

## Article

# Integrating Tree Species Identity and Diversity in Particulate Matter Adsorption

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**Abstract:** The amount of PM bound by tree canopies depends on leaf traits, but also the leaf area available, both of which are dependent on tree identity. We investigated four species (*Acer platanoides* L., *Tilia cordata* Mill., *Quercus robur* L., *Carpinus betulus* L.) grown in monocultures and in two and four species polycultures. The amount of PM on the leaves of these species was determined by washing and fractionation of the PM into PM<sub>2.5</sub>, PM<sub>10</sub> and PM<sub>100</sub> size classes using a filtering method. The leaf area index was estimated by litter collection. The amount of PM<sub>2.5</sub> per m<sup>2</sup> leaf area was significantly higher in *T. cordata* compared to *Q. robur* and *A. platanoides*, and in *C. betulus* compared to *A. platanoides*. The leaf area index in monocultures was similar for all species except *T. cordata* which was considerably lower. Overyielding of LAI was shown in the two species polyculture of *T. cordata* and *A. platanoides*, and also in the four species polyculture. In polyculture, higher amounts of PM were determined in the two species polyculture of *Q. robur* and *C. betulus* and also in the four species polyculture. The result show that both tree identity and mixture influence the amount of PM in the canopy, and this is related to tree leaf traits, and also to overyielding of LAI in the polyculture.



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## 1. Introduction

Ambient air pollution is linked to a plethora of human health problems, and is one of the leading five health risks worldwide, especially affecting urban populations [1,2]. Airborne particulate matter (PM) is an important group of air pollutants composed of particles with a wide range of sizes, from less than 2.5 µm (PM<sub>2.5</sub>) to up to 100 µm (PM<sub>100</sub>). In the EU-27 countries, 307,000 premature deaths are caused by PM<sub>2.5</sub> pollution alone [3]. Biological effects of these small inhalable particles encompass immune response, cytotoxicity, and mutagenicity, causing stroke, heart disease, lung cancer, chronic obstructive pulmonary disease, and acute respiratory infections [4].

Trees and forest ecosystems have a direct effect on air quality regulation, and can significantly reduce air pollutant concentrations, therefore reducing detrimental effects on human health [5]. Trees can reduce PM through physical binding on leaf surfaces [6,7], but also on structural elements such as branches and stems [8,9]. Between tree species, considerable differences have been shown in the amount of PM bound by leaves [7,10], differing in amount, but also in the size class bound [11,12]. The binding to leaves is regulated by microstructural leaf traits such as the density of leaf trichomes [13], leaf wettability [10,14], high surface roughness and high quantities of epicuticular waxes [15,16]. Macrostructural traits such as shorter petiole length [17,18], leaf size [14,19] and the arrangement of leaves [17,20] also strongly affect PM binding. In addition to micro- and macrostructural leaf traits, the absolute leaf area available for binding of PM [15,21] and

the density of leaves within the crown [19,22] affect the PM binding capacity of trees. In the present work, we investigated four tree species: Norway maple (*Acer platanoides* L.), small leaf lime (*Tilia cordata* Mill), common oak (*Quercus robur* L.) and hornbeam (*Carpinus betulus* L.), grown in monoculture and polycultures. The leaf surface of *T. cordata* is characterized by a high density of trichomes [10,23,24], and a high number of small leaves [19], characters that should promote binding of PM [4]. However, [7,25,26] considered *T. cordata* as a less efficient species for PM binding; whereas [27] classified *T. cordata* as especially good in capturing fine particle matter, but poor in capturing coarse particles. Both *Q. robur* and *C. betulus* were rated as high mitigating species [26]; however, another other investigation ranked *C. betulus* only slightly above the average [7]. *A. platanoides* has large leaves [28] and smooth epicuticular waxes [18], and accordingly, has been classified as a less efficient PM capturing species [7,26].

The amount of leaves within the canopy is normally expressed as the leaf area index (LAI), the total one-sided leaf area in m<sup>2</sup> per m<sup>2</sup> ground surface area [29]. The LAI provides information about the forest canopy structure [30] and allows scaling up of leaf level results to local, regional and global levels. Leaf area index can be determined by both direct methods through determination of litter fall [31] and through indirect methods such as remote sensing [32]. The size of the LAI is tree species dependent [33].

Particularly in the face of climate change, interest in tree diverse forests to maintain ecological stability has increased [34,35]. Tree species biodiversity promotes productivity [34,36] and the provision of ecosystem functioning [37]. The promotion of growth is due to both facilitation and reduction of competition between trees [36,38]. Although promotion of productivity, particularly if measured as aboveground productivity, is common [34,36], the outcome of polycultures is environmentally context dependent, and subject to selection effects of tree identity. For young tree diversity experiments, ref. [39] reported that 80% of the diversity effects were driven by selection effects and the remaining 20% were influenced by complementarity effects. Thus, the species identity of trees in polyculture is important, but also the degree of function dissimilarity between the component tree species [40]. Increased productivity in polyculture is often expressed as overyielding [41], determined as the observed productivity against a predicted productivity, and calculated from the component trees grown in monocultures. In tree polycultures, overyielding has been shown for many growth or biomass parameters, such as above ground biomass [42], fine roots [43], but also leaf biomass and thus, LAI [44,45]. However, little is known about whether polyculture, and subsequent potential changes in LAI and canopy structure, could influence binding of PM.

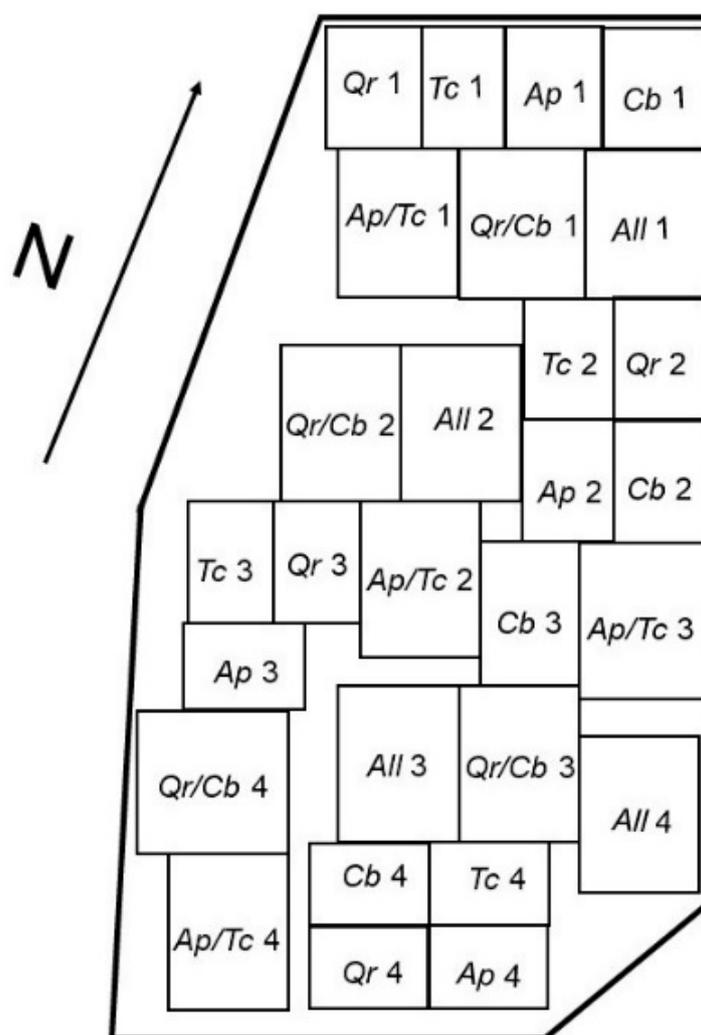
In the work presented, we quantified LAI and PM accumulation on leaf surfaces of four common European tree species with different leaf and crown traits, grown as monocultures or 2 and 4 species polyculture at the biodiversity experimental site B-Tree, Austria. Specifically, we determined whether growth in polyculture influences the amount of PM, in the classes PM<sub>100</sub>, PM<sub>10</sub> and PM<sub>2.5</sub>, that could be removed from the leaves. We tested the hypotheses: (1) trees with small and rough leaves will have a greater PM accumulation than trees with large, smooth leaves, (2) LAI will greatly modify the total amount of PM accumulated per tree, and (3) growth of trees in polyculture affects LAI and thus, total canopy PM accumulation.

## 2. Material and Methods

### 2.1. Study Area

All work was carried out at the B-Tree experimental site (48°19′03.2″ N 16°04′01.0″ E) which is part of the TreeDivNet platform, a worldwide network of tree biodiversity experiments [46]. The B-Tree site was established in 2013 with the tree species *Acer platanoides* L. (*Ap*), *Tilia cordata* Mill. (*Tc*), *Quercus robur* L. (*Qr*), *Carpinus betulus* L. (*Cb*), representing a typical warm low-land forest composition. The trees were planted at a density of 10,000 trees ha<sup>-1</sup> in a hexagonal pattern, which ensured a 100 cm spacing between all adjacent trees. No thinning or any other treatment has been carried out to date. The tree

species were planted in both polycultures and monocultures in four blocks (Figure 1). The mixed stands consist of two species polycultures *Ap* and *Tc* (*ApTc*) or *Qr* and *Cb* (*QrCb*), or a four species (*All*) polyculture. In two species polycultures, each species contributes 50% of the total number of trees; in the four species plots, each species contributes 25% of the total number of trees. Every combination and monoculture has four replicates, and the replicates are rotated within each block to ensure no one species has a similar position in the blocks. The plot sizes are 81 m<sup>2</sup> for single species plots and 196 m<sup>2</sup> for two and four species plots (Figure 1). At the time of planting, the saplings were ca. 30–40 cm high. At the time of sampling in September 2020, the mean heights of the trees were 5.5 ± 1.6 m (*A. platanoides*), 3.6 ± 1.2 m (*T. cordata*), 4.2 ± 1.1 m (*Q. robur*) and 4.1 ± 1.0 m (*C. betulus*). The surrounding area of the B-Tree site is mainly agricultural farmland.



**Figure 1.** Schematic overview of the plot arrangement of the TreeDivNet experimental site B-Tree. The four species, *Acer platanoides* L. (*Ap*), *Tilia cordata* Mill. (*Tc*), *Quercus robur* L. (*Qr*), *Carpinus betulus* L. (*Cb*), are replicated as monocultures, two species polycultures *ApTc* and *QrCb* and as four species polycultures *All*. Numbers show the block number.

The annual precipitation in Tulln an der Donau is 597 mm, with a mean annual temperature of 9.5 °C [47]. In addition, weather data for the accumulation period of the 7 September until 12 September 2020 were obtained from a local weather station (Adcon A733, Department for Crop Sciences, University for Life Sciences and Natural Resources Vienna) adjacent to the B-Tree site. The recorded wind speed at the site had a mean of 1.9 ± 0.4 m·s<sup>-1</sup> with a maximum 2.4 m·s<sup>-1</sup>. Wind speeds lower than 5 m·s<sup>-1</sup> [48] or

$11 \text{ m}\cdot\text{s}^{-1}$  [49] should not have an effect on leaf accumulated particulate matter. Ambient air temperature was measured 2 m above ground with a mean of  $16.1 \pm 2.5 \text{ }^\circ\text{C}$ . Ground level concentration of particulate matter measured at the station Dürnröhr, 12 km from the B-Tree site, was generally low, with a mean 24 h  $\text{PM}_{10}$  and  $\text{PM}_{2.5}$  concentration of  $17 \pm 3.1 \text{ }\mu\text{g}\cdot\text{m}^{-3}$  and  $6.8 \pm 2.4 \text{ }\mu\text{g}\cdot\text{m}^{-3}$ , respectively (accessed at [50]). The last heavy rain event before sampling occurred in the night from 6 to 7 September with a total precipitation of 18.8 mm.

## 2.2. Leaf Sampling

Leaves were collected on 12 September 2020. To exclude edge effects, leaves were taken from trees located toward the center of each plot. A pair of scissors was used to carefully cut single leaves or small branches from the outermost crown layer [15,19,27]. Three randomly chosen vital, dominating or co-dominating tree individuals, distributed around the center of the plot, were sampled. Sampling height varied based on the different stand height of the sampled plots. Contact between other objects and harvested leaves were avoided or minimized. From each species, sufficient leaves were collected to give a total one-sided leaf area of 300–400  $\text{cm}^2$ . This range of leaf area was chosen to reduce the risk of filter blockage during particle size fractionation. In the polycultures, one mixed leaf sample per plot was harvested proportional to the number of trees in the plot. In the polycultures, leaves were taken to give a leaf area of 150–200  $\text{cm}^2$  or 75–100  $\text{cm}^2$  per species for two or four species polycultures, respectively. After the leaf area was determined, the leaf area per species in the two species polycultures deviated 9.6% from the target value of 50% leaf area per species, and 6.6% in the four species polyculture from the target value of 25% per species. The petioles were removed [51] with a pair of scissors before carefully sliding the leaves into the paper bags. The harvested leaves were immediately brought back to the laboratory, and stored in a fridge at  $4 \text{ }^\circ\text{C}$  [19] until further analysis. Leaf washing was carried out two days after sample acquisition. Optically healthy leaves with no sign of insect or disease damage were used whenever possible. However, at the time of sampling, some of the leaves of *Q. robur* and *T. cordata* already showed signs of infestation with *Erysiphe alphetoides* Griffon & Maubl. and *Cercospora microsora* Sacc., respectively.

## 2.3. Determination of Leaf Area Index (LAI)

The leaf area index (LAI) was directly estimated from leaves collected in 108 leaf litter traps, similar to the approach of [52]. Litter traps were standard planting pots ( $\text{Ø}$  33 cm) with perforated bottoms. Within the monoculture and mixed stands, three and five leaf litter traps representing 0.26  $\text{m}^2$  and 0.43  $\text{m}^2$ , respectively, were randomly distributed throughout each plot. Tent pegs were used to fix the pots to the ground. The leaves were collected biweekly during the main abscission period (August–December), and monthly until budbreak of the next vegetation period, to capture the retained leaves of the marcescent *Q. robur* and *C. betulus*. The collected leaf litter was dried at  $38 \text{ }^\circ\text{C}$ . Before further processing, the species identity of the leaves in each trap was checked; only rarely were leaves from neighboring plots found in the leaf litter traps during the whole collecting season.

## 2.4. Quantitative Analysis of PM

For analysis of PM, the leaves were removed from the paper bag using tweezers and put into glass containers with 250 mL deionized distilled water ( $18.2 \text{ M}\Omega \text{ cm}$  @  $25 \text{ }^\circ\text{C}$ , 3 ppb TOC). The glass containers were closed with a lid, put into an end-over-end rotating shaker (GFL Rotating Shaker 3040, Gesellschaft für Labortechnik GmbH, Burgwedel, Germany) for 10 min, and were then placed in a 120 Watt ultrasonic bath for another 3 min (BRANSONIC 220, Branson Ultrasonics Corporation, Brookfield, WI, USA) [15,53]. The washing solution samples which could not be analyzed right away were stored in 300 mL plastic bottles in a fridge at  $4 \text{ }^\circ\text{C}$ . Before filtration, the stored solutions were shaken for at least 8 h (170 RPM) on a plate shaker (VWR Incubated Microplate Shaker, VWR International, Radnor, PA, USA) and 20 s with a vortex shaker, and finally 10 s per hand by the same person.

Prior to the first weighing step, the filters were equilibrated in a weighing room, at a temperature of 20 °C and at an 20% relative humidity, for at least 24 h. For each filtration sample, three PM filters and three reference filters of all PM classes (10, 2.5 and 0.2 µm) were prepared. The two filter groups per sample were stored in labelled petri dishes. The filters did not touch each other in the petri dishes. Nitril gloves and tweezers were used for all steps. After stabilization, the filters were sequentially passed through an anti-static ionizer (CEM Anti-static ionizer, CEM Corporation, Matthews, NC, USA) to avoid electrostatic charges on the filters, and then weighed to a precision of 0.00001 g on a balance (Sartorius SECURA125-1S, Sartorius Lab Instruments GmbH & Co., KG, Goettingen, Germany). Each filter was weighed a minimum of 3 times, and the mean of the best corresponding values taken to calculate the final weight.

Particulate matter was divided into 3 diameter classes (10–100 µm, 2.5–10 µm, 0.2–2.5 µm) using a sequential fractionation. In total, four different types of filters were used for the filtration process. First, the solutions were passed through a 100 µm stainless steel sieve to remove coarse particles > 100 µm. Thereafter, the solution was sequentially passed through a 10 µm cellulose filter (Whatman Type 91, GE Healthcare UK Limited, Little Chalfont, UK) to capture the 10–100 µm PM class; third, a 2–3 µm wet-strengthened cellulose filter (Sartorius Grade 1291, Sartorius Stedim Biotech, Goettingen, Germany) for the 2.5–10 µm PM class; and finally, a 0.2 µm cellulose acetate membrane filter (Sartorius Type 111, Sartorius Stedim Biotech, Goettingen, Germany) for the 0.2–2.5 µm PM class. For every filtration step, the filters were placed in a 47 mm glass filter funnel and stopper support assembly (Cytiva, Whatman, Little Chalfont, UK), and placed under vacuum using a vacuum pump (KNF Meter Laboport N820.3, METER Group AG, Munich, Germany) with a distribution manifold. The glass funnels were covered with filter papers to avoid dust contamination during filtering. To avoid additional contamination between filtration steps, the glass funnels and Erlenmeyer flasks were rinsed with deionized distilled water (18.2 MΩ cm @ 25 °C, 3 ppb TOC), which was then added back to the solution for the next filtration step. The leaf washing methods from [16] were adapted according to [13,54,55]. The PM loaded filters were dried and equilibrated for 24 h in the weighing room at constant relative humidity and air temperature, as described above. The equilibrated PM filters and reference filters were passed through the ionizer device again and weighed a second time, as described above. The weight difference was automatically adjusted for the relative humidity bias provided by the reference filters.

To obtain an estimation of the effect of any possible variation due to conditions in the weighing room, the whole weighing procedure was repeated two times. No statistical differences were found between the values obtained on the different days. After final development of the measuring protocol, it was noticed that it is in stark congruence with the protocol from [54]. This means that six filters were weighed two times each to get the weight difference from before and after each filtration process, and the relative humidity bias caused by humidity changes in the weighing room.

### 2.5. Determination of Leaf Area and Specific Leaf Area

The leaf surface area was determined by scanning the samples with a flatbed scanner using the leaf area analysis software WinFoliaPro (2014). To calculate the SLA, 50 freshly fallen leaves per species were collected in autumn 2020. The leaves were dried for 48 h at 80 °C in a drying chamber, and the dry mass of the leaf samples was determined and used to calculate the specific leaf area (SLA) as leaf area cm<sup>2</sup> per g dry mass. The specific leaf area (SLA) was used to calculate plot wise LAI in m<sup>2</sup>·m<sup>-2</sup>.

### 2.6. Calculations

Plot level PM retention was derived from direct double-sided leaf-surface PM retention measurements, upscaled using LAI values from the respective species combination plots. The values correspond to mg·m<sup>-2</sup> ground area for the five-day rain free PM accumulation period before leaf sampling.

To determine the effect of growing species in polyculture on LAI, the measure values were compared and a predicted value calculated from the single species plots. For the two species plots, each species was assumed to contribute 50% of the value of the single species plots; for the four species plots, 25% of the value of the single species plots. This calculation is directly analogous to calculation of over-yielding used in biomass calculations [56,57]. For estimation of the effects of polyculture on PM retention on a plot area scale, two values of predicted retention were calculated for the values obtained from the monocultures. One value, predicted nominal leaf area (pn), was calculated using the PM binding per leaf area of the monocultures and a leaf area index of 50:50 for two species polycultures, or 25:25:25:25 for four species polyculture. The value predicted actual leaf area (pa) was calculated using the PM binding per leaf area of the monocultures and the measured leaf area of each species from the polyculture plots.

### 2.7. Statistical Analysis

Values were entered in a prepared Excel sheet which automatically calculated the humidity bias and the corrected filtered net PM load per sample and PM class. Further data wrangling and analysis was conducted in R [58]. As between the two weighing runs of the loaded filters no statistical differences were found, these two datasets of raw data were then pooled to average the variance of the weighing steps. This pooled dataset was then used for further statistical tests and analysis.

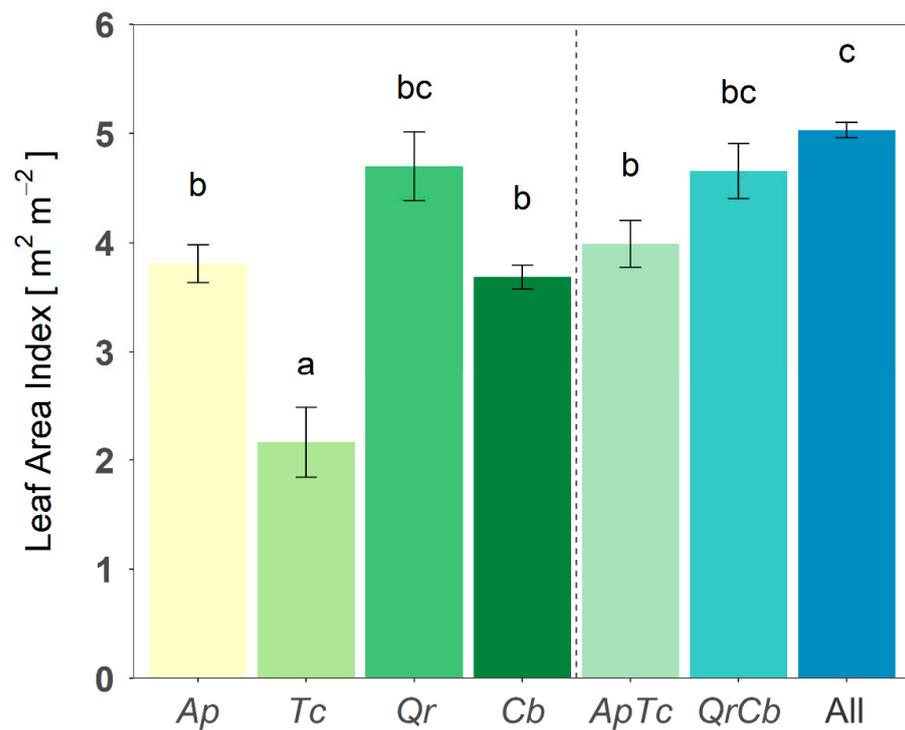
One-way ANOVAs were carried out along a species diversity gradient of 1, 2 and 4 species: *Ap*, *Tc*, *Qr*, *Cb*, *ApTc*, *QrCb* and All, respectively (one-way ANOVA, \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). The statistical analysis was carried out in the same way for LAI and PM analysis, latter split up into  $PM_{100}$ ,  $PM_{10}$ ,  $PM_{2.5}$  and  $\Sigma PM$  analysis. Tukey's HSD test ( $p < 0.05$ ) was used to assess the significance of pairwise comparisons between plot combinations. Residuals of the models were checked for normality using a Shapiro–Wilk test ( $p < 0.05$ ). To test for homogeneity of variances, a Levene test ( $p < 0.05$ ) was applied to the datasets. Null hypothesis was not rejected for any test.

Additionally, Student's *t*-tests for LAI and all three PM classes between the measured and the predicted nominal values (or/pn) of PM binding of the different PM classes were run, as well as for measured and predicted actual values (or/pa) for the PM classes (unpaired *t*-test, one-way ANOVA, \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). The null hypothesis for Shapiro–Wilk normality tests of the subsamples was not rejected.

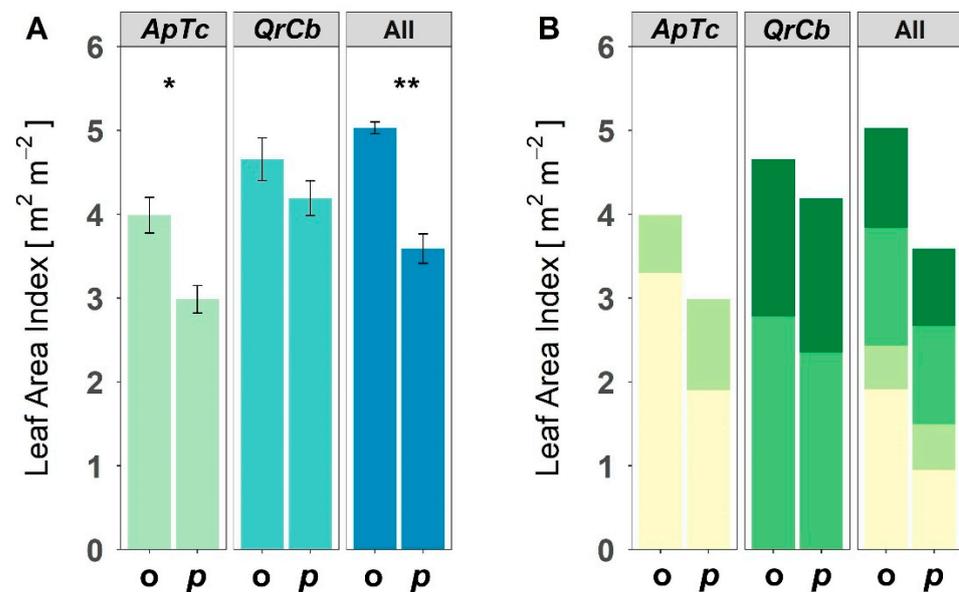
## 3. Results

### 3.1. Leaf Area Index

In the monocultures, the leaf area index (LAI) of *T. cordata* was significantly lower compared to the other three species (Figure 2, Table 1). The two species polycultures of *T. cordata* and *A. platanoides* (*ApTc*) showed a significantly higher LAI than that of the monocultures with *T. cordata*, but not the monocultures of *A. platanoides*. In the *ApTc* polyculture, the measured total LAI exceeded the predicted (Figure 3A), and the contribution from *A. platanoides* to the total LAI increased significantly from a predicted 64% to the observed 82% (Figure 3B). In the two species polyculture of *Q. robur* and *C. betulus* (*QrCb*), the total LAI was not different to either of the monocultures (Figure 2) and did not differ to the predicted value (Figure 3A). The four species polycultures (All) showed the highest LAI and were significantly different to all monocultures and polycultures, except those containing *Q. robur* (Table 1). The measured LAI was 40% higher than the predicted value and was significantly different (Figure 3A). The higher LAI in the four species polyculture is due to a significantly higher contribution of *A. platanoides*. The observed LAI of *Q. robur*, *C. betulus* and *T. cordata* singly was not greater than the predicted value (Figure 3B). However, the sum LAI of *Q. robur* and *C. betulus* in All was significantly higher ( $p < 0.05$ ) in the observed ( $2.6 \text{ m}^2 \cdot \text{m}^{-2}$ ) compared to the predicted ( $2.1 \text{ m}^2 \cdot \text{m}^{-2}$ ).



**Figure 2.** Differences in mean Leaf Area Index (LAI) in  $\text{m}^2 \cdot \text{m}^{-2}$  of monocultures (*Ap*, *Tc*, *Qr*, *Cb*), two species polycultures (*ApTc*, *QrCb*) and a four species polyculture (*All*) consisting of the four species *Acer platanoides* (*Ap*), *Tilia cordata* (*Tc*), *Quercus robur* (*Qr*), *Carpinus betulus* (*Cb*). Each bar represents the mean LAI of four replicates. Error bars refer to the standard error (SE) of the mean. Letters mark significant differences of the mean LAI between compositions (Table 1). Significant differences in the monocultures occurred only between *T. cordata* and the other species, see Table 1.



**Figure 3.** (A) Observed (o) and predicted (p) leaf area index (LAI) and (B) the contribution of each species to the LAI in two species polycultures (*ApTc*, *QrCb*), and the four species polyculture (*All*) of the four species *A. platanoides* (*Ap*), *T. cordata* (*Tc*), *Q. robur* (*Qr*), *C. betulus* (*Cb*). Shown are means and standard error of the LAI. Asterisks show significant differences determined using an unpaired t-test (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ).

**Table 1.** Corresponding ANOVA results for the plot-wise LAI results shown in Figure 2 (one-way ANOVA).

Pairs	<i>p</i> -Value	Pairs	<i>p</i> -Value
<i>Tc</i> – <i>Ap</i>	<0.001	<i>Ap</i> –All	<0.05
<i>Tc</i> – <i>Qr</i>	<0.001	<i>Tc</i> –All	<0.001
<i>Tc</i> – <i>Cb</i>	<0.01	<i>Cb</i> –All	<0.01
<i>Tc</i> – <i>QrCb</i>	<0.001	<i>ApTc</i> –All	<0.05
<i>Tc</i> – <i>ApTc</i>	<0.001		

### 3.2. Particulate Matter

Particulate matter removed from leaves per m<sup>2</sup> leaf area of all four species is shown in Table 2. For the PM<sub>100</sub> and PM<sub>10</sub> size classes, no significant differences were found between the tree species, even though in the PM<sub>100</sub> class the amount removed from *T. cordata* exceeded that of *A. platanoides* by a factor of 3. In the PM<sub>2.5</sub> class, the amount of PM per m<sup>2</sup> was significantly higher in *T. cordata* compared to *Q. robur* and *A. platanoides*, and in *C. betulus* compared to *A. platanoides*. The amount of PM washed from leaves of *T. cordata* was twice that of *Q. robur*, and 3 times that of *A. platanoides*.

**Table 2.** Particulate matter (PM) fractions removed from leaves of *A. platanoides* (*Ap*), *T. cordata* (*Tc*), *Q. robur* (*Qr*), *C. betulus* (*Cb*) for the PM classes PM<sub>100</sub>, PM<sub>10</sub>, PM<sub>2.5</sub>. Data shown in mg PM·m<sup>−2</sup> leaf area ± standard error (SE). The letters mark the significant differences of an ANOVA performed within each PM class (one-way ANOVA, *p* < 0.05).

	<i>Ap</i>	<i>Tc</i>	<i>Qr</i>	<i>Cb</i>
<b>PM<sub>100</sub></b>	38.0 ± 6.5	130.9 ± 40.0	60.4 ± 8.8	91.4 ± 20.7
<b>PM<sub>10</sub></b>	9.7 ± 3.3	11.7 ± 5.1	15.9 ± 2.9	22.0 ± 3.9
<b>PM<sub>2.5</sub></b>	4.1 ± 1.3 <sup>a</sup>	14.2 ± 2.4 <sup>b</sup>	6.7 ± 1.5 <sup>a,c</sup>	12.9 ± 1.6 <sup>b,c</sup>

### 3.3. Particulate Matter Canopy Storage

The amount of PM stored in the canopy of the monocultures and the polycultures is shown in Table 3. For the large particulate matter fraction PM<sub>100</sub>, for the observed value of PM removed from the leaves, no significant differences between species in the monocultures or within the polycultures could be identified. However, *Ap* and *ApTc* showed the lowest values of PM<sub>100</sub> removed from the leaves compared to the other species in monocultures or other polycultures. Similarly, no significant differences were shown between the observed, predicted nominal and predicted actual values, indicating that the PM retention function of a single tree species in a polyculture did not differ from its function in a monoculture.

For the PM<sub>10</sub> class, again, no significant differences in the amounts of PM removed were shown between species in the monocultures. Similarly, between the monocultures and the respectively polycultures, no significant differences were shown, even though the amount removed for the *QrCb* polyculture was numerically greater than both of the species in monoculture. The larger amount of PM<sub>10</sub> removed from the leaves in the *QrCb* polyculture is clearly seen when the observed, predicted nominal and predicted actual values are compared. There was a significant difference between observed and predicted nominal and predicted actual in the *QrCb* polyculture. The observed value was significantly higher than both the predicted values, indicating a higher than expect value of PM in the polyculture compared to the respective monocultures.

**Table 3.** Mean PM<sub>100</sub>, PM<sub>10</sub>, PM<sub>2.5</sub> and ΣPM removed from leaves per square meter stand area (mg·m<sup>-2</sup>) and standard error (SE) of the monocultures and polycultures. The row observed shows measured values of PM removed from leaves of the monocultures and an approximately 50:50 leaf area mixture of the two species in two species polyculture or 25:25:25:25 in 4 species polyculture (All). Predicted nominal shows values of PM binding calculated for the polycultures from PM binding of the monocultures *Ap*, *Tc*, *Qr* and *Cb* and using the same leaf areas as in observed. Predicted actual shows values of PM binding calculated for the polycultures from PM binding of monospecific plots and the measured LAI values in the polyculture plots. Within a PM class, in rows, different lower-case letters indicate significant differences between monocultures and polycultures. In columns, different capital letters indicate significant differences between the observed and predicted values. (one-way ANOVA,  $p < 0.05$ ). *A. platanoides* (*Ap*), *T. cordata* (*Tc*), *Q. robur* (*Qr*), *C. betulus* (*Cb*).

PM <sub>100</sub>	Monocultures				Polycultures		
	<i>Ap</i>	<i>Tc</i>	<i>Qr</i>	<i>Cb</i>	<i>ApTc</i>	<i>QrCb</i>	All
observed	144 ± 26 a	279 ± 84 a	277 ± 24 a	333 ± 68 a	148 ± 35 a/A	358 ± 67 a/A	288 ± 50 a/A
predicted nominal					215 ± 39 A	316 ± 45 A	318 ± 47 A
predicted actual					210 ± 23 A	353 ± 77 A	327 ± 40 A
PM <sub>10</sub>	Monocultures				Polycultures		
	<i>Ap</i>	<i>Tc</i>	<i>Qr</i>	<i>Cb</i>	<i>ApTc</i>	<i>QrCb</i>	All
observed	36 ± 10 a	23 ± 10 a	74 ± 14 ab	81 ± 15 ab	52 ± 21 ab/A	114 ± 8 b/A	58 ± 20 ab/A
predicted nominal					31 ± 5 A	82 ± 4 B	66 ± 6 A
predicted actual					43 ± 13 A	88 ± 7 B	71 ± 8 A
PM <sub>2.5</sub>	Monocultures				Polycultures		
	<i>Ap</i>	<i>Tc</i>	<i>Qr</i>	<i>Cb</i>	<i>ApTc</i>	<i>QrCb</i>	All
observed	16 ± 6 b	32 ± 9 ab	32 ± 9 ab	48 ± 7ab	35 ± 13 ab/A	61 ± 12 a/A	62 ± 3 a/A
predicted nominal					24 ± 5 A	41 ± 8 A	37 ± 4 B
predicted actual					24 ± 5 A	43 ± 9 A	40 ± 6 B
ΣPM	Monocultures				Polycultures		
	<i>Ap</i>	<i>Tc</i>	<i>Qr</i>	<i>Cb</i>	<i>ApTc</i>	<i>QrCb</i>	All
observed	196 ± 18 a	344 ± 96 ab	382 ± 27 ab	463 ± 76 ab	235 ± 49 a/A	533 ± 75 b/A	407 ± 52 b/A
predicted nominal					270 ± 44 A	439 ± 47 A	421 ± 48 A
predicted actual					277 ± 19 A	484 ± 85 A	437 ± 37 A

For the smallest PM<sub>2.5</sub> class, again, no significant differences in the amounts of PM removed were shown between species in the monocultures, even though the amount of PM<sub>2.5</sub> removed from the crowns of *C. betulus* was three times that removed from *A. platanoides*. In the *QrCb* and All polycultures, the observed values of PM<sub>2.5</sub> were 50% greater than the predicted nominal or predicted actual values. For the All four species polyculture, the increase was significant different, indicating a higher amount of PM<sub>2.5</sub> than could be predicted from the monocultures. For the sum of all the PM size classes (ΣPM Table 3), no significant differences between species in the monocultures could be identified. The polyculture *QrCb* showed the highest value for PM removed from leaves, with significant differences compared to the monoculture *Ap* and the polyculture *ApTc*. No differences were found between observed and predicted for any of the polycultures.

#### 4. Discussion

##### 4.1. PM per Unit Leaf Area

The amount of PM retained in the crowns of trees is a function of the leaf area [15,27], and the macromorphological and micromorphological properties of leaves [13,18,19,59]. At a unit leaf level, in the four species investigated in our study, there were few differences in the amount of PM removed from the leaves for the total PM, and the different size classes. The most striking difference was the higher amounts of PM<sub>2.5</sub> removed from the leaves of

*T. cordata* and *C. betulus* compared to *A. platanoides*. In a study of 22 tree species in Norway and Poland, ref. [7] showed that both *T. cordata* and *A. platanoides* were among the species with the lowest PM accumulation levels. In contrast, ref. [60] rated *A. platanoides* and *T. cordata* as moderate, *C. betulus* and *Q. robur* as high air pollution mitigation species. Ref. [19] investigated the same four species as in this study. Contradictorily, they identified *C. betulus* as one of the lowest PM retaining species after *Q. robur*, *T. cordata* and *A. platanoides*. In the wind tunnel experiment of [61], *T. cordata* was among the best species capturing NaCl and talcum particles. The leaf saturation isothermal remanent magnetization (SIRM) analysis of [10] showed *T. cordata* and *Q. robur* with high, and *C. betulus* with low magnetic particle deposition. These comparisons indicate that often, which species binds the highest amount of PM is context dependent. In our study, the trees have been exposed to a relatively low atmospheric level of PM.

Again, at the unit leaf area level, ref. [27] reported high accumulation of PM<sub>2.5</sub> on leaves of *T. cordata*, which was related to the presence of a high number of trichomes on the leaves. It is known that leaf hairiness increases PM capture efficiency [10,23,24]. In contrast to *T. cordata*, leaf epicuticular waxes on *A. platanoides* are relatively smooth [28], and the leaves relatively large (mean area 56.8 cm<sup>2</sup>), which could explain the lower levels of PM found on this species. Broadleaf species with smooth surfaces, such as *A. platanoides*, tend to have lower PM accumulation [18] than species with rough leaf surface properties [13,23,62,63]. High leaf wettability, as shown for *Q. robur*, also positively influences PM retention [10], but is a debatable factor [19]. Tree species with a large number of small leaves have been shown to have the highest PM retention [19]. PM capture also increases with a finer and more complex structure of the foliage [13,23,64]; thus, PM capture tends to be greater in conifers. In the four species we investigated, *C. betulus* had the smallest individual leaf area (20.8 cm<sup>2</sup>) compared to the other species. The arrangement of leaf veins has also been shown to influence PM accumulation. Ref. [19] stated that most PM captured on the leaf surfaces of *Q. robur* and *T. cordata*, was concentrated close to the leaf midribs. Thus, the deep ridges of the leaf veins [65–67] in *C. betulus* may also contribute to the high PM accumulation in all diameter classes in this species.

#### 4.2. Leaf Area Index

The leaf area available to capture PM was quantified through the leaf area index. In the monocultures, the LAI was similar in all species except *T. cordata*, which had almost only half the LAI of the other species. In polyculture, overyielding of LAI was shown in both the *ApTc* mixture and the four species mixture (All). In both polycultures, the overyielding was driven by an increase in the leaf area of *A. platanoides*, but in All, also by an increase in *C. betulus* and *Q. robur*. Overyielding of leaf biomass has been shown in other studies in subtropical [45] and tropical forests [44]. Overyielding in mixtures is a relatively common phenomenon for a number of biomass parameters [43,68]. However, overyielding does not always occur [52] as diversity effects are strongly driven by functional identity of the component species in the polyculture [39], but also trait differences between the species [68]. Overyielding occurs due to (i) reduced competition and (ii) facilitation between species [41,68]. Mixtures containing species enabling ecological coalescence due to having different traits in crown, root and growth characteristics results in facilitation and competition reduction. This can clearly be seen in the overyielding of LAI in the *ApTc* and All polycultures, but not in the *QrCb* polyculture where the species have similar traits.

#### 4.3. PM and LAI

On the basis of leaf area among the tree species, the highest amount of PM<sub>2.5</sub> was removed from leaves of *T. cordata* and *C. betulus*. However, when the amount of PM in the crowns of the trees is calculated on the basis of LAI, there were no significant differences among the species, due to the equalizing effect of the different LAI of the species. For example, the low levels of PM retained on the smooth leaves of *A. platanoides* is compensated by the higher LAI compared to the other species. A similar compensator effect of LAI has

been shown for other tree species growing in urban environments [15]. Ref. [15] showed that the trees with the high canopy density removed the highest amounts of PM. At the B-Tree site, *A. platanoides* is the height dominant species with a mean height of 5.5 m across the plots, and the monoculture LAI of  $3.8 \text{ m}^2 \cdot \text{m}^{-2}$  is composed of ca. 670 individual leaves. In comparison, the average height of *C. betulus* is 3.6 m and LAI ( $3.7 \text{ m}^2 \cdot \text{m}^{-2}$ ) is composed of ca. 1800 individual leaves, thus having a much denser and more complex crown.

The importance of considering LAI is clearly seen in the polycultures, where overyielding and competition or facilitation between the tree species affects the contribution of each species. The four species polyculture had a higher than predicted amount of  $\text{PM}_{2.5}$ , which may be due to the LAI overyielding particularly of *C. betulus*. However, the two species polyculture of *Q. robur* and *C. betulus* had the highest levels of total PM and higher than predicted amount of  $\text{PM}_{10}$ , without a significant change in LAI. In the *Q. robur* and *C. betulus* polyculture, the two species partition the crown space with *Q. robur* in the upper crown and *C. betulus* in the lower crown, thus increasing the complexity and density of the effective crown volume. The amount of PM in the crowns of the *QrCb* polyculture was significantly higher than the *ApTc*, thus emphasizing the importance of species identity in mixtures [39].

#### 4.4. Species Selection and Human Health

In the polycultures, the most significant increase in removal of PM occurred in  $\text{PM}_{2.5}$  class, which is considered to be the most detrimental for human health [69]. Airborne  $\text{PM}_{2.5}$  are of practical relevance to ambient air quality management worldwide, given the vast epidemiological and toxicological evidence on adverse health effects [1,2]. Thus, the correct choice of trees for PM removal has direct human health implications. In this study, leaves were collected 5 days after a heavy rain event (18.8 mm). Even rainfall events of 10 mm or less can remove a significant amounts of PM loading [70–72], thus resetting the system to allow new accumulation. During the vegetation period 2020, at Tulln rainfall events of more than 10 mm occurred 13 times; thus, the potential PM removal is greater than the 5-day value calculated. To date, most frequently occurring tree species in global cities are commonly not the best choice in removing  $\text{PM}_{2.5}$  [73]. However, there is great potential for using selected tree species and mixtures to yield the most effective air cleaning potential in condensed urban areas, suggesting the need to rethink traditional urban greening projects accompanying climate change mitigation strategies. So far, species-specific canopy structures have hardly been integrated in considerations in urban vegetation planning and air quality management.

## 5. Conclusions

Trees play a vital role in removing PM and are ultimately an aid to human health and wellbeing. Leaf properties and canopy architectural trait of the leaf area index (LAI) have a significant influence on PM retention, the degree of which differs between the PM classes. Our work shows that estimation of the amount of PM retained on leaves does not truly reflect the potential PM binding of trees, as the LAI strongly influences the amount of PM retained per tree. An example of this can be seen for *A. platanoides*, which has a low binding capacity per leaf area, but a high LAI. In polycultures, LAI is influenced by competition or facilitation of the component tree species in the polyculture. LAI overyielding can result in greater PM removal. In addition, PM retention appears to be increased by the complexity of the crown, the reasons for which are currently unknown, as shown in the *Q. robur* and *C. betulus* polyculture.

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