Science of the Total Environment 922 (2024) 171306

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Evaluation of *Orthotrichum lyellii* moss as a biomonitor of diesel exhaust

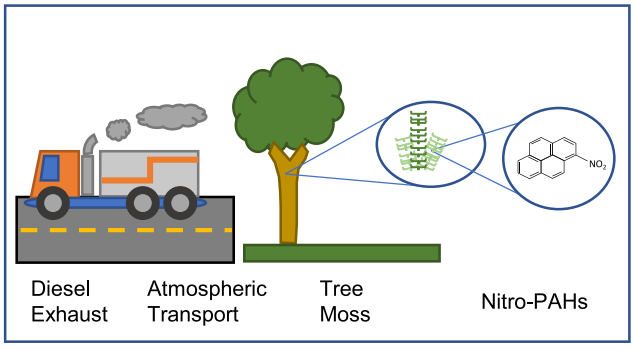
Christopher Zuidema [a,b], Michael Paulsen [a], Christopher D. Simpson [a], Sarah E. Jovan [c,*]

[a] Department of Environmental and Occupational Health Sciences, University of Washington, 4225 Roosevelt Way NE, Seattle, WA 98105, USA
[b] Pacific Northwest Research Station, USDA Forest Service, 400 N 34th St., Seattle, WA 98103, USA
[c] Pacific Northwest Research Station, USDA Forest Service, 1220 SW 3rd Ave., Suite 1410, Portland, OR 97204, USA

HIGHLIGHTS

- Diesel exhaust is an important urban air pollutant and poses risk to human health.
- Nitrated polycyclic aromatic hydrocarbons can be used as markers of diesel exhaust.
- Tree moss takes up pollutants in the atmosphere, including nitro-PAHs.
- Nitro-PAH concentrations in moss are correlated to greenness and roadways.
- *Orthotrichum lyellii* moss can be used as a biomonitor of diesel exhaust.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Pavlos Kassomenos

Keywords:
Epiphytic moss
Bioindicator
Nitrated polycyclic aromatic hydrocarbons
Air toxics
Traffic-related air pollution
Urban air pollution

ABSTRACT

Exhaust from diesel combustion engines is an important contributor to urban air pollution and poses significant risk to human health. Diesel exhaust contains a chemical class known as nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) and is enriched in 1-nitropyrene (1-NP), which has the potential to serve as a marker of diesel exhaust. The isomeric nitro-PAHs 2-nitropyrene (2-NP) and 2-nitrofluoranthene (2-NFL) are secondary pollutants arising from photochemical oxidation of pyrene and fluoranthene, respectively. Like other important air toxics, there is not extensive monitoring of nitro-PAHs, leading to gaps in knowledge about relative exposures and urban hotspots. Epiphytic moss absorbs water, nutrients, and pollutants from the atmosphere and may hold potential as an effective biomonitor for nitro-PAHs. In this study we investigate the suitability of *Orthotrichum lyellii* as a biomonitor of diesel exhaust by analyzing samples of the moss for 1-NP, 2-NP, and 2-NFL in the Seattle, WA metropolitan area. Samples were collected from rural parks, urban parks, residential, and commercial/industrial areas ($N = 22$ locations) and exhibited increasing concentrations across these land types. Sampling and laboratory method performance varied by nitro-PAH, but was generally good. We observed moderate to moderately strong correlation between 1-NP and select geographic variables, including summer normalized difference vegetation index (NDVI) within 250 m ($r = -0.88$, $R^2 = 0.77$), percent impervious surface within 50 m ($r = 0.83$, $R^2 = 0.70$), percent high development land use within 500 m ($r = 0.77$, $R^2 = 0.60$), and distance to nearest secondary and connecting road ($r = -0.75$, $R^2 = 0.56$). The relationships between 2-NP and 2-NFL and the geographic variables were generally weaker. Our results suggest *O. lyellii* is a promising biomonitor of diesel

* Corresponding author.
E-mail address: sarah.jovan@usda.gov (S.E. Jovan).

https://doi.org/10.1016/j.scitotenv.2024.171306
Received 8 December 2023; Received in revised form 11 February 2024; Accepted 25 February 2024
Available online 27 February 2024
0048-9697/Published by Elsevier B.V.

exhaust, specifically for 1-NP. To our knowledge this pilot study is the first to evaluate using moss concentrations of nitro-PAHs as biomonitors of diesel exhaust.

1. Introduction

Diesel exhaust is a major component of urban air pollution (Yin et al., 2010). Originating from combustion engines, diesel exhaust is a complex mixture of gases and particles; these particles consist of a carbon core with hundreds of compounds adsorbed to the surface (Kittelson, 1998; Lim et al., 2021). Approximately 80–95 % of diesel exhaust particle mass is ≤1.0 μm aerodynamic diameter (Kerminen et al., 1997; Kittelson, 1998; Lim et al., 2021; McDonald et al., 2004; US EPA, 2002) and is therefore capable of penetrating deep into the alveolar region of the human respiratory system (Brain and Valberg, 1979; Darquenne, 2020; Schwab and Zenkel, 1998). Human exposure to diesel exhaust is associated with a range of deleterious health effects including oxidative stress; inflammation; pulmonary, immunological, and cardiovascular effects; and cancer (IARC, 2014; Kagawa, 2002; Long and Carlsten, 2022; Sydbom et al., 2001).

While there are many constituents of diesel exhaust, polycyclic aromatic hydrocarbons (PAHs), and in particular nitrated PAHs (nitro-PAHs), have drawn interest due to their adverse human health effects (Bandowe and Meusel, 2017). 1-nitropyrene (1-NP) is the most abundant particle-associated nitro-PAH in diesel exhaust (US EPA, 2002). Because of its enrichment in diesel exhaust, 1-NP has been proposed as a marker for diesel exhaust particulate matter (Bamford et al., 2003; Miller-Schulze et al., 2007; Riley et al., 2018; Scheepers et al., 1995; Schulte et al., 2015). Related nitro-PAHs 2-nitropyrene (2-NP) and 2-nitrofluoranthene (2-NFL) are secondary pollutants resulting from photochemical oxidation of primary pollutants pyrene and fluoranthene, respectively (Arey et al., 1986; Sweetman et al., 1986). Due to the geographically diffuse nature of the formation of 2-NP and 2-NFL, they may exhibit less fine-scale spatial variability compared to 1-NP, which is produced at the combustion source.

Despite the importance of 1-NP as a traffic-related air pollutant with adverse human health effects, there is not widespread long-term monitoring of this and many other air toxics. For example, in the US the main network monitoring hazardous air pollutants is the National Air Toxics Trends Stations (NATTS), which is comprised of only 27 sites across the country (Strum and Scheffe, 2016; US EPA, 2022). Furthermore, benzo(*a*)pyrene and naphthalene are the only PAHs that must be measured at NATTS (many other PAHs remain unmeasured) and no nitro-PAHs are required. As a consequence, there are extensive gaps in knowledge about the intraurban variability of such air toxics and researchers have turned to modeling to characterize human exposure (Isakov et al., 2007, 2009; Morello-Frosch et al., 2001; Schulte et al., 2015). An additional strategy, one well suited for initial screenings and identification of pollution "hotspots," is the use of moss biomonitors.

Epiphytic mosses possess underdeveloped vascular tissue and outer cuticles, readily collecting water, nutrients, and pollutants from the atmosphere (Varela et al., 2023). Moss has been used for decades as a biomonitor (Chaudhuri and Roy, 2023); specifically for radionuclides (Belivermiş and Çotuk, 2010; Jenkins et al., 1972; Wattanavatee et al., 2017), heavy metals (Donovan et al., 2016; Giordano et al., 2010; Jovan et al., 2022; Messager et al., 2021; Rühling and Tyler, 1968), and PAHs (Ares et al., 2009; Jovan et al., 2021; Vuković et al., 2015; Zechmeister et al., 2006). While pollutant concentrations in air contribute to moss pollutant concentrations (including intra-, inter-, and extracellular), these relationships are complex and mediated by many biological and environmental factors, hampering the use of one matrix to directly predict the other (Boquete et al., 2020; Varela et al., 2023). There may be additional challenges with detecting nitro-PAHs in moss that carry over from air samples, including low concentrations (pg/m^3 in ambient air) and extensive laboratory processing for adequate detection limits (Bandowe and Meusel, 2017; Miller-Schulze et al., 2007). Despite these potential limitations, moss biomonitors are used to characterize urban pollutant variability and identify areas of high concentration. Moss biomonitoring studies in Portland, OR, for example, identified a previously unknown cadmium emission source (Donovan et al., 2016) and linked PAHs with neighborhood-scale patterns in road density and tree canopy (Jovan et al., 2021).

In this proof-of-concept study, we examine the suitability of a pollution-tolerant moss, *Orthotrichum lyellii*, as a biomonitor of nitro-PAHs, with a focus on 1-NP as a marker for diesel exhaust pollutants. To our knowledge, this is the first study attempting to detect nitro-PAHs in moss for use as a biomonitor. We describe our sampling and analytical approach, assess laboratory method performance, summarize nitro-PAH concentrations in moss using descriptive statistics across several land use types, and characterize univariate relationships between nitro-PAHs in moss and a number of geographic and land-use variables. Using *O. lyellii* as a biomonitor of diesel exhaust may be an innovative tool to characterize an important traffic-related air pollutant exposure in urban settings.

2. Methods

2.1. Sampling

Our sampling goal was to capture a gradient of 1-NP concentrations. Therefore, we intentionally sought areas in the Seattle, WA metropolitan area likely to contain variation in 1-NP concentration. We sampled in a rural park (a "regional wildland park"), urban parks, residential neighborhoods, and commercial and industrial areas. We also targeted sampling in the Georgetown and South Park neighborhoods of Seattle, which have extensive commercial and industrial development patterns and have been previously described as impacted by diesel emissions (Schulte et al., 2015).

We collected *O. lyellii* samples generally following established protocols for heavy metals (Donovan et al., 2016; Jovan et al., 2022), but modified for 1-NP as informed by our previous experience with air samples (Miller-Schulze et al., 2007; Schulte et al., 2015). Samples were collected from deciduous hardwood trees (including species of maple, sycamore, and alder) 1–3 m from ground height using fresh nitrile gloves. We collected field duplicates immediately after the primary sample in the same fashion (with a change in gloves) to examine sampling repeatability. Duplicate sites were chosen without preconceived bias other than the presence of sufficient moss biomass. The samples were initially stored in washed, heat sterilized, and dried glass jars, with the amount of moss approximately filling the 16 oz. (470 mL) container. In the field, samples were kept on ice until they could be transported to the laboratory for refrigerated storage at 4 °C. Samples were collected August 22–24, 2022 under typical summer season conditions in this area – warm and dry. The temperature highs for sample collection days were 25–28 °C and relative humidity was 47–57 %; there was full sun (maximum solar radiation of approximately 850 W/m^2) and no precipitation. Climate normals for the area include a maximum daily temperature of 25 °C and a 15.3 % probability of precipitation.

2.2. Laboratory preparation & analysis

Initial laboratory preparation also followed established methods for heavy metals. Briefly, the upper half to two-thirds of the moss stems were trimmed with scissors and the bases discarded. The trimmed samples were then transferred to 50 mL polypropylene centrifuge tubes and stored at −20 °C.

Samples were then freeze dried under vacuum (Labconco Freezone Triad, model 740040, Kansas City, MO). Freeze drying samples is a common technique used for the preparation of geological and biological material for analysis of PAHs (Ju et al., 2022; Soursou et al., 2023), with analyte recoveries equivalent to air drying and superior to oven drying (Beriro et al., 2014). Freeze dried samples were ground with a ceramic mortar and pestle and then 200 mg portions were spiked with d_9–1-NP (Midwest Research Institute, Kansas City, MO) as an internal standard, extracted with 10 mL methylene chloride, vortex-mixed, and sonicated. Extracts were filtered, evaporated under nitrogen, and then cleaned using silica solid phase extraction. Silica-cleaned extracts were evaporated again, reconstituted, and filtered through a 0.2 μm polytetrafluoroethylene (PTFE) filter. The final extracts were analyzed by two-dimension high-performance liquid chromatography tandem mass-spectrometry (2-D HPLC-MS/MS) using internal standard calibration, as described previously (Miller-Schulze et al., 2007, 2010).

To evaluate laboratory performance, we estimated the limit of quantitation (LOQ) based on laboratory method blanks. The method blanks were prepared by spiking the extraction tube with internal standard and running it through the entire sample preparation process alongside the moss samples. The nitro-PAH concentration was quantified for each blank, and the mean plus three times the standard deviation (SD) of the blanks was calculated to determine LOQ.

To measure recovery of the nitro-PAHs, 200 mg aliquots of freeze dried, ground moss samples were spiked with 100 pg of each target analyte (1-NP, 2-NP, 2-NFL), for a concentration of 500 pg/g. These samples were collected from two locations (one rural park, one commercial/industrial), from which we made two unspiked and two spiked samples and were therefore analyzed in duplicate. Laboratory blanks were also spiked in duplicate for comparison with spiked moss. Laboratory duplicates were prepared by analyzing separate 200 mg portions of the moss from a single field sample. Nitro-PAH concentrations of the unspiked samples were subtracted from the spiked samples to calculate recoveries for each nitro-PAH.

2.3. Data analysis

We categorized the moss sampling locations into land use types (rural parks, urban parks, residential, and commercial/industrial) and prepared descriptive statistics and boxplots of nitro-PAHs in moss samples for each category. We also mapped the nitro-PAH concentrations to display their spatial distribution.

We hypothesized that road and roadway sources and high development would be associated with higher nitro-PAH concentrations in moss, and that less developed forested areas would have lower nitro-PAH concentrations. To investigate these relationships, we plotted and calculated the Pearson correlation (r) and coefficient of determination (R^2) between the log-transformed nitro-PAH concentrations in moss and 12 geographic variables. These geographic variables were either proximity variables or buffer variables (Young et al., 2016). Proximity variables describe the distance to a geographic feature, such as distance to roadways, truck routes, or commercial areas. The proximity variables were also log-transformed. Buffer variables measure features within specified radii, for example, the population within a 1 km radius, the length of roads within a 500 m radius, or the percent impervious surface within a 50 m radius. For buffer variables we selected the smallest reasonable buffer sizes that would provide adequate variability among sample locations. The geographic variables we considered are summarized in Table 1, and details, including their data sources, are further described in Table S1. The values of the geographic variables at sample locations were calculated using ArcGIS (ESRI, West Redlands, CA) after compiling source data into shapefiles.

Data analysis was carried out in R version 4.2.2.

Table 1
Selected geographic variables and their source. See Table S1 for further details on data sources.

Geographic variable	Data source
Distance to A3 roadways[a]	TeleAtlas Dynamap
Sum of road lengths within 500 m	TeleAtlas Dynamap
Distance to truck routes	Bureau of Transportation Statistics
Sum of truck routes within 1 km	Bureau of Transportation Statistics
Distance to commercial areas	USGS
Population within 1 km	US Census
Summer NDVI[b] within 250 m	University of Maryland
Percent impervious surface within 50 m	MRLC National Landcover Dataset
Percent mixed forest land use within 500 m	MRLC National Landcover Dataset
Percent low development land use within 500 m	MRLC National Landcover Dataset
Percent medium development land use within 500 m	MRLC National Landcover Dataset
Percent high development land use within 500 m	MRLC National Landcover Dataset

[a] A3 roadways: defined by US Census Feature Class Codes as secondary and connecting roads.
[b] NDVI: normalized difference vegetation index; a measure of photosynthetic activity (i.e., "greenness").

3. Results

3.1. Sampling & laboratory performance

We collected 27 samples from 22 locations, which included two field duplicates and three field blanks. An additional three laboratory blanks, four laboratory duplicates and three duplicate laboratory spikes (one method and two sample spikes) were analyzed. Analytical performance differed among the three nitro-PAHs, though calibration curves were linear ($R^2 > 0.98$) over the range 1 to 100 pg/mL. The mean ± SD assay recovery and precision based on spiked samples ($N = 6$) were: 1-NP 97 ± 18 %, 2-NP 89 ± 14 %, and 2-NFL 80 ± 3 %. Among field duplicates, 2-NP had the lowest mean absolute percentage differences between primary and duplicate samples (8.9 %), followed by 1-NP (13 %) and 2-NFL (16 %). For laboratory duplicates, 1-NP had the lowest mean absolute percentage difference (7.7 %), while 2-NP and 2-NFL had larger differences (32 and 22 %, respectively) and variability (i.e., SD of the mean absolute differences, 46 % and 34 %, respectively) compared to 1-NP (7.9 %).

All three nitro-PAHs were detected in method blanks as follows (mean ± SD): 1-NP 17 ± 10 pg/g, 2-NP 3.9 ± 2.8 pg/g, 2-NFL 20 ± 6.5 pg/g. The three nitro-PAHs were also detected at similar levels in the field blanks: 1-NP 18 ± 15 pg/g, 2-NP 4.7 ± 2.3 pg/g, and 2-NFL 22 ± 2.1 pg/g. Estimated LOQs for the three nitro-PAHs were: 1-NP 47 pg/g, 2-NP 12 pg/g, and 2-NFL 40 pg/g. For 1-NP the LOQ was about half the minimum value observed in the moss samples, for 2-NP the LOQ was about a third of the minimum concentration observed, and for 2-NFL the LOQ was comparable to the minimum concentration in the moss samples (details in Section 3.2).

3.2. Nitro-PAHs by land type

Sampling locations with land type are shown in Fig. 1, and a summary of the analytical results by land type is presented in Table 2. Of the 22 primary samples, 10 were collected from urban parks, 8 were from commercial/industrial areas, 3 were from residential areas, and 1 was from a rural park where we expected to find lower pollutant levels. Generally, nitro-PAH concentrations increased across land types, such that rural park < urban park < residential < commercial industrial (Fig. 2). There was an exception to this general trend, where the median

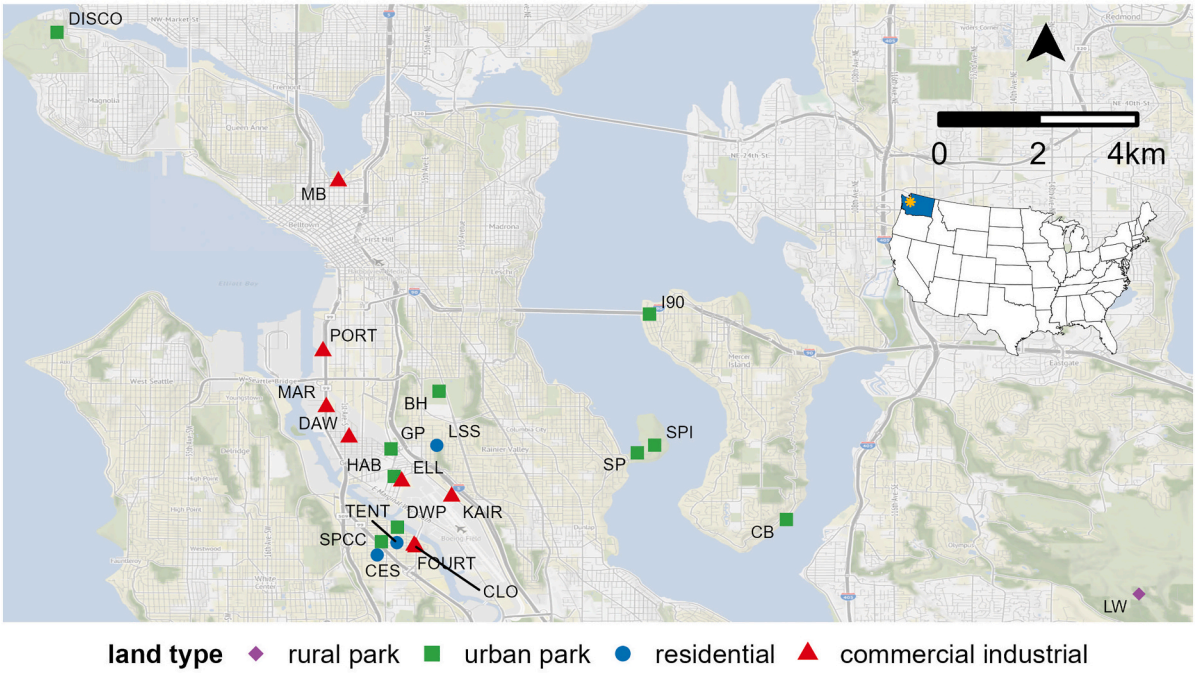


Fig. 1. Moss sampling locations in Seattle, WA, and nearby areas, covering a variety of land types.

Table 2
Descriptive statistics of nitro-PAHs in moss (pg/g).

Land Type	N	Mean ± SD	Median (Min, Max)
1-NP			
Rural Park	1	101	101 (101,101)
Urban Park	10	285 ± 163	262 (104, 561)
Residential	3	379 ± 147	321 (270, 546)
Commercial Industrial	8	1281 ± 236	1280 (982, 1600)
2-NP			
Rural Park	1	38	38 (38, 38)
Urban Park	10	116 ± 61	97 (36, 213)
Residential	3	124 ± 32	112 (100, 161)
Commercial Industrial	8	334 ± 158	282 (195, 678)
2-NFL			
Rural Park	1	379	379 (379, 379)
Urban Park	10	1523 ± 704	1514 (544, 2879)
Residential	3	1418 ± 482	1336 (983, 1936)
Commercial Industrial	8	2393 ± 864	2338 (954, 3997)

2-NFL concentration in urban parks was marginally greater than in residential areas.

Individual sample results (Fig. 2, bottom row), indicate a clear difference in 1-NP between samples collected from commercial/industrial locations, with concentrations ≥982 pg/g, compared to samples collected from other land use types where concentrations were ≤561 pg/g. 2-NP and 2-NFL did not exhibit such distinct differences among land used types. For 2-NFL especially, commercial/industrial concentrations were more comparable to those observed in rural and urban park and residential samples, and in some instances were less than concentrations observed in park and residential samples.

Nitro-PAH concentrations varied spatially over the sampling region (Fig. 3), with concentrations in Seattle's industrial core higher than outlying areas. There were some differences in the patterns observed among the three nitro-PAHs. For example, there was an area of high 1-NP in commercial areas of the city's south that was not as pronounced for 2-NP or 2-NFL. Overall, the nitro-PAH with the highest concentration in moss was 2-NFL, followed by 1-NP and 2-NP.

3.3. Geographic variables

The univariate relationships between log-transformed moss nitro-PAH concentrations and geographic variables are shown in Figs. S1 (1-NP), S2 (2-NP), and S3 (2-NFL). A subset of these with the strongest relationships for 1-NP are shown (with 2-NP and 2-NFL) in Fig. 4. The strongest relationship with 1-NP observed was with summer normalized difference vegetation index (NDVI) within 250 m ($r = -0.88$, $R^2 = 0.77$). Other geographic variables with the next strongest relationships with 1-NP were percent impervious surface within 50 m ($r = 0.83$, $R^2 = 0.70$), percent high development land use within 500 m ($r = 0.77$, $R^2 = 0.60$), and log distance to A3 road ($r = -0.75$, $R^2 = 0.56$). The relationships between 2-NP and 2-NFL and the geographic variables were generally weaker, though we did observe a moderately strong relationship between 2-NP and summer NDVI within 250 m ($r = -0.77$, $R^2 = 0.59$).

4. Discussion

In this pilot study we assessed the suitability of a common epiphytic moss for use as a biomonitor of diesel-related air pollution. We observed good sampling and laboratory method performance, with agreement between field duplicates, blank concentrations and LOQs at or below nitro-PAH concentrations in moss, and analyte recoveries between 80 and 97 %. We also observed a concentration gradient of nitro-PAHs over various land types and moderately strong relationships between select geographic variables and 1-NP. For these reasons we believe that measurements of 1-NP in *O. lyellii* hold promise as a suitable biomonitor of atmospheric concentrations of diesel exhaust.

Relationships between geographic variables and the nitro-PAHs, especially 1-NP, were intuitive and consistent with our hypothesis of higher concentrations associated with roadways and higher land development versus lower levels in less developed forested areas. Trees and vegetation are well-known scavengers of atmospheric PAHs (Huang et al., 2018; Mukhopadhyay et al., 2020) and may include 1-NP. Furthermore, 1-NP has been observed to correlate highly with traffic volume (Hayakawa et al., 1995b). While we were unable to obtain traffic volume data for this study (e.g., vehicles per day), roadway variables like ours are used as proxies for describing roadway emissions

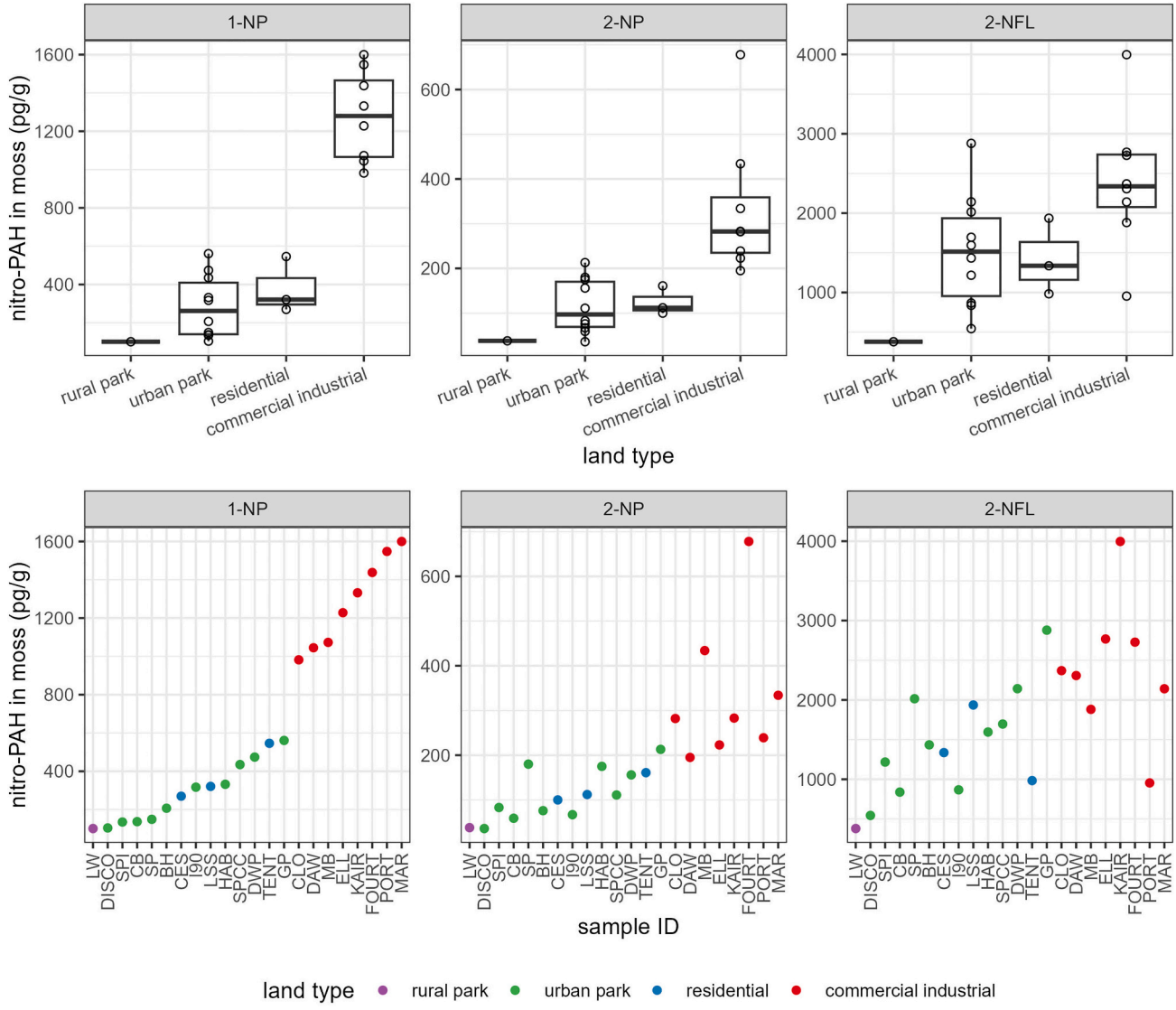


Fig. 2. Summary of nitro-PAH concentrations in moss grouped by land type (top row) and shown by individual sample (bottom row, ordered by 1-NP concentration).

(Kwon et al., 2006; Noth et al., 2011). The strong positive correlation we observed with impervious surfaces ($r = 0.83$) also follows; impervious surfaces include roadways, which may drive the relationship of 1-NP concentrations in moss with vehicular traffic, as well as areas like parking lots, for example, which may not be an important source of diesel exhaust but lack the mitigating effects of vegetation.

Our results are also consistent with prior research examining concentrations of non-nitrated PAHs in urban *O. lyelli* samples. Compounds with 3–4 rings, including pyrene, were negatively correlated with tree canopy cover and positively correlated with roadway variables (Jovan et al., 2021). In the present study, however, our sample size was much smaller ($N = 22$ versus 346), limiting us to univariate analysis. That, and the observational nature of this pilot, means the relationships we observed do not imply causation, and do not account for other influences. For example, the moderately strong relationship between nitro-PAHs and summer NDVI may simply be a consequence of the fact that rural areas are subject to fewer diesel emissions and also tend to be greener. In any case, results are encouraging for expanding the research to build more robust, informative multiple linear regression models.

Given these nitro-PAHs arise from their non-nitrated PAHs, it follows that nitro-PAH concentrations are observed at concentrations one to three orders lower than parent PAHs (Albinet et al., 2007; Bamford and Baker, 2003). The physical, chemical and transport behaviors of PAHs and nitro-PAHs in the atmosphere are complex. Atmospheric pyrene and fluoranthene will exist in both the vapor and particulate phases due to their vapor pressures (4.5×10^{-6} mmHg and 9.22×10^{-6} mmHg, respectively, at 25 °C) (PubChem, 2024c, 2024b). In the vapor phase, pyrene and fluoranthene are both readily subject to photochemical degradation with half-lives of approximately 8 h (PubChem, 2024c, 2024b), potentially producing 2-NP and 2-NFL. 2-NP and 2-NFL have low vapor pressures (approximately 1×10^{-13} mmHg) (ChemSpider, 2024b, 2024a) and exist in the atmosphere almost exclusively bound to particles.

In contrast to parent PAHs (e.g., pyrene), once emitted from a primary source, 1-NP will exist only in the particle phase in the atmosphere due to its lower vapor pressure (8.3×10^{-8} mmHg at 25 °C) (Albinet et al., 2006; PubChem, 2024a). Furthermore, the strong sorption interactions between nitro-PAHs and diesel particulate matter further limit their evaporation from the particle surface. Previous studies have demonstrated that nitro-PAHs exist in the small size fractions of particulate matter (Hayakawa et al., 1995a; Riley et al., 2018; Wietzoreck et al., 2022).

While 1-NP, 2-NP, and 2-NFL can be photolyzed in the particulate phase, they can also exhibit stability, even permitting long-range transport (Lammel et al., 2017; PubChem, 2024a), which is reflected in the large range of degradation times (e.g., half-life or residence time)

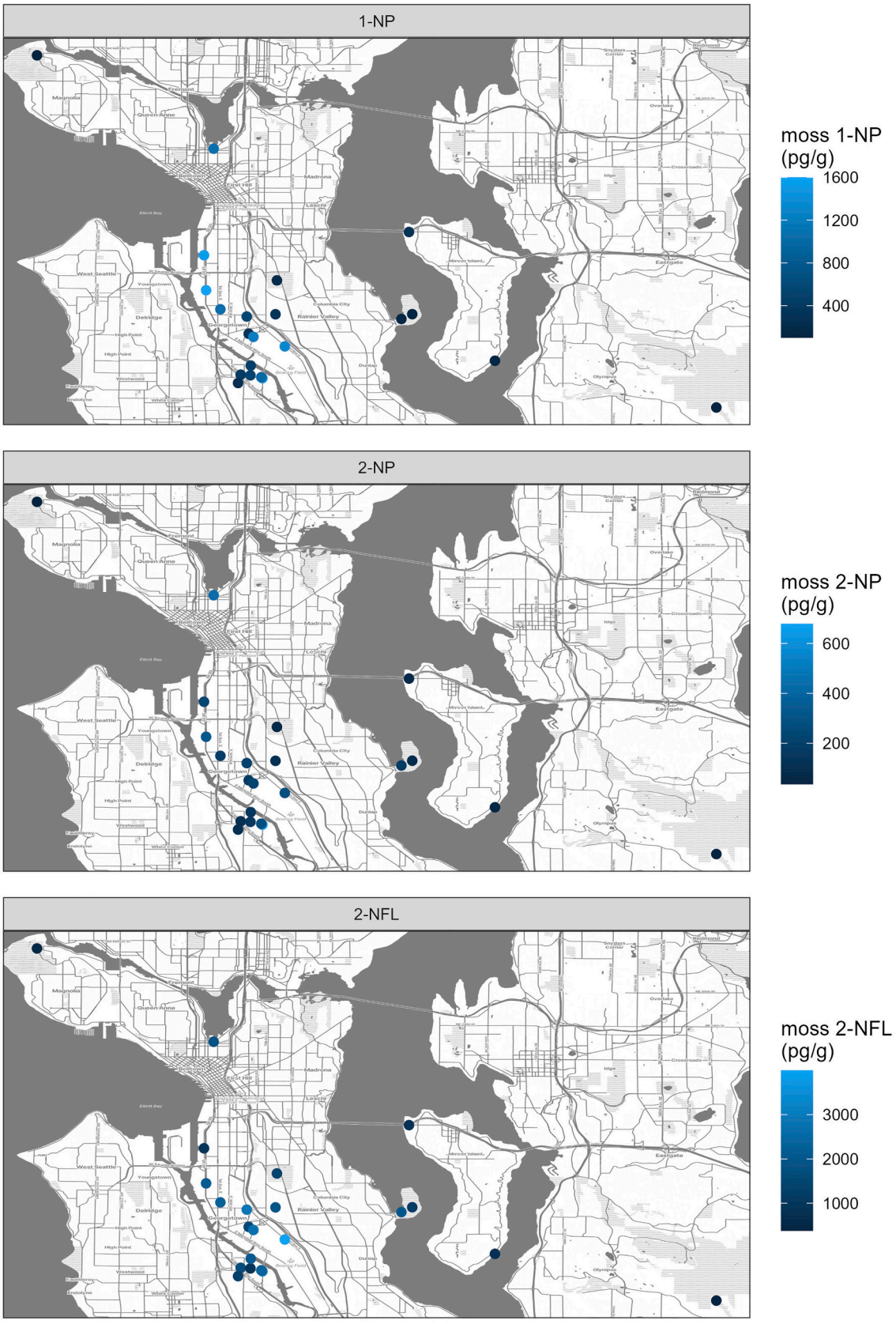


Fig. 3. Spatial distribution of nitro-PAH concentrations in moss in Seattle, WA and nearby areas.

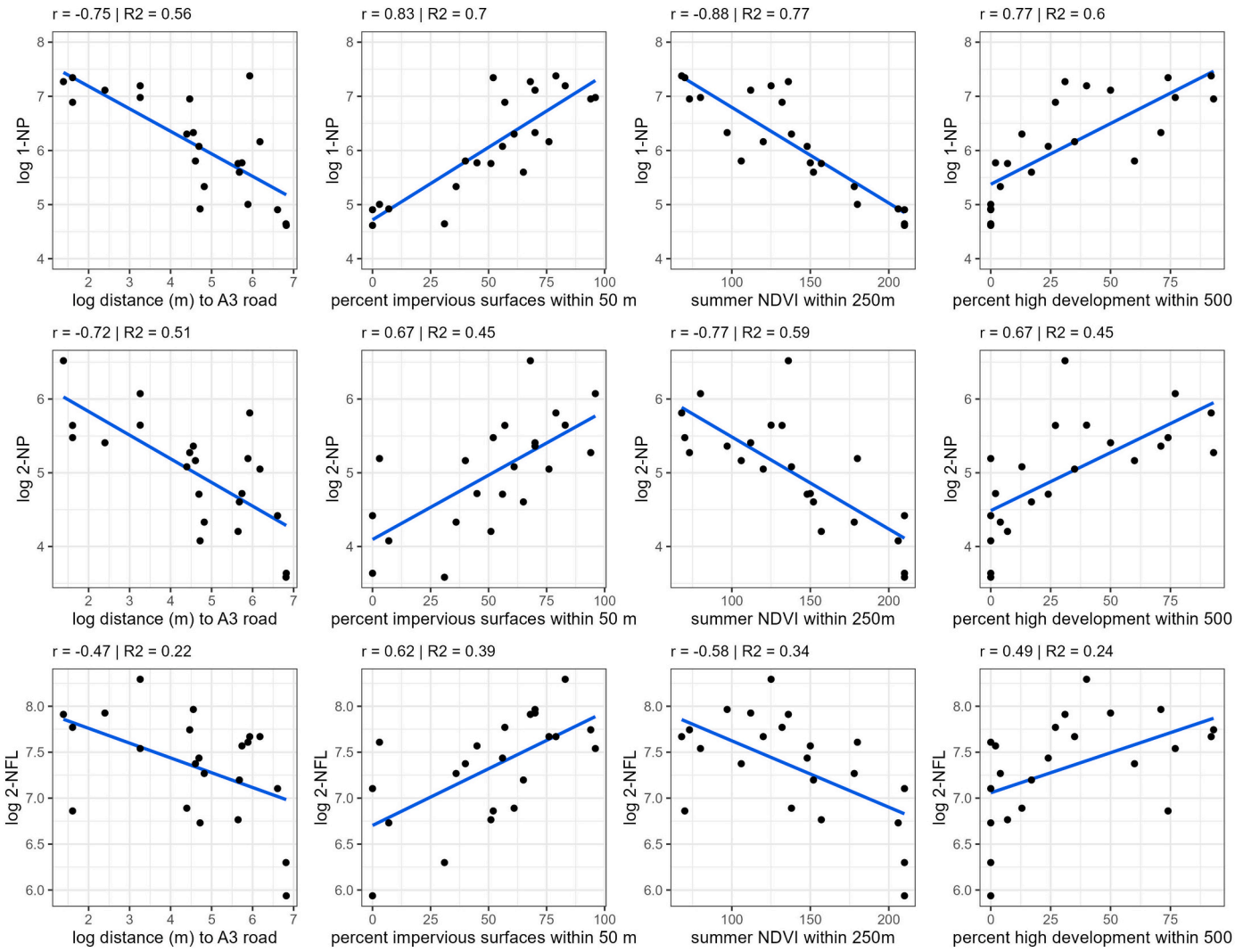


Fig. 4. Pearson Correlation between nitro-PAH concentrations in moss and geographic variables.

of these nitro-PAHs from 1 h to 50 days (Fan et al., 1996; Koizumi et al., 1994; Wilson et al., 2020). This stability depends on the intensity of solar radiation and the nature of the surface that the nitro-PAHs are adsorbed to (Fan et al., 1996). The rate of photolysis is much lower when nitro-PAHs are adsorbed onto particulates, as would be the case in the air (Fan et al., 1996). As with aerosols generally, PAHs and nitro-PAHs in the particle phase are subject to both wet and dry deposition, removing them from the atmosphere.

Broadly, these complex dynamics occur behind a backdrop where, qualitatively, 1-NP is emitted to the atmosphere as a primary combustion pollutant from localized combustion sources. On the other hand, 2-NP and 2-NFL form as secondary pollutants after some degree of transport away from those combustion sources, acting conceptually as more diffuse "sources." In light of these dynamics, it is logical that associations between the geographic variables and 2-NP and 2-NFL mirrored those seen with 1-NP, albeit with weaker correlations resulting from spatial distributions of 2-NP and 2-NFL that are less localized than for 1-NP, and hence less well correlated with fine-spatial-scale geographic variables.

In addition, these nitro-PAHs are susceptible to some amount of photodegradation on the moss prior to sample collection, especially during sunny weather which is typical in summer when we collected samples. Concentrations of 1-NP in moss may therefore under-represent emissions to air. Furthermore, it is unknown whether the nitro-PAHs we detected were deposited on the surface of the moss or taken up into tissue and what potential differences in photodegradation there may be between these conditions. Weather was a significant predictor of PAHs in moss in a prior study (Jovan et al., 2021), so it's possibly important for 1-NP concentrations as well. Adding to these complexities is that no data are available from which to predict degradation times for particle-associated nitro-PAHs adsorbed to moss. In this scenario, it is likely that the nitro-PAH residence times on moss are longer than in the atmosphere due to reduction of solar irradiance experienced by particles on the moss due to shadows cast by the moss itself and the trees to which the moss is attached.

This pilot study has informed several opportunities for future research in this area. We plan to collect longitudinal samples at a subset of these original 22 locations. This will provide important information on the temporal variability of nitro-PAHs in *O. lyellii* and the differences across land types. For example, if there is little variation in concentrations of nitro-PAHs over the course of a year, that is good evidence that moss samples collected at different times are comparable or can be pooled for data analysis. We collected only one moss sample from a rural park; while originally intended to serve as a regional "background" sample, in the future, we plan to collect more samples from this land type to gain understanding about the variability of nitro-PAHs in areas that are relatively less impacted by diesel exhaust emissions.

We also plan to expand this pilot and collect samples in a wider geographic scale to map the spatial variability in nitro-PAHs and potentially identify areas of high concentration within Seattle. Additionally, future sample collection from a wider geographic area may be

able to capture a gradient that varies on a greater geographic scale, such as those important for secondary pollutants 2-NP and 2-NFL. A greater number of samples will also permit a multiple linear regression modeling approach, which may provide better insights into the relationships between pollutant concentrations and geographic variables. Our experience in this study indicates in these future sampling efforts that collecting samples, moderately compacted, into 50 mL centrifuge tubes will provide sufficient moss mass for nitro-PAH analysis, easing collection and sampling impact (i.e., the amount of moss removed from trees).

Ultimately, the concentration of nitro-PAHs in air are important for assessing human health risk. To understand the connections between concentrations of nitro-PAHs in moss and concentrations in air, we are planning future research collecting co-located moss and air samples.

CRediT authorship contribution statement

Christopher Zuidema: Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Michael Paulsen:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Christopher D. Simpson:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Sarah E. Jovan:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Christopher D. Simpson reports financial support was provided by US National Institute of Environmental Health Sciences. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This research was funded by the US Department of Agriculture (USDA) Forest Service, Pacific Northwest Research Station Joint Venture Agreement number 22-JV-11261979-054 and the US National Institute of Environmental Health Sciences (NIEHS) grant number P30ES007033 and was supported in part by an appointment to the US Forest Service (USFS) Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the US Department of Energy (DOE) and the USDA. ORISE is managed by ORAU under DOE contract number DE-SC0014664. All opinions expressed in this paper are the authors' and do not necessarily reflect the policies and views of USDA, NIEHS, DOE, or ORAU/ORISE.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171306.

References

Albinet, A., Leoz-Garziandia, E., Budzinski, H., Villenave, E., 2006. Simultaneous analysis of oxygenated and nitrated polycyclic aromatic hydrocarbons on standard reference material 1649a (urban dust) and on natural ambient air samples by gas chromatography–mass spectrometry with negative ion chemical ionisation. J. Chromatogr. A 1121, 106–113. https://doi.org/10.1016/j.chroma.2006.04.043.

Albinet, A., Leoz-Garziandia, E., Budzinski, H., Villenave, E., 2007. Polycyclic aromatic hydrocarbons (PAHs), nitrated PAHs and oxygenated PAHs in ambient air of the Marseilles area (South of France): concentrations and sources. Sci. Total Environ. 384, 280–292. https://doi.org/10.1016/j.scitotenv.2007.04.028.

Ares, A., Aboal, J.R., Fernández, J.A., Real, C., Carballeira, A., 2009. Use of the terrestrial moss Pseudoscleropodium purum to detect sources of small scale contamination by PAHs. Atmos. Environ. 43, 5501–5509. https://doi.org/10.1016/j.atmosenv.2009.07.005.

Arey, J., Zielinska, B., Atkinson, R., Winer, A.M., Ramdahl, T., Pitts, J.N., 1986. The formation of nitro-PAH from the gas-phase reactions of fluoranthene and pyrene with the OH radical in the presence of NOx. Atmos. Environ. 20, 2339–2345. https://doi.org/10.1016/0004-6981(86)90064-8.

Bamford, H.A., Baker, J.E., 2003. Nitro-polycyclic aromatic hydrocarbon concentrations and sources in urban and suburban atmospheres of the Mid-Atlantic region. Atmos. Environ. 37, 2077–2091. https://doi.org/10.1016/S1352-2310(03)00102-X.

Bamford, H.A., Bezabeh, D.Z., Schantz, M.M., Wise, S.A., Baker, J.E., 2003. Determination and comparison of nitrated-polycyclic aromatic hydrocarbons measured in air and diesel particulate reference materials. Chemosphere 50, 575–587. https://doi.org/10.1016/S0045-6535(02)00667-7.

Bandowe, B.A.M., Meusel, H., 2017. Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) in the environment – a review. Sci. Total Environ. 581–582, 237–257. https://doi.org/10.1016/j.scitotenv.2016.12.115.

Belivermiş, M., Çotuk, Y., 2010. Radioactivity measurements in moss (Hypnum cupressiforme) and lichen (Cladonia rangiformis) samples collected from Marmara region of Turkey. J. Environ. Radioact. 101, 945–951. https://doi.org/10.1016/j.jenvrad.2010.06.012.

Beriro, D.J., Vane, C.H., Cave, M.R., Nathanail, C.P., 2014. Effects of drying and comminution type on the quantification of Polycyclic Aromatic Hydrocarbons (PAH) in a homogenised gasworks soil and the implications for human health risk assessment. Chemosphere 111, 396–404. https://doi.org/10.1016/j.chemosphere.2014.03.077.

Boquete, M.T., Ares, A., Fernández, J.A., Aboal, J.R., 2020. Matching times: trying to improve the correlation between heavy metal levels in mosses and bulk deposition. Sci. Total Environ. 715, 136955 https://doi.org/10.1016/j.scitotenv.2020.136955.

Brain, J.D., Valberg, P.A., 1979. Deposition of aerosol in the respiratory tract. Am. Rev. Respir. Dis. 120, 1325–1373. https://doi.org/10.1164/arrd.1979.120.6.1325.

Chaudhuri, S., Roy, M., 2023. Global Ambient Air Quality Monitoring: Can Mosses Help? Environ Dev Sustain, A systematic meta-analysis of literature about passive moss biomonitoring. https://doi.org/10.1007/s10668-023-03043-0.

ChemSpider, 2024a. 2-Nitrofluoranthene. Available: http://www.chemspider.com/Chemical-Structure.24000.html [accessed 17 February 2024].

ChemSpider, 2024b. 2-Nitropyrene. Available: http://www.chemspider.com/Chemical-Structure.12544.html [accessed 17 February 2024].

Darquenne, C., 2020. Deposition mechanisms. J. Aerosol Med. Pulm. Drug Deliv. 33, 181–185. https://doi.org/10.1089/jamp.2020.29029.cd.

Donovan, G.H., Jovan, S.E., Gatziolis, D., Burstyn, I., Michael, Y.L., Amacher, M.C., et al., 2016. Using an epiphytic moss to identify previously unknown sources of atmospheric cadmium pollution. Sci. Total Environ. 559, 84–93. https://doi.org/10.1016/j.scitotenv.2016.03.182.

Fan, Z., Kamens, R.M., Hu, J., Zhang, J., McDow, S., 1996. Photostability of nitro-polycyclic aromatic hydrocarbons on combustion soot particles in sunlight. Environ. Sci. Technol. 30, 1358–1364. https://doi.org/10.1021/es9505964.

Giordano, S., Adamo, P., Spagnuolo, V., Vaglieco, B.M., 2010. Instrumental and biomonitoring of heavy metal and nanoparticle emissions from diesel engine exhaust in controlled environment. J. Environ. Sci. 22, 1357–1363. https://doi.org/10.1016/S1001-0742(09)60262-X.

Hayakawa, K., Kawaguchi, Y., Murahashi, T., Miyazaki, M., 1995a. Distribution of nitropyrenes and mutagenicity in airborne particulates collected with an Andersen sampler. Mutat. Res. Lett. 348, 57–61. https://doi.org/10.1016/0165-7992(95)00046-1.

Hayakawa, Kazuichi, Tsuyoshi, Murahashi, Mizuka, Butoh, Motoichi, Miyazaki, 1995b. Determination of 1,3-, 1,6-, and 1,8-Dinitropyrenes and 1-nitropyrene in urban air by high-performance liquid chromatography using Chemiluminescence detection. Environ. Sci. Technol. 29, 928–932. https://doi.org/10.1021/es00004a012.

Huang, S., Dai, C., Zhou, Y., Peng, H., Yi, K., Qin, P., et al., 2018. Comparisons of three plant species in accumulating polycyclic aromatic hydrocarbons (PAHs) from the atmosphere: a review. Environ. Sci. Pollut. Res. 25, 16548–16566. https://doi.org/10.1007/s11356-018-2167-z.

IARC, 2014. Diesel and gasoline engine exhausts and some nitroarenes. IARC Monogr. Eval. Carcinog. Risks Hum. 105, 9–699.

Isakov, V., Irwin, J.S., Ching, J., 2007. Using CMAQ for exposure modeling and characterizing the subgrid variability for exposure estimates. J. Appl. Meteorol. Climatol. 46, 1354–1371. https://doi.org/10.1175/JAM2538.1.

Isakov, V., Touma, J.S., Burke, J., Lobdell, D.T., Palma, T., Rosenbaum, A., et al., 2009. Combining regional- and local-scale air quality models with exposure models for use in environmental health studies. J. Air Waste Manage. Assoc. 59, 461–472. https://doi.org/10.3155/1047-3289.59.4.461.

Jenkins, C.E., Wogman, N.A., Rieck, H.G., 1972. Radionuclide distribution in olympic national park, Washington. Water Air Soil Pollut. 1, 181–204. https://doi.org/10.1007/BF00187706.

Jovan, S.E., Monleon, V.J., Donovan, G.H., Gatziolis, D., Amacher, M.C., 2021. Small-scale distributions of polycyclic aromatic hydrocarbons in urban areas using geospatial modeling: a case study using the moss *Orthotrichum lyellii* in Portland,

Oregon, U.S.A. Atmos. Environ. 256, 118433 https://doi.org/10.1016/j.atmosenv.2021.118433.
Jovan, S.E., Zuidema, C., Derrien, M.M., Bidwell, A.L., Brinkley, W., Smith, R.J., et al., 2022. Heavy metals in moss guide environmental justice investigation: a case study using community science in Seattle, WA, USA. Ecosphere 13, e4109. https://doi.org/10.1002/ecs2.4109.
Ju, Y.-R., Chen, C.-F., Wang, M.-H., Chen, C.-W., Dong, C.-D., 2022. Assessment of polycyclic aromatic hydrocarbons in seafood collected from coastal aquaculture ponds in Taiwan and human health risk assessment. J. Hazard. Mater. 421, 126708 https://doi.org/10.1016/j.jhazmat.2021.126708.
Kagawa, J., 2002. Health effects of diesel exhaust emissions—a mixture of air pollutants of worldwide concern. Toxicology 181–182, 349–353. https://doi.org/10.1016/S0300-483X(02)00461-4.
Kerminen, V.-M., Mäkelä, T.E., Ojanen, C.H., Hillamo, R.E., Vilhunen, J.K., Rantanen, L., et al., 1997. Characterization of the particulate phase in the exhaust from a diesel car. Environ. Sci. Technol. 31, 1883–1889. https://doi.org/10.1021/es960520n.
Kittelson, D.B., 1998. Engines and nanoparticles: a review. J. Aerosol Sci. 29, 575–588. https://doi.org/10.1016/S0021-8502(97)10037-4.
Koizumi, A., Saitoh, N., Suzuki, T., Kamiyama, S., 1994. A novel compound, 9-Hydroxy-1-nitropyrene, is a major photodegraded compound of 1-nitropyrene in the environment. Arch. Environ. Health 49, 87–92. https://doi.org/10.1080/00039896.1994.9937459.
Kwon, J., Weisel, C.P., Turpin, B.J., Zhang, J. (Jim), Korn, L.R., Morandi, M.T., et al., 2006. Source proximity and outdoor-residential VOC concentrations: results from the RIOPA study. Environ. Sci. Technol. 40, 4074–4082. https://doi.org/10.1021/es051828u.
Lammel, G., Mulder, M.D., Shahpoury, P., Kukučka, P., Lišková, H., Přibylová, P., et al., 2017. Nitro-polycyclic aromatic hydrocarbons – gas–particle partitioning, mass size distribution, and formation along transport in marine and continental background air. Atmos. Chem. Phys. 17, 6257–6270. https://doi.org/10.5194/acp-17-6257-2017.
Lim, J., Lim, C., Jung, S., 2021. Characterizations of size-segregated ultrafine particles in diesel exhaust. Aerosol Air Qual. Res. 21, 200356 https://doi.org/10.4209/aaqr.200356.
Long, E., Carlsten, C., 2022. Controlled human exposure to diesel exhaust: results illuminate health effects of traffic-related air pollution and inform future directions. Part. Fibre Toxicol. 19, 11. https://doi.org/10.1186/s12989-022-00450-5.
McDonald, J.D., Barr, E.B., White, R.K., 2004. Design, characterization, and evaluation of a small-scale diesel exhaust exposure system. Aerosol Sci. Technol. 38, 62–78. https://doi.org/10.1080/02786820490247623.
Messager, M.L., Davies, I.P., Levin, P.S., 2021. Low-cost biomonitoring and high-resolution, scalable models of urban metal pollution. Sci. Total Environ. 767, 144280 https://doi.org/10.1016/j.scitotenv.2020.144280.
Miller-Schulze, J.P., Paulsen, M., Toriba, A., Hayakawa, K., Simpson, C.D., 2007. Analysis of 1-nitropyrene in air particulate matter standard reference materials by using two-dimensional high performance liquid chromatography with online reduction and tandem mass spectrometry detection. J. Chromatogr. A 1167, 154–160. https://doi.org/10.1016/j.chroma.2007.08.026.
Miller-Schulze, J.P., Paulsen, M., Toriba, A., Tang, N., Hayakawa, K., Tamura, K., et al., 2010. Exposures to particulate air pollution and nitro-polycyclic aromatic hydrocarbons among taxi drivers in Shenyang, China. Environ. Sci. Technol. 44, 216–221. https://doi.org/10.1021/es802392u.
Morello-Frosch, R., Pastor, M., Sadd, J., 2001. Environmental justice and Southern California's "Riskscape": the distribution of air toxics exposures and health risks among diverse communities. Urban Aff. Rev. 36, 551–578. https://doi.org/10.1177/10780870122184993.
Mukhopadhyay, S., Dutta, R., Das, P., 2020. A critical review on plant biomonitors for determination of polycyclic aromatic hydrocarbons (PAHs) in air through solvent extraction techniques. Chemosphere 251, 126441. https://doi.org/10.1016/j.chemosphere.2020.126441.
Noth, E.M., Hammond, S.K., Biging, G.S., Tager, I.B., 2011. A spatial-temporal regression model to predict daily outdoor residential PAH concentrations in an epidemiologic study in Fresno, CA. Atmos. Environ. 45, 2394–2403. https://doi.org/10.1016/j.atmosenv.2011.02.014.
PubChem, 2024a. 1-Nitropyrene. Available: https://pubchem.ncbi.nlm.nih.gov/compound/21694 [accessed 26 January 2024].
PubChem, 2024b. Fluoranthene. Available: https://pubchem.ncbi.nlm.nih.gov/compound/9154 [accessed 29 January 2024].
PubChem, 2024c. Pyrene. Available: https://pubchem.ncbi.nlm.nih.gov/compound/31423 [accessed 29 January 2024c].
Riley, E.A., Carpenter, E.E., Ramsay, J., Zamzow, E., Pyke, C., Paulsen, M.H., et al., 2018. Evaluation of 1-nitropyrene as a surrogate measure for diesel exhaust. Ann. Work Expos. Health 62, 339–350. https://doi.org/10.1093/annweh/wxx111.
Rühling, Å., Tyler, G., 1968. Ecological approach to the lead problem. Bot. Not. (Sweden) 121, 3.
Scheepers, P.T.J., Martens, M.H.J., Velders, D.D., Fijneman, P., Van Kerkhoven, M., Noordhoek, J., et al., 1995. 1-nitropyrene as a marker for the mutagenicity of diesel exhaust-derived particulate matter in workplace atmospheres. Environ. Mol. Mutagen. 25, 134–147. https://doi.org/10.1002/em.2850250207.
Schulte, J.K., Fox, J.R., Oron, A.P., Larson, T.V., Simpson, C.D., Paulsen, M., et al., 2015. Neighborhood-scale spatial models of diesel exhaust concentration profile using 1-nitropyrene and other nitroarenes. Environ. Sci. Technol. 49, 13422–13430. https://doi.org/10.1021/acs.est.5b03639.
Schwab, J.-A., Zenkel, M., 1998. Filtration of particulates in the human nose. Laryngoscope 108, 120–124. https://doi.org/10.1097/00005537-199801000-00023.
Soursou, V., Campo, J., Picó, Y., 2023. Revisiting the analytical determination of PAHs in environmental samples: an update on recent advances. Trends Environ. Anal. Chem. 37, e00195 https://doi.org/10.1016/j.teac.2023.e00195.
Strum, M., Scheffe, R., 2016. National review of ambient air toxics observations. J. Air Waste Manage. Assoc. 66, 120–133. https://doi.org/10.1080/10962247.2015.1076538.
Sweetman, J.A., Zielinska, B., Atkinson, R., Ramdahl, T., Winer arthur, M., Pitts, J.N., 1986. A possible formation pathway for the 2-nitrofluoranthene observed in ambient particulate organic matter. Atmos. Environ. (1967) 20, 235–238. https://doi.org/10.1016/0004-6981(86)90230-1.
Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandström, T., Dahlén, S.-E., 2001. Health effects of diesel exhaust emissions. Eur. Respir. J. 17, 733–746.
US EPA, 2002. Health Assessment Document For Diesel Engine Exhaust.
US EPA, 2022. Air Toxics | Ambient Monitoring Technology Information Center | US EPA. Available: https://www3.epa.gov/ttnamti1/airtoxpg.html [accessed 1 November 2022].
Varela, Z., Boquete, M.T., Fernández, J.A., Martínez-Abaigar, J., Núñez-Olivera, E., Aboal, J.R., 2023. Mythbusters: unravelling the pollutant uptake processes in mosses for air quality biomonitoring. Ecol. Indic. 148, 110095 https://doi.org/10.1016/j.ecolind.2023.110095.
Vuković, G., Urošević, M.A., Pergal, M., Janković, M., Goryainova, Z., Tomašević, M., et al., 2015. Residential heating contribution to level of air pollutants (PAHs, major, trace, and rare earth elements): a moss bag case study. Environ. Sci. Pollut. Res. 22, 18956–18966. https://doi.org/10.1007/s11356-015-5096-0.
Wattanavatee, K., Krmar, M., Bhongsuwan, T., 2017. A survey of natural terrestrial and airborne radionuclides in moss samples from the peninsular Thailand. J. Environ. Radioact. 177, 113–127. https://doi.org/10.1016/j.jenvrad.2017.06.009.
Wietzoreck, M., Kyprianou, M., Musa Bandowe, B.A., Celik, S., Crowley, J.N., Drewnick, F., et al., 2022. Polycyclic aromatic hydrocarbons (PAHs) and their alkylated, nitrated and oxygenated derivatives in the atmosphere over the Mediterranean and Middle East seas. Atmos. Chem. Phys. 22, 8739–8766. https://doi.org/10.5194/acp-22-8739-2022.
Wilson, J., Octaviani, M., Bandowe, B.A.M., Wietzoreck, M., Zetzsch, C., Pöschl, U., et al., 2020. Modeling the formation, degradation, and spatiotemporal distribution of 2-nitrofluoranthene and 2-nitropyrene in the global atmosphere. Environ. Sci. Technol. 54, 14224–14234. https://doi.org/10.1021/acs.est.0c04319.
Yin, J., Harrison, R.M., Chen, Q., Rutter, A., Schauer, J.J., 2010. Source apportionment of fine particles at urban background and rural sites in the UK atmosphere. Atmos. Environ. 44, 841–851. https://doi.org/10.1016/j.atmosenv.2009.11.026.
Young, M.T., Bechle, M.J., Sampson, P.D., Szpiro, A.A., Marshall, J.D., Sheppard, L., et al., 2016. Satellite-based NO2 and model validation in a national prediction model based on universal kriging and land-use regression. Environ. Sci. Technol. 50, 3686–3694. https://doi.org/10.1021/acs.est.5b05099.
Zechmeister, H.G., Dullinger, S., Hohenwallner, D., Riss, A., Hanus-Illnar, A., Scharf, S., 2006. Pilot study on road traffic emissions (PAHs, heavy metals) measured by using mosses in a tunnel experiment in Vienna, Austria. Environ. Sci. Pollut. Res. 13, 398–405. https://doi.org/10.1065/espr2006.01.292.