



Relationship between foliar polycyclic aromatic hydrocarbons (PAHs) concentrations and plant traits: Intra-canopy variability for a broadleaf species in an urban environment

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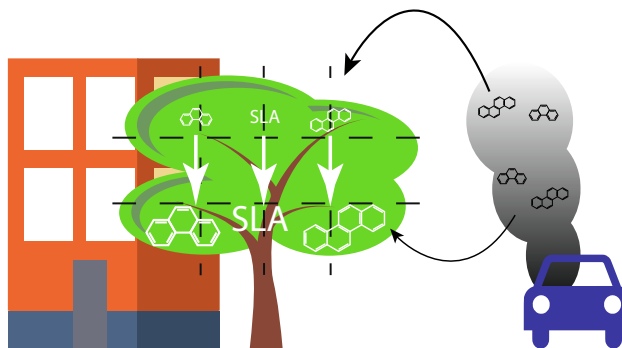
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HIGHLIGHTS

- Intra-canopy variation of PAH concentrations is not negligible.
- This variation is associated with the position of the leaves within the canopy.
- Leaf height is the main source of variation in PAH concentrations.
- Lower leaves take up more PAHs than upper leaves.
- PAHs levels depend on leaf morphology, exposure, and distance to the emission.

GRAPHICAL ABSTRACT



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ABSTRACT

The emission of potentially harmful compounds, including polycyclic aromatic hydrocarbons (PAHs), and the resulting air pollution is a serious problem in modern cities. It is therefore important to develop mitigation strategies, such as “smart” planting of trees that act as sinks for PAHs. However, the intra-individual (within-tree) variability in leaf PAH concentrations remains unknown. In this paper, we studied 15 ornamental apple trees (*Malus × moerlandsii* ‘Profusion’) growing on a main street in a medium-sized city in Galicia (NW Spain). We determined the PAH concentrations at 12 canopy positions in each tree (2 orientations and 2 distances from the trunk at 3 heights), measured various ecological traits (specific leaf area [SLA], $\delta^{13}\text{C}$, stomatal density, fatty acid contents and leaf hairiness) and analyzed the variability in traits within the canopy in relation to PAH concentrations. We observed high intra-individual variability in the PAH concentrations and the leaf traits. Statistical analyses revealed that leaf height was the main source of variability both in the PAH concentrations and in the traits, mainly due to the leaf morphology, particularly to the SLA. Therefore, the ideal vegetation to remove PAHs would be high leaf biomass trees, not too tall and with a high proportion of shade leaves.

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1. Introduction

Air pollution is a major environmental problem and has important effects on ecosystems and human health (Kampa and Castanas, 2008), in particular, in urban areas (EPA, 2023; Piracha and Chaudhary, 2022). The exposure to air pollution is responsible for approximately 300,000 premature deaths every year in Europe (EEA, 2022) and is estimated to cause over 4 million premature deaths annually, globally (World Health Organization, 2022). Air pollution is increasing along with the rapid rise in the urban population and 68 % of the world's population is expected to live in urban areas by 2050. This would represent an almost threefold increase in the world's urban population (United Nations Department of Economic and Social Affairs Population Division, 2019), which highlight the need to address urban development challenges in a sustainable manner. Therefore, it is essential to develop local strategies to reduce pollution in cities.

Plants are known to accumulate gaseous contaminants and particulate matter (PM) (Terzaghi et al., 2013). “Smart” tree planting, involving the use of trees as green filters to remove pollutants from the air, has thus been proposed as a measure to reduce pollution (Nowak et al., 2014). However, for this approach to be effective, more information about the mechanisms of the uptake of air pollutants in trees is required (Janhäll, 2015). This information could then be used to develop models and make informed decisions for selecting the types of trees that should be planted in urban environments, considering the capacity of the trees to remove pollutants from the air. Polycyclic aromatic hydrocarbons (PAHs) are included among the compounds that contribute to the deterioration of air quality in cities and that can be mitigated by trees (Wagrowski and Hites, 1997). PAHs are known carcinogens and primarily originate from the incomplete combustion of fossil fuels (IARC, 2010). The main sources of PAH emissions in urban areas include road traffic and building heating systems (Krugly et al., 2014).

Numerous studies have analyzed PAH concentrations in trees, mainly in the leaves (e.g. Ray et al., 2021; Pleijel et al., 2022); however, many aspects of the uptake of these pollutants by trees remain unknown. Some studies have compared foliar PAH concentrations (in different cohabiting species) in relation to leaf traits, with contradictory findings. For example, variable results have been obtained regarding the relationship between specific leaf area (SLA) and PAH concentrations, depending on the considered PAH compound. For heavy molecular weight (HMW) PAHs, Howsam et al. (2001) reported that an increase in SLA increases PAH uptake, while Tian et al. (2019) found the opposite, and Pleijel et al. (2022) did not observe any effect of SLA. The variable findings can probably be attributed to the complex interactions between traits -which determine the PAH concentration in leaves- and the difficulty in isolating the effects of individual traits. Thus, a novel approach to the study of the relationship between PAHs and traits is to focus on a single species; this will minimize the number of interactions between traits because the intra-specific (inter-individual) variability in some traits is lower than the inter-specific variability (Jiang et al., 2020).

Most researchers (with the exception Murakami et al., 2012) have ignored intra-individual variability in foliar PAH concentrations in relation to leaf position. Intra-individual variability is of paramount importance in modular organisms such as plants, where there may be greater variation within the same individual than between individuals of the same species (Herrera, 2009). This is a major restriction to accurately scaling up the concentration of PAHs from the leaves sampled to the total concentration in the tree, hindering estimation of the ability of plants to remove pollutants from the air.

Nowadays, modelling studies (e.g. Wania and McLachlan, 2001; Undeman et al., 2009; Trapp, 2015; and Terzaghi et al., 2017) generally use a bulk or generic plant/air partition coefficient (KPA) and/or particle deposition velocity to evaluate PAH and PM capture and ignore “within the canopy” variability of traits such as leaves position, SLA and Leaf Area Index (LAI), among others. Stochastic versions of such deterministic simulations are lacking due to the lack of measurements of

the variability. Therefore, it is essential to characterize the magnitude and patterns of intra-individual variability and to establish standardized sampling designs to provide a representative estimate of PAH concentrations in the whole individual.

The main aims of the present study were to determine i) the intra-individual variability in foliar PAH concentrations and ii) the relationship between this variability and leaf traits (SLA, stomata density, gas exchange, cuticular waxes and hairiness) and leaf position (height, distance to the trunk and orientation). In addition to these aims, a further objective was to propose guidelines for selecting the most suitable trees and tree canopy treatments (such as pruning) that could be conducted to enhance PAH capture.

2. Material and methods

2.1. Sampling and sample processing

Sampling was conducted on 10–11 May 2021, on a main street in A Coruña (see Fig. A.1), a city in northern Galicia (NW Spain). The average elevation of the city is 21 m a.s.l., ranging from 0 to 291 m. The city occupies an area ca. 39 km² and has a population of ca. 245,000 inhabitants (2021). The climate in the area is oceanic, with an average annual temperature of 14.8 °C, mean precipitation of 1014 mm distributed across 130 rainy days (>1 mm) per year (climatological normal values in Coruña between 1981 and 2010; <http://aemet.es/>) and a wind speed at 10 m height of 5 m s⁻¹ (mean value between 2007 and 2021; <http://www.meteogalicia.gal/>). The sampled street is one of the main one-way streets in the city, almost 1 km long, with three traffic lanes and cars parked on both sides. The slightly sloping street has an average traffic intensity of 12,262 vehicles/day.

The species selected for this study was an ornamental apple tree (*Malus × moerlandsii* ‘Profusion’). This selection was made because the trees sampled met the characteristics that we considered relevant and necessary for the study, both in terms of location (the characteristics of the street where the trees were located and their arrangement in it) as well as the characteristics of the trees themselves (their age, foliage, canopy height...). Samples were obtained from 15 apple trees, of age at least 15 years, growing on the pavements on both sides of the road (see Fig. A.1). The average dimensions of the trees were as follows: canopy bottom height, 2.3 m; canopy top height, 7.3 m; canopy diameter, 5.4 m; and trunk diameter at breast height (DBH), 13.8 cm. Twelve samples were taken in each tree, i.e.: at three heights (from the bottom, middle and top of the canopy), two orientations (towards the road and towards the buildings) and at two distances from the trunk (near and far from the trunk) (see Fig. 1). Between 15 and 25 leaves were sampled at each point. The sampled leaves were wrapped in aluminum foil, placed in zip lock bags, which were then sealed and stored at 5 °C. The leaves were subsequently analyzed to measure different leaf traits and to determine the foliar PAH concentrations.

2.2. Trait measurements

The traits measured in this study are of interest in relation to understanding PAH loading mechanisms in tree leaves. The SLA is defined as the ratio of leaf surface area to leaf dry weight. Thus, for a given weight of leaf, higher SLA values will indicate larger areas available for deposition. Some authors (e.g. Desalme et al., 2013; Dias et al., 2016) have suggested that the opening of stomata during gas exchange may contribute to the diffusion of PAHs. To evaluate this possibility, the stomata density and $\delta^{13}\text{C}$ (a proxy for the intensity of gas exchange) were measured. The isotopic discrimination capacity of plants is determined by the length of time that stomata are open, which determines gas exchange and photosynthetic activity (carbon fixation). Thus, high $\delta^{13}\text{C}$ values correspond to low gas exchange and vice versa. The best known way in which PAHs bind to leaves is sorption to the hydrophobic cuticle (Barber et al., 2004). This phenomenon was evaluated by measuring the

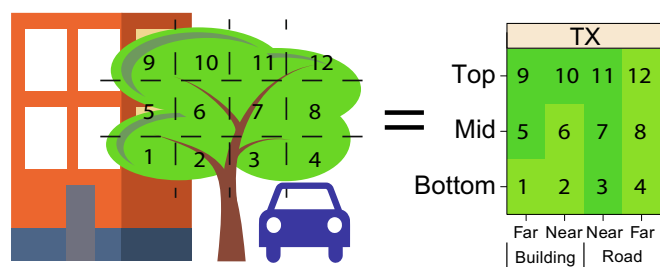


Fig. 1. Sketch showing the position of the 12 points studied in each tree (left) and the corresponding representation that will be used hereafter in this paper (right). The different shades of green indicate different hypothetical concentrations of PAHs at the sampled positions within the tree.

fatty acid content of leaves. Finally, the presence of trichomes on leaves, i.e., the pilosity, can increase the roughness of the leaf surface and modify the leaf boundary layer, thus altering the capacity of leaves to retain PAHs.

2.2.1. SLA

From each of the 180 samples, between 7 and 10 (depending on sample availability) fresh leaves were selected at random. The petioles were removed, and the projected area of each leaf was measured with a LI-3100 AREA METER scanner (LI-COR®). The leaves were then dried at 100 °C in an oven (Digitronic-TFT model; J.P. Selecta®) until constant weight and weighed with a precision balance (model C-3000CB; Cobos®).

2.2.2. Stomata density

One leaf was selected from each sample, and three impressions of the abaxial surface were made with nail varnish. A photograph of each impression was taken (CX41 Olympus® microscope), and stomata were counted over an area of 0.777 mm². The stomatal density was estimated as the average of the counts on the three impressions.

2.2.3. Gas exchange- $\delta^{13}\text{C}$ VPDV %

Leaves selected for carbon isotopic determination were dried at 100 °C until constant weight, before being homogenized in a ball mill (MM 400; Retsch®) at 12,000 rpm. The homogenized samples were stored in hermetically sealed glass vials at 20 °C in darkness until analysis. Carbon isotopic composition was determined by the Research Support Service (SAI, University of Coruña) with an EA1108 elemental analyzer (Carlo Erba Instruments) coupled to a MAT253 isotope ratio mass spectrometer (Thermo Finnigan) and a ConFloIII interface (Thermo Finnigan). The $\delta^{13}\text{C}$ ratios are expressed in parts per thousand (‰) relative to VPDV (Vienna Pee Dee Belemnite) according to standard notation ($\delta^{13}\text{C} = [(\text{R}_{\text{sample}} / \text{R}_{\text{standard}}) - 1] \times 1000$, where R is the ratio $^{13}\text{C}/^{12}\text{C}$).

2.2.4. Fatty acid concentration in cuticular waxes

The fatty acid concentrations ($\mu\text{g g}^{-1}_{\text{fw}}$) in waxes were determined from a total of 60 samples, so that for each of the 12 locations on the tree, 5 samples were semi-randomly selected from the 15 samples (trees) available. Fresh leaves (0.2 g) were immersed in 2 mL of ethyl acetate and then sonicated for 2 min in an ultrasound bath (50 Hz, 25 °C). A 0.2 mL sample of the obtained extract was then diluted in ethyl acetate to a final volume of 1 mL. A 0.1 mL sample of this solution was evaporated to dryness under a gentle stream of nitrogen. The remaining solid was reconstituted in 0.5 mL of methyl tributyl ether (MTBE), and the fatty acids were derivatized by reaction with trimethyl sulfonium hydroxide (TMSH) according to a previously optimized procedure (Gómez-Brandón et al., 2008). After derivatization, the solution was filtered (0.22 μm) and analyzed by gas chromatography-mass spectrometry (GC-MS), in a chromatograph (Agilent 7890A) coupled to a mass

spectrometer (Agilent 5975C) and an autosampler (Agilent 7693) (Palo Alto, CA, USA). Separation was carried out on a Zebron Semivolatiles column (30 m $\times$ 0.25 mm i.d. $\times$ 0.25 μm film thick) from Phenomenex (Torrance, CA, USA). The GC-MS instrumental parameters were previously optimized to achieve optimal separation and an unequivocal identification of the target compounds (García-Jares et al., 2017).

The fatty acids determined in the samples were decanoic, myristic, palmitic, heptadecanoic, stearic, oleic, linoleic, and linolenic acid.

2.2.5. Characterization of leaf pilosity

In order to characterize the leaf pilosity, we took optical microscopy and electron microscopy pictures. Two leaves were selected from each tree -from the bottom and the top of the canopy- and were photographed in a stereoscopic microscope (Leica M205FA, equipped with a Leica DFC7000T CCD camera), in brightfield and fluorescence mode, with the ET GFP filter (ex: 470/40 nm, em: 525/50 nm) at 20 $\times$ optical magnification. To get a better idea of the leaf structure, scanning electron microscopy (SEM) photomicrographs of two leaves were acquired, at different magnifications, with an electron microscope (SEM Zeiss EVO LS15). The morphology, size, and density of the trichomes were determined by visual inspection.

2.3. PAH determination in leaves

Each sample was dried in a paper envelope at 25 °C in a universal precision oven (model Digitronic-TFT; J.P. Selecta®), before being homogenized in an ultra-centrifugal mill (ZM 200; Retsch®) at 12,000 rpm and finally stored in hermetically closed glass vials at 20 °C in darkness until PAH analysis.

The following PAHs were analyzed: acenaphthylene (Acy), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (Ant), fluoranthene (Ft), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Chry), benzo(b+j)fluoranthene (B(b+j)F), benzo(k)fluoranthene (BkF), benzo(e)pyrene (BeP), benzo(a)pyrene (BaP), dibenzo(a,h)anthracene (DBaH), indeno(1,2,3-cd)pyrene (IP) and benzo(ghi)perylene (BghiP). The foliar contents of these PAHs were measured by the Applied Analytical Chemistry research group (QANAP, University of Coruña), with the MSPD-PTV-GC-MS/MS method (optimized for *Quercus robur*) developed by this group. The method involves matrix solid-phase dispersion extraction followed by programmed temperature vaporization-gas chromatography-tandem mass spectrometry (for a brief summary of the method and the equipment used see (De Nicola et al., 2017; for further details, see De Nicola et al., 2016). In cases where the concentration was below the limit of quantification, the middle-bound approach (0.5*LOQ) was applied.

2.3.1. Quality assurance/quality control (QA/QC)

Instrumental and procedural blanks, i.e. surrogates spiked on the C₁₈ without matrices, were systematically evaluated (De Nicola et al., 2016). Quality control of the complete procedure and quantification was performed using labelled PAHs as surrogate standards. Chromatographic control was performed by injection of two standard solutions containing all target PAHs for each batch. Peak identification and integration were performed by Excalibur 2.0.7 software from Thermo Fisher Scientific Inc. The LOQs for each PAH correspond to those reported for *Q. robur* (De Nicola et al., 2016), and generally comprised between 0.13 and 1.46 ng/g dw.

2.4. PM measurement

It was not possible to measure particulate matter (PM) in the air at the same time as the leaves were sampled. The PM levels were sampled in 2023, on the same dates and under similar meteorological conditions. The concentrations of PM₁₀ and PM_{2.5} were determined with an M2000 2nd Generation Multi-functional Air Quality Detector (Temtop®), at each of the 12 points in trees 1, 2, 9, 10, 11, 12 and 14, on 23 and 24 May

2023. The lack of leaves on the other trees meant that measurements could not be performed. However, we made measurements at all trees at a height of 1.70 m, close to the road and close to the buildings. The lack of leaves on some of the trees may have been due to pruning work carried out in the street. Tree number 15 was found to be dead.

2.5. Data analysis

In order to elucidate the differences between the different positions within the tree, different statistical analyses were performed according to the characteristics of the data (see Fig. A.2). In summary, the normality and homoscedasticity of the data were tested. When the data were not normally distributed, the optimal Box-Cox or Yeo-Johnson power transformation was applied. The interaction between canopy height, distance from the trunk and orientation (towards the building or the road) and their main effects were tested by ANOVA or permutation tests. Finally, linear regression models were fitted with the significant variables where the data allowed (normality and homoscedasticity). All tests were performed on PAHs with <10 % observations below the LOQ. The significance level for all tests was 0.05.

All statistical analyses were performed using different functions and packages in RStudio version 4.1.1 (RStudio Team, 2021). The shapiro.test function (stats package) was used to test for normality, and the leveneTest function (car package; Fox and Weisberg, 2019) was used to test for homoscedasticity. The Box-Cox transformation was performed with the boxcox function (MASS package; Venables and Ripley, 2002) and the interactions and main effects were determined using the aov (stats package) and perm.fact.test (asbio package; Aho, 2022) functions. The respective post hoc analyses were performed with the TukeyHSD function (stats package) and the pairwisePermutationTest function (rcompanion package; Mangiafico, 2022). Finally, the models were fitted using the lm function (stats package).

To determine the relationships between the PAHs and to observe how the trees clustered according to PAH concentrations, we performed a principal component analysis (PCA) and grouped the trees with a K-means algorithm. The mean values of the 12 concentrations of PAHs determined in each tree were used as input data in the analysis. The PCA was performed with the prcomp function (stats package). The functions of the factoextra package (Kassambara and Mundt, 2020) and the corrplot function (corrplot package; Wei and Simko, 2021) were used to visualize the results. Clustering was performed with the fviz_nbclust and eclust function (factoextra package).

Finally, the relationships between PAHs and traits were determined by Spearman correlations, implemented with the cor function (stats package). The significance of each correlation was determined with the cor.mtest function (corrplot package). The same package was used to visualize the results.

3. Results and discussion

3.1. PAH concentrations in leaves

The total PAH concentrations in leaves ranged from 57 to 472 ng/g dw (see Table A.1 for each sample). These results were in line with those reported in the literature for other plant species located in urban and semiurban areas in Europe (De Nicola et al., 2014; Fasani et al., 2016; Franzetti et al., 2020; Giráldez et al., 2022; Terzaghi et al., 2015a). Although our results are not directly comparable with them because they were not determined in the same spring/summer period, did not use deciduous trees, or were not located next to roads, they can give an idea of the range of concentrations. For example, Pleijel et al. (2022) in 2018 measured PAHs in leaves/needles of 11 species in the city of Gothenburg (Sweden), 800 m away from the main road, showing concentrations that ranged from 15 ng/g dw for *Fagus orientalis* to 160 ng/g dw for *Pinus nigra*. De Nicola et al. (2014) found 200 ng/g dw to 7800 ng/g dw in *Quercus ilex* leaves collected in Naples city (Italy) from 1998

to 2009, while the concentrations in Terzaghi et al. (2015b) in *Cornus mas* and *Acer pseudoplatanus* leaves, sampled in a semiurban site located in Como (Italy) in 2007, ranged from 30 to 170 ng/g dw. These numbers are more than one order of magnitude lower than those measured in megacities such as Shanghai (Liang et al., 2017; Yin et al., 2020).

In the present study, the most abundant PAH was Chry, which represented 17 % of the PAH fingerprint, followed by Ft (14 %) and Pyr (13 %). The sum of the isomers BbF and BbF (B(b+j)F) also accounted for 17 % of the total PAHs. The concentrations of Acy, Ace, Fl, Ant, BaP and DBaH were below the LOQ in several samples (>10 %) (Figs. A.3-A.8).

3.1.1. Inter- and intra-individual variation in PAH concentrations

The concentrations of PAHs differed between trees (inter-individual) and leaf positions (intra-individual) (Table A.1). Overall, both types of variation are of the same order of magnitude (Fig. 2). The differences in total PAH concentrations are illustrated in Fig. 2A; the foliar concentrations were about two times higher in trees 7, 8, 9, 11 and 12 than in the other trees. Differences between positions were also observed (Fig. 2B), mainly in relation to canopy height, with apparently higher concentrations in the lower areas of the canopy than in the upper areas.

3.1.2. Differences in PAH concentrations

The results of the statistical analyses (see Fig. A.2) used to test for differences in the PAH concentrations in the leaves according to their position in the trees are shown in Table 1. In most cases, there were significant differences (p -value <0.05) in the concentrations in relation to height in the canopy and distance from the trunk. Leaves lower in the canopy and closer to the trunk had significantly higher concentrations than those higher and further away from the trunk. Leaf orientation towards the road or buildings was only significant for BghiP, with leaves facing the road having higher concentrations of this compound than leaves facing buildings. The adjusted R^2 of the linear regression models performed with the significant variables are also shown in Table 1. The range is 0.14 to 0.48, with an average R^2 of 0.26 for individual compounds.

3.2. PAH concentration and tree clustering

PCA was used to examine patterns in PAH concentrations and relationships among the different compounds. The inclusion of PAHs with a high percentage (>10 %) of observations below the LOQ altered the natural variability of the data, so we decided to remove these compounds (Acy, Ace, Fl, Ant, BaP and DBaH) from the analysis (Fig. 3). All variables are well represented by 2 components, which explained ca. 80 % of the variation. The first component (Dim1 in Fig. 3) discriminated between trees with higher total PAH concentration and lower Phe concentration, and trees with lower total PAH concentrations and higher Phe concentrations. The presence of higher total concentrations is presumably associated with higher exposure to pollution sources (vehicles). The second component (Dim2 in Fig. 3) mainly explains part of the variability of Phe and BghiP and, to a lesser extent, seems to slightly discriminate the other PAHs according to whether they are associated with the gaseous phase or particulate matter. Nonetheless, the 3-ring PAHs produced different results than the other compounds, probably due to their higher volatility or the existence of an unknown secondary source contributing additional 3-ring PAHs to trees at the beginning of the street. The BghiP also produced slightly different results, as although it seems to follow the general trend of most PAHs (positive values in Dim1), it seems to be better represented by Dim2. On the basis of these results, we differentiated two clusters of trees (Fig. 3C): group 1 (Fig. 3D; trees 1 to 6, 10, and 13 to 15), with lower total PAH concentrations, but higher concentrations of light PAHs; and group 2 (Fig. 3D; trees 7 to 9, 11 and 12), with higher total PAH concentrations. These two groups of trees can be clearly observed in Fig. A.9, in which total PAHs, Chry (representative heavy molecular weight PAHs) and Phe (representative of the 3-ring fraction) concentrations are shown for each sample.

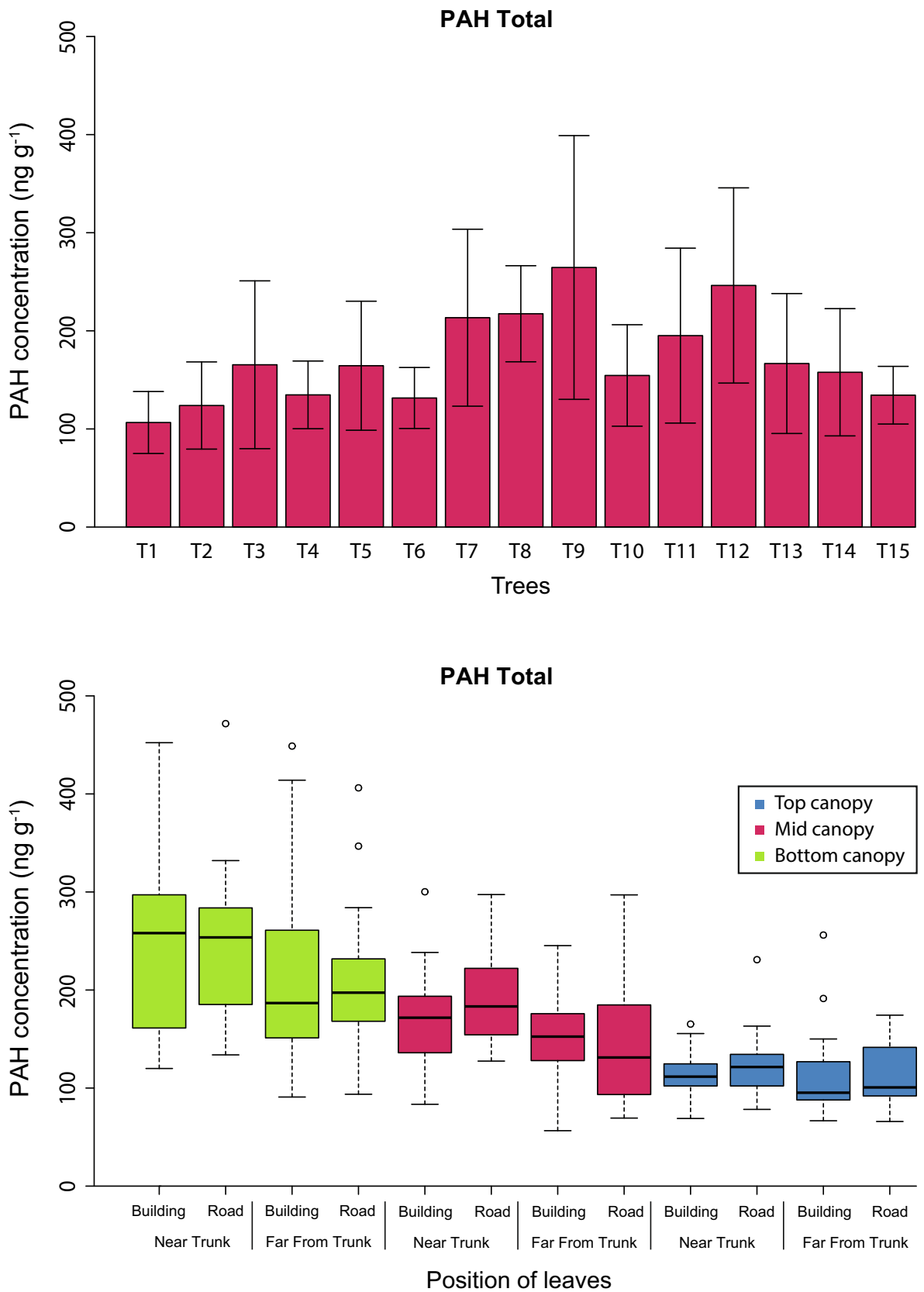


Fig. 2. A: Bar chart of the total PAH concentrations (ng g^{-1}) in each of the sampled trees. The error bars represent the standard deviation. B: Box plot of the total PAH concentration (ng g^{-1}) in each of the 12 sampled points within the tree canopy ($n = 15$).

Table 1

Statistical analysis results on the differences in PAH concentrations in leaves according to their position in the canopy ($n = 180$, i.e., all trees). The significance level (α) selected was 0.05 for all analyses.

PAH	Test	Interaction	Main effect	Post hoc	Adjusted R ^{2*}
Phe	A	–	C.H. T.D.	Bot>Mid, Bot>Top N > F	0.18
Ft	A	–	C.H.	Bot>Mid, Bot>Top	0.14
Pyr	A	–	C.H. T.D.	Bot>Mid, Bot>Top, Mid > Top N > F	0.34
BaA	A	–	C.H. T.D.	Bot>Mid, Bot>Top, Mid > Top N > F	0.21
Chry	A	–	C.H. T.D.	Bot>Mid, Bot>Top, Mid > Top N > F	0.26
B(b+j)F	A	–	C.H. T.D.	Bot>Mid, Bot>Top, Mid > Top N > F	0.31
BkF	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.19
BeP	P	–	C.H. T.D.	Bot>Mid, Bot>Top, Mid > Top N > F	–
IP	P	–	C.H. T.D.	Bot>Mid, Bot>Top, Mid > Top N > F	–
BghiP	A	–	C.H. T.D.	Bot>Mid, Bot>Top, Mid > Top N > F	0.48
3rings	A	–	O. C.H.	R > B Bot>Mid, Bot>Top	0.15
4rings	A	–	T.D. C.H.	N > F Bot>Mid, Bot>Top, Mid > Top	0.26
5rings	A	–	T.D. C.H.	N > F Bot>Mid, Bot>Top, Mid > Top	0.29
6rings	A	–	T.D. C.H.	N > F Bot>Mid, Bot>Top, Mid > Top	0.37
Total	A	–	T.D. C.H.	N > F Bot>Mid, Bot>Top, Mid > Top	0.38

A, ANOVA; P, permutation; C.H., Canopy Height; T.D., Trunk Distance; O, Orientation; –, indicates no significant interaction; Bot, Bottom canopy; Mid, Mid canopy; Top, Top canopy; N, Near to the trunk; F, Far from the trunk; R, Road orientation; B, Building orientation.

* Adjusted R² of the regression model constructed with the significant variables.

3.3. Intra-individual differences in PAHs by tree cluster and PM concentrations

3.3.1. Differences in PAH concentrations for each tree cluster

The results in Table 1 include all samples from all trees; however, as the concentrations of PAHs in the trees are not homogeneous and considering the clustering results in Fig. 3, the statistical analyses were repeated by separating the trees into two groups (Tables A.2 and A.3). In the first group (Table A.2), the results (significant differences, i.e. p -value < 0.05) were similar to those reported in Table 1. The differences were mainly between canopy height and distance from the trunk. The R² values were also very similar (range 0.12 to 0.45; mean 0.27). In the second group (Table A.3), the differences were only related to canopy height, and differences according to distance from the trunk were only observed for Phe. The R² values of this group were much higher (range 0.32 to 0.59; mean 0.50). Orientation seems to have little effect in both cases, and significant differences were only detected for BghiP (Table A.2). An interaction between orientation and distance from the trunk was also detected for BaA (Table A.2). The observed differences between the two groups can probably be explained by the fact that group 2 is smaller and more homogeneous. In addition, the trees in group 2 are closer to each other, and they are therefore subject to less variability than the trees in group 1, which are distributed along the entire length of the street. These two factors could explain why the explanatory power of the linear models is higher in group 2. Note that BaP is included in group 2 analysis but not in group 1 due to the high percentage of samples < LOQ.

3.3.2. Differences in PM concentrations

The concentrations obtained for atmospheric PM_{2.5} and PM₁₀ in the different parts of the canopy and at a height of 1.70 m are shown in Fig. A.10. As can be seen from both figures and the test results (Table A.4), there does not seem to be any significant difference related

to the different parts of the tree. This result was surprising, as previous findings and the results obtained for the PAH concentrations in the leaves suggested the opposite. In fact, several authors (see Hofman et al., 2013) have detected decreases in PM concentration with height. The following factors may explain this result: i) the time between sampling, the distribution of PAHs and PM is spatial and temporal, so by measuring at different times it is likely that environmental factors have been affected, e.g. the structure and cover of the trees, which has changed, affecting the barrier effect and their filtering capacity; ii) the wind turbulence that can occur in a street canyon, which may contribute to homogenizing the concentration of pollutants at different heights (De Nicola et al., 2013); and iii) single point measurements, which may not capture the differences that accumulate over time.

3.4. Trait variability

3.4.1. Inter- and intra-individual trait variability

The results for the different traits considered (SLA, $\delta^{13}\text{C}$, stomata density, concentration of different fatty acids in the leaf cuticle and leaf pilosity) are shown in Figs. 4 and A.11–A.15. These figures show that the inter-individual variability (Figs. A.11 and A.13) is lower or similar to the intra-individual variability (Figs. 4 and A.12). In fact, after calculating the inter-individual coefficient of variation (CV) of the traits and comparing it with the mean intra-individual CV, we found the former to be similar to (e.g. linolenic acid: inter-CV = 2.1 % and intra-CV = 2.0 %) or lower than (e.g. SLA: inter-CV = 6.8 % and intra-CV = 18.9 %) the latter.

The position of the leaves within the tree canopy seems to account for a large part of the intra-individual variability. Thus, the most exposed leaves (sun leaves, top canopy) seem to have a lower SLA, higher stomata density and higher $\delta^{13}\text{C}$ (Fig. 4) than the least exposed leaves (shade leaves, bottom canopy). These results are not surprising considering the variation in these variables in relation to height and

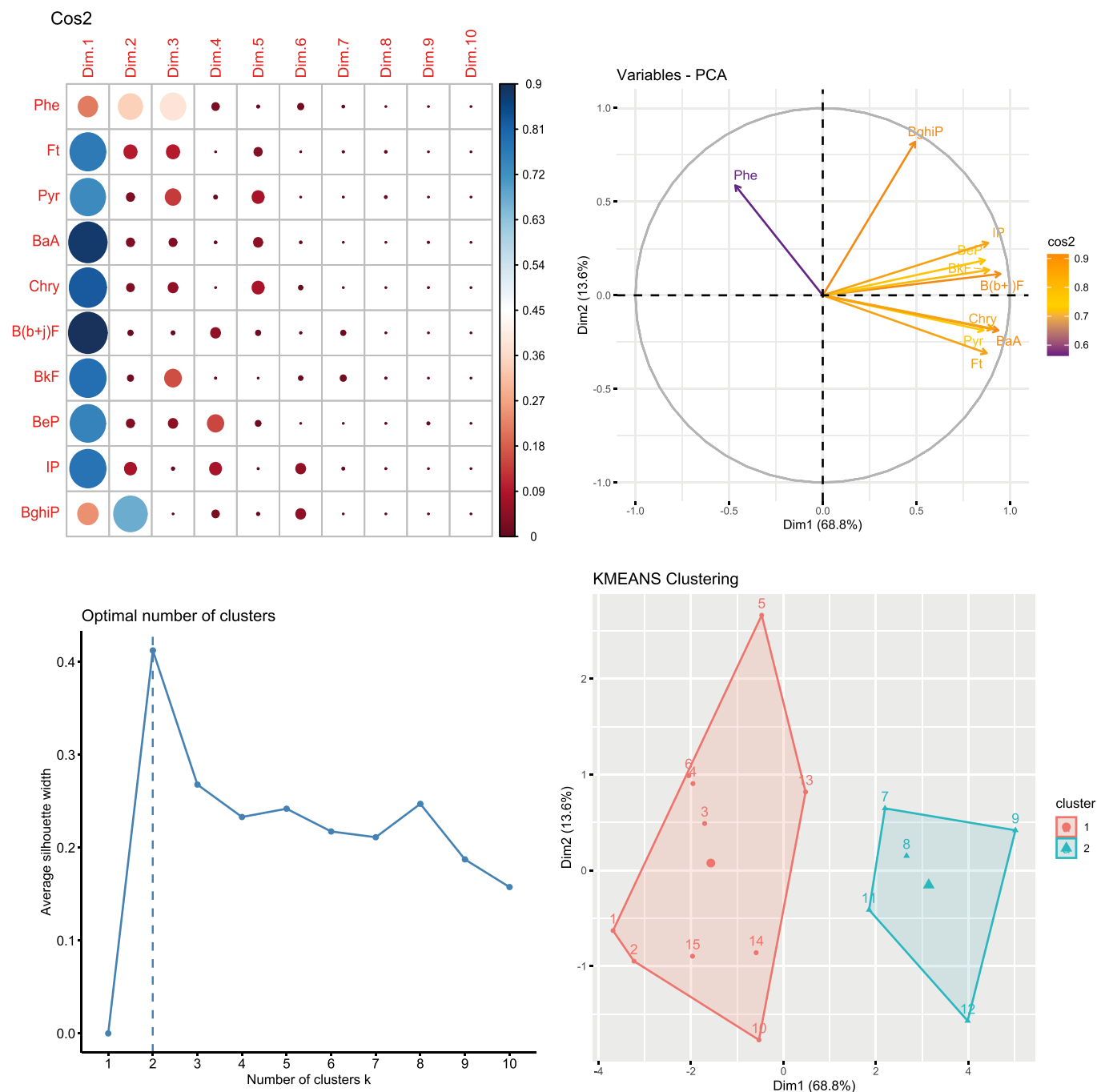


Fig. 3. Results obtained for PAHs for which <10 % of the observations were below the LOQ. From left to right and from top to bottom. A: Cosine-squared representation (Cos2), indicating the quality of representation of the variables by each of the PCA dimensions. B: Variable correlation plot. Positively correlated variables are grouped together, and negatively correlated variables are placed in opposite quadrants. The length of the arrows indicates the quality of representation of the variables (the longer the arrows, the better). C: Optimal number of clusters indicated by the dashed line. D: Result of clustering the trees with a KMEANS algorithm.

light exposure within the tree canopy (Vinod et al., 2023). The SLA indicates the leaf area per gram of leaf and is generally higher in low light conditions (Liu et al., 2016), i.e. leaves that are not as exposed to sun-light (leaves in the lower part of the canopy and close to the trunk). The $\delta^{13}\text{C}$ represents the $^{13}\text{C}/^{12}\text{C}$ isotope ratio, which is modified by the positive discrimination of ^{12}C against ^{13}C that occurs in C fixation processes in leaves. This discrimination is higher when stomata are open, with a continuous flow of CO_2 , than when stomata are closed. Although the maximum stomatal conductance tends to increase with tree height, the stomata of sun leaves are more likely to close due to increased transpiration, which increases the $\delta^{13}\text{C}$ value (Vinod et al., 2023).

Stomatic density (stomata per unit area) is an indicator of possible gas exchange (although this can vary depending on the size of the stomata and duration of opening) and also tends to increase with height and light exposure (Camargo and Marenco, 2011). The fatty acid concentration (Fig. A.12) does not seem to depend on leaf position. In terms of pilosity, the leaves do not seem to be characterized by having many trichomes, which are filiform and mainly located on the leaf veins (Figs. A.14-A.15). A considerable difference between the number of trichomes on the bottom canopy leaves (less trichomes) and top canopy leaves (more trichomes) was observed by the naked eye. In addition, there also appeared to be more trichomes on the adaxial side than on the abaxial

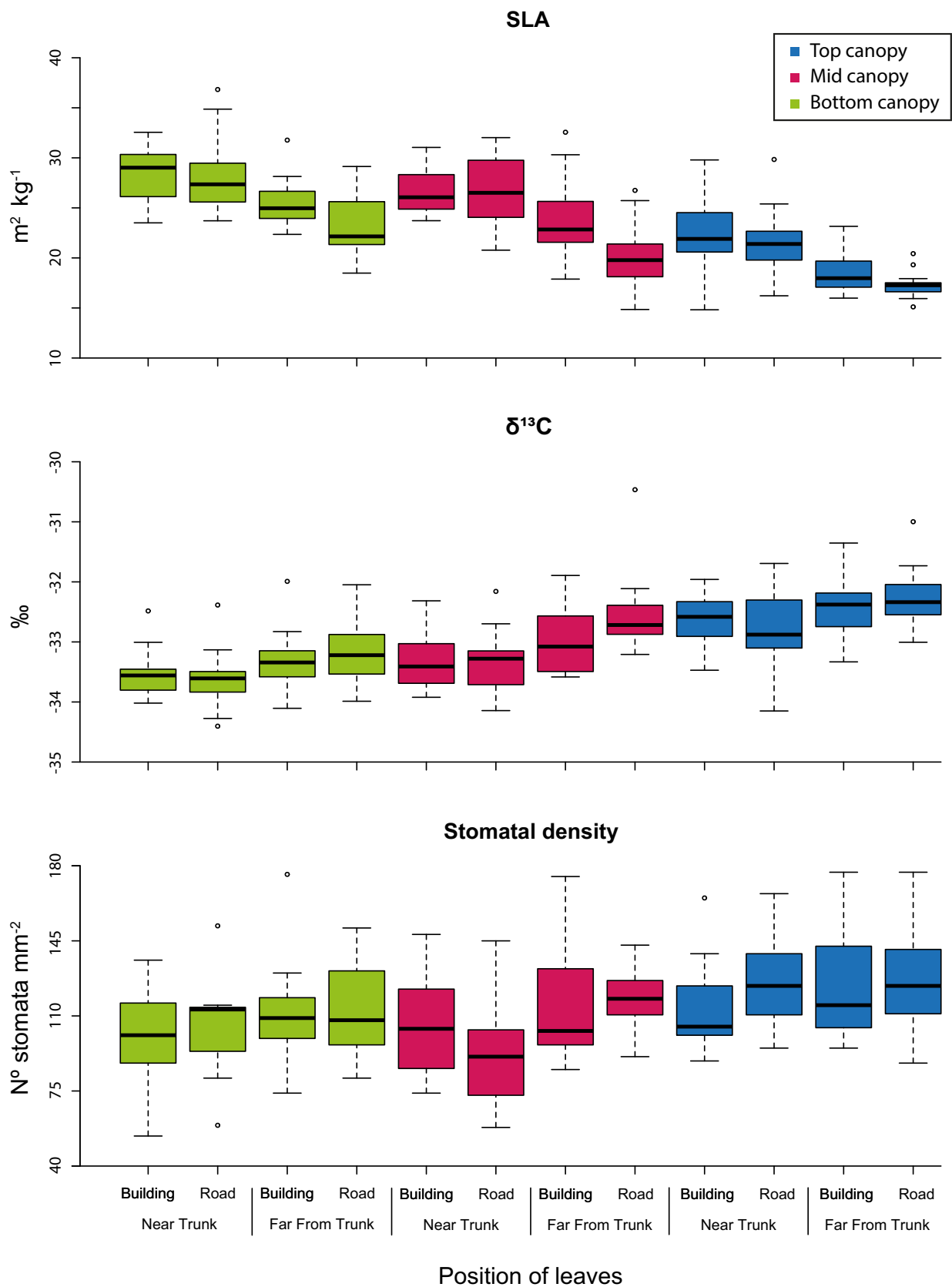


Fig. 4. Box plot of the SLA, $\delta^{13}C$ and stomatal density in each of the 12 sampled points within the tree canopy.

Table 2
Statistical analysis results on differences in leaf traits (SLA, $\delta^{13}\text{C}$, stomatal density and different fatty acids) according to their position in the canopy (all samples from all trees were used). The significance level (α) selected was 0.05 for all analyses.

PAH	Test	Interaction	Main effect	Post hoc	Adjusted R ^{2*}
SLA	A	T.D. O.	C.H.	Bot>Mid, Bot>Top, Mid < Top	0.58
$\delta^{13}\text{C}$	A	T.D. O.	C.H.	Bot<Mid, Bot<Top, Mid < Top	0.40
Stomata density	A	—	C.H. T.D.	Bot<Top, Mid < Top N < F	0.13
Heptadecanoic Ac.	A	—	—	—	—
Decanoic Ac.	A	—	—	—	—
Oleic Ac.	A	—	—	—	—
Linoleic Ac.	P	—	—	—	—
Linolenic Ac.	P	—	—	—	—
Myristic Ac.	A	—	—	—	—
Stearic Ac.	A	—	—	—	—
Palmitic Ac.	A	—	—	—	—

A, ANOVA; P, permutation; C.H., Canopy Height; T.D., Trunk Distance; O, Orientation; —, indicates no significant interaction; Bot, Bottom canopy; Mid, Mid canopy; Top, Top canopy; N, Near to the trunk; F, Far from the trunk; R, Road orientation; B, Building orientation.

* Adjusted R² of the regression model constructed with the significant variables.

side. This is not surprising as the position of trichomes varies greatly between species (Amada et al., 2023). However, it is not uncommon that, as in this case, there are more trichomes on leaves that are more exposed to sunlight (Vinod et al., 2023), where adaxial trichomes are associated, among others, with reflectance and protection functions against damaging ultraviolet-B (UV-B) radiation (Bickford, 2016). Trichomes can have many functions and can even influence gas exchange fluxes (Amada et al., 2023), in the trees under study, the trichomes were sparse and mainly located on the veins, and their effect on PAH uptake was not expected to be very important and was probably limited to retaining particulate matter, as seen in the lower left photo in Fig. A.15.

3.4.2. Intra-individual trait differences

The differences in traits among the different leaf positions in the trees were also tested. The results for the whole data set are shown in Table 2, while the results for each of the two groups of trees are shown in Tables A.5 and A.6. Significant differences were found in all cases for SLA, $\delta^{13}\text{C}$ and stomatal density. The R² (ca. 0.5) indicates that much of the variability in SLA and $\delta^{13}\text{C}$ depends on the position of the leaf on the tree. Regarding stomatal density, the position seems to have little influence (R² below 0.2). For fatty acids, no significant differences were detected between positions, only in group 1 for myristic acid, with a higher concentration in leaves close to the trunk.

3.5. Correlations between PAH concentrations and traits

It is well known that correlations generally reveal the strength and direction of the relationship between two variables, but do not indicate causality. Disentangling causal relationships among variables can be problematic in this field of study, in which a huge number of sources of variability must be considered. Numerous factors can affect the concentration of PAHs in tree leaves, and these factors can also interact and/or blend with each other. In addition to this complication, the variability among different tree species among trees of the same species (especially if they grow in different environments), and the variability within individual trees must be considered. By designing this study on trees of the same species located in the same street, we greatly reduced the variability in these traits, eliminating interspecific variability and limiting inter-individual variability while evaluating intra-individual variability. We were thus able to reduce the dimensions to be taken into account in this problem, allowing us to draw more robust conclusions from the results of the statistical tests applied.

The Spearman correlations that proved significant (p -value <0.05) for the PAHs determined and the traits measured, are shown in Fig. 5. The correlations clearly show a strong inverse relationship between SLA

and $\delta^{13}\text{C}$ and, to a lesser extent, the nature of the relationship (inverse for SLA and direct for $\delta^{13}\text{C}$) of these variables with stomatal density. Likewise, most of the PAHs were positively correlated with SLA and negatively correlated with the other two traits. Most of the cuticle fatty acids were not significantly correlated with any variable except for other cuticle fatty acids. Considering the strong correlations between the traits

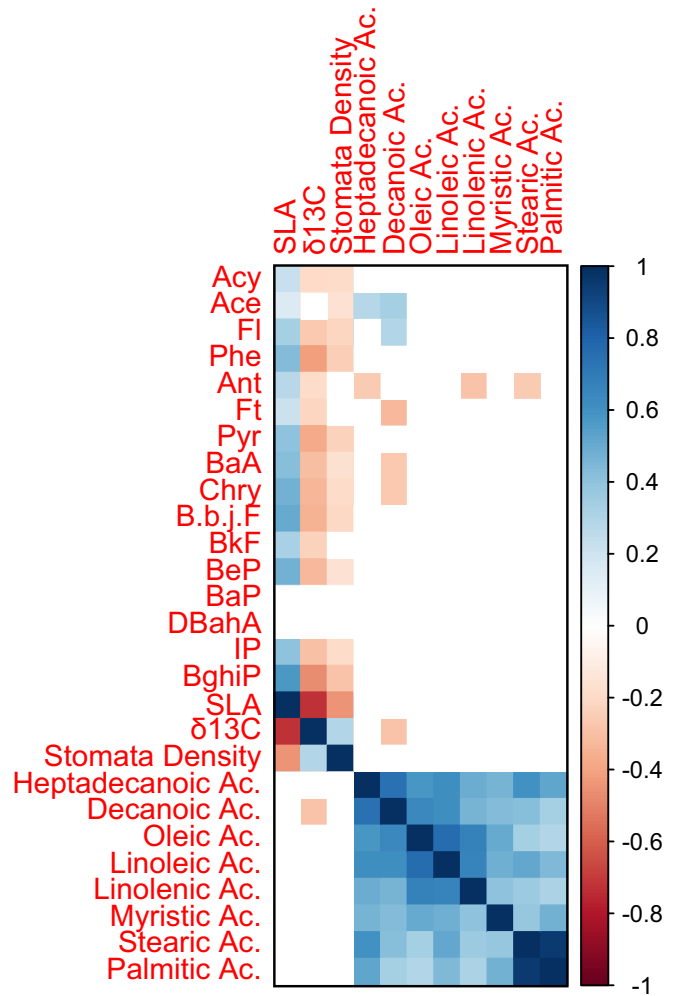


Fig. 5. Significant correlations (p -value <0.05) between PAH concentrations and traits.

Table 3

Statistical analysis results on differences in SLA-corrected PAH concentrations (PAH concentration/SLA) in leaves according to their position in the canopy (n = 180, i. e., all trees). The significance level (α) selected was 0.05 for all analyses.

PAH	Test	Interaction	Main effect	Post hoc	Adjusted R ^{2*}
Phe	A	–	C.H.	Bot>Mid, Bot>Top	0.06
Ft	A	–	C.H.	Bot>Mid	0.05
			O.	R > B	
Pyr	A	–	C.H.	Bot>Mid, Bot>Top	0.13
			O.	R > B	
BaA	A	–	C.H.	Bot>Top	0.07
Chry	P	–	C.H.	Bot>Top	–
B(b+j)F	A	–	C.H.	Bot>Mid, Bot>Top	0.12
BkF	A	–	C.H.	Bot>Mid, Bot>Top	0.12
BeP	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.12
IP	A	–	C.H.	Bot>Mid, Bot>Top	0.09
BghiP	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.31
			T.D.	N > F	
			O.	R > B	
3rings	A	–	C.H.	Bot>Mid, Bot>Top	0.05
4rings	A	–	C.H.	Bot>Mid, Bot>Top	0.07
5rings	A	–	C.H.	Bot>Mid, Bot>Top	0.12
6rings	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.17
			O.	R > B	
Total	A	–	C.H.	Bot>Mid, Bot>Top	0.14

A, ANOVA; P, permutation; C.H., Canopy Height; T.D., Trunk Distance; O, Orientation; –, indicates no significant interaction; Bot, Bottom canopy; Mid, Mid canopy; Top, Top canopy; N, Near to the trunk; F, Far from the trunk; R, Road orientation; B, Building orientation.

* Adjusted R² of the regression model constructed with the significant variables.

related to PAHs concentration (SLA, $\delta^{13}\text{C}$ and stomatal density) it appears that these depend on one main source of variability, the leaf position, which modifies the traits according to the vertical gradient and light exposure. However, although the three traits vary according to these gradients, SLA seems to have the greatest influence on PAH uptake. This is the trait for which the highest correlations were obtained (0.16 to 0.58) and, furthermore, if gas exchange through the stomata would play a central role, differences should be clearly observed in the correlations with the lighter and heavier PAHs, which was not the case. The inverse correlation with stomatal density was probably due to (Prigioniero et al., 2022) the stomata being closed or, at least, not open sufficiently to significantly influence the concentration of PAHs.

3.6. Intra-individual differences in PAH concentrations expressed by leaf surface area

Considering the correlation between PAHs and SLA (Fig. 5) it is possible that some of the differences that we observed in Tables 1, A.2 and A.3 could be attributed to the concentrations being expressed in terms of mass ($\mu\text{g g}^{-1}$), without taking the leaf surface area into account. Therefore, we repeated the analyses with the data divided by the SLA, i. e., by expressing the concentration of PAHs expressed in terms of leaf surface area ($\mu\text{g m}^{-2}$). The results for all trees and for groups 1 and 2, are shown in Tables 3, A.7 and A.8. The most remarkable changes in the results are the disappearance of most of the differences due to the distance of the leaves from the trunk and the decrease in the explanatory power (R^2) of the models. In other words, a large part of the variation observed in the first analyses can be attributed to leaf morphology. This is consistent with the findings of other studies (Terzaghi et al., 2015b) showing the influence of SLA on the uptake of PAHs by trees.

3.7. Final remarks

In this study we have shown the importance of leaf morphology, mainly SLA, in explaining the variation in foliar PAH concentrations. (Terzaghi et al., 2015b) previously showed that the temporal variability of SLA could influence the PAH uptake rate of leaves of the same species during the growth season and therefore variations of SLA in time should be considered when evaluating the multimedia fate of chemicals using models accounting for a vegetation compartment (Terzaghi et al., 2017).

However, the intraspecies (inter-individual) spatial variability was not considered yet.

Some researchers (Jouraeva et al., 2002) recommend expressing foliar PAH concentrations per unit of surface area rather than express them per unit of mass when comparing results for different plant species. Despite following this recommendation (Tables 3, A.7 and A.8), we still observed a significant decrease in leaf PAH concentrations due to leaf height. This observation is consistent with that of (Murakami et al., 2012) who reported a similar decrease, mainly associated with the distance from the source of emission of PAHs (cars). However, these researchers also detected differences between leaves over the road and those over the pavement, which we did not observe. This difference is probably due to the fact that the trees in the aforementioned study have “no obstacles to limit air circulation”, while in the present study the buildings caused a street canyon effect that could enhance local air mixing. Finally, it is important to note that, although the distance from the PAH emission sources may be the main factor accounting for the observed variation between heights (once the effect of SLA is removed), other factors also play a role, such as the barrier effect of leaves and leaf illumination/temperature, because of both their influence on SLA (Gara et al., 2018) and their role in the volatilization/degradation-deposition balance of PAHs (Terzaghi et al., 2020). Interestingly, leaf pilosity is associated with higher uptake and retention of PM-associated PAHs (Prigioniero et al., 2023) and therefore we would expect higher concentration in exposed leaves; however, the slightly higher trichome density does not seem to be able to counteract the effect of factors that decrease PAH concentration with height.

Based on the findings of this study, although a mass balance of PAHs in the air-plant-soil environment was not performed, it is clear that not only SLA would be important as factor driving the uptake but also the leaf area index (LAI, m^2/m^2), which together compose plant biomass. In other terms to significantly reduce air concentrations in urban environments different measures would be needed: emission reduction, and mitigation measures such as the increase of plant foliar biomass in the streets. Rows of trees with high leaf biomass should be planted along roads in cities. Low trees are preferable for a given biomass, as lower leaves capture more pollutants (and remove them directly from the air that pedestrians breathe). These trees should have a dense canopy with a high proportion of shade leaves, characterized by a corresponding large SLA. This would increase the uptake of pollutants such as PAHs and

possibly PM. Therefore, we recommend selecting species with a specific morphology and pruning practices that favour the maintenance of this morphology. Furthermore, as recommended by other authors (e.g. De Nicola et al., 2017), it is better to select perennial species that can remove pollutants throughout the year, especially in winter, when fossil fuel combustion is higher and the planet boundary layer decreases in height for large portion of the day, leading to an increase in the concentration of pollutants in the air of cities.

4. Conclusions

- This research study the intraindividual variability in foliar PAH concentrations in trees by limiting interspecific variation and reducing interindividual variation.
- The intraindividual variability is proved to be significant and should be considered in designing sampling schemes or contamination model development.
- The main source of intraindividual variability is leaf height (bottom, mid or top canopy), with lower leaves taking up more PAHs than higher leaves.
- This depends on leaf morphology (SLA), distance to the emission source (cars), the barrier effect and illumination/temperature of the leaves.
- Therefore, the ideal vegetation to remove PAHs from the air in urban environments similar to the one of this study would be leafy trees (with a high leaf biomass), not too tall and with a high proportion of shade leaves.

CRediT authorship contribution statement

Pablo Giráldez: Formal analysis, Investigation, Visualization, Writing – original draft. **Zulema Varela:** Conceptualization, Funding acquisition, Investigation, Resources, Writing – original draft. **Antonio Di Guardo:** Supervision, Writing – review & editing. **Elisa Terzaghi:** Supervision, Writing – review & editing. **Maria Celeiro:** Investigation, Writing – review & editing. **Carmen Garcia-Jares:** Funding acquisition, Investigation, Writing – review & editing. **J. Ángel Fernández:** Conceptualization, Resources, Validation, Visualization, Writing – review & editing. **Jesús R. Aboal:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing.

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.

Data availability

Most of the data is available in the supplementary material, and the rest will be available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.173698>.

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