



# Leaf ecological traits (morphology and gas exchange) and polycyclic aromatic hydrocarbons concentrations in shrubs and trees: A meta-analysis approach<sup>☆</sup>

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## ARTICLE INFO

### Keywords:

Green filter  
Atmospheric pollution  
Specific leaf area  
PAH  
Air pollution  
Leaves characteristics  
Leaf functional traits

## ABSTRACT

The leaves of trees and shrubs can capture atmospheric pollutants such as polycyclic aromatic hydrocarbons (PAHs), and the capacity of uptake depends on the leaf traits. Although numerous studies have measured PAH concentrations in leaves of woody plants and the variability in leaf traits, few have investigated the relationship between these factors. We conducted a literature review to summarize the available information on this topic and found that five types of leaf traits have been studied, with those associated with leaf morphology and gas exchange being the most common. However, the results of the studies are often contradictory. To address these discrepancies, we conducted a meta-analysis to examine how PAH uptake by woody species is affected by leaf ecological traits associated with morphology (leaf area, specific leaf area [SLA], leaf thickness and leaf width/length ratio [W/L]) and with gas exchange (stomatal conductance, leaf carbon isotopic signature [ $\delta^{13}\text{C}$ ] and stomatal density). The meta-analysis included studies involving at least two different species with comparable PAH concentrations. Many of the studies did not examine the relationship between ecological traits and PAH concentration, and those that did often involve different traits. We therefore used the TRY Plant Trait Database data as the standard source of trait data. Relationships were analyzed by determining differences regarding PAHs and traits and calculating Spearman correlations and their significance. The leaf morphology traits were more closely correlated with PAH concentrations than the gas exchange traits. Thus, morphological traits such as SLA and leaf area can be considered significant predictors of PAH uptake, especially for particulate-associated PAHs. Gas exchange traits showed less consistent correlations, indicating the complexity of factors influencing PAH uptake in leaves. This study highlights the importance of considering multiple leaf traits in order to better understand and predict PAH uptake in woody plants.

## 1. Introduction

Trees can remove polycyclic aromatic hydrocarbons (PAHs) from the atmosphere, as there is general scientific consensus regarding the existence of atmosphere-plant (mainly leaves) partition coefficients and the capacity of plants to adsorb/absorb PAHs (see e.g. Kömp & Mclachlan, 1997; Giráldez et al., 2022). This is further demonstrated by hundreds of articles using chamaephyte and phanerophyte species in biomonitoring atmospheric PAHs. However, the PAH loading process is complex and is

determined by the ecological characteristics of the leaf (i.e. leaf ecological traits). Leaf ecological traits are largely determined by the conditions and resources available in their environment and show adaptive responses (see e.g. Reich et al., 1999; Wright et al., 2004). Nevertheless, the way in which these traits interact with atmospheric PAHs is still the subject of scientific debate (less than two dozen studies, all conducted in the present century, most in the last ten years). Moreover, given the huge differences in the molecular size of the different PAHs and their dispersion forms (gaseous vs. particulate), the

<sup>☆</sup> This paper has been recommended for acceptance by Eddy Y. Zeng.

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interactions are expected to be different for each.

The leaf ecological traits studied in relation to PAH concentrations can be divided into five categories: i) general leaf morphology; ii) leaf gas exchange; iii) ultrastructure of the leaf surface; iv) physical characteristics of cuticle; and v) chemical composition of the cuticle. Various studies have considered leaf ecological traits included in the general leaf morphology category, such as the type of leaf (simple or compound) and its orientation (Terzaghi et al., 2013; Domingos et al., 2015; Dias et al., 2016); width/length ratio (Tian et al., 2019); leaf area (Howsam et al., 2000; Jouraeva et al., 2002, 2006; Domingos et al., 2015; Dias et al., 2016; Yang et al., 2017; Zhao et al., 2018); specific leaf area (Howsam et al., 2001; Müller et al., 2001; Jouraeva et al., 2002, 2006; Tian et al., 2008, 2019; Wang et al., 2008; Domingos et al., 2015; Dias et al., 2016; De Nicola et al., 2017) and leaf thickness (Domingos et al., 2015; Dias et al., 2016). Studies involving traits related to gaseous exchange include stomatal density (Wang et al., 2008; Fellet et al., 2016; De Nicola et al., 2017; Yang et al., 2017; Tian et al., 2019; Prigioniero et al., 2022, 2023), stomatal surface area (Tao & Hornbuckle, 2001; Prigioniero et al., 2022, 2023) and stomatal conductance (De Nicola et al., 2017). Studies on the ultrastructure of the leaf surface have considered the epidermal cell shape and roughness (Howsam et al., 2001; Tian et al., 2008, 2019; Murakami et al., 2012; Terzaghi et al., 2013; Domingos et al., 2015; Dias et al., 2016); leaf hairiness (pubescence: presence, distribution, morphology and size) (Howsam et al., 2000, 2001; Wang et al., 2008; Terzaghi et al., 2013; Domingos et al., 2015; Dias et al., 2016; De Nicola et al., 2005, 2017; Prigioniero et al., 2022, 2023), and roughness and morphology of the epicuticular waxes (amorphous and crystalloid structures) (Terzaghi et al., 2013; Domingos et al., 2015; Dias et al., 2016; De Nicola et al., 2017). Studies concerning the physical characteristics of cuticles have considered the thickness (Wang et al., 2008; Terzaghi et al., 2013; Fellet et al., 2016; Prigioniero et al., 2022, 2023) and the surface free energy (Tian et al., 2019). Regarding the chemical composition, different studies have examined the lipid concentration (Howsam et al., 2001; Jouraeva et al., 2002; Tian et al., 2008; Wang et al., 2008; Murakami et al., 2012; Mętrak et al., 2016; De Nicola et al., 2011), the concentration of cuticle aliphatic compounds (cutin and waxes) (Li et al., 2010), the esterification index (Prigioniero et al., 2022, 2023), the cuticular/epicuticular wax concentration (Jouraeva et al., 2002, 2006; Yang et al., 2017; Tian et al., 2019), the concentration of hydrophobic compounds in the wax (Terzaghi et al., 2013; Domingos et al., 2015) and the polar and dispersion components (Tian et al., 2019).

Two conclusions can be drawn after examining all the previously cited studies: i) the findings are sometimes contradictory and vary according to the compounds; and ii) the leaf ecological traits most commonly studied are those associated with leaf morphology, although some attention has also been given to leaf traits associated with gas exchange. In addition, some of the studies analyze the concentrations of PAHs in leaves from trees or shrubs of more than one species occurring in the same sample site, which could enable comparison of the findings. However, the leaf ecological traits of the species involved have not been documented or studied. Nevertheless, combining the data from these articles with the existing information in the databases on leaf ecological traits (e.g. the TRY Plant Trait Database) enabled us to conduct a meta-analysis focusing on the current questions about the role of leaf ecological traits in the PAH load in leaves of woody species.

Therefore, in the present study, after compiling the PAH concentrations determined in species coexisting at the same sites, we performed a meta-analysis to examine how PAH uptake by woody species is affected by leaf ecological traits associated with leaf morphology (i.e. leaf area, specific leaf area, leaf thickness and leaf width/length ratio) and gas exchange (i.e. stomatal conductance per leaf area, leaf carbon isotope signature [ $\delta^{13}\text{C}$ ] and stomatal density).

## 2. Material and Methods

The bibliographical review was conducted at the end of 2022 using the Scopus document search tool with the search terms "pah AND leaf". A total of 524 documents were initially selected. Two successive screenings were then carried out. In the first, articles written in English and containing useful information on the concentrations of one or more PAHs in phanerophyte leaves were selected, and those concerning herbaceous plants, those written in other languages and those not reporting numerical values of concentrations were disregarded. In the second screening, articles involving at least two different plant species and comparable PAH concentrations were selected as follows: i) tree/shrubs in the same sampling site or close to each other; ii) trees/shrubs affected by the same sources of contamination; iii) samples collected in the same period; and iv) leaves of the same age (to ensure the same length of exposure to the sources of contamination). From these articles, we compiled a dataset with the average concentrations provided for each of the 16 US EPA PAHs (see Table A.1). In cases where the exact concentration was not reported in the article but was represented by figures, the concentrations were estimated using the WebPlotDigitizer tool (<http://apps.automeris.io/wpd/>). In addition to the 16 individual PAHs (see Table A.1), we also calculated the total sum of PAHs, the sum of PAHs according to their number of rings (2–6) and the sum according to the molecular weight: low (LMW, PAHs with 2–3 rings), medium (MMW, PAHs with 4 rings) and high (HMW, PAHs with 5–6 rings). Different ways have been used to group PAHs according to their molecular weight (e.g. Amodu et al., 2013; De Nicola et al., 2017; Mukhopadhyay et al., 2020; Pleijel et al., 2022; Singh et al., 2023), but the approach used here is one of the most common (e.g. Zhang et al., 2020; Ray et al., 2021; Yang et al., 2022). The sums were only computed when values were available for each of the individual PAHs.

Many of the selected studies either did not study the relationship between leaf traits and PAH concentrations or did not consider the same traits. We therefore decided to use the traits included in the TRY Plant Trait Database (Kattge et al., 2020) as a standard source of data for the species in our database. Although this database includes an enormous number of traits, many of these have no known relationship with PAHs concentration and in other cases there was not enough information for the species studied. Most of the traits included in the databases were related to leaf morphology and gas exchange, which as highlighted above are those that have received most attention in the existing literature. Finally, we considered 7 traits (Table 1), 6 from the TRY Plant Trait Database and 1 (ratio leaf width/length) calculated from another two. In cases where there were different measurements for the same trait and species, we used the median as the statistical reference value.

For each article examined, we identified the comparable samples (samples collected at the same place and time) and calculated the differences between the PAH concentrations in each pair of species and the differences between their traits. By comparing trees sampled at the same place and time, we ensure that they are subjected to the same environmental variables and therefore diminish the differential effect they might have on PAH uptake. Still, if our aim was to predict or model the

**Table 1**  
Traits selected for analysis.

Type of trait	Name of trait	Unit of measurement
General leaf morphology	Leaf area (in case of compound leaves: leaf, petiole excluded)	mm <sup>2</sup>
	Leaf area per leaf dry mass (specific leaf area, SLA or 1/LMA): petiole excluded	mm <sup>2</sup> mg <sup>-1</sup>
	Leaf thickness	mm
	Ratio leaf width/length	–
	Stomatal conductance per leaf area	mmol m <sup>-2</sup> s <sup>-1</sup>
Gas exchange	Leaf carbon isotope signature (delta 13C)	‰
	Stomatal density	n° stomata mm <sup>2</sup>

uptake of PAHs by leaves, we could not ignore environmental conditions, but when studying the relationship between PAH concentrations and trait values considering differences between comparable data this is not necessary. Once we obtained the differences between PAHs and between traits, we used them to calculate Spearman correlations between them and determined the significance levels. We only considered relevant those correlations that were significant ( $p < 0.05$ ) and had an absolute value greater than 0.20. In addition, as each correlation corresponded to a different number of observations, we also computed the number of data pairs for each. All statistical analyses were performed with RStudio version 4.1.1 (RStudio Team, 2021).

### 3. Results

We selected 24 articles (including 67 species and 162 sampling sites) in order to construct the dataset. We then calculated the differences by subtracting the corresponding value (PAH concentration or value of the trait) of species 2 from species 1, for a total of 480 comparisons (Table B.1).

Based on these comparisons, we calculated the correlations (Table 2) and the number of pairs available for each (Table 3). We found significant correlations for all traits, with absolute correlation values varying between 0.17 and 0.80. However, most of the absolute values are below 0.50 and the higher values generally correspond to a small number of available pairs of observations (see Table 3). The available comparisons vary widely, ranging from 17 to 168 pairs (Table 3).

Fig. 1 shows an example of the Spearman correlations. The graph shows the rankings of the differences obtained for the sum of 6 rings PAHs and leaf thickness, the line representing the perfect correlation ( $-1$ ) and, in color, whether the differences of the observations for the PAH and for trait vary in the same direction (green) or not (red), i.e. whether the sign of the difference is the same or different. Ties in rankings were resolved by using the median value (midrank), as this is the same resolution as the Spearman correlation implemented in R.

### 4. Discussion

#### 4.1. Leaf morphology

Leaf morphology determines some purely physical characteristics of

leaves in relation to PAH uptake and accumulation. In general, the variables under study affect the area available for adsorption of PAHs, either exclusively (i.e. leaf area) by considering the mass (i.e. SLA and leaf thickness) or through the modification of the boundary layer thickness (i.e. leaf width/length ratio).

##### 4.1.1. Leaf area

Some authors have studied the effect of leaf area ( $\text{cm}^2$ ) on foliar concentrations of PAHs (Domingos et al., 2015; Dias et al., 2016; Howsam et al., 2000; Jouraeva et al., 2002; Jouraeva et al., 2006; Yang et al., 2017; Zhao et al., 2018). In this case, the increase in concentrations is unequivocally explained by the increase in adsorption surface area regardless of mass, so that it affects both PAHs emitted in the gas phase and particularly those in the particulate phase. Our results show that concentrations of 4-, 5- and 6-ring compounds (with the only exceptions of BbF, BaP and Ind) are positively and significantly correlated with leaf area, as well as with MMW,  $\Sigma 6$ -rings PAHs and  $\Sigma$ PAHs. These results are based on a large number of observations ( $n$  ranging between 45 and 69, except for DbA –  $n = 29$ ; Table 3), and they can therefore be considered robust. Thus, leaf area affected those PAHs that are mainly deposited together with particles ( $\log K_{OA} > 10$ ; Giráldez et al., 2022), confirming the initial hypothesis that the concentration of particle-bound PAHs deposited on leaf surfaces is related to leaf area. The results obtained contrast with some previous findings, as several authors did not find any relationship between this variable and  $\Sigma$ PAHs (which also includes gas-phase PAHs) for *Croton floribundus*, *Piptadenia gonoacantha* and *Astronium graveolens* (Domingos et al., 2015; Dias et al., 2016), *Tilia x euchlora* and *Pyrus calleryana* (Jouraeva et al., 2002) and *Salix matsudana* (Zhao et al., 2018).

##### 4.1.2. Specific leaf area (SLA)

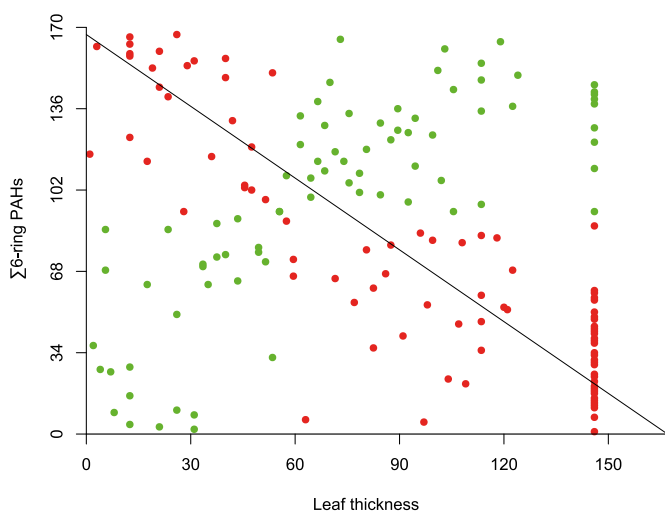
SLA ( $\text{cm}^2 \text{g}^{-1}$ ) was the leaf trait most frequently studied in relation to PAH concentrations (De Nicola et al., 2017; Domingos et al., 2015; Dias et al., 2016; Howsam et al., 2001; Jouraeva et al., 2002; Jouraeva et al., 2006; Müller et al., 2001; Tian et al., 2008; Tian et al., 2019; Wang et al., 2008). The initial hypothesis has a physical justification: as the surface area available for adsorption increases relative to the leaf weight (which implies a larger surface available for adsorption for the same leaf weight, Simonich & Hites, 1994), the concentration of PAHs will also increase, as the concentration includes the weight in the denominator. This will

**Table 2**  
Spearman correlations, significant at  $p$ -value  $\leq 0.05$ .

PAH	Leaf area	SLA	Leaf thickness	Ratio leaf width/length	Stomatal conductance	$\delta^{13}\text{C}$	Stomatal density
Naph				-0.29		0.26	
Acy		0.29					
Ace			-0.21				
Flu							
Phe			0.33	-0.32		0.30	-0.60
Ant			0.24				
Fla	0.40	0.17					-0.77
Pyr	0.37	0.25		0.22			-0.61
BaA	0.49	0.36	-0.29	0.48	0.25		
Chry	0.43	0.41	-0.31	0.31	0.24		-0.71
BbF		0.27	-0.24	0.43			
BkF	0.31	0.33	-0.33	0.30	0.36		
BaP		0.22			0.18		-0.53
Ind		0.22					
DbA	0.63	0.44		0.29			
BghiP	0.43	0.34	-0.20	0.47	0.21	-0.37	
$\Sigma$ LWM					0.29		
$\Sigma$ MMW	0.33	0.23	-0.16	0.28			-0.80
$\Sigma$ HWM		0.28	-0.23	0.42			
$\Sigma$ 2rings				-0.29		0.26	
$\Sigma$ 3rings							
$\Sigma$ 4rings	0.33	0.23	-0.16	0.28			-0.80
$\Sigma$ 5rings		0.21		0.30			
$\Sigma$ 6rings	0.32	0.40	-0.26	0.28	0.21	-0.27	
$\Sigma$ PAHS	0.35	0.28		0.36		-0.27	

**Table 3**  
Number of pairs of available values for each significant correlation.

PAH	Leaf area	SLA	Leaf thickness	Ratio leaf width/length	Stomatal conductance	$\delta^{13}\text{C}$	Stomatal density
Naph				74		58	
Acy		63					
Ace			122				
Flu							
Phe			168	123		107	17
Ant			162				
Fla	69	139					18
Pyr	69	138		124			18
BaA	66	131	160	109	133		
Chry	67	135	165	121	137		17
BbF		114	138	100			
BkF	58	126	156	112	128		
BaP		135			137		17
Ind		90					
DbA	29	67		71			
BghiP	45	86	137	77	91	61	
$\Sigma$ LWM					81		
$\Sigma$ MMW	67	132	161	117			17
$\Sigma$ HWM		94	138	80			
$\Sigma$ 2rings				74		58	
$\Sigma$ 3rings							
$\Sigma$ 4rings	67	132	161	117			17
$\Sigma$ 5rings		94		80			
$\Sigma$ 6rings	69	117	167	103	119	87	
$\Sigma$ PAHS	45	112		91		75	



**Fig. 1.** Plot showing the rankings of the differences obtained for each variable. Each point represents a comparison of two comparable observations. The differences between the sums of 6-ring PAHs and between the leaf thickness of both observations were calculated and the rank of each of these differences was computed for each variable. The green dots represent cases in which the difference in both variables has the same sign (both are positive or negative), while the red dots represent cases in which the differences have opposite signs. The line represents a perfect negative correlation ( $-1$ ). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

particularly affect the PAHs associated with particles, with a  $\log K_{\text{OA}} > 10$  (Jouraeva et al., 2002). However, a second hypothesis considers that the increase in SLA will imply a lower molecular attraction capacity of PAHs, as the decrease in leaf thickness may be associated with a reduction in the thickness of the leaf wax layer, which could lead to a decrease in the concentration of waxes responsible for the sorption of PAHs (Tian et al., 2019). In this case, higher SLA values will imply lower PAH concentrations.

The results obtained in the present study show positive and significant correlations for all MMW and HMW PAHs and also  $\Sigma$ MMW,  $\Sigma$ HMW,

$\Sigma$ 4-rings,  $\Sigma$ 5-rings and  $\Sigma$ 6-rings PAHs, confirming the first hypothesis (Table 2). These results are based on a large number of observations ( $n$  ranging between 63 and 139, Table 3), and they can therefore be considered solid. Other authors have reached the same conclusion for these PAHs. For instance, Jouraeva et al. (2002) detected the same pattern for half of the MMW and all HMW PAHs studied in *Tilia x euchlora* and *Pyrus calleryana* leaves, all with  $\log K_{\text{OA}} > 10$ , except for phenanthrene ( $\log K_{\text{OA}} = 9.20$  according to Jouraeva's calculations). De Nicola et al. (2017) observed the same effect in *Pinus pinaster* vs. *Quercus robur* leaves for PAHs with more than 3 rings, except for Ace, and Müller et al. (2001) reached the same conclusion comparing *Melaleuca* sp. with grasses leaves for B(a)P, Per, Ind and B(ghi)P.

Nevertheless, many other authors only detected positive relationships between SLA and some of the MMW or HMW PAHs, thus only partly confirming the first hypothesis. For example, Howsam et al. (2001) reported correlations between the SLA and the concentrations of 5- and 6-rings PAHs in *Quercus robur* and *Quercus petraea* leaves and 4- and 5-ring PAHs in *Fraxinus excelsior* leaves; and Tian et al. (2019) reported significant positive correlations in a group of 8 species for MMW. By contrast (and by way of exception), significant negative correlations between the SLA and HMW PAHs have also been reported, by Tian et al. (2019), who based the second hypothesis on their findings. Furthermore, an absence of correlation has been reported by Domingos et al. (2015) and Dias et al. (2016) for some of the species indicated above (lending more weight to other leaf traits such as epicuticular wax composition and leaf surface structure) or in Wang et al. (2008) for *Gingko biloba*, *Prunus persica*, *Sophora japonica*, *Prunus cerasifera* and *Syringa pекinensis*. The first hypothesis was also confirmed in the present meta-analysis by the absence of a relationship between the concentrations of LMW PAHs other than Acy and SLA, which is consistent with the results of Howsam et al. (2001) and Jouraeva et al. (2002) and is only contradicted (again exceptionally) by Tian et al. (2019), who found that LMW PAHs were affected by SLA.

Regarding the  $\Sigma$ PAHs, the relationship was also significant and positive, as previously reported by Tian et al. (2008) for *Pinus massoniana*, *Cunninghamia lanceolata*, *Eucalyptus citriodora*, *Acacia auriculaeformis*, *Acacia mangium* and *Schima superba*. By contrast, a negative correlation between  $\Sigma$ PAHs and SLA (Tian et al., 2019) and also no correlation (Domingos et al., 2015; Dias et al., 2016; Wang et al., 2008) have also been reported.

#### 4.1.3. Leaf thickness

Leaf thickness (mm) is closely related to SLA, which may explain why the relation to PAH concentrations has scarcely been studied (Domingos et al., 2015; Dias et al., 2016; in the species above indicated). Assuming a constant surface area, an increase in leaf thickness implies a decrease in SLA, leading to a decrease in the adsorption surface for the same leaf weight. This should lead to a reduction in particle-bound PAHs concentrations, as in the previous case. However, the increase in thickness could also be related to an increase in the concentration of waxes responsible for the sorption of PAHs (Tian et al., 2019) or to an increase in the proportion of intercellular spaces in the spongy parenchyma. Thus, the increase in leaf thickness could lead to an increase in PAH concentrations, both those deposited in the particulate phase and particularly those associated with the gas phase. The results obtained, although not as consistent as for SLA, suggest a similar pattern. For the individual 4-, 5- and 6-ring PAHs (with some exceptions that showed no significance, i.e. Fla, Pyr, BaP, Ind and DbA), and the  $\Sigma$ MMW,  $\Sigma$ HMW,  $\Sigma$ 4-rings and  $\Sigma$ 6-rings, significant, inverse correlations were obtained. Regarding the 3-ring PAHs, Phe and Ant were significantly, positively correlated with leaf thickness. These results are based on a large number of observations ( $n$  ranged between 122 and 168, Table 3), and they can therefore be considered solid. Thus, it appears that as leaf thickness increases (and therefore the SLA decreases), the concentrations of particle-bound PAHs with 4 or more rings will decrease. At the same time, the concentration of some of the 3-ring PAHs (gaseous phase) increases, either due to higher wax concentration, or by larger proportion of intercellular spaces in the parenchyma. The result for the sum of PAHs was not significant. In general, these results are consistent with previous findings, as Domingos et al. (2015) and Dias et al. (2016) reported empirical evidence supporting that the decrease in thickness produces an increase in the total concentration of PAHs.

#### 4.1.4. Ratio leaf width/length

The leaf width/length ratio ( $\text{cm cm}^{-1}$ ) has generally been neglected (Tian et al., 2019). These authors hypothesized that as the leaf width/length ratio (W/L) increases, the capacity to intercept dew drops also increases, which would wash the surface of the leaves and lead to low accumulation of PAHs. On the other hand, the thickness of the boundary layer can also vary; as the W/L ratio decreases, the thickness of the boundary layer increases, thus decreasing the gas exchange processes and decreasing the concentration of PAHs in the gas phase. The results obtained in the present meta-analysis show that light PAHs (2 and 3 rings) related to the gas phase, such as Naph and Phe, are significantly and negatively correlated with W/L. However, for the heavier PAHs of 4, 5 and 6 rings (Table 2), the respective correlation is positive. These results are based on a large number of observations ( $n$  ranged between 71 and 124, Table 3), and they can therefore be considered robust. Thus, the results obtained are consistent with a change in the boundary layer thickness. These findings are partly in agreement with those reported by Tian et al. (2019), who found that leaf W/L ratio was positively correlated with LMW and MMW, but not with  $\Sigma$ PAHs or HMW.

## 4.2. Leaf gas exchange

Gas exchange in plants is physiologically regulated by control of stomatal closure. Greater gas exchange can increase the intake of gas-phase PAHs into the stomatal cavity, potentially increasing the transfer of the compounds into the parenchyma. As access of particles through stomata is thought to be negligible, the selected variables should not be related to the concentrations of particle-bound PAHs. Conversely, gaseous exchange may favor volatilization of PAHs from the stomatal chamber or mesophyll (Tian et al., 2019). Therefore, all the variables studied are proxies which attempt to describe the magnitude of the leaf gas exchanges. Using leaf traits as proxies for gas exchange has the advantage that the traits are less variable over time and

methodologically easier to determine than direct gas exchange; however, they can also introduce noise into the data.

#### 4.2.1. Stomatal conductance (per leaf area)

The relationship between stomatal conductance and PAHs has only been addressed once in the published literature (De Nicola et al., 2017; species specified above). The lack of studies is probably due to the methodological difficulty of measurement, even though this relationship best describes the gas exchange defined as the transfer of water vapor or  $\text{CO}_2$  through the stomata. According to the above authors, as the stomatal conductance increases (per leaf area,  $\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), gas exchange and the concentration of PAHs linked to the gas-phase bioconcentration also increase. Significant correlations were obtained in a very small number of cases, in which they were always positive, e.g. for some MMW and HMW PAHs (i.e. BaA, Chry, BkF, BaP BghiP, and  $\Sigma$ 6-rings PAHs). These correlations are based on a high  $n$  (80–137, Table 3). This result does not support the previously stated hypothesis, in which higher gas exchange would favor the uptake of gas phase PAHs, and there is no apparent causal relation between stomatal conductance and particle-bound PAHs concentration. This contrasts with previous findings, as De Nicola et al. (2017) demonstrated that stomatal conductance was related to an increase in  $\Sigma$ 2-rings and  $\Sigma$ 3-rings PAHs, in accordance with the proposed hypothesis.

#### 4.2.2. Leaf carbon isotope signature ( $\delta^{13}\text{C}$ )

This variable has not previously been used in comparative studies about the relationship between gas exchange and PAH concentration. However, this isotopic ratio has been shown to be a suitable proxy for gas exchange. The greater affinity of the photosynthetic enzyme RuBisCo for  $^{12}\text{CO}_2$  than for  $^{13}\text{CO}_2$  and the greater diffusion of the former isotope through the stomata leads to differences in the  $^{13}\text{C}/^{12}\text{C}$  ratio in plants and in the atmosphere. This variation is captured by the  $\delta^{13}\text{C}$  (Farquhar et al., 1989). However, under lower stomatal conductance (greater stomatal closure) depletion of  $^{12}\text{CO}_2$  leads to the use  $^{13}\text{CO}_2$ , yielding higher  $\delta^{13}\text{C}$  values. Thus, as  $\delta^{13}\text{C}$  is negatively correlated with gas exchange, we would expect it to be negatively correlated with the concentration of gas-associated PAHs. Nevertheless, only positive correlations were observed for Naph, Phe and  $\Sigma$ 2-ring PAHs, and negative correlations were observed for BghiP,  $\Sigma$ PAHs and  $\Sigma$ 6-ring PAHs. These correlations are based on a high  $n$  (58–107, Table 3). These results are not consistent with the main hypothesis claiming that higher gas exchange would favor the uptake of gas phase PAHs. Only two LMW PAHs were positively correlated with  $\delta^{13}\text{C}$ , partly supporting the hypothesis by Tian et al. (2019) that PAH volatilization from the stomatal chamber or mesophyll increases with gas exchange. There is no hypothesis to support the result for 6-ring PAHs and sums of PAHs. Although light PAHs have some weighting within the  $\Sigma$ PAHs, we do not believe that this is sufficient to explain the observed correlation between  $\delta^{13}\text{C}$  and the  $\Sigma$ PAHs.

#### 4.2.3. Stomatal density

Given the ease of measurement, the relationship between stomatal density ( $\text{stomata/mm}^2$ ), as a proxy for gas exchange, and PAH concentrations is the most commonly studied (De Nicola et al., 2017; Fellet et al., 2016; Prigioniero et al., 2022; Prigioniero et al., 2023; Tian et al., 2019; Wang et al., 2008; Yang et al., 2017). According to these authors, as the stomata density increases, gas exchange and the PAHs associated with the gas-phase also increase. The absence of any relationship between the previous gas exchange proxies and the concentration of light PAHs established a low expectation for their correlation with stomatal density, but apart from gas exchange, the increase in this parameter is related to a decrease in wax concentration (Tian et al., 2019). Furthermore, its use as a proxy for gas exchange is not straightforward as stomatal density does not consider the morpho-physiological characteristics of stomata (such as their size, protective structure -as could be trichomes- or location) or the rate of opening, and therefore it

does not determine stomatal conductance (De Nicola et al., 2017; Yang et al., 2017). The number of significant correlations was small and always negative. Significant correlations were found for half of the 4-ring PAHs (i.e. Fla, Pyr and Chry) and their sum, as well as the sum of the MMW and exceptionally for PAHs with other number of rings (Phe and BaP). These correlations are based on a low  $n$  (17–18, Table 3), which casts doubt on the reliability of the findings. In the previous literature, as in this meta-analysis, no relationship between stomatal density and  $\Sigma$ PAHs concentration was usually found (De Nicola et al., 2017, Tian et al., 2019; Wang et al., 2008; Yang et al., 2017; all species studied cited above, except in the last, *Cinnamomum camphora*), except by Fellet et al. (2016) (who studied leaves of *Elaeagnus x ebbingei*, *Ilex aquifolium*, *Laurus nobilis*, *Ligustrum japonicum*, *Photinia x fraserii* and *Viburnum lucidum*). However, in contrast to our meta-analysis, Tian et al. (2019) observed a relationship between stomatal density and LMW and MMW PAH concentrations, supporting the starting hypothesis in this section. A study by Prigioniero et al. (2022) with *Chamaerops humilis*, *Citrus x aurantium*, *Magnolia grandiflora*, and *Quercus ilex* differentiated the upper and lower leaf surfaces, from which they also selectively removed waxes. In the study, the upper leaf surface (with waxes) was significantly and positively correlated with LMM. In addition, the lower leaf surface (with waxes and without them) was significantly and positively correlated with HMM and negatively with LMM (with waxes). Both this and the present meta-analysis coincide in finding a relationship between PAHs associated with particulate matter and stomatal density, although no hypothesis for the relationship was proposed. Prigioniero et al. (2023) obtained similar results in a study including 28 species, detecting positive correlations between stomatal density on the upper leaf surface and the concentration of MMM and LMM PAH in dewaxed leaves.

#### 4.3. Study limitations and conclusions

This paper provides a comprehensive review of the current knowledge on the relationship between leaf traits and leaf PAH concentrations. It also provides further information obtained through a meta-analysis. However, without detracting from its value, we must be aware of the limitations of the study, many of which stem from the nature of the data available, particularly the trait data. Unlike the data on PAH concentrations, the trait data do not correspond to the trees studied but are median values of the data available for each species in the TRY Plant Trait Database. This obviates the natural variability in this type of data and leads to some pseudoreplication, which introduces many ties in the Spearman correlations, thus affecting the exact  $p$ -values. Another problem arises when assessing the importance of each species in the comparisons, as some species are included much more often, and some studies carry out many more comparisons than others.

It is interesting to note that PAH uptake by tree leaves is influenced by environmental conditions. Thus, although we minimize its effect in the analyses by making comparisons between “comparable” individuals (samples collected by the same authors in the same place, time period, with leaves of the same age and exposed to the same sources of contamination), in some cases interactions may occur. That is, it could happen that in one climate (or set of environmental conditions) species 1 captures more than species 2 and in another it is the other way around. However, such a scenario would only have the effect of increasing noise when calculating correlations. This would make more difficult to find significant correlations but would not invalidate the ones we have found. The same would be true for all factors that could affect the concentrations measured in each of the studies. Although the conditions are heterogeneous between studies, they are standardized in each of the comparisons. It is important to emphasize that our goal is not to accurately model the process by which PAHs are uptaken by tree leaves (this would require the inclusion of numerous environmental variables), but to examine the relationship between the concentration of PAHs in leaves with their ecological traits. Therefore, the non-assessment of environmental conditions cannot be considered a limitation of the study.

The results obtained for the traits related to leaf morphology are much more robust and are more consistent with the main hypotheses than those related to gas exchange. This may be due to several factors, including two main candidates. First, the morphological traits are more consistent possibly because they are less sensitive than gas exchange traits to intraindividual and intraspecific variability caused by the environmental conditions. Second, the morphological traits are measured directly, while gas exchange is estimated by proxy measures (gas exchange traits). Although there is a large variability in both intraindividual and interindividual traits (e.g. Giráldez et al., 2024; Herrera, 2009; Vinod et al., 2023), gas exchange is generally much more variable than morphological traits, and the effect of disregarding this variability in the analysis may therefore be much more pronounced. Although we can deduce from the results that gas exchange is not of great importance in relation to PAH uptake, it seems logical to think that it will probably contribute to the load of light, gas-phase PAHs. However, it is possible that these traits alone are not as decisive and are affected by other traits, as we focused on individual traits and many of these uptake processes may be multifactorial.

Despite these limitations, this study provides a complete view of the current knowledge on the relationship between leaf traits and leaf PAH concentrations. Moreover, it reinforces existing hypotheses on the relationship between leaf morphological traits and the different types of PAHs, while also challenging some of the hypotheses relating PAHs and gas exchange. Future studies on the effect of gas exchange should apply more careful and detailed approaches and consider the multifactorial aspects of PAH uptake.

#### CRedit authorship contribution statement

**Pablo Giráldez:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Antón Vázquez-Arias:** Writing – review & editing, Investigation, Data curation. **Flavia De Nicola:** Writing – review & editing, Investigation. **J. Ángel Fernández:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Jesús R. Aboal:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization.

#### Declaration of competing interest

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

#### Acknowledgements

P. Giráldez and A. Vázquez-Arias are grateful to the Spanish Ministerio de Ciencia, Innovación y Universidades for grants awarded within the Programa de Formación de Profesorado Universitario (FPU 2018 [grant number FPU18/04134] and FPU 2019 [grant number FPU19/01989]).

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2024.125337>.

#### Data availability

Data will be made available on request.

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