



Variability between Hirst-type pollen traps is reduced by resistance-free flow adjustment

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Abstract During an international campaign organized in Munich (Germany) in 2021 to test the performance of automatic pollen traps, we ran four manual Hirst-type pollen traps in parallel. All 4 Hirst-type pollen traps were set and monitored on a weekly basis for the entire campaign to 10 L/min using the same standard hand-held rotameter. Afterwards, a hand-held heat-wire anemometer (easyFlux®) was used additionally to obtain the correct flow without internal resistance. Uncorrected pollen concentrations were 26.5% (hourly data) and 21.0% (daily data) higher than those obtained after correction with the easyFlux®. After mathematical flow correction, the average coefficient of variation between the four Hirst traps was 42.6% and 16.5% (hourly and daily averages, respectively) for birch and 36.8% and 16.8%

(hourly and daily averages, respectively) for grasses. When using the correct flow of each pollen trap (i.e. the resistance free anemometer measured flow), for hourly values, the median standard deviation across the traps for the eight pollen types was reduced by 28.2% ($p < 0.001$) compared to the uncorrected data. For daily values, a significant decrease in the median standard deviation (21.6%) between traps was observed for 7 out of 8 of the pollen types, ($p < 0.05$ or lower). We therefore recommend continuing to calibrate Hirst-type pollen traps with standard hand-held rotameters to avoid changing the impacting characteristics of the instruments, but simultaneously also measure with resistance-free flow meters to be able to apply flow corrections to the final pollen concentrations reported. This method improved the accuracy of the final results.

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1 Introduction

Pollen measurements are routinely performed at more than 600 stations worldwide according to the pollen monitoring map of the world (www.zaum-online.de/pollen) (Buters et al., 2018). The large majority of these sites use pollen traps that sample a known volume of air (Hirst, 1952), which at the time of development was a milestone in Aerobiology since it allowed standardization and thus comparison between aerobiological studies. Over the last decade, another milestone has been achieved through the development of automatic pollen monitoring devices (Oteros et al., 2015; Crouzy et al., 2016; Šaulienė et al., 2019; Sauvageat et al., 2020; Tešendić et al., 2020). Hirst-type pollen traps are, however, still widely used and often serve as a basis for comparison against the automatic devices at the sites (Maya-Manzano et al., 2023; Tummon et al., 2021) where such instruments are installed (about 40 in Europe).

Within the framework of the EUMETNET Auto-Pollen Programme (Clot et al., 2020) and the ADOPT COST Action, an international intercomparison was organised in Munich, Germany (Maya-Manzano et al., 2023). The aim of this campaign was to evaluate the capabilities of the large range of automatic pollen monitors that are currently available. Four Hirst-type manual traps were run in parallel and analysed in detail to provide a baseline of the current manual standard.

To ensure that the Hirst-type traps provide reliable data, basic procedures were defined and standardized (Galán et al., 2014; EN16868:2020). This includes maintaining certain essential calibrations related to the sample collection and counting methods. For example, the speed at which the drum rotates inside the trap was standardized at 2 mm/hour (Hirst, 1952). Cleaning of the instrument's inlet is also a standardized procedure before starting collection. Besides this, other factors can also disturb the incoming airflow, and it is recommended to keep a minimum of 2 m distance from other

devices, 200 m from other buildings, and 3 m from roof edges to limit turbulences (Oteros et al., 2019) and a canyon effect (Peel et al., 2014). Other factors such as the adhesive used (Maya-Manzano et al., 2018), the area of the sample counted (Sikoparija et al., 2011), or the human operator making the counts (Oteros et al., 2020; Sikoparija et al., 2017) also influence the values obtained. Furthermore, not all networks provide data clustered in the same hourly periods (Galán et al., 2014).

The Hirst-type traps suck air at a standardized rate of 10 L/min. It is assumed that their pumps do not age and lose power over time. Loss of aspiration power is a potential problem because the instruments commonly used to calibrate the airflow have an internal airflow resistance, which normally goes unnoticed if the pump still functions correctly. However, if the pump is weak and the airflow is calibrated using a device (often a hand-held rotameter) with an internal resistance, the pump together with the hand-held rotameter will not manage to suck in 10 L/min and the operator thus falsely increases the flowrate to compensate. This results in errors averaging 33% (range 5–72%) according to Oteros et al. (2017). This potentially affects the measurements of different pollen taxa differently. When working with the common hand-held rotameters, the differences in daily pollen concentrations between traps were reported to be 23% for birch (Buters et al., 2012) and 20% for Poaceae (Buters et al., 2015). This error can easily be mitigated by using a resistance-free flow measurement to calibrate the traps (Oteros et al., 2017).

Some authors have reported higher variability in hourly (Tormo Molina et al., 2013) or two-hourly pollen concentration data (Adamov et al., 2021) compared to daily averages. It is possible that compensating for airflow errors at these temporal resolutions may decrease the variability and thus make the data more comparable. The aim of this work is; (1) to provide an overview of the environmental conditions of the AutoPollen-ADOPT international intercomparison campaign. (2) to evaluate the hourly and daily variability between Hirst-type traps when calibrated using hand-held rotameters and when a correction of the flow is applied using resistance-free flow measurements. We also discuss the differences in measured airflow using five different flowmeters.

2 Materials and methods

2.1 Pollen sampling

Four Hirst-type traps (Burkard Manufacturing Co Ltd) were placed on a rooftop 10.5 m above ground level at the Helmholtz Zentrum München (11.5956°E, 48.2208°N, Munich, Germany), as part of the EUMETNET AutoPollen-COST ADOPT instruments intercomparison campaign (Maya-Manzano et al., 2023), which ran from 3rd March to 14th July 2021. The clocks inside the traps were calibrated using a Witschi Watch Expert IV device (Witschi, 2022) and weekly tests were run until less than $\pm 2\%$ error was obtained (± 3.4 h over a total of 168 h per week).

To ensure the traps were affected by as little turbulence as possible, they were located 3 m from the edge of the building, with their inlet 1.7 m above roof level, in a 12 m long and 5 m wide rectangular disposition. The four traps were labelled A, B, C and D, with the devices A and B closer to each other, and C and D also closer to one another. Melinex tape coated with vaseline adhesive was used to capture the particles. The tapes were replaced every 7 days and then cut into 24 h fragments and embedded using glycerine-gelatine with safranin. Pollen was analysed under 400 \times magnification along 4 longitudinal transects, covering 15.8% of the total surface following the minimum recommendations ($> 10\%$) of the European standard EN 16868:2020. Hourly data were assessed by using the same methodology explained by Galán et al., (2007). Because of the possible influence that the adjacent vegetation could have on the obtained data, the surrounding tree species were mapped in a 500 m radius from the sampling site.

2.2 Flow correction calculations

At the beginning of the campaign, the airflow of the traps was set to 10 L/min using a Burkard Manufacturing rotameter. Airflow was monitored weekly and adjusted if necessary.

On 14th July 2021, we tested five different flowmeters to check their differences. These included Burkard Manufacturing Co Ltd, Burkard Scientific Ltd, and Lanzoni Srl rotameters, as well as an in-house-designed resistance-free, externally-calibrated flow meter called RFF and an easyFlux® resistance-free flow meter (Figure S1, Supplementary material).

The two resistance free flowmeters were externally calibrated by ESZ calibration laboratories, Munich, Germany. From these measurements, we obtained the correction factor to adjust the average of the flow measured with the Burkard Scientific rotameter which was used throughout the campaign.

The average flow values for the traps were 14.43 ± 0.32 L/min for A, 13.38 ± 0.26 L/min for B, 13.21 ± 0.25 L/min for C and 13.39 ± 0.25 L/min for D, as determined with the calibrated easyFlux® flowmeter.

2.3 Weather data

The meteorological data for Munich were obtained from the German National Meteorological Service (DWD, 2021) using the R package *rdwd* (Boessenkool, 2021). Data from the München-Stadt station located 7.5 km away from the sampling site were acquired. The following parameters were analysed at hourly resolution: rainfall (mm), air temperature (°C), relative humidity (%), wind speed (m/s), wind direction (°), sunshine duration (hourly sum, in minutes), and air pressure (hPa) (Figure S2, Supplementary material).

2.4 Statistical analysis

The Main Pollen Season (hereafter, MPS) was calculated using the 95% method averaged over all the Hirst traps that were working at any given time ($n=2-4$) with the *AeRobiology* R package (Rojo et al., 2019a). For the statistical analysis, only the time steps when all four traps recorded pollen data and concentrations were > 10 pollen grains/m³ were included for both, hourly and daily basis. This threshold was set as described by Adamov et al. (2021), due to the high uncertainty of Hirst traps for such low pollen concentrations (Adamov et al., 2021; Rojo et al., 2020). The hourly pollen concentrations per pollen type were also averaged and reported as daily pollen concentrations and subjected to the same statistical analysis. After checking normality with the Shapiro–Wilk test, hourly data were processed and treated for the eight dominant pollen types: *Pinus*, *Betula*, Poaceae, Urticaceae, Taxaceae/Cupressaceae, *Fraxinus*, *Quercus* and *Carpinus*. Other pollen types, such as *Ambrosia*, *Alnus*, *Artemisia*, *Corylus*, *Fagus*, *Picea*, *Plantago*, *Populus* or *Tilia*, were also counted

but they were not recorded in sufficiently high concentrations to warrant further analysis. In the case of *Corylus*, we missed its MPS and thus did not include it in the analysis. Also unrecognized pollen type (labelled as Varia) was counted.

The Coefficient of Variation (%) between the four Hirst traps was calculated for each pollen type using corrected pollen concentrations. The standard deviation of pollen concentrations across the four traps was calculated for each time step with and without the flow correction and these differences were evaluated using the Wilcoxon signed rank-Test for paired samples. To compare the differences between traps for pollen concentrations at hourly and daily resolutions, the Friedman test (Friedman, 1940) was applied, (e.g. Maya Manzano et al. 2017a; Picornell et al., 2019). When statistical significance was found, it was followed by a Wilcoxon signed-rank test for pairwise comparisons with *p*-value corrections using the Bonferroni method. Spearman's rank coefficient between traps was also calculated following the same methodology to ensure paired values as a statistical parameter to check the similarity between traps and Bland–Altman plots (Altman & Bland, 1983) were drawn to evaluate the agreement between pairs of measurements for all traps and the existence of possible systematic errors, with a 95% Confidence Interval represented by the mean difference ± 1.96 * standard deviation. Finally, to calculate differences between the flow measured by the flowmeters, the Kruskal–Wallis test (Kruskal & Wallis, 1952) was carried out for multiple unpaired samples (5 groups). When a *p*-value < 0.05 was obtained, a Dunn's post-hoc test (Dunn, 1964) was applied, again with *p*-value corrections using the Bonferroni method. Statistical differences in all cases were reported when *p*-values were < 0.05.

3 Results

3.1 Surrounding vegetation

Eleven tree taxa of aerobiological interest were identified within a 500 m radius surrounding the Hirst traps (Fig. 1A). The predominant taxa, with 506 specimens was *Pinus* (especially *Pinus sylvestris* and *Pinus nigra*), followed by *Betula* spp. (mainly *Betula pendula*), *Carpinus* spp. (mainly *Carpinus betulus*),

and *Platanus* spp., with 73, 57, and 38 specimens, respectively. For the rest of the taxa, the number of specimens was lower and ranged from 4 (*Taxus baccata*) to 14 specimens (*Corylus* spp., mainly *Corylus avellana*). Their geolocation provides an idea of their distribution and how their proximity to the traps may affect the results obtained (Fig. 1B). For instance, some specimens of *Taxus baccata*, *Corylus* spp. or *Carpinus* spp. were very close (< 200 m) to the sampling site and are thus likely to be overrepresented in the pollen counts. Additionally, sources close to the traps could result in an inhomogeneous distribution of pollen that may have impacted one trap more than the others.

Moreover, the background coloured in light-green in Fig. 1B represents a large surface covered by grass pastures, with Poaceae spp., Urticaceae spp., *Plantago* spp. and Asteraceae spp. amongst other herbaceous taxa present.

3.2 Meteorological data

The results for wind direction show that the four traps were predominantly facing the west and south-west during the observational period (Figure S2A, Supplementary material). Rainfall was for the most part under 10 mm/day, except for late in the campaign when precipitation rates were over 20 mm/day for four days (Figure S2B, Supplementary material). Starting in early spring and ending in mid-summer, the air temperature increased over the measurement period reaching 25 °C during the second half of the campaign. The maximum temperature observed was 31.6 °C while the minimum was –4.1 °C (Figure S2C, Supplementary material). The average relative humidity was 65.5% over the entire campaign, varying on average around 41% over the day between the lowest and highest humidity (Figure S2D, Supplementary material).

3.3 Pollen sampling and differences between Hirst traps

The pollen concentrations obtained during the MPS show that *Pinus* was the most abundant pollen type, followed by *Betula* and Poaceae (Fig. 2). The main characteristics of the MPS for each counted pollen type is presented in Table S1 (Supplementary material). Time series' of hourly and daily pollen

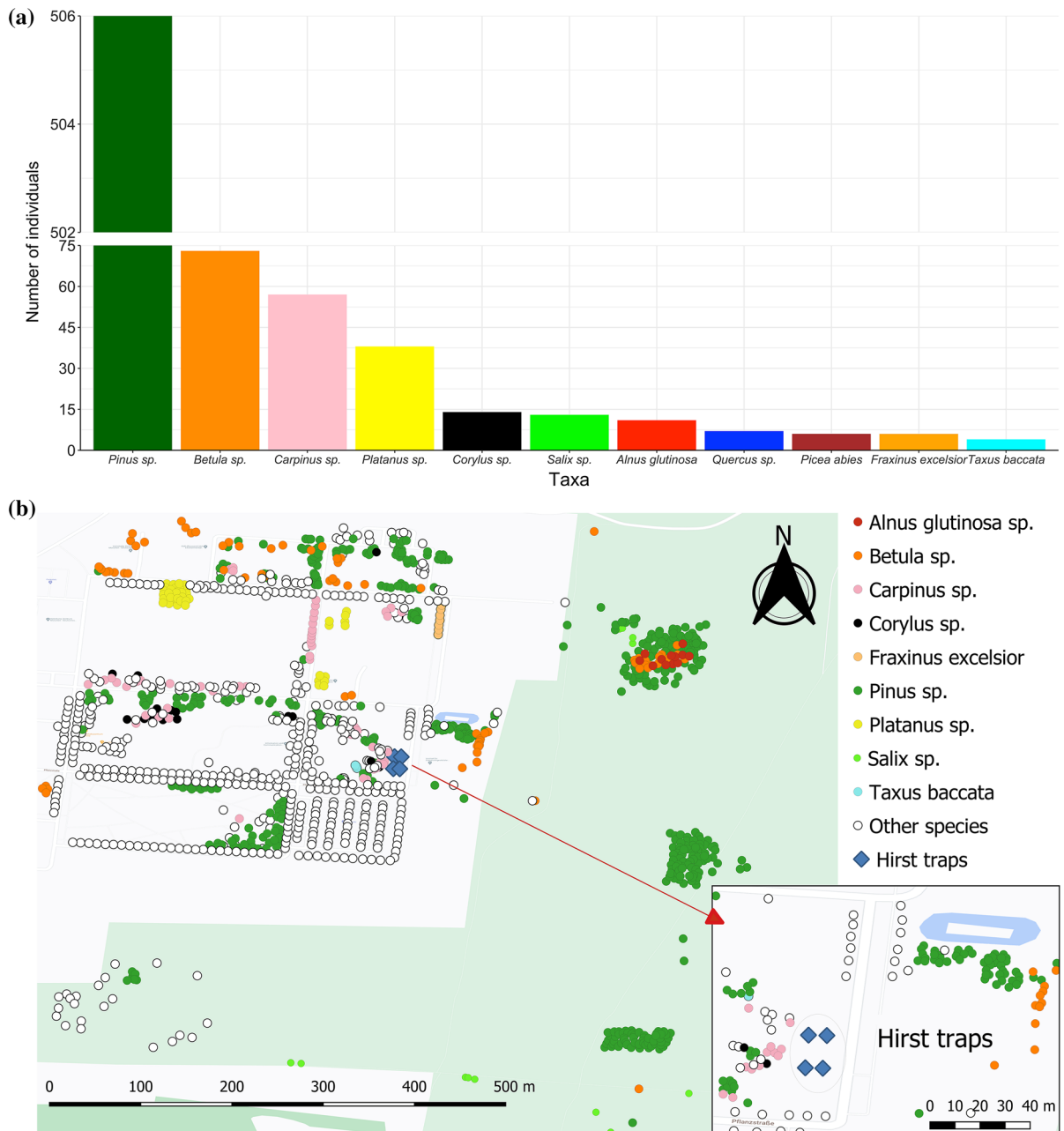


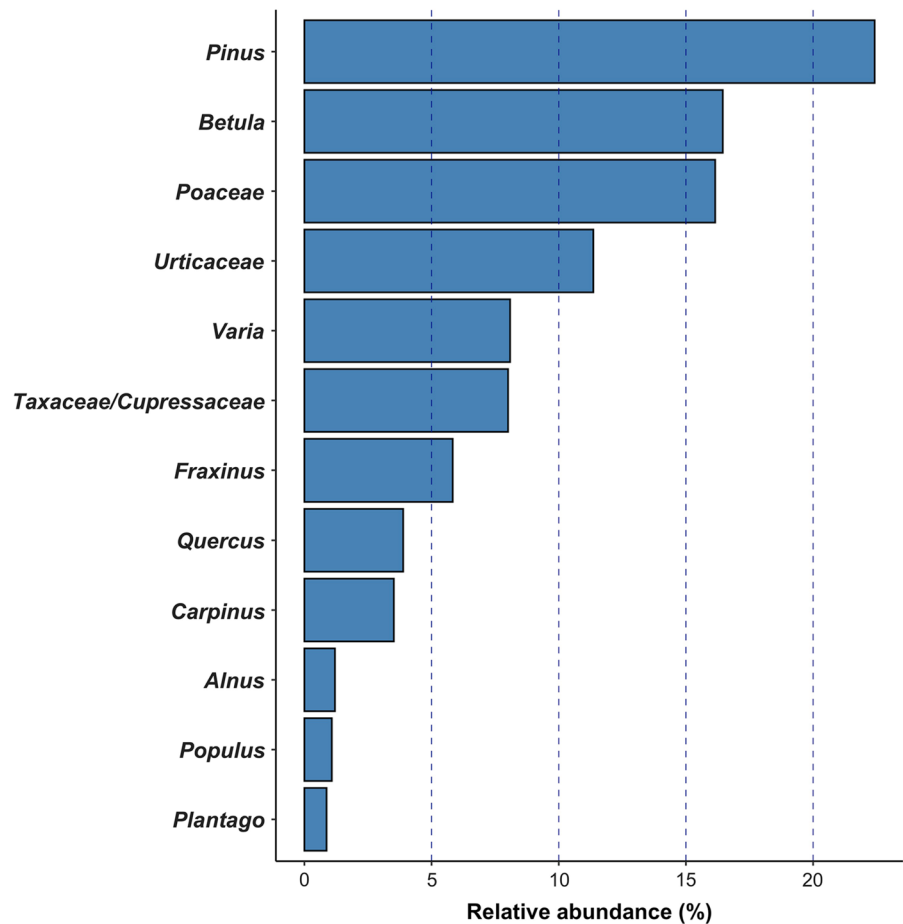
Fig. 1 Species distribution surrounding the sampling site. **A** The number of individuals for different aerobiologically-relevant species. Note the gap in the y-axis for *Pinus sp.* **B** Locations of these trees within a 500-m radius of the sampling site

(Hirst-type traps shown as blue diamonds), with a zoom on the measurement site. Background colour: white = Helmholtz Zentrum München and other buildings, green = natural areas

concentrations are shown in Fig. 3A and B while the pollen concentrations per wind direction for the eight pollen types are shown in Figure S3 (Supplementary material). Uncorrected pollen concentrations were

on average 26.5% and 21.0% higher (hourly data and daily data) than the resistance-free flow corrected concentrations. The average coefficient of variation between the 4 Hirst type (after correction) was pollen

Fig. 2 Relative abundance (%) of pollen taxa. Relative abundance (in %) of airborne pollen in the Hirst-type pollen traps during the intercomparison campaign 2021 for the major pollen types. Data are averaged over the entire sampling period from 3 March to 15 July 2021



species dependent and also higher for hourly pollen concentrations than for daily pollen concentrations ($40.5 \pm 2.5\%$ vs. $19.0 \pm 5.1\%$, Table 1).

When comparing pairs of traps per pollen type (after flow correction), statistically significant differences between the traps were observed in all cases for the hourly data, except for *Taxaceae/Cupressaceae* (Table 2). For all possible combinations (48, since we have 8 pollen types and 6 different combinations between the traps), 23 combinations (47.9% cases) were significantly different, but not always between the same traps. Trap A was involved in 11 of the cases with significant differences, trap B in 15, trap C in 9, and trap D in 10 of the cases. As additional information, A and B were counted by the same counter, but C and D were examined by another counter (but from the same laboratory). When the daily data were compared, the Friedman test showed significant differences between traps for four pollen types (5 cases

out of 48 or 10.4%): *Pinus*, *Poaceae*, *Urticaceae* and *Quercus*. In all the 48 cases tested, A and C were different in 2 cases, trap B on 3 occasions and trap D 3 times. The average pollen concentrations for each pollen trap and the average standard deviations for the analysed period are displayed in Table 3. Bland–Altman plots (Fig. 4) showed that the agreement between pairs of traps was lower for hourly pollen concentrations (Fig. 4a) than for daily pollen concentrations (Fig. 4b), which the most of values fell within the confidence intervals. The mean differences (bias) were reasonably closer to zero, with the points distributed most as a “triangle” according to the random relative errors, with no irregular patterns (systematic bias). The higher deviations were seen for those pollen taxa that reached the higher concentrations, such as *Pinus*, *Poaceae*, *Taxus* or *Urticaceae*. Other pollen taxa (*Betula*, *Fraxinus*, *Carpinus* or *Quercus*) did not show so clear patterns, with bigger differences

between measurements that can be reached also at medium pollen concentrations.

3.4 Differences between Flowmeters and reduction of variability by correcting the flow

The flow values obtained for each trap using the rotameters and resistance free-flowmeters showed that there are no significant differences between the Burkard Scientific, Lanzoni, and RFF (Fig. 5). The Burkard Manufacturing flowmeter always measured the lowest values, while the easyFlux® showed the highest values and were significantly different ($p < 0.05$) compared to the four other devices. We chose the easyFlux® to correct the flow since this instrument is resistance-free and is cheaper and easier to handle than the RFF. As a side note, all the flowmeters, with the exception of the Burkard Manufacturing device, showed that trap A had the highest flow rates.

When the observations were corrected for erroneous flow using the easyFlux® flowmeter, all the standard deviations between traps were reduced (Fig. 6). For the hourly data, differences were statistically significant ($p < 0.001$) for all cases, with the median concentration also being reduced by 28.2% on average (ranging from 23.5% for Urticaceae to 33.2% for *Quercus*). For the daily averages, 7 out of the 8 pollen types show significantly different values ($p < 0.05$ or lower) and with median values being reduced by 21.6% on average across the 8 pollen types (ranging from a reduction of 5.5% for *Carpinus* to 34.6% for *Pinus*). The daily values for the Taxaceae/Cupressaceae pollen type were the only exception in which the variability did not significantly decrease after the flow correction, but this is likely due to the low number of values considered in this case (5 values).

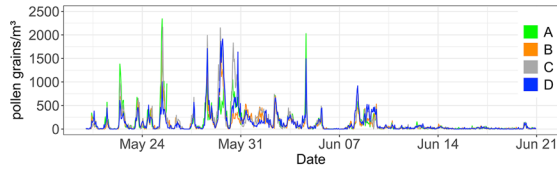
4 Discussion

When comparing with a previous study carried out in Munich (Rojo et al., 2020), the influence of surrounding vegetation can be noticed in pollen types such as *Pinus* or Poaceae. They reported the mean for pollen monitoring over ten years (2006–2016) for two stations of Munich (located in city centre at 6.3 and 11.9 km distance from the current location). For *Pinus*, the APIn was lower than 2000 pollen*day/m³

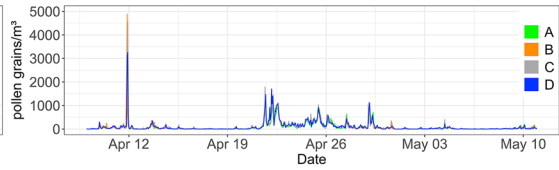
for both stations and around 2000 pollen*day/m³ for Poaceae. In our study *Pinus* is two times higher (4211 pollen*day/m³) and Poaceae reached a higher pollen integral too (3017 pollen*day/m³). Regarding other types such as *Betula* or *Fraxinus* pollen, our location obtained two and three-five times lower pollen (3091 for *Betula* and 1090 pollen* day/m³ for *Fraxinus*) than the compared two stations (around 7500–7700 pollen*day/m³ for *Betula* and 3200–5000 pollen*day/m³ for *Fraxinus*). The comparison between the surrounding vegetation and the relative abundance of pollen concentrations during the sampling period can provide a general idea on how pollen sources could influence the measured pollen concentrations. In our work, the two main taxa present in the area within 500 m of the sampling site (*Pinus* spp. and *Betula* spp.) were also the most abundant pollen types in the observations, although not in the same proportion. Despite many *Pinus* trees being close to the traps, the predominant wind direction was from the west and southwest meaning that not much pollen was directly transported from the trees, which were mostly located to the east of the sampling location. The influence of vegetation type together with dominant wind patterns on observed pollen concentrations has been reported by other authors (Rojo et al., 2015; Maya-Manzano et al. 2017b). Since the traps were located on a flat rooftop 10.5 m above the ground, this reduces the influence of close-by pollen sources that are not large, for example herbaceous plants and grasses, and should serve to provide a more homogenous representation of airborne pollen than if the site were located at ground level (Rojo et al., 2019b). However, since some species such as *Carpinus betulus*, *Corylus avellana*, *Taxus baccata* and *Tilia platyphyllos* are located to the west, southwest, and northwest of the sampling site, they are likely to have influenced the measurements to some extent when the wind blew from these directions (predominant in the studied area). The different pollen types and their predominant wind direction patterns over the campaign are shown in Figure S3.

The comparison between the four traps showed the extent of the variability between them (Fig. 6), especially for the higher time resolutions, as highlighted in former studies (Adamov et al., 2021; Tormo Molina et al., 2013). Interestingly, the devices did not show a systematic bias between them, according to the captured behaviour shown by the Bland Altman

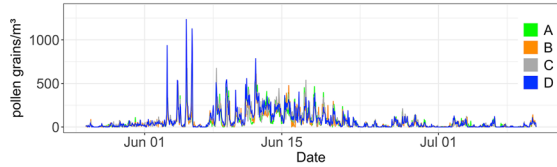
(a) *Pinus* concentrations (Hourly data)



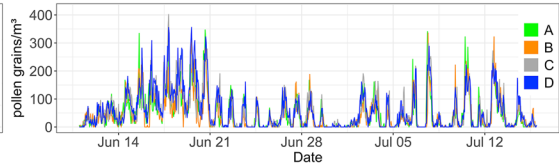
Betula concentrations (Hourly data)



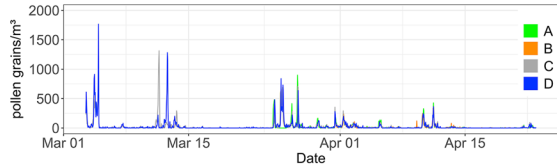
Poaceae concentrations (Hourly data)



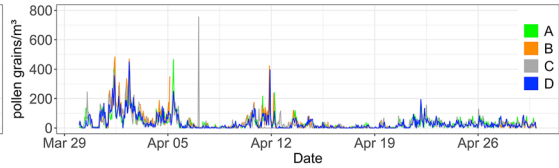
Urticaceae concentrations (Hourly data)



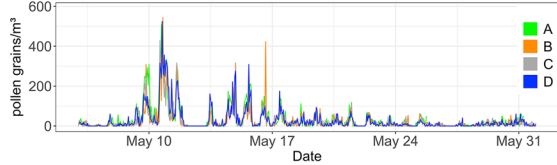
Taxaceae/Cupressaceae concentrations (Hourly data)



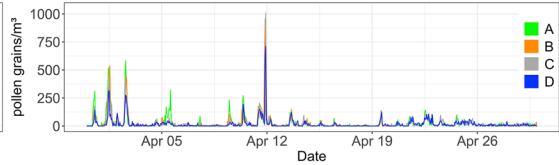
Fraxinus concentrations (Hourly data)



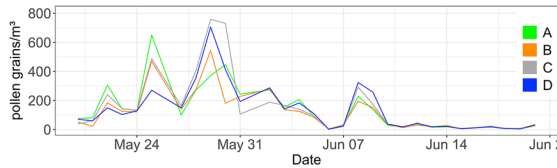
Quercus concentrations (Hourly data)



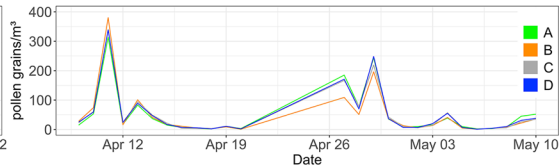
Carpinus concentrations (Hourly data)



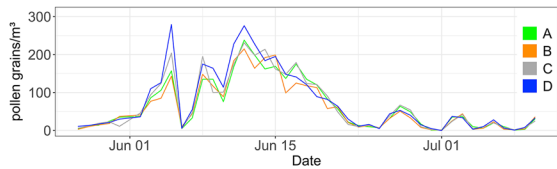
(b) *Pinus* concentrations (Daily data)



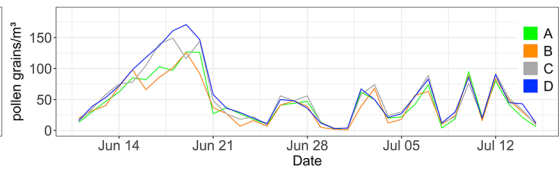
Betula concentrations (Daily data)



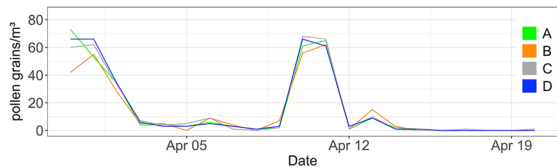
Poaceae concentrations (Daily data)



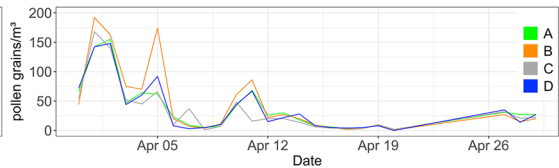
Urticaceae concentrations (Daily data)



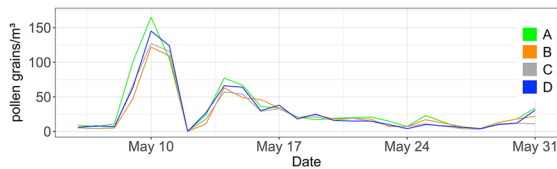
Taxaceae/Cupressaceae concentrations (Daily data)



Fraxinus concentrations (Daily data)



Quercus concentrations (Daily data)



Carpinus concentrations (Daily data)

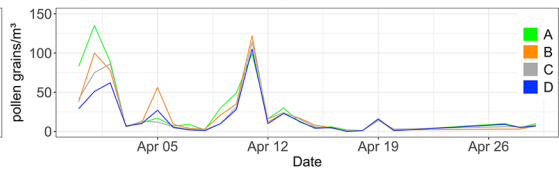


Fig. 3 Time series. Time series of hourly **A** and daily **B** observations for the 8 main pollen types from the four Hirst traps (**A**, **B**, **C** and **D**) after individual mathematical flow correction to 10 L/min

plots (Fig. 4). However, these plots also showed a higher variability in hourly data regarding daily data, as reported by the former studies. The careful design of the experiments, including the calibration of measurement instruments (calibration of clocks rotation, external calibration of resistance-free flowmeters and periodical checking of flow rate) used in our research, together with good practices to minimize the influence of external factors (ensure the distance to the borders in the rooftop to avoid turbulences, to elevate the sampling point at a minimum height and also above the ground level, the pollen analysis being performed within the same laboratory, etc.) are the only measure possible to reduce bias.

Differences were taxa dependent, with, for instance, daily coefficients of variation after correction of 16.5% for *Betula* and 16.8% for Poaceae, which are lower than reported in previous studies (23% *Betula* and 20% Poaceae, (Buters et al., 2012, 2015)) at the same location but without carrying out flow corrections. An additional source of variability is likely to stem from the different sampling flow of each pollen trap. While the flow rate was controlled by adjusting the flow of each trap with a rotameter every week, following the European standard (EN 16868:2020), Oteros et al. (2019) showed that the air-flow resistance of the rotameter itself impacted the flow adjustment and this may influence traps differently, in part because their individual pumps react differently. It is possible that the traps used in this study do not represent the entire spectrum of differences seen across such instruments since we report a smaller range than the 5–72% observed by Oteros et al. (2017), who measured 19 different traps instead of the 4 in our study.

The differences found between rotameter brands (Fig. 5) are in agreement with Oteros et al. (2017). From our results, a low air-flow resistance flowmeter is recommended, which could be an electronic flowmeter like the easyFlux®. Our in-house RFF would be a solution too, however, it is more cumbersome to work with and thus more prone to handling errors. Indeed, statistically significant differences were found between the easyFlux® and RFF ($p < 0.05$), possibly

because of handling errors. Interestingly, the RFF showed results more similar to the standard rotameters (Burkard Scientific and Lanzoni). It is very challenging to estimate the internal resistance of the easyFlux® or the RFF and although both are externally calibrated and certified, some small differences might remain. Nevertheless, from the design of both instruments, this is likely to be significantly smaller than the resistance of the rotameters tested. The higher sensitivity of the resistance-free flowmeters compared to the standard rotameters is also evident through the higher flow measured for trap A, which was practically undetected by the two Burkard rotameters (Fig. 5). In practice, it would mean that since most stations are usually calibrated with Burkard rotameters, nearly all traps are erroneously calibrated, confirming the work of Oteros et al. (2017).

By correcting for the flow errors, we reduced the standard deviation by about 28% (Fig. 6). Even if the four traps were originally set to 10 L/min, the correction using the more accurate resistance-free flow rate resulted in significantly different values and less variability between traps. The power of applying this correction can be clearly observed for Trap A that was found to suck in 1L/min more than the three other traps. By applying the correction factor, differences from the other traps are essentially removed. These results support the statement from Oteros et al. (2017) recommending that the measured flow rate and type of flowmeter used are reported. This would improve the accuracy of the Hirst-type measurements

Table 1 Coefficient of variation between Hirst-type pollen traps after flow correction

Pollen type	Hourly average CV (%)	Daily average CV (%)
<i>Pinus</i>	42.4	21.4
<i>Betula</i>	42.6	16.5
Poaceae	36.8	16.8
Urticaceae	37.0	17.3
Taxaceae/Cupressaceae	41.3	10.7
<i>Fraxinus</i>	39.8	23.8
<i>Quercus</i>	40.9	18.0
<i>Carpinus</i>	43.5	27.5
Mean of means	40.5	19.0
SD of means	2.5	5.1

Average Coefficient of Variation (%) across the four traps for hourly and daily values

Table 2 Correlations between the pairs of Hirst-type pollen traps after flow correction

Pair	Pinus (n = 352)		Betula (n = 215)		Poaceae (n = 519)		Urticaceae (n = 402)		Taxaceae/Cupressaceae (n = 74)		Fraxinus (n = 175)		Quercus (n = 163)		Carpinus (n = 94)	
	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho
A-B	<0.001	0.82	<0.001	0.59	0.666	0.77	1.000	0.67	>0.05	0.71	0.152	0.68	<0.001	0.62	<0.05	0.72
C-D	0.161	0.85	1.000	0.68	1.000	0.79	0.642	0.69	>0.05	0.65	1.000	0.73	1.000	0.71	<0.01	0.75
A-C	1.000	0.81	1.000	0.69	<0.05	0.77	<0.001	0.65	>0.05	0.67	0.606	0.68	<0.01	0.77	0.297	0.71
B-D	<0.001	0.87	<0.001	0.69	<0.001	0.80	<0.001	0.68	>0.05	0.64	<0.001	0.76	0.467	0.67	<0.01	0.79
A-D	1.000	0.85	1.000	0.75	<0.001	0.76	<0.001	0.68	>0.05	0.78	1.000	0.69	<0.01	0.74	<0.001	0.64
B-C	<0.001	0.83	<0.05	0.57	<0.001	0.78	<0.001	0.69	>0.05	0.53	<0.01	0.73	1.000	0.68	1.000	0.70
Friedman test	p < 0.001		p < 0.001		p < 0.001		p < 0.001		p = 0.09		p < 0.05		p < 0.001		p < 0.001	
Pair	Pinus (n = 26)		Betula (n = 15)		Poaceae (n = 30)		Urticaceae (n = 28)		Taxaceae/Cupressaceae (n = 5)		Fraxinus (n = 14)		Quercus (n = 15)		Carpinus (n = 9)	
	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho
A-B	0.371	0.94	>0.05	0.89	0.828	0.96	1.000	0.93	-	-	>0.05	0.87	0.353	0.81	>0.05	0.87
C-D	1.000	0.95	>0.05	0.99	1.000	0.96	1.000	0.95	-	-	>0.05	0.75	0.151	0.83	>0.05	0.88
A-C	1.000	0.95	>0.05	0.93	1.000	0.95	<0.05	0.96	-	-	>0.05	0.70	0.068	0.77	>0.05	0.88
B-D	0.261	0.94	>0.05	0.98	<0.01	0.95	<0.01	0.92	-	-	>0.05	0.89	1.000	0.89	>0.05	0.92
A-D	1.000	0.95	>0.05	0.94	0.089	0.94	<0.01	0.93	-	-	>0.05	0.91	0.780	0.91	>0.05	0.93
B-C	0.062	0.91	>0.05	0.98	0.121	0.94	<0.05	0.93	-	-	>0.05	0.69	1.000	0.88	>0.05	0.73
Friedman test	p < 0.05		p = 0.17		p < 0.05		p < 0.001		Not shown		p = 0.20		p < 0.05		p = 0.23	

(A) Hourly values and (B) daily values. Only timestamps when all four traps were running and when pollen concentrations were > 10 pollen/m³ were considered. Friedman test results are shown, and when the *p*-value was <0.05, results from a post hoc analysis (Wilcoxon-rank test with Bonferroni correction for the *p*-value) are displayed. *R*/*rho* is the result from the Spearman rank coefficient test

Table 3 Pollen concentrations for each of the four pollen traps

Hourly data											
<i>Pinus</i>			<i>Betula</i>			Poaceae			Urticaceae		
Trap	Average	Sd	Trap	Average	Sd	Trap	Average	Sd	Trap	Average	Sd
(A)											
A	240	304	A	122	286	A	121	113	A	86	62
B	203	263	B	117	371	B	114	98	B	84	60
C	279	383	C	125	343	C	130	116	C	98	66
D	244	311	D	125	322	D	137	140	D	104	70
Mean	242	316	Mean	122	330	Mean	126	117	Mean	93	64
Taxaceae/Cupressaceae			<i>Fraxinus</i>			<i>Quercus</i>			<i>Carpinus</i>		
Trap	Average	Sd	Trap	Average	Sd	Trap	Average	Sd	Trap	Average	Sd
A	77	86	A	79	72	A	97	88	A	122	156
B	68	74	B	92	86	B	78	87	B	96	130
C	78	80	C	75	71	C	80	81	C	98	128
D	79	73	D	77	68	D	86	86	D	81	107
Mean	75	78	Mean	81	74	Mean	85	85	Mean	99	130
Daily data											
<i>Pinus</i>			<i>Betula</i>			Poaceae			Urticaceae		
Trap	Average	Sd	Trap	Average	Sd	Trap	Average	Sd	Trap	Average	Sd
(B)											
A	158	158	A	82	92	A	92	64	A	55	33
B	135	137	B	78	97	B	87	61	B	53	31
C	175	207	C	83	91	C	98	70	C	64	39
D	159	162	D	85	94	D	106	80	D	67	44
Mean	157	166	Mean	82	93	Mean	96	69	Mean	60	37
Taxaceae/Cupressaceae			<i>Fraxinus</i>			<i>Quercus</i>			<i>Carpinus</i>		
Trap	Average	Sd	Trap	Average	Sd	Trap	Average	Sd	Trap	Average	Sd
A	57	15	A	58	42	A	51	44	A	59	44
B	49	14	B	71	62	B	41	34	B	54	39
C	58	14	C	51	47	C	41	37	C	46	37
D	59	14	D	58	43	D	46	41	D	39	29
Mean	56	14	Mean	59	48	Mean	45	39	Mean	49	37

Average and standard deviation values are shown (pollen grains/m³) for (A) hourly values and (B) daily values during the studied timestamps. Values were calculated only when all four traps were running and when pollen concentrations were > 10 pollen/m³

and allow a higher level of standardization within and across networks.

Since the flow rate is essential to estimate the total volume of air sampled, the underestimation of flow resulting from the hand-held rotameters means that the reported pollen concentrations are higher than they should be (in our case a 26.5 and 21% for hourly

and daily resolution). This obviously has potentially significant consequences for end users, for example, allergy sufferers who are expected to have symptoms above certain concentration thresholds. The correction with simple resistance-free sensors has two advantages: more accurate values are reported and differences between instruments, for example,

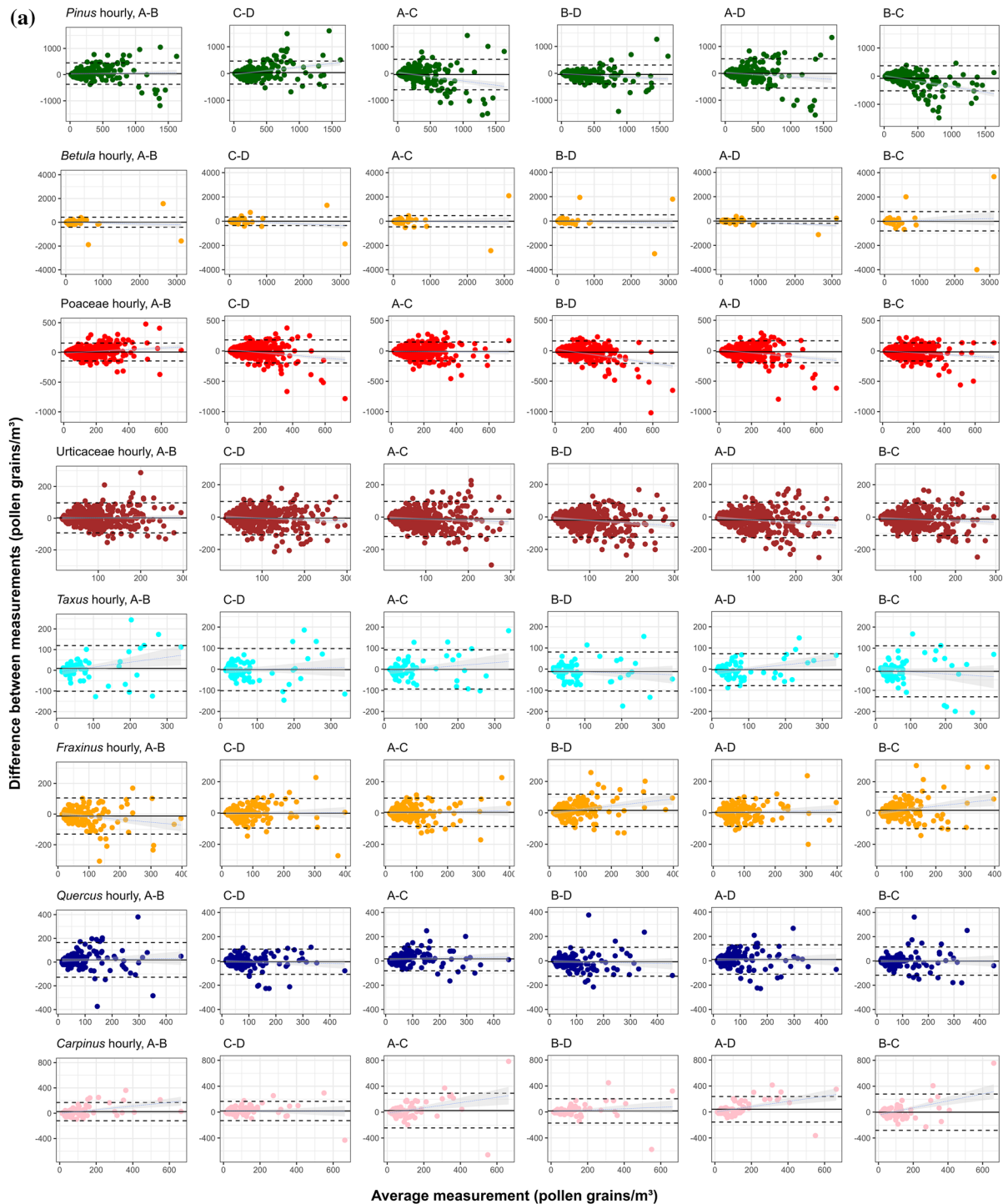


Fig. 4 Bland–Altman plots. Plots of agreement between pairs of measurements for hourly **A** and daily **B** observations for the 8 main pollen types from the four Hirst traps (**A**, **B**, **C** and **D**) after individual mathematical flow correction to 10 L/min.

Black solid line shows the mean difference (bias) between both measurements. Dashed black lines showed the upper and lower threshold for confidence intervals (95%, mean difference \pm 1.96 times the standard deviation)

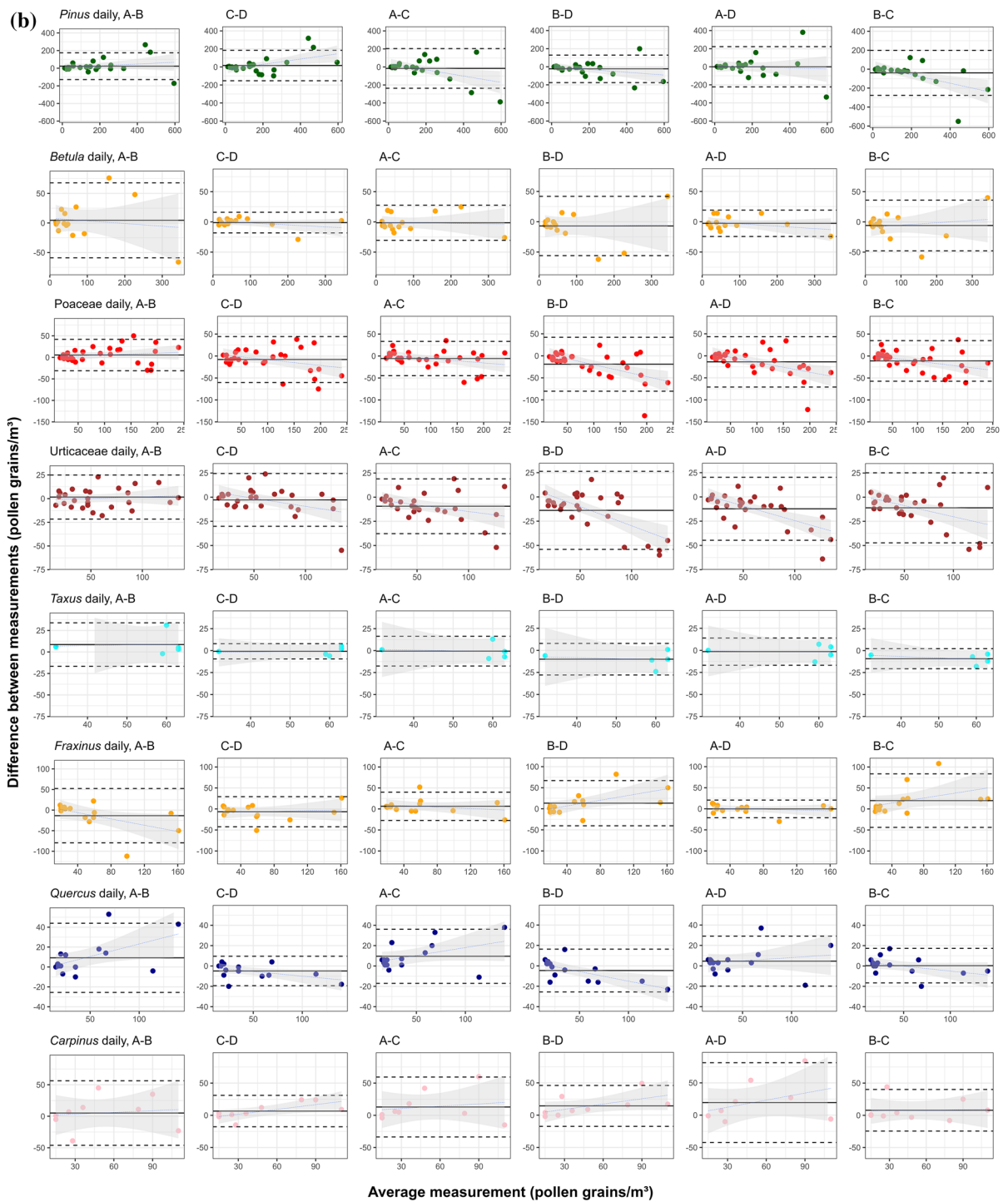
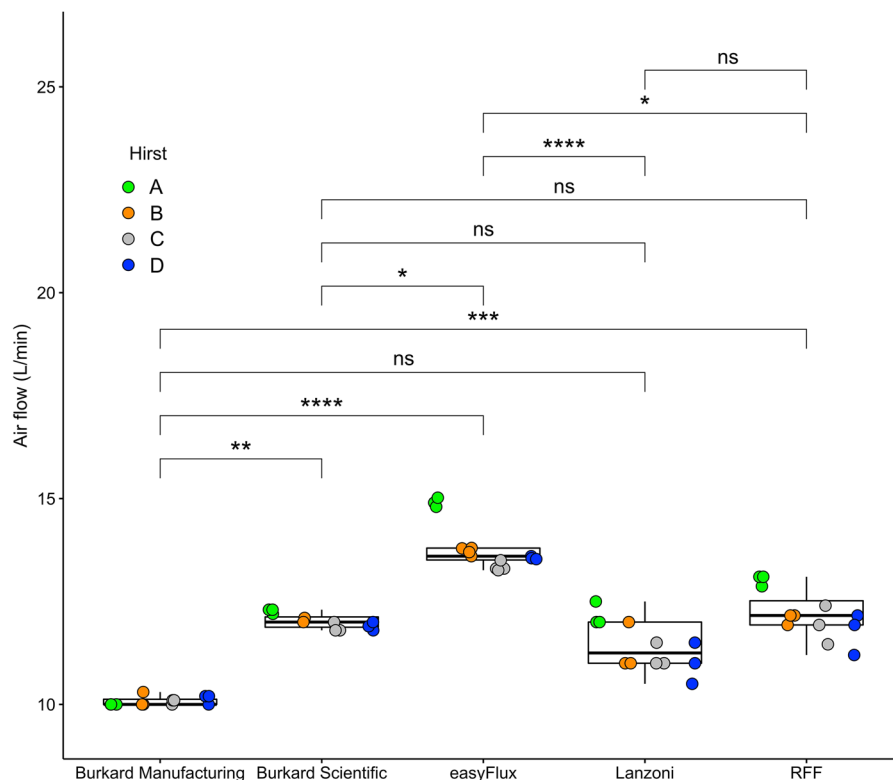


Fig. 4 (continued)

due to differences in pump age, are corrected for and will result in more standardized measurements. The recommended weekly control routine does not need

to be changed as long as the hand-held rotameter is calibrated against a resistance-free measurement and flow values corrected accordingly when calculating

Fig. 5 Airflow measured by 5 different flowmeters. The tests were carried out on 14 July 2021. Burkard Scientific Ltd, Burkard Manufacturing Co Ltd, and Lanzoni Srl are all rotameters with internal resistance. RFF (resistance free flow measurement) and easyFlux® are heat anemometers. The anemometers have considerably less internal flow resistance than the rotameters. Results for a post-hoc analysis (Dunn test with Bonferroni correction for the p -values) are shown after a Kruskal Wallis test ($p < 0.0001$) was performed. ns, *, **, *** and **** represent p -values of > 0.05 , ≤ 0.05 , ≤ 0.01 , ≤ 0.001 and ≤ 0.0001 , respectively



airborne pollen concentrations. This calibration should be repeated at regular intervals.

Adapting the “old” flow, i.e. reducing the flow to a real 10 L/min, is likely to change the impacting efficacy (lower flow results in less impaction), thus affecting the time series. In the case of existing stations, we recommend correcting for the flow only afterwards, when the pollen concentrations are calculated. However, if a new station is established the correct flow should be used from the start. As almost all Hirst-type traps have been running with a “wrong” flow, we suggest setting a new trap at 13 L/min using resistance free flow measurements to maintain similar impacting characteristics as the other traps, using this flow to calculate the pollen/m³.

5 Conclusions

The EUMETNET AutoPollen – COST ADOPT international intercomparison campaign took place in Munich, Germany, from 3 March to 14 July 2021. As part of this campaign, four Hirst-type pollen traps were run in parallel according to the European

standard (CEN16868:2020), close enough to assume homogenous measurement conditions. The variability between Hirst-type traps is a critical issue that needs to be considered in aerobiological studies and here we assess the reduction in this variability resulting from correcting air flow differences. Even after correction, we found that 47.9% of the possible combinations between four different pollen traps and eight pollen types were significantly different when hourly averages were considered. For daily values, the variability was lower, with 10.4% of cases being significantly different from one another. However, according to the Bland Altman analysis, systematic bias between the devices were not detected. We showed that a proportion of these differences is related to erroneous flow measurements, which can easily be corrected for. By doing so, the variability (based on the difference between medians standard deviations) was reduced on average by 28.2% for the eight pollen types for hourly values (ranging from 23.5% for *Urticaceae* to 33.2% for *Quercus*) and 21.6% for daily values (reduction of 5.5% for *Carpinus* to 34.6% for *Pinus*). As a result, the average coefficient of variation between the four traps was lower than in previous studies (averages of

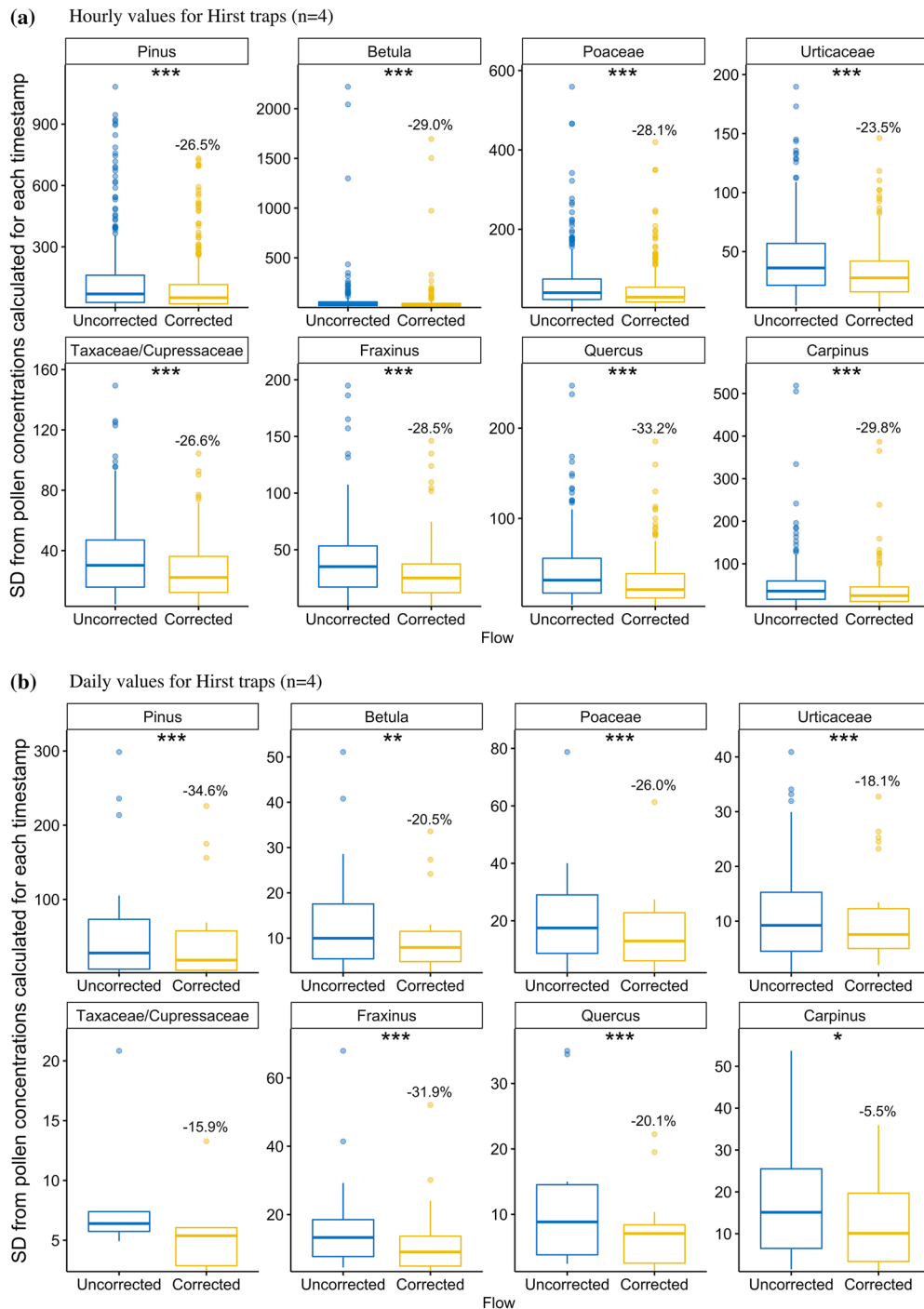


Fig. 6 Effect of resistance-free flowmeter correction. Standard deviation between the measurements of the 4 different traps, with and without additional easyFlux® flow correction. The variability for hourly **A** and daily data **B** before correction are shown for the eight most abundant pollen types (Taxaceae/

Cupressaceae having $n=5$). Only periods when all four Hirst traps had values > 10 pollen/m³ were considered. *, ** and *** represent p -values of ≤ 0.05 , ≤ 0.01 and ≤ 0.001 , respectively. P -values > 0.05 remain without asterisk

$40.5 \pm 2.5\%$ for hourly observations and $19.0 \pm 5.1\%$ for daily values) but depended on the pollen taxa (daily coefficient of variation of 16.5% for *Betula* and 16.8% for Poaceae). In order to provide more precise airborne pollen concentrations, we recommend that flow is corrected using more accurate values obtained from resistance-free flowmeters. This will also make the results between stations more comparable, especially important in pollen monitoring networks.

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Author's Contribution MMT (lead), JMM (equal), JB (equal) and FT (equal) wrote the main manuscript. JMM (lead), MMT (lead), JB (equal), FT (equal), LG (equal) and BC (equal) prepared the figures and tables. JMM (lead), MMT (equal) and JB (equal) performed the data curation and formal analysis, including visualization and software. FT and BC (lead) and JB (supporting) obtained the funding acquisition and project administration. FT (equal), BC (equal), JB (lead) and LG (supporting) supervised the work. All authors reviewed and edited the manuscript.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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