cleaned with AMPure XP beads, indexed in a second PCR, quantified, equimolarly pooled, quality checked, and sequenced on an Illumina MiSeq (2 × 300 bp). Supplementary technical details are included in Appendix I.

2.3.2. Bioinformatics and filtering

Sequencing data were processed using two independent pipelines: a custom Galaxy-based workflow producing ZOTUs (UNOISE2) and a DADA2 workflow producing ASVs. Reads were merged, primer-trimmed, quality filtered, denoised, and chimera-checked. Taxonomic assignment was performed using MegaBLAST against an NCBI ITS database, applying identity thresholds of 97% (species), 95% (genus), and 90% (family), with lowest common ancestor assignment where needed. Tag-jumping was estimated using negative controls and corrected (0.3% per sequence variant). Additional filtering removed contaminants, low-abundance sequences, and non-target taxa, followed by LULU clustering to merge erroneous variants while retaining closely related taxa. Supplementary technical details are included in Appendix I.

2.4. Data analysis

Rarefaction curves were generated using the vegan package in R [42] to analyze whether sequencing depth was sufficient to reveal the majority of biological diversity. Differences in total airborne pollen concentrations of trees, forbs, and grasses between the green street and the grey streets detected with microscopy were tested using Wilcoxon signed-rank tests paired on sampling moment in base R version 4.2.3 [43]. Local pollen counts were compared to regional pollen monitoring data collected with the rooftop mounted Hirst-type sampler at LUMC. Pollensniffer samples from the green street and grey neighborhood sites were collected at street level over 30 min at a flow rate of 9 L min^{-1}, whereas regional measurements at the Leiden monitoring station were obtained using a Hirst-type volumetric sampler operating continuously for 24 h at 10 L min^{-1} at 30 m altitude. To facilitate graphical comparison between local Pollensniffer samples and regional Hirst-type measurements, pollen counts were converted to airborne pollen concentrations (pollen m^{-3}) by standardizing counts to the sampled air volume. Because Pollensniffers and Hirst-type samplers differ in sampling efficiency and sampling regime, these concentrations should be interpreted as approximate rather than directly equivalent measures of airborne pollen abundance. Monthly averages, ranges, and sample sizes were subsequently calculated from the normalized concentration values. Paired sample comparisons were made of airborne pollen concentrations of moderately to highly allergenic tree genera, as well as for selected genera (*Platanus* and *Sambucus*) that were abundant in the local flora and exhibited distinct, well-defined flowering periods during the sampled timeframe.

nrITS2 read counts were converted to Relative Read Abundances (RRA) in Microsoft Excel in order to compare them to the relative abundances of microscopic pollen counts on whole cover slips. RRAs represent the proportion of reads for each taxon present in a sample out of the total reads in a sample. To determine the taxonomic resolution of each bioinformatics pipeline, we compared the total number of unique species identified by each pipeline.

Semi-quantification using DNA reads was performed by regressing RRA values against relative abundance of microscopic pollen counts using least squares regression of the lm function in base R (v4.2.3; Team, R. Core, 2013). Regressions were performed for all taxa identified using microscopic analysis, adopting the maximum taxonomic level achieved using this method.

Finally, to visualize the (dis)similarity of the pollen identifications and quantifications in samples from the green street and grey neighborhood during different seasons, the Bray-Curtis dissimilarity index was calculated using the RRA values of nrITS2 between each pair of samples using the vegdist function of the vegan package in R [42]. These values were ordinated using Principal Coordinate Analyses (PCoA) and visualized with the ordiplot function in vegan, grouped per locality and per season. The statistical significance of the differences between these variables was tested using a permutational multivariate analysis of variance (perMANOVA) with 999 permutations, using the adonis function in vegan.

2.5. Assessment of pollen origin from public and private green spaces

To evaluate whether airborne pollen originated primarily from public or private spaces, we combined plant observations in private gardens with taxonomic identifications obtained through nrITS2 metabarcoding of airborne pollen samples.

Plant composition in private residential gardens within the Noorderkwartier study area was assessed using publicly available photographs of gardens from real estate advertisements. The study area comprised 874 residential addresses, of which approximately half included private gardens. Of these houses, 23% were owner-occupied, while the remainder were rental properties. A total of 67 individual plants, representing 34 unique taxa identified at family, genus, or species level, were observed and recorded across 23 private gardens that were considered representative of typical private garden flora within the study area.

Plant taxa observed in gardens and detected with nrITS2 metabarcoding were classified as predominantly wild or ornamental based on their most likely ecological status in urban environments. Floristic guides and reference databases were consulted for classification based on the probable source of pollen. *Heukels' Flora van Nederland* [31] and the *Flora van Nederland* database were consulted to identify taxa known to occur spontaneously in the Netherlands, whereas the *Dutch vascular plant checklist* [44] was used to confirm native status. The Global Register of Introduced and Invasive Species (GRIIS) database [45] was consulted to identify non-native taxa. GRIIS provides curated, country-level information on species reported as introduced or invasive, based on expert review and published sources. Taxa were classified as wild when they are regularly reported outside cultivation in urban settings, even if they are also cultivated. Taxa primarily confined to managed plantings and lacking evidence of frequent spontaneous occurrence were classified as ornamental. For taxa known to occur both as wild and as cultivated ornamentals, classification was based on their predominant local ecological or horticultural context. Taxa with putative ambiguous status and the rationale for their classification are provided in Table S2.

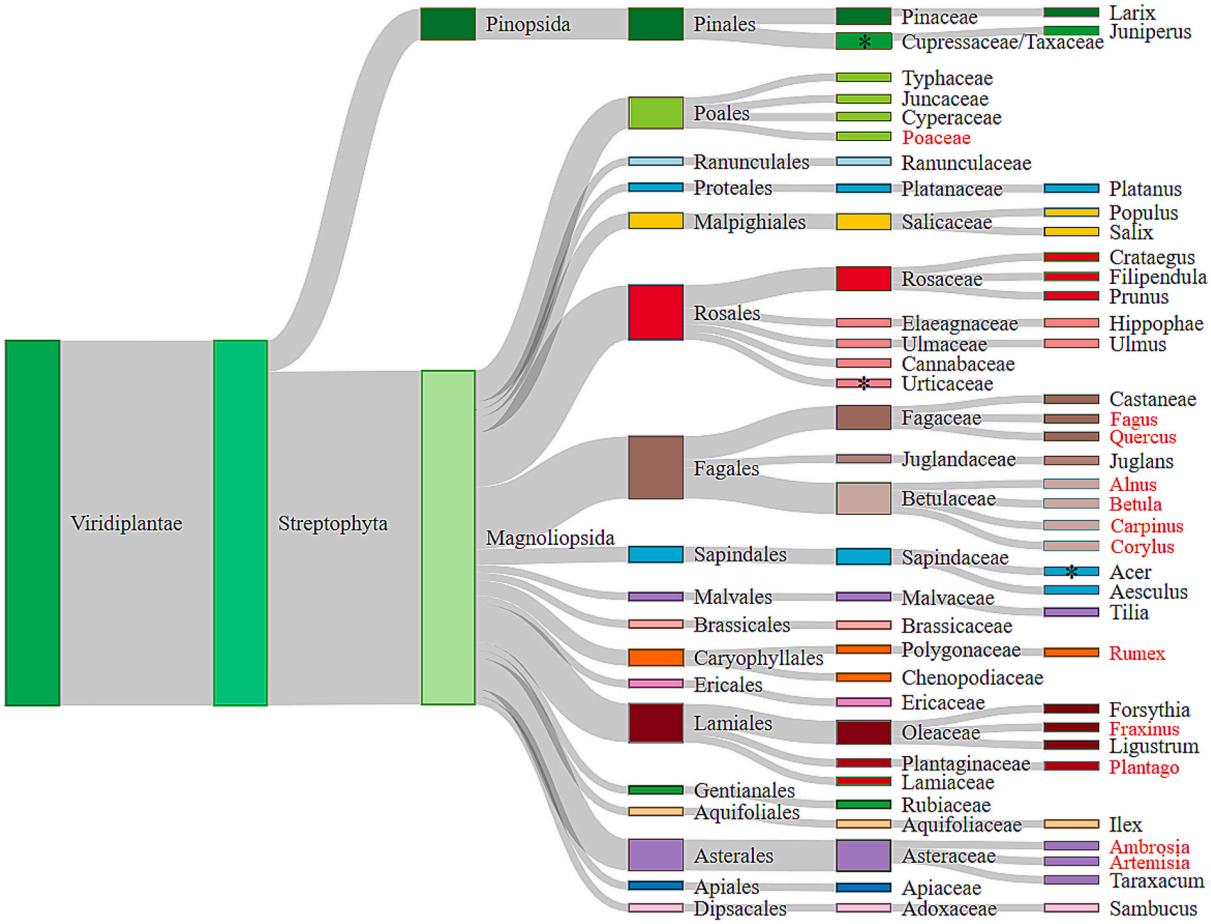

Fig. 2. Sankey plot of results of morphological pollen analyses using microscopy. Red names indicate moderately to highly allergenic taxa. Asterisks indicate taxa that include both allergenic and non-allergenic species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Microscopic identification and quantification

A total of 37,738 pollen grains were identified and quantified based on morphology in 74 microscope slides from the green street, representing 28 genera across 30 families. In the grey neighborhood, 22,909 pollen grains were identified and quantified from 81 slides, representing 29 genera across 27 families. In total, 31 genera from 31 families were identified across both localities, as some pollen types were identified only to the family level (Fig. 2). None of the pollen grains were identified to the species level. Samples from the green street contained on average more pollen grains than those from the grey neighborhood. The number of families slightly exceeded the number of genera because some pollen types could only be assigned to the family level due to morphological similarity among genera.

The taxa *Hippophae* (sea buckthorn), *Ilex* (holly), Juncaceae (rush family), and Rubiaceae (bedstraw family) were uniquely detected at the green street using microscopy. The taxa *Forsythia* (Easter tree), *Prunus* (cherry), *Taraxacum* (dandelion), Rosaceae (rose family) and Typhaceae (cattail family) were uniquely detected in the grey neighborhood.

Based on monthly average pollen concentrations, peak pollen season for both localities ranged from March to August, consistent with spring and summer months when most plants disperse pollen (Fig. 3). Airborne pollen concentrations were low at both localities in winter from October to January, and also in July. Pollen concentrations were consistently higher at the green street compared to the grey neighborhood from March until December, with the largest differences occurring from March until May (Fig. 4). A Wilcoxon signed-rank test of log-transformed pollen counts paired on sampling moment indicated that pollen concentrations in the grey streets were 36% lower than in the green street ($p < 0.0001$). A difference plot of log-transformed pollen counts is included in Fig. S7. Regional pollen concentrations showed a similar pattern to street-level measurements during spring. However, street-level concentrations exceeded regional concentrations during summer and winter, with Urticaceae comprising most of the detected pollen during these periods. Street-level monitoring further revealed substantial daily fluctuations in pollen abundance among neighborhoods, as well as clear differences compared to regional monitoring data (Fig. S9). Figures based on the original, unnormalized pollen counts are provided in the Supplementary Material (Fig. S6 and S8) and show patterns similar to those observed for normalized pollen concentrations.

A total of 15 out of 35 identified taxa were moderately to highly allergenic. The percentage of airborne pollen being allergenic was highest in winter and early spring for both localities, and lowest during late summer and early autumn (Fig. 5). Percentages from November until December are based on low average pollen concentrations. High allergenicity percentages in January and February were mainly due to *Alnus* (alder) pollen. In March, November, and December, the proportion of allergenic pollen was higher at the green street compared to the grey neighborhood. The opposite was the case from April until October. Higher pollen allergenicity percentages at the green street in March were mainly due to high *Betula* (birch) and Cupressaceae/Taxaceae (cypress and yew family) concentrations. Higher pollen allergenicity percentages in the grey neighborhood from May through July were mainly due to high *Sambucus* (elderberry) and Urticaceae (nettle) pollen concentrations at the green street. Higher allergenicity percentages at the green street in November and December were mainly due to Poaceae, *Artemisia* (wormwood), and *Alnus* pollen. Overall, 32.7% of the total sampled yearly pollen load at the green street was allergenic, whereas this was 41.4% in the grey neighborhood. At the regional monitoring station, a notable 58.6% of the sampled pollen load was allergenic.

Trees were the main sources of airborne allergenic pollen in winter and spring, and grasses and forbs in summer (Fig. 6). Pollen of trees, grasses, and forbs was observed in very small quantities from October until December after the main flowering period. Tree and forb pollen concentrations were higher at the green street than at the grey neighborhood, whereas grass pollen concentrations were similar between sites. Grass pollen concentrations were low in both localities throughout the sampling period.

3.2. DNA metabarcoding

Illumina sequencing of 164 nrITS2 samples resulted in 19.7 M read-pairs. After quality filtering, merging, correction for equimolar pooling of negative controls, and bioinformatics post-processing, 12.1 M read-pairs remained. Per sample read abundance was 60,858 ± 6365. Six samples were discarded because they did not yield any reads. Rarefaction curves (Fig. S10 and S11) showed that most samples approached an

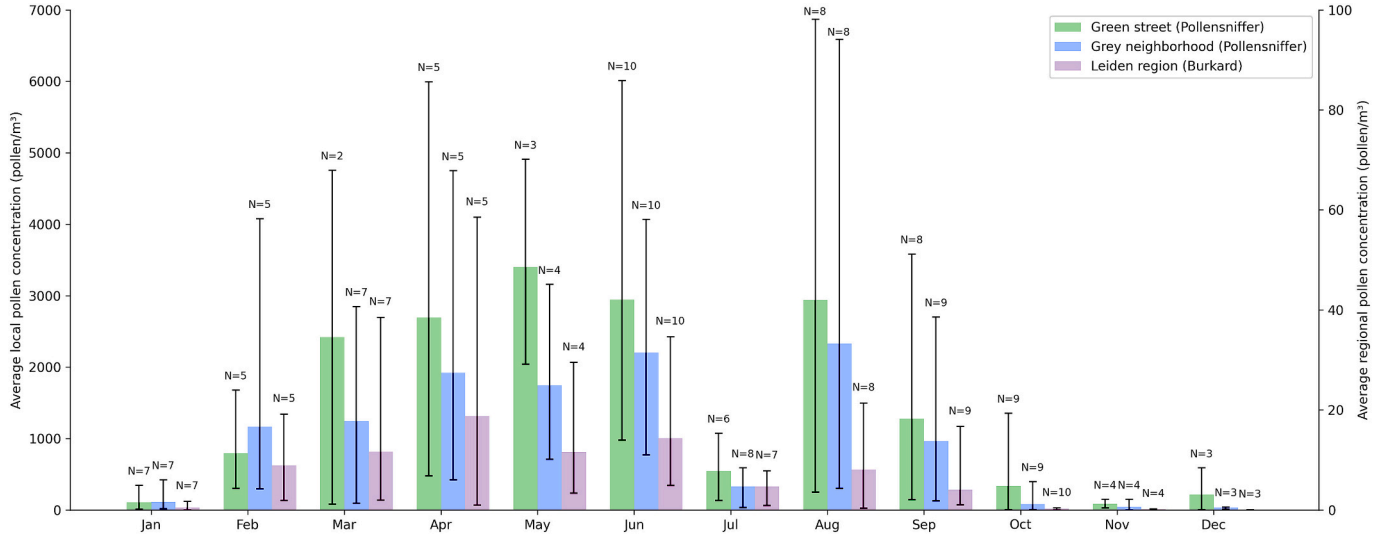

Fig. 3. Average monthly pollen concentrations at the green street (green bars), the grey neighborhood (blue bars), and the regional Leiden monitoring station (purple bars), measured from March 2023 until March 2024. Pollen counts were standardized to sampled air volume and expressed as airborne pollen concentrations (pollen m^{-3}) to facilitate comparison between Pollensniffer and Burkard (Hirst-type) sampler measurements. Vertical black lines indicate the range of the monthly pollen concentrations. Sample sizes are noted above the range bars. Regional concentrations were included only for days when Pollensniffer data were collected. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

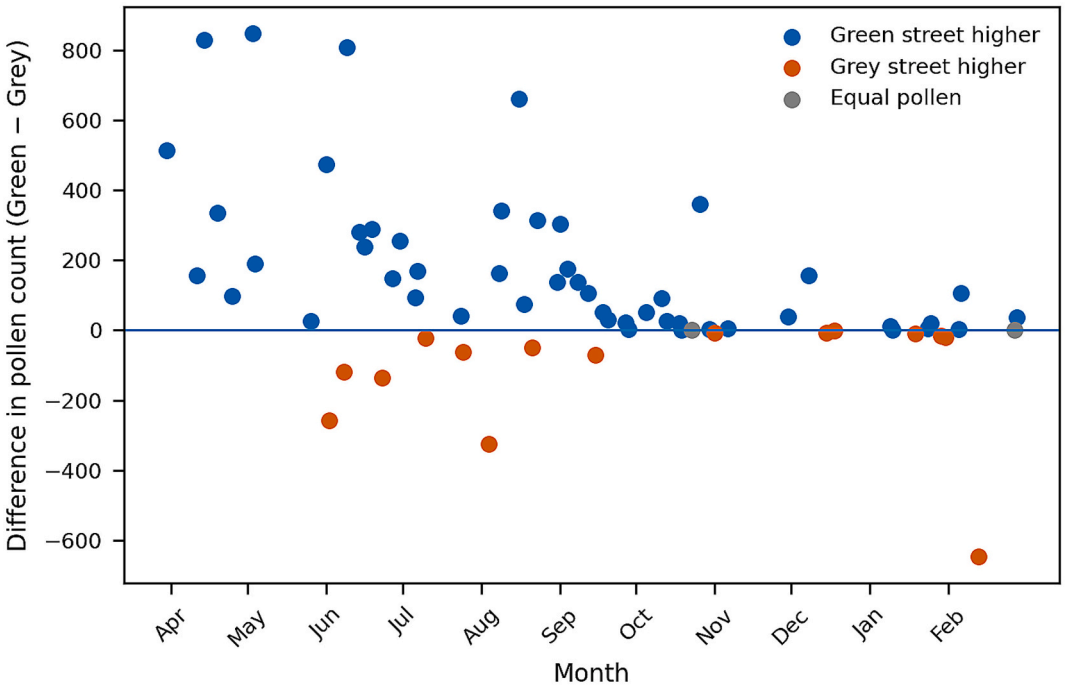

Fig. 4. Difference plot of pollen counts between the green street and grey streets paired on sampling moment in Leiden between March 2023 and March 2024. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

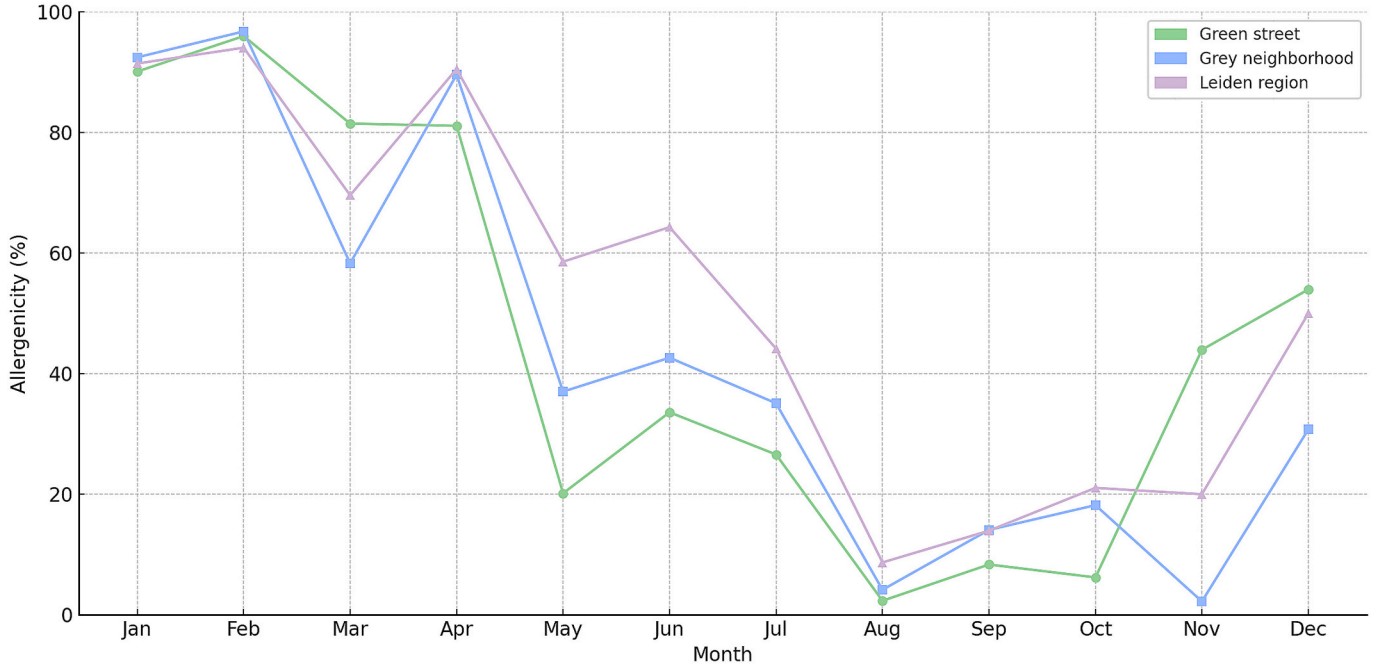

Fig. 5. Line graph showing the monthly proportion of allergenic pollen in the total pollen load at the green street (green line), the grey neighborhood (blue line), and the regional monitoring station (purple line) measured from March 2023 until March 2024. Pollen samples were analyzed using microscopy. Note that average pollen concentrations were low in November, December and January. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

asymptote at their current sequencing depth, suggesting that the sequencing effort was sufficient to capture the majority of the detectable biodiversity. Mean GC-content of nrITS2 amplicons was 59.87 ± 5.7%.

The ZOTU pipeline identified 233 species across 221 genera and 76 families, whereas the ASV pipeline identified 322 species across 216 genera and 70 families. Families identified uniquely by the ZOTU pipeline were Actinidiaceae (kiwi family), Moringaceae (horseradish tree family), Polemoniaceae (Jacob's ladder family), Potamogetonaceae (pondweed family), Primulaceae (primrose family), and Santalaceae (sandalwood family). The Typhaceae (cattail) family was uniquely identified by the ASV pipeline. At the green street, a total of 172 taxa were uniquely detected with DNA metabarcoding, 3 taxa were uniquely detected with microscopy, and 40 taxa were detected by both methods (Fig. S12). In the grey neighborhood, 169 taxa were uniquely detected

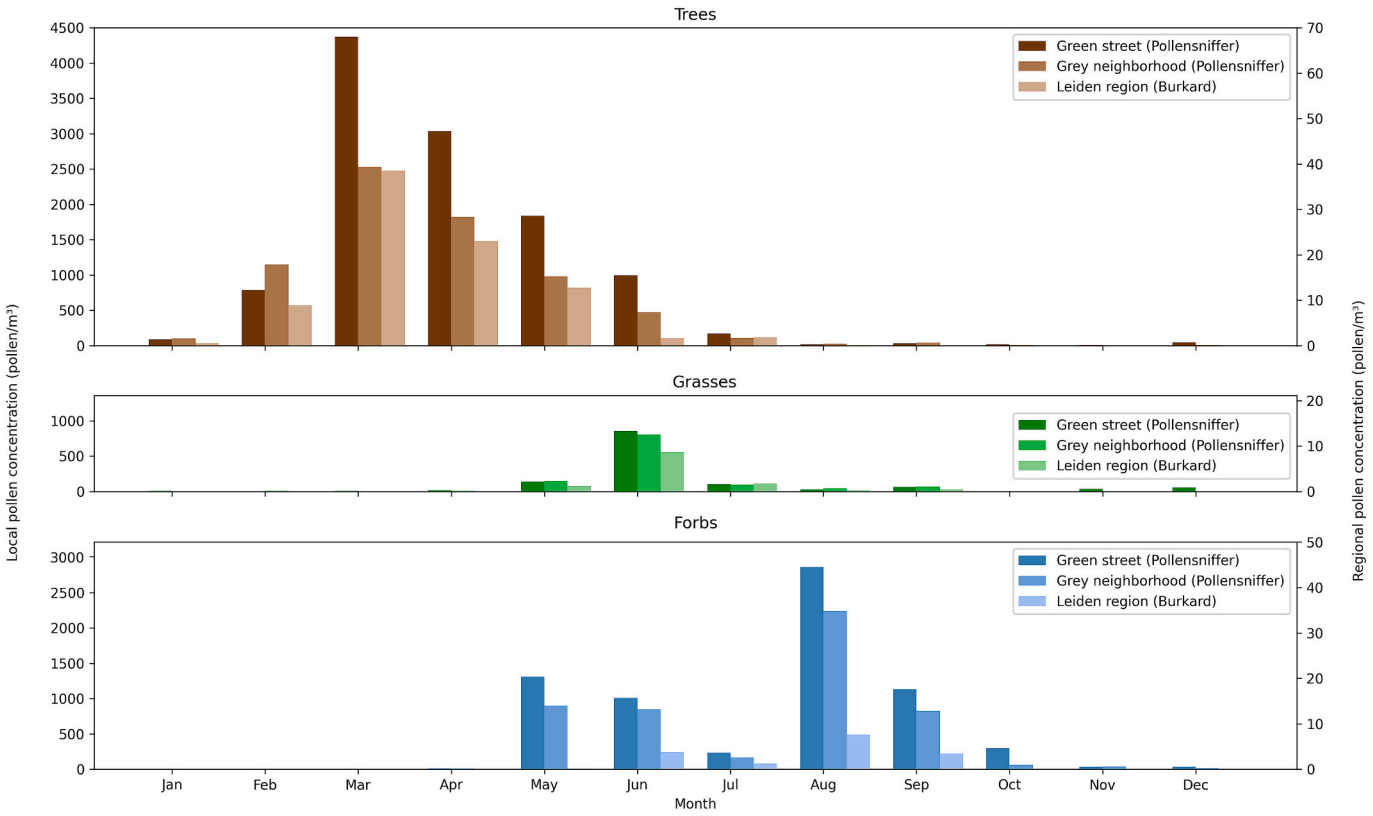

Fig. 6. Average monthly pollen concentrations (pollen m^{-3}) of trees (brown), grasses (green), and forbs (blue) at the green street, the grey neighborhood, and the regional Leiden pollen monitoring station, measured from March 2023 until March 2024 and analyzed using microscopy. Local samples were collected using Pollensniffers and regional samples using a Burkard (Hirst-type) sampler. Pollen counts were standardized to sampled air volume and expressed as airborne pollen concentrations (pollen m^{-3}). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with DNA metabarcoding, 4 taxa were uniquely detected with microscopy, and 41 taxa were detected by both methods. DNA metabarcoding additionally detected 79 taxa exclusive to the green street and 74 taxa exclusive to the grey neighborhood. *Larix* (larch) and *Ligustrum* (privet) were uniquely detected by microscopy in both localities, whereas Juncaceae (rush family) was uniquely detected in the green street and *Forsythia* and Typhaceae were uniquely detected in the grey neighborhood.

3.3. Local pollen signals

A perMANOVA of Bray-Curtis dissimilarities based on RRA from the complete nrITS2 dataset revealed significant differences in pollen composition among samples collected in different seasons but not between sampling locations within Leiden (Fig. S13 and S14). Reads assigned to *Urtica* spp. accounted for 92% of the total variation, reflecting the strong presence of its pollen in both the green and grey localities. *Urtica* (stinging nettles) plants are commonly present along canals in both localities.

Comparisons of airborne pollen concentrations among moderately to highly allergenic tree genera, as well as two non-allergenic genera with substantial airborne presence (*Platanus* and *Sambucus*), revealed genus-specific differences (Table 1; see Table S3 for full numerical results). For *Alnus* and *Quercus*, higher pollen concentrations tended to occur at the site with greater plant abundance, although no statistically significant differences between sites were detected. In contrast, significant associations were found for *Platanus* and *Sambucus*, both of which occurred exclusively along the green street. Despite the higher abundance of *Betula* and *Fraxinus* trees in the grey neighborhood, their pollen concentrations were higher at the green street. We did not record whether all individual plants flowered during the sampling period. Time series of pollen counts for these genera at both localities are shown in Fig. S15.

Table 1
Genus-specific paired comparisons of pollen concentrations between the green street (G.S.) and the grey neighborhood (G.N.) during genus-specific flowering periods between March 2023 and March 2024. Comparisons were performed using Wilcoxon signed-rank tests on paired sampling dates (W stat). The *p*-value corresponds to a one-sided test assessing whether pollen counts were significantly higher at one site than the other. Asterisks indicate significant p-values.

Genus	Individuals within 100 m	Mean pollen counts	W stat	p-value
Alnus	G.S. < G.N.	G.S. < G.N.	11	0.344
Betula	G.S. < G.N.	G.S. > G.N.	18	0.326
Carpinus	G.S. < G.N.	G.S. < G.N.	14	0.531
Corylus	G.S. < G.N.	G.S. = G.N.	16.5	0.417
Fagus	G.S. > G.N.	G.S. > G.N.	26	0.461
Fraxinus	G.S. < G.N.	G.S. > G.N.	12.5	0.273
Platanus	G.S. > G.N.	G.S. > G.N.	12.5	0.001 *
Quercus	G.S. < G.N.	G.S. < G.N.	24	0.360
Sambucus	G.S. > G.N.	G.S. > G.N.	17	< 0.001 *

3.4. Seasonal pollen patterns and regional comparison

Observations of flowering *Betula* spp. (birch) and *Acer* spp. (maple) trees in the Netherlands were reported on national nature platforms from March through October. In contrast, airborne *Betula* spp. pollen was detected by microscopy in our street-level samples until December, extending far beyond its main flowering season in spring, whereas DNA reads of birch pollen were detected until November (Fig. 7). *Acer* spp. pollen was observed microscopically until July and again in September and October, whereas DNA reads were detected in samples collected up

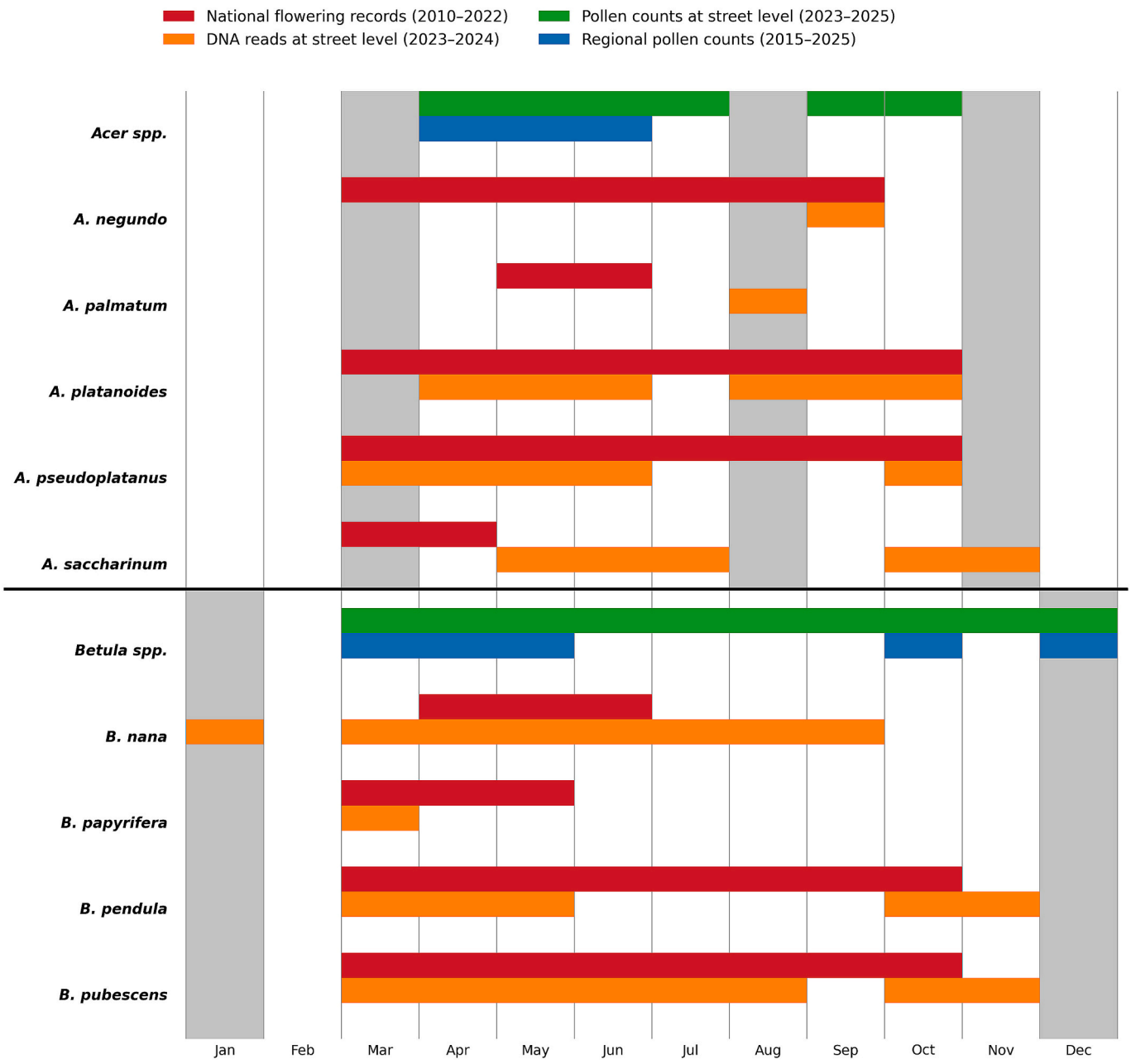

Fig. 7. Seasonal detection of airborne *Acer* and *Betula* pollen at the green street and the grey neighborhood over 12 months across methods, compared with reported national flowering observations [46], Plantenvinder (n.d.)) [47] and regional pollen count records from the past decade. Discrepancies between pollen counts and nrITS2 reads at street level are marked in grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to November. Additional targeted sampling conducted beneath *Acer* and *Betula* trees from September to December further confirmed the presence of airborne pollen grains of both taxa during this period, particularly for *Betula* (Table S1).

Because birches and maples were detected substantially in multiple months outside their flowering season, these genera were analyzed in more detail. The ASV pipeline identified four species of *Betula* (birch) occurring in Leiden: *B. nana, B. papyrifera, B. pendula,* and *B. pubescens*, whereas the ZOTU pipeline identified only one *Betula* species: *B. pendula* (Fig. 8). *B. nana* is an exotic dwarf birch that is cultivated in the Hortus botanicus Leiden. The other *Betula* species are either native (*B. pendula, B. pubescens*) or ornamentals (*B. papyrifera*) and all planted in Leiden in the public domain. The ASV pipeline identified six *Acer* (maple) species: *A. campestre*, *A. negundo*, *A. platanoides*, *A. pseudoplatanus*, *A. palmatum*, and *A. saccharinum*. The ZOTU pipeline identified five *Acer* species: *A. campestre*, *A. negundo*, *A. platanoides*, *A. pseudoplatanus*, and *A. saccharinum*. All these maple species occur in Leiden in the public domain.

3.5. Allergenicity of private and public green

Private gardens covered 21.6% of the total surface area along the green street and 20.8% within the grey neighborhood. Among 67 plants observed across 23 gardens, 45 (67.2%) were classified as ornamental and 20 (32.8%) as wild (Fig. 9). Of the ornamental plants, 6.7% were considered allergenic, predominantly including olive trees and ornamental grasses. In contrast, 40.1% of wild plants were classified as allergenic, largely represented by taxa belonging to the Betulaceae

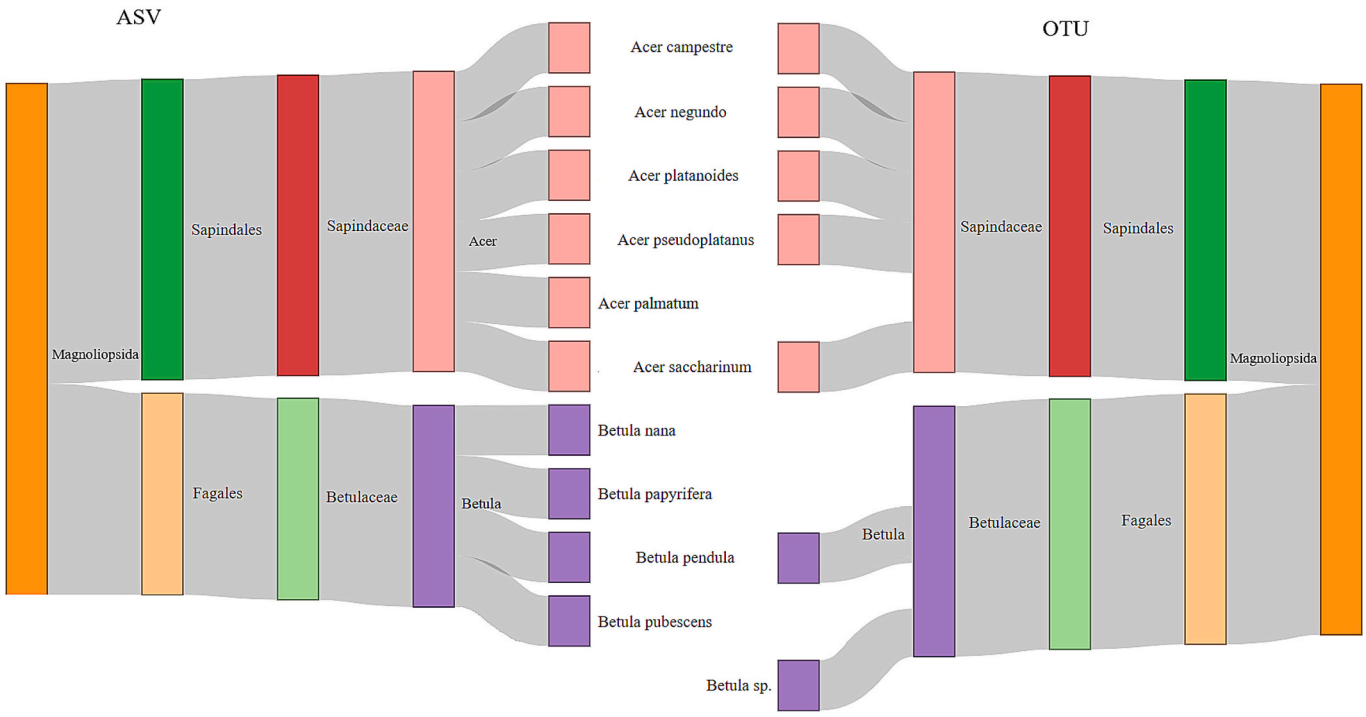

Fig. 8. Sankey plots of *Acer* and *Betula* species detected with DNA barcodes at the green street and the grey neighborhood, analyzed by our bioinformatics pipelines as either Amplicon Sequence Variants (ASVs) or zero-radius Operational Taxonomic Units (ZOTUs). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

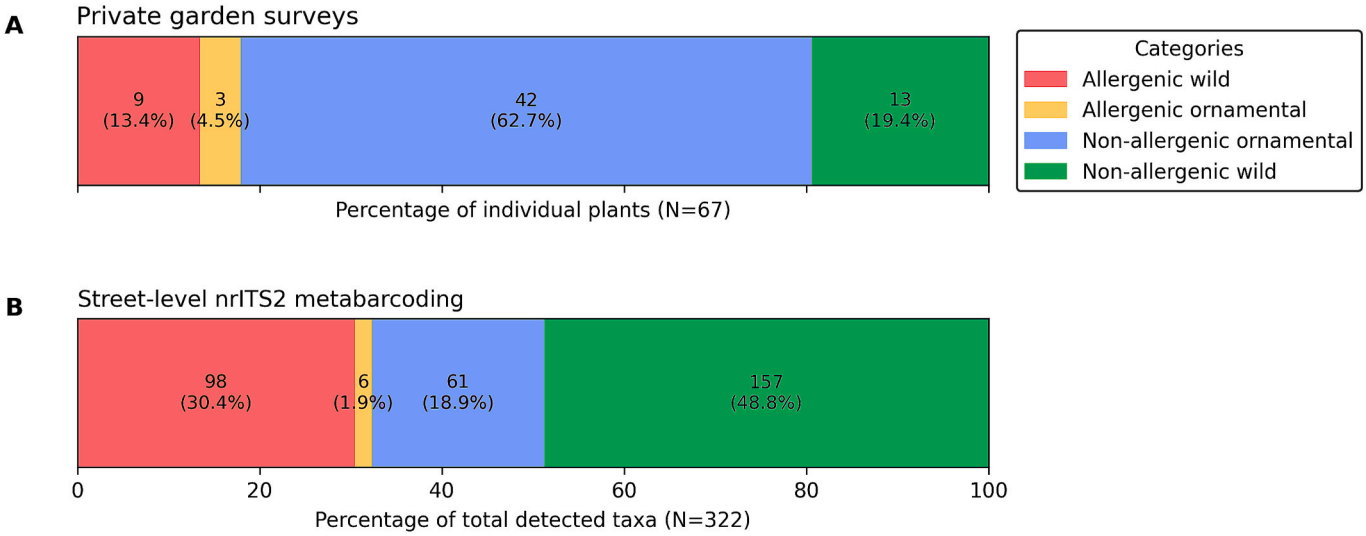

Fig. 9. Stacked bar plots showing plant status and allergenicity in the studied area in Leiden. (A) Individual plants recorded in garden surveys in the grey neighborhood in 2025. (B) Total plant taxa detected using street-level nrITS2 metabarcoding over a 12-month period at the green street and the grey neighborhood. Values within bar segments indicate the number of individuals (A) or detected taxa (B), and their corresponding percentage of the total. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

family. See Table S4 for the full data.

A total of 67 out of the 322 taxa (20.8%) detected with nrITS2 metabarcoding in the study areas were annotated as ornamental plants. A total of 104 taxa (32.3%) were weakly to highly allergenic, of which 6 (5.8%) were ornamental plants. Allergenic ornamental plants detected were *Callitropsis nootkatensis* (Nootka cypress), *Sorghastrum nutans* (Indiangrass), *Morus nigra* (black mulberry), *Miscanthus sinensis* (Chinese silver grass), *Olea* (olive), and *Cortaderia* (pampas grass). None of these species have been observed as wild or rewilding in the Netherlands in recent years and were also not listed as invasive species in the GRIIS database.

Based on relative read abundances, ornamental plants contributed to the airborne allergenic pollen load only during spring and summer (Fig. 10). Their overall contribution was low, with a maximum relative read abundance of 3% in May. The majority of reads originated from street trees, grasses, and forbs present in public spaces.

3.6. Semi-quantification

Alnus (alder), *Betula*, *Castanea* (chestnut), *Fraxinus* (ash), *Sambucus*,

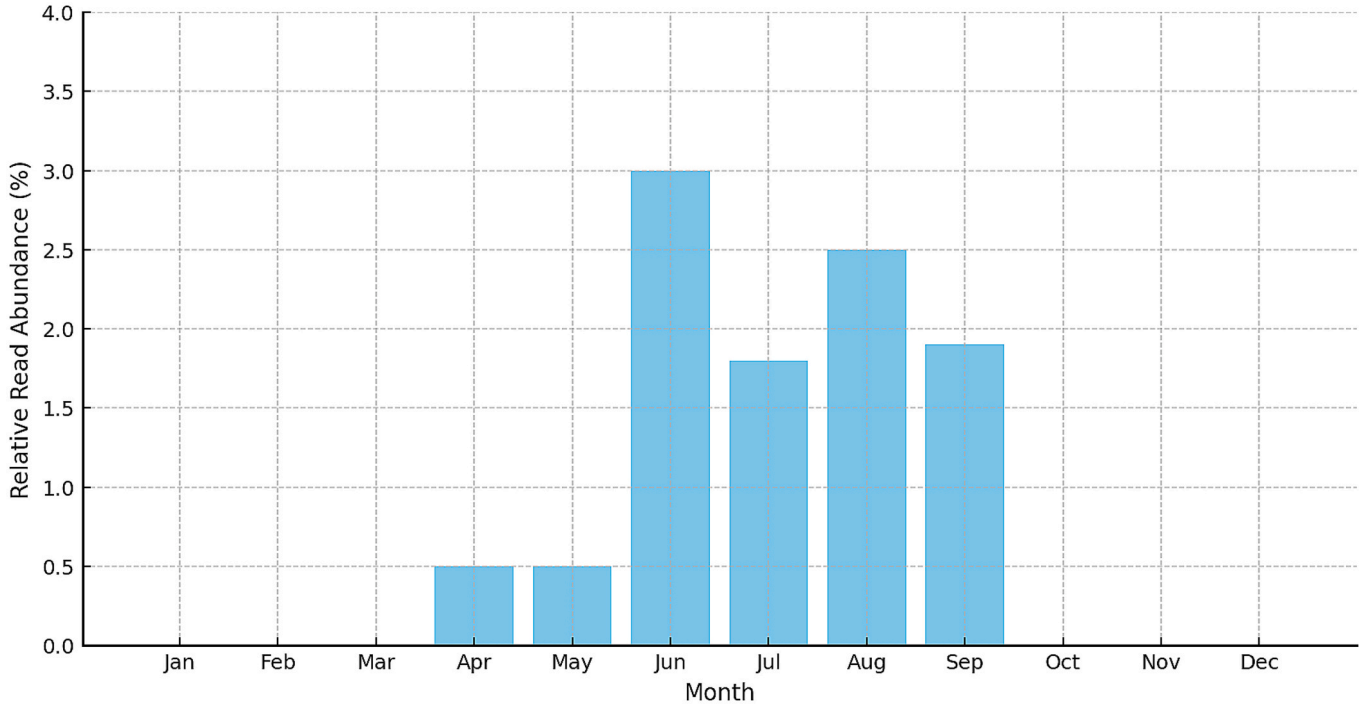

Fig. 10. Bar plot showing monthly relative read abundances of allergenic ornamental plants detected with nrITS2 DNA metabarcoding of air sampled in the grey neighborhood and the green street between March 2023 and March 2024. Scale shown only up to 4%. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and Urticaceae showed a strong and significant correlation between relative DNA read abundances and relative microscopic abundances (Appendix IV). *Carpinus* (hornbeam), Cupressaceae/Taxaceae, *Fagus* (beech), Poaceae, *Quercus* (oak), and *Rumex* (sorrel) showed a weak but significant correlation. *Acer*, *Aesculus* (horse chestnut), *Artemisia*, Asteraceae (daisy family), Brassicaceae (mustard family), Cannabaceae (hemp family), Chenopodiaceae (goosefoot family), Ericaceae (heather family), *Plantago* (plantain), *Platanus* (sycamore), *Populus* (poplar), *Prunus*, Ranunculaceae (buttercup family), *Salix* (willow) and *Ulmus* (elm) showed no significant correlation.

4. Discussion

4.1. Urban pollen exposure is highly local and correlated with public green spaces

Previous studies have shown clear differences in allergenic airborne pollen taxonomic composition during spring, summer, and autumn between cities using microscopy [21,22]. These differences were confirmed for spring and autumn using nrITS2 metabarcoding [36]. Our study examined variation at a finer, intra-urban scale using year-round street-level monitoring by applying both microscopy and DNA metabarcoding. Street-level monitoring previously revealed local variation in airborne pollen using microscopy [30]. With nrITS2 metabarcoding, we found no significant differences in overall pollen composition between urban neighborhoods (Bray-Curtis analysis). However, microscopy revealed that total airborne pollen loads from trees and forbs differed significantly between green and grey urban localities. The higher airborne pollen concentrations in the green street likely reflect the greater density and diversity of local plants, including threes, shrubs and forbs. Vegetation inventories showed substantially higher tree density in the green street compared with the streets in the grey neighborhood, increasing the proximity and abundance of local pollen sources. Grass pollen concentrations were low at both localities, likely due to regular mowing. We also detected differences in the proportion of allergenic pollen taxa and in taxa occurring uniquely at each site. Comparisons between local Pollensniffer measurements and regional Hirst-type monitoring should be interpreted with caution. Although pollen counts were standardized to sampled air volume (pollen m^{-3}), the two sampler types differ in both sampling efficiency and sampling regime. Previous work showed that Pollensniffers capture more pollen per sampled air volume than Hirst-type samplers [30], and Pollensniffers sampled for 30 min during daytime whereas the Hirst-type sampler operated continuously over 24 h. Consequently, normalized concentrations provide an approximate rather than directly equivalent comparison between local and regional pollen measurements. These results suggest that although urban airborne pollen composition is broadly similar across neighborhoods, factors such as design and management of local public green spaces strongly influence urban airborne allergenic pollen abundance. In addition to plant composition and abundance, urban morphology, such as street canyons, building layout, and street orientation, may also influence local pollen dispersion patterns through its effects on airflow and turbulence [48], although these processes were not explicitly investigated in this study.

In terms of taxonomic resolution, nrITS2 metabarcoding enabled identification to finer taxonomic levels than light microscopy, which was generally limited to the genus or family level. We compared microscopy with nrITS2 DNA metabarcoding and evaluated the performance of both ZOTU- and ASV-based bioinformatic pipelines. Zero-radius Operational Taxonomic Units (ZOTUs) with standard settings were clustered at 97% read sequence similarity, whereas Amplicon Sequence Variants (ASVs) treat each unique read sequence as a distinct entry. Using the ASV pipeline, we detected a larger number of plant species, including several allergenic taxa present in Leiden that could not be distinguished with the ZOTU pipeline. We therefore recommend using ASVs for nrITS2 metabarcoding of pollen when the research objective requires species-level identification. Importantly, nrITS2 metabarcoding enabled species-level resolution for several taxa, allowing differentiation between ornamental and wild plant species. While ornamental and wild species may occur in both public and private areas, this distinction becomes informative in the context of local flora. Garden surveys indicated that flora in private gardens largely consisted of

ornamental plant species, with relatively few allergenic taxa belonging to species that commonly occur in the wild. Although private gardens occupied a similar proportion of the total surface area in both sampling localities, pollen load and allergenicity differed, suggesting that plants in public spaces are the main sources of allergenic pollen. Classification with nrITS2 metabarcoding further showed that urban allergenic pollen originated predominantly from wild plant species, whereas ornamental plants contributed only marginally. Together, these observations suggest that airborne allergenic pollen detected in this study is more likely associated with publicly managed green spaces than with private gardens. These findings indicate that allergenic pollen exposure at the street level is strongly shaped by the composition and management of publicly maintained urban green infrastructure. This highlights municipal planting design and management as important leverage points for reducing exposure to allergenic pollen. Because airborne pollen may disperse beyond its immediate source area, source attribution in this study should be interpreted as an inference derived from local urban green structure rather than direct spatial tracing of pollen origin. However, the correspondence between local vegetation composition and detected pollen taxa, together with the observed neighborhood-scale differences, suggests that nearby vegetation contributed substantially to local pollen exposure.

Standardized household income percentiles indicated a stable socio-economic contrast between regions. Noorderkwartier remained below the national average (≈40th percentile), whereas Levendaal-West consistently ranked above average (≈56th percentile) throughout 2014–2019 [49]. Accordingly, the grey neighborhood, characterized by a lower socio-economic status than the green street, contained less urban green space and a higher proportion of impervious surfaces. Consequently, heat stress was higher in the grey neighborhood [37]. Visible urban green mitigates heat stress and promotes both physical and mental health [15,50]. The airborne pollen load in the grey neighborhood contained a higher proportion of pollen from allergenic plant species. These findings indicate that heat stress, allergenic pollen exposure, and the health benefits of urban green are unequally distributed, to the disadvantage of residents with lower socioeconomic status.

Many municipalities worldwide are adopting new greening agendas, as research has shown the benefits of urban green spaces on human health, microclimates, and other aspects [50]. Several design guidelines have been developed to help planners optimize these green space benefits. An increasingly popular approach is the 3–30-300 rule, which proposes that every resident should be able to see at least three trees from their home, that 30% of public spaces should have tree canopy cover, and that everyone should live within 300 m of a green space [51]. These initiatives promote planting of more trees, and therefore create opportunities for planners and designers to integrate allergenicity as a design criterion. Changes in public spaces will have the greatest impact, as they cover larger areas and typically contain more allergenic species than private gardens. However, clear and evidence-based guidelines are needed to support such efforts. When implementing urban greening strategies, allergenicity should be carefully considered. Planting highly allergenic trees should be avoided near busy or densely populated areas, as there is a local effect to pollen exposure risk. Large numbers of moderately allergenic species should also be avoided, as well as weakly allergenic trees that may become more allergenic under future climate conditions. Tree species selection should be guided by local allergenicity guidelines based on clinical research [27]. It is also important to take climate change into account, as some endemic species may no longer be able to survive, particularly in urban areas, which tend to be hotter than the surrounding rural areas due to the urban heat island effect [52,53]. In such cases, the use of non-allergenic exotic tree species from slightly warmer regions or paleorelict species that are adapted to higher temperatures should be considered.

Although native tree species typically support higher richness of specialist herbivores, growing evidence indicates that non-native trees can nevertheless provide substantial ecosystem services, particularly in human-modified landscapes. Studies in urban and peri-urban environments have shown that non-native trees can contribute comparably to regulating services such as climate moderation, carbon sequestration, and water interception, and can also support meaningful levels of biodiversity, particularly among generalist arthropods, birds, and microbial communities [54,55].

Previous studies have shown that semi-quantification of airborne grass pollen using DNA metabarcoding correlates to hay fever medication sales [56]. Exposure risk is shaped by allergenic plant abundance and their flowering phenology. Future diachronic studies could further improve interpretation of temporal pollen dynamics by incorporating direct phenological observations of allergenic plant species. Exposure to allergenic grass pollen can be reduced through regular mowing. However, depending on the mowing regime, mowing may harm other biodiversity living in and on grasses [57]. Our results suggest that mowing strategies could be more effectively targeted based on the presence and phenology of allergenic plant taxa. In areas where grasses and allergenic forbs are absent or rare, intensive mowing aimed at reducing pollen exposure may be unnecessary. In contrast, in locations dominated by abundant allergenic plants, aligning mowing schedules with shifting flowering phenology, rather than applying fixed schedules, could help reduce pollen release. Where feasible, replacing grass lawns with mixtures of non-allergenic or insect-pollinated forbs may further reduce pollen exposure risk while simultaneously supporting biodiversity.

4.2. Extended allergenic pollen season

Our street-level sampling results revealed substantial late-season presence of airborne pollen and plant DNA during autumn of species that typically flower in spring. To further evaluate whether late-season DNA detections could plausibly originate from pollen, we conducted targeted pollensniffer sampling beneath *Acer* and *Betula* trees during autumn. The results show that allergenic airborne pollen can be present locally outside the main flowering season and can therefore be a plausible source of the DNA reads detected during this period. These patterns may be consistent with secondary flowering events. However, these observations should be interpreted with caution, as airborne pollen and DNA alone do not allow us to distinguish active male flowering from other sources, such as pollen persistence in the local environment, wind-driven resuspension, or contributions to airborne DNA from fruit-bearing individuals or resuspended plant material.

Secondary flowering has been increasingly documented for several insect-pollinated species, including *Acer pseudoplatanus, Acer platanoides, Berberis eurybracteata, Magnolia kobus, Prunus serrulata, Rhododendron* spp. and *Viburnum rhytidophyllum* (Table S5). In contrast, reports of secondary flowering in wind-pollinated plants remain rare [58]. Nevertheless, accumulating evidence suggests that this phenomenon also occurs in wind-pollinated trees. Sporadic out-of-season flowering has been observed for *Betula* spp. and *Acer negundo*, and second blooms have been observed for *Quercus ilex* (holm oak) in Spain [58]. Although these second blooms were documented under warmer climatic conditions than those currently prevailing in the Netherlands, ongoing climate warming may increase the likelihood of similar events occurring in northwestern Europe. In addition, several wind-pollinated genera, including *Alnus*, *Corylus* and *Quercus,* show climate-driven shifts in flowering phenology according to national nature observation platforms [59], further highlighting the sensitivity of wind-pollinated trees to changing climatic conditions.

Resolving the origin of late-season airborne pollen signals will require integrated approaches that combine under-tree pollen sampling, DNA metabarcoding of the same samples, and direct phenological monitoring of male flowers. Such coordinated observations would allow more robust discrimination between active flowering, pollen carryover effects, and environmental redistribution processes. If secondary flowering in wind-pollinated allergenic species were to be confirmed, this

would have important implications for public health. A secondary flowering period could substantially extend the pollen season, potential allergen exposure and the duration of allergy symptoms among sensitive individuals.

These findings provide key input for the development of a decision support system aimed at supporting municipalities in designing urban green infrastructure that balances climate adaptation benefits with the reduction of allergenic pollen exposure.

5. Conclusions

In this study we combined traditional microscopy and DNA metabarcoding to quantify and identify street-level urban airborne pollen. DNA metabarcoding provided higher taxonomic resolution than microscopy, enabling more precise identification of airborne pollen taxa and a detailed characterization of their community composition. Our results demonstrate that airborne pollen concentrations are highly localized, with significant differences observed between neighborhoods within a city. Although variations in pollen composition between individual streets were less pronounced than those between cities, local plants strongly influence both total and allergenic pollen concentrations in urban air. Street-level monitoring further revealed substantial daily fluctuations in pollen abundance among neighborhoods, as well as clear differences compared to regional monitoring data. Using microscopy and nrITS2 metabarcoding, we detected earlier and prolonged flowering periods, and airborne allergenic tree pollen far outside the main flowering season. Urban trees are the main sources of local airborne allergenic pollen in winter, spring and autumn, and roadside grasses in summer. Our combined garden observations and metabarcoding results indicate that allergenic airborne pollen is largely associated with wild plant species, whereas ornamental species prevalent in private gardens appear to make a smaller contribution. Urban planners and landscape designers should therefore prioritize non-allergenic plant species in public green spaces and avoid planting highly allergenic species in densely populated or heavily frequented areas. These findings highlight the importance of selecting non-allergenic plant species to avoid allergenic pollen exposure risk in densely populated or highly frequented urban areas.

CRediT authorship contribution statement

Nemi Dorst: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Maarten Hogeweij:** Writing – review & editing, Investigation, Formal analysis. **Aukje Clercx:** Investigation, Formal analysis. **Dominique de Boer:** Writing – review & editing, Investigation, Formal analysis. **Ilse Thijssen:** Writing – review & editing, Investigation, Formal analysis. **Leon van Leeuwen:** Investigation, Formal analysis. **Martijn van de Velde:** Investigation, Formal analysis. **Arjen G.C.L. Speksnijder:** Writing – review & editing, Supervision, Funding acquisition. **Gijs M. Gerrits:** Writing – review & editing, Validation. **Laurens Sparrius:** Writing – review & editing, Resources. **Letty A. de Weger:** Writing – review & editing, Resources. **Kevin Raaphorst:** Writing – review & editing, Supervision, Funding acquisition. **Marcel Polling:** Writing – review & editing, Supervision. **Barbara Gravendeel:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cacint.2026.100434.

Data availability

Data will be made available on request.

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