Biodiversity, abundance, seasonal and diurnal airborne pollen distribution patterns at two different heights in Augsburg, Germany

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ABSTRACT

Airborne pollen are the most important aeroallergens worldwide. Because of climate change, pollen seasonality and abundance have been altering significantly, raising the fundamental question: when and how much is the pollen exposure increasing? To answer this, we applied a multi-resolution study design, from bi-hourly to yearly scale, investigating the diversity, abundance and temporal occurrence of airborne pollen.

The whole spectrum of airborne pollen concentrations was registered during 2015–2017, using a 7-day recording Hirst-type volumetric trap. Monitoring took place at ground-level, where we mostly commute and reside, and at the 'gold-standard' rooftop-level (12 m above ground level), at resolutions: A) bi-hourly, B) daily. The biodiversity and the relative abundance of all taxa were assessed, and the first pollen season calendars, along with circadian calendars, for Augsburg, Germany, were developed.

More than 40 pollen types were identified, of which 13 were the most abundant (>0.5% relative abundance each, accounting for a total of 91.8%). Biodiversity did not present any striking differences between heights, with pollen from Urticaceae, *Betula* and Poaceae representing consistently more than half of the regional atmospheric biodiversity. At rooftop-level, pollen abundances often appeared to be higher, particularly for *Betula*, *Picea* and *Quercus*. The main pollen season extended from March to October, with the highest peak occurring April–May. At rooftop-level, the pollen seasons of most taxa were observed earlier and the overall seasons were longer. Within the day, higher pollen concentrations were observed either at midday to early afternoon (Urticaceae, Poaceae, *Plantago* and mostly taxa at ground-level) or night to early morning, frequently with multi-modal diurnal patterns (*Betula*, *Fraxinus* and mostly taxa at rooftop-level).

Our findings reveal that generalisation of abundance and temporal distribution patterns between ground-level and 'gold-standard' rooftop-level pollen measurements should be intensively reconsidered. While the pollen

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diversity and abundance may be well represented within this height range, the temporal occurrence is not, with pollen vertical variability being more important than originally anticipated. Hence, we need to reassess when and how much the relevant pollen exposure is increasing.

1. Introduction

Airborne pollen grains are released by anemophilous plants into the atmosphere as part of their reproductive process, but, at the same time, they also comprise a major source of airborne allergens that can cause immunological responses and symptoms manifested as allergic conjunctivitis, allergic rhinitis and allergic bronchial asthma, as well as organic dust toxic syndrome (Bauchau and Durham, 2004; Pointner et al., 2020; Traidl-Hoffmann et al., 2009). Pollen-induced respiratory allergic reactions represent a growing global health problem with differences in the clinical manifestation amongst patients from different countries and with an important socioeconomic cost (Bhattacharyya, 2012; Bousquet et al., 2013).

The intensity of the symptoms in allergic individuals depends on the aeroallergens patients are exposed to, in terms of airborne abundance and duration of exposure (Cecchi et al., 2010; Raulf et al., 2014). The clinical relevance of pollen exposure is not standardised and concluded upon, because of the personalised character of the exposure and the associated health impacts, as highlighted by (de Weger et al., 2013). Nonetheless, many aerobiological researches have been performed worldwide to determine pollen distribution and concentrations, especially in urban areas (e.g. (Galán et al., 2016; Gioulekas et al., 2004; Rojo et al., 2016), where more people reside and where they tend to be more affected by pollen-induced respiratory allergy compared to rural areas (D'Amato, 2000). Apart from urbanity, expectedly many other co-factors, like chemical air pollutants, interact with pollen grains and are documented to be associated with a higher incidence of allergic respiratory diseases (D'Amato et al., 2007). Actually, under ongoing climate change scenarios, continuous increases in incidence and severity of allergic diseases have been reported, with frequencies even 50% in children (Annesi-Maesano et al., 2012; To et al., 2012), and such alterations are mainly attributed to the effects of climate change (D'Amato et al., 2010; Fairweather et al., 2020; Heuson and Traidl-Hoffmann, 2018).

Climate change has been responsible for changes in biodiversity and species richness and influences allergic sensitization (Beggs, 2004). Recent aerobiological studies (Beggs, 2010; Damialis et al., 2019; Ziello et al., 2012; Ziska et al., 2019) have shown that climate change has been influencing aeroallergens by increasing airborne pollen quantities and shifting earlier the pollen season worldwide, as well as changing the geographical prevalence of plants. Experiments mimicking future climate conditions predict an even worse scenario for the inflammatory potential of pollen grains (Rauer et al., 2020). The above makes the need for assessment of pollen exposure even greater, as existing information may be already obsolete.

Given this inter-relationship between allergic diseases and airborne pollen abundance and occurrence, it is crucial to determine the factors driving the environmental exposure risk to the sensitised human population. These can be, but not limited to, different prevalent aeroallergen species because of variable local vegetation, differing micro-climatic conditions across biogeographical regions, urbanity and heat island effects, and meteorological parameters like precipitation and wind vectors (Damialis et al., 2019). The whole process of release, transport and pollen deposition are determined by multiple co-factors, which can explain the variance detected in the same species, not only between countries, but also even within the same country and among different years (García-Mozo, 2017; Plaza et al., 2016).

To provide an estimate of the variability of airborne pollen abundance and seasonality, aeroallergen monitoring stations are usually established locally. These operate continuously and can acquire aerobiological data on a bi-hourly scale and, thus, determine the prevalence of different pollen types seasonally and intra-daily. Such data may be invaluable for the avoidance of exposure and for the most efficient management of allergic symptoms. In combination with the abovementioned environmental conditions, predictive models may be developed (e.g. (Helbig et al., 2004; Oteros et al., 2015a,b; Sofiev and Bergmann, 2012). On an hourly scale, definitely such predictions are still a challenge, but there are promising results already towards this direction (Muzalyova et al., 2021).

On the other hand, pollen concentration varies during a day and the methods for predicting maximum peaks when pollen exposure is higher and allergy risk increased have still not been established (Grewling et al., 2016). The variation in diurnal pollen patterns is particularly clear in multi-species pollen types such as Poaceae or in cases with multiple vegetation sources. It is generally accepted that high pollen concentration is during daylight when it is sunny and the average temperature is the highest (Alcázar et al., 1999; Dahl et al., 2013). However, some studies have shown that airborne pollen concentrations may just as well be increased during cooler night-time intervals (Grewling et al., 2016).

To evaluate seasonality, pollen calendars have been constructed around the world to visualise the biodiversity and the temporal abundance of airborne pollen. Most frequently, a wide variety of pollen types have been presented usually on a daily or 7-10-day timescale. Nonetheless, based on data availability and human resources there are still many locations that do not yet possess such information. In Germany, a pollen calendar for 16 types of pollen has been constructed for North and central Germany (Werchan et al., 2018). However, for the city of Augsburg, one of the most populated in the State of Bavaria in south Germany, a pollen diversity calendar has never been established so far.

Particularly in an era of fast and dramatic environmental alterations, mostly because of the ongoing climate change, prevailing biodiversity as well temporal changes in sensitive biological and environmental parameters, like flowering and biomass abundance, may comprise bioindicators of this climatic change. In terms of changing biodiversity, to keep track of newly introduced or expanded, potentially invasive, plant species, it is necessary to thoroughly investigate spatially a region for the most abundant and representative taxa over time, and specifically compare against either neighbouring areas or with similar bioclimatic regions to detect possible differences in a timely manner. Finally, frequent updates of existing pollen diversity will keep relevant information up to date, as shifts and changes in pollen abundances are well documented to be fast and large (Ziello et al., 2012). This would be particularly important for the study area, Augsburg, in south Bavaria, and in vicinity to the German Alps, since average air temperature has been historically increasing significantly during 1961–2017 (Der Deutsche Wetterdienst. Climate Data Center, 2020); in contrast, the total sum of precipitation per year registered in the city in the same period shows a decreasing trend.

The aim but also novelty of the study was to assess biodiversity and abundance, as well as temporal occurrence, with a multi-resolution approach, referring to the whole airborne biodiversity of the region and from the short-term (circadian) to the mid-term (yearly) pollen periodicity and abundance in the atmosphere of Augsburg, southern Germany. This will be the first time that such information on the diversity, abundance and seasonality of airborne pollen is disseminated for the city of Augsburg, Bavaria, Germany. To achieve the maximum possible reliability but also neutrality, another objective was to re-visit general assumptions and hypotheses in airborne pollen circulation when developing pollen diversity chronology. Namely, especially in everyday practice for allergy management, pollen exposure is considered more relevant clinically at ground-level, where also allergic individuals commute, live and work (Šikoparija et al., 2018), whereas, temporally speaking, pollen peaks are mostly anticipated at midday (Alcázar et al., 1999; Ščevková et al., 2015; Tosunoglu and Bicakci, 2015). So, here we re-consider such assumptions aiming at the 'multi-resolution pollen calendar toolkit', moreover, keeping in mind that ongoing climate change may modify patterns in a quick, unpredictable way (Ziello et al., 2012; Ziska et al., 2019). This could also reflect in the allergic symptoms of the sensitised population of the study area, citizens or visitors, as is the case of extreme weather events like thunderstorm asthma (Damialis et al., 2020; Straub et al., 2021). The presented results will hopefully improve prevention and treatment of allergy problems and comprise the basis for enhancing everyday clinical practice.

2. Methods

2.1. Sampling site

Augsburg lies at the merging of two Alpine rivers, Lech and Wertach, on the northern foothills of a high terrace. It is the third-largest city in Bavaria with a population of 300,000 inhabitants, with 885,000 in the metropolitan region.

Augsburg owes the characteristic juxtaposition of plants belonging to different European areas in terms of flora in particular to the Lech river. Non-native plant species settled along it, as the result of the earlier changing climate. The potential natural vegetation of the lower terrace is the cinquefoil-pine-oak forest (Potentillo-Quercetum), which represents the typical final forest community for the gravel areas of the foothills of the Alps that are remote from the groundwater. Depending on the local soil conditions, the transition to the typical oak-hornbeam forests (Galio-Carpinetum) is fluid, and existing pine-oak forests may have emerged as a result of grazing and thus already represent a cultivated forest form through browsing and nutrient deprivation (Hiemeyer, 1978). On the shallow, stony soils of the sparse pine-oak forests and extensive heaths, species-rich limestone lawns developed, whose species composition reflects the special natural location of the Lechfeld.

Augsburg's climate is oceanic (Köppen climate classification: Cfb) or, following the 0 °C isotherm, a humid continental climate (Dfb) The mean annual temperature is 13.2 °C, and the mean annual rainfall is 766.9 mm (30-year averages, 1981–2010, Der Deutsche Wetterdienst. Climate Data Center, 2020). The wettest month (with the highest rainfall) is July (99.7 mm). The driest month (with the lowest rainfall) is February (36.6 mm).

2.2. Pollen monitoring and data analysis

Airborne pollen has been monitored using Hirst-type volumetric traps (Hirst, 1952) in Augsburg from March 2015 to October 2017. The first device was located in the southeast of Augsburg City (Landesamt für Umwelt, LfU – 48°19′33.6N, 10°54′10.8"E) at 1.5 m above ground level (agl). To assess potential differences from ground-level height, where we mostly commute and reside, and the commonly employed monitoring at rooftop-level (approximately 12 m agl), we processed measurements from a second site situated in the same location with a horizontal distance of less than 50 m.

In brief (as in more detail in (Damialis et al., 2007) the trap is equipped with a vacuum pump drawing 10 L of air per minute through a thin orifice. Air particles are trapped on an adhesive-coated transparent plastic tape, supported on a clockwork-driven drum, which moves at a speed of 2 mm per hour making a complete revolution in a week. The tape is then removed, cut in seven equal parts each representing a 24-h period, and mounted onto microscope slides. All pollen grains were identified by expert aerobiologists under a light microscope at a magnification of x400, transversely in 12 traverses. Counts (2-hourly or daily) are expressed as mean pollen concentrations per m^3 of air. The general procedure followed is established across the world and conforms with the minimum requirements as suggested by the European Aerobiology Society (Galán et al., 2014).

To develop the pollen calendars for Augsburg, we used Rstudio (Core Team, 2018), R version 3.6.1. and the specific AeRobiology R package and its visualisation (Rojo et al., 2019b). The latter elaborates on the standard pollen calendar development technique as published by D'Amato and Spieksma (1991). Daily mean concentrations (pollen grains/m^3) were used to calculate 10-day (Julian calendar) arithmetical means corresponding to one third of the month. Data were then grouped by dates to compute the 10-day arithmetical mean for the study period as a whole (2015–2017), for 31-day months. The last group contained 11 days, as recommended by (Gutiérrez et al., 2006), while February was taken as a 29-day month, the last group comprising 9 days.

Results were expressed in a graph in which each 10-day mean corresponded to an exponential frequency class, following (Stix and Ferretti, 1974). In the pollen chronology, taxa were ordered chronologically, by the timing of maximum peaks. Only those taxa displaying a minimum 10-day mean equal to or greater than 1 pollen grain/m^3 were included, but limited to 15 in the graph.

To assess the diurnal pollen distribution pattern, apart from calculating the summed concentration for each two-hourly interval in total pollen concentration per year of the study (as well as for all years of the study), we also calculated the frequency of cases during which there were any counts of pollen grains. The combination of both pointed out more clearly whether the prevailing diurnal patterns were due to a systematic pattern repeated frequently over the course of each year, or because of isolated incidents of abrupt and high concentrations as the potential result of unique weather events.

Regarding the seasonality of the pollen seasons and the determination of the onset, peak, end and duration of the seasons, we defined main pollen season (MPS) following Andersen (1991) method. This method includes 95% of seasonal total pollen concentration, starting on the day on which 2.5% of the annual pollen integral was recorded and ending on the day on which 97.5% of the annual pollen integral was registered.

To conclude on significant differences between sampling heights, but also regarding universal patterns among all taxa, General Linear Models (GLMs) were adopted, and full factorial ANOVAs and ANCOVAs were run, followed by Bonferroni posthoc tests. In particular, the pollen concentrations (dependent variable) were checked against categorical independent factors, like the taxon and the site (here differing height), and covariates like years and bi-hourly intervals. The normality of data was checked beforehand with Kolmogorov-Smirnov test, which confirmed the suitability of GLMs.

3. Results

More than 40 pollen types were detected in the local biodiversity of the atmosphere of Augsburg (Fig. S1 (rooftop-level) and Fig. S2 (ground-level)). Of them, 13 were the most abundant (>0.5% relative abundance) and accounted for 91.8% of the total. Pollen from Urticaceae, *Betula* and Poaceae alone accounted for more than half of the atmospheric biodiversity, from both rooftop measurements (57.2% of the total pollen; Fig. S1) and ground measurements (55.2% of the total pollen; Fig. S2). The rest of the monitored pollen types did not differ significantly in terms of diversity, but they did in terms of ranking compared to their relative abundance (Figs. S1, S2). Since we started monitoring in March every year, some pollen types' seasons were not fully included in the recorded biodiversity and seasonality, namely *Alnus* and *Corylus*.

The sampling height seemed to play a significant role for specific taxa, when checked in a factorial design (factorial ANOVA, $p < 0.05$). In terms of abundance, at rooftop-level, eight out of the 13 taxa examined exhibited higher pollen concentrations, mainly *Betula, Picea*, *Pinus* and *Quercus*. The rest 5 of 13 taxa were higher at ground-level monitoring station, mainly for the taxa of *Fraxinus*, *Carpinus* and *Plantago* (Fig. 1). Overall, herbaceous plants accounted for less than 40% on average of

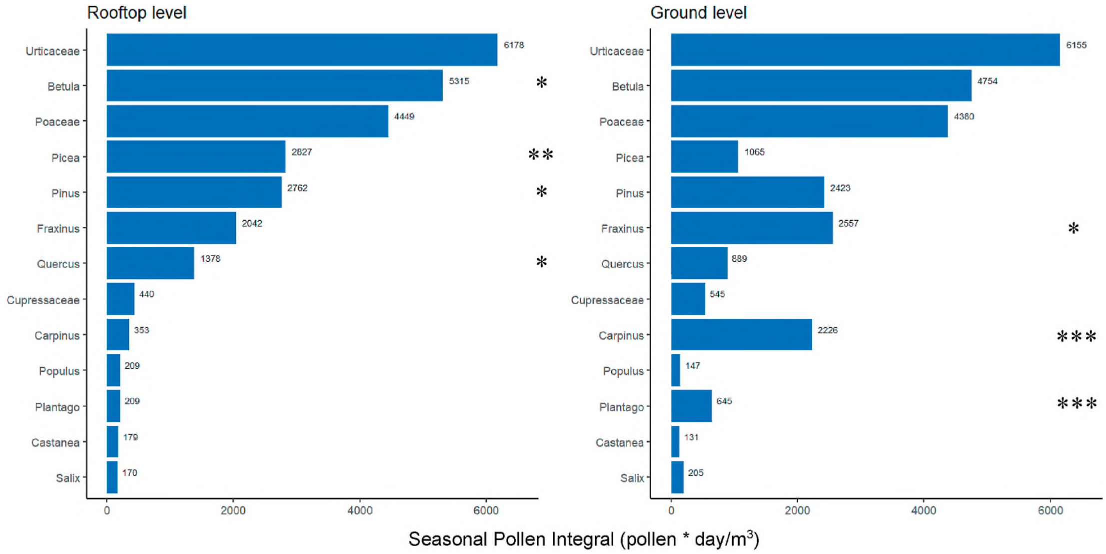

Fig. 1. Seasonal Pollen Integral of the 13 most abundant airborne pollen types in Augsburg at rooftop-level (left) and ground-level (right) during 2015–2017 (March–October).
Asterisks denote significant differences (factorial ANOVA, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$), with the location of the asterisk indicating the height with the highest abundance.

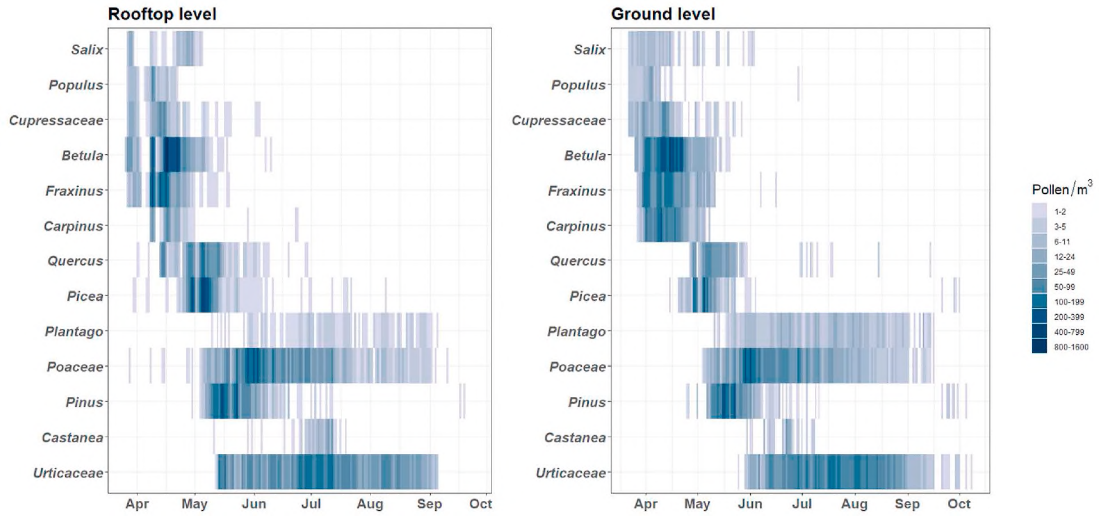

Fig. 2. Pollen calendars in Augsburg, Germany. The average seasonal distribution is given for the 13 most abundant pollen types during 2015–2017 (March–October).

the total annual pollen integral, with pollen from Urticaceae, Poaceae and *Plantago* being the most dominant, in descending order (Figs. S1, S2, 1).

Considering the seasonality of the pollen period (Fig. 2), the main pollen season extended from March to October, but with the highest concentrations observed in April and May, accounting for more than 30% of the total average every year. These peaks refer to some of the most abundant pollen types (Fig. 1), like *Betula*, *Fraxinus*, *Picea* and *Pinus*. Urticaceae and Poaceae, being also with the highest seasonal integral, exhibited the longest pollen seasons, lasting on average four months (Fig. 2). During late autumn and winter months, we observed the lowest pollen concentrations, frequently with days with a total absence of airborne pollen.

When comparing the pollen seasons between the two monitoring sites, at rooftop-level and ground-level (Fig. 2), we can see that those taxa flowering early in spring seem to have low variation in the onset date between the two sampling heights. But as long as we get deeper in spring (after mid-April), pollen from rooftop-level are observed earlier in the season, like for *Quercus*, *Picea*, Poaceae, *Castanea* and Urticaceae, with an average earlier season start of three weeks (Fig. 2). The only exception is *Carpinus* pollen, which seems to begin its season about half a month later at rooftop-level. Hence, *Carpinus* pollen season, together with that of *Fraxinus*, are the only pollen seasons that display a shorter duration at higher sampling height (Fig. 2); at the same time, these two pollen types are the ones with the biggest abundance differences too (Fig. 1). Moreover, the duration of the season is longer at rooftop-level for most of the woody taxa, namely *Betula*, *Quercus*, *Picea*, *Castanea* (about 3–5 weeks), while it gets shifted (about 3–4 weeks) but not longer for the herbaceous Poaceae and Urticaceae (Fig. 2).

Regarding the diurnal variability of airborne pollen concentrations (Fig. 3), the overall circadian peak was observed approximately at 2pm (median and average) and a significant difference was found in pollen concentrations between the interval 10:00–22:00 (higher) compared to the night-morning interval 22:00–10:00 (lower) (full factorial ANOVA,

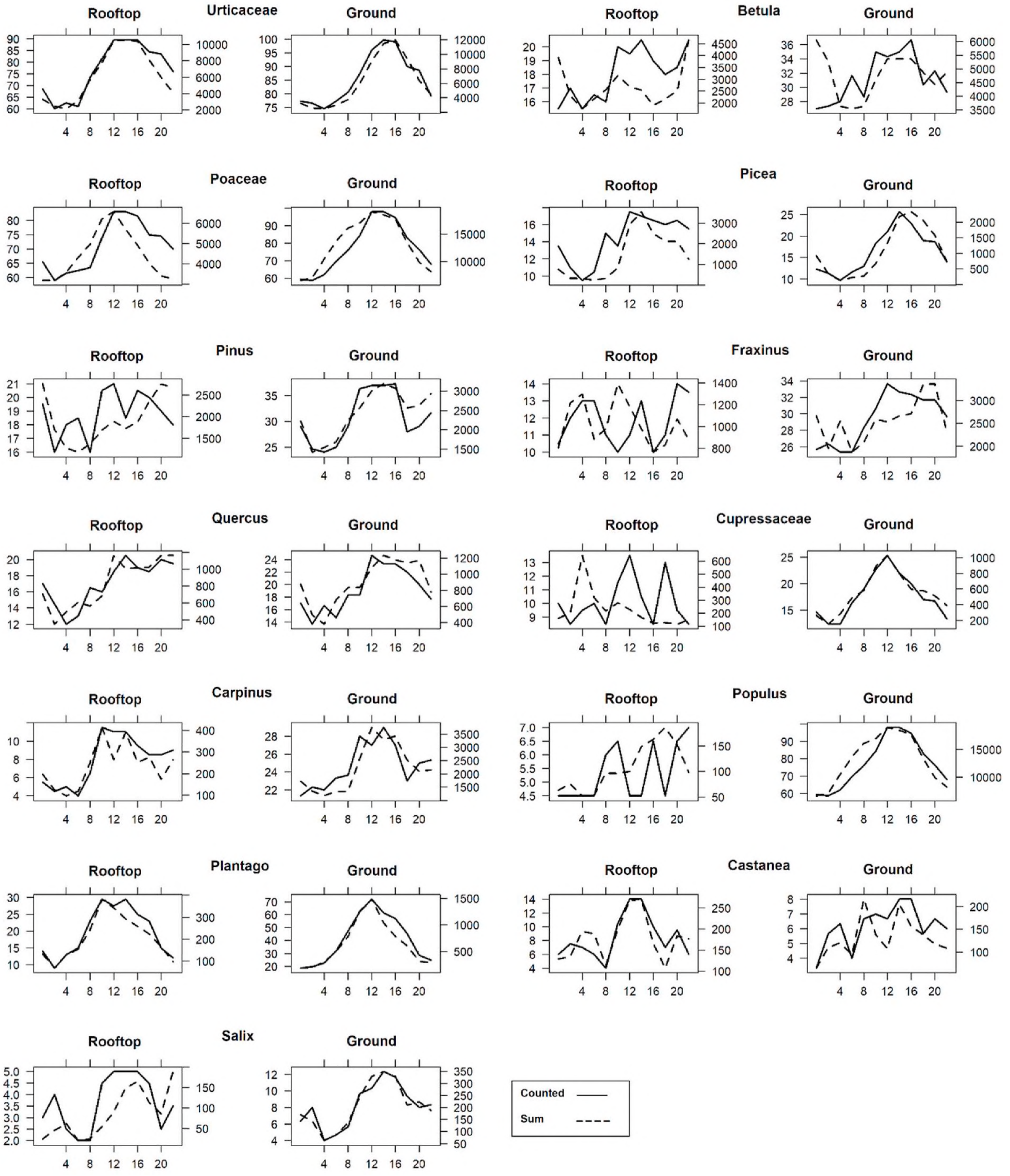

Fig. 3. Pollen atmospheric circulation for the 13 most abundant pollen types in Augsburg during 2015–2017 (March–October). *X*-axis shows the sampling bi-hourly interval displayed on a 24-h scale. The primary *Y*-axis (left) shows the frequency of pollen occurrence for each bi-hourly interval (solid line). The secondary *Y*′-axis (right) displays the Seasonal Pollen Integral for each bi-hourly sampling interval (dashed line). The different taxa are presented in descending order depending on their maximum bihourly atmospheric abundance. Hence, different scales are used in *Y*-axes.

$p < 0.001$, after Bonferroni correction), even though this was significantly also altering among different pollen types. As seen in Fig. 3, the first pollen distribution diurnal pattern exhibited the pollen peak during midday-afternoon and this was the case for all the herbaceous taxa (Urticaceae, Poaceae and *Plantago*), for several woody taxa only at ground-level (*Picea*, *Pinus*, Cupressaceae, *Populus*, *Salix*), and for *Carpinus*, the pollen taxon with the highest abundance difference between ground-vs. rooftop-measurements (Fig. 1) and the only taxon with a later onset at rooftop-level (Fig. 2). The second pollen diurnal variability pattern consisted of pollen types that consistently showed a late evening to early morning peak in pollen abundances, as was the striking case of *Betula*. The rest of the cases comprised more complex, multi-modal circadian pollen distribution patterns, which was mostly the case for woody taxa particularly at rooftop-level, like *Pinus*, Cupressaceae, *Populus*, *Picea* and *Salix*. So as to separate repeated incidents of higher pollen abundance at specific time intervals per day vs. isolated high peaks because of extreme weather events, we plotted (Fig. 3) the frequency of pollen occurrence (solid line) vs. the pollen sum per 2-hourly interval per day across an average year (dashed line). The only taxa that were consistently alike between pollen occurrence and pollen peaks and also between rooftop- and ground-level abundances were the three herbaceous ones, viz. Urticaceae, Poaceae and *Plantago*.

When trying to quantify potential differences between rooftop-vs. ground-level abundances at the diurnal scale, we found that pollen measurements were consistently earlier in nine out of the total 13 taxa examined (median, average and mode equal to −2 h), compared to the ground-level measurements (Fig. 4). The taxa with the highest difference in the diurnal pollen peak occurrence between the two sampling stations at different heights were those of *Fraxinus*, *Populus* and Cupressaceae (Fig. 4), which were also among the ones with the most distinct multi-modal circadian pollen distribution patterns especially at rooftop-level (Fig. 3).

4. Discussion

Regular dissemination of pollen distribution patterns comprises a necessary toolkit of preventive allergy management in allergic individuals, whether it refers to short-term (hourly) measurements or seasonal ones (daily). Keeping updated the daily and seasonal biodiversity and distribution patterns is literally the cornerstone of prophylaxis and management of allergic diseases until today. Especially during the ongoing climate change, data are continuously altering, seasons are shifted, prolonged or both, pollen abundances have been increasing, biodiversity is changing (Ziello et al., 2012; Ziska et al., 2019). For this reason, it is today timelier and more important than ever to provide the public with high-quality, updated, complete data, with a multi-resolution analysis, from diurnal to long-term atmospheric circulation patterns of pollen abundances.

Airborne pollen diversity has been reported to reflect approximately more than 40 different taxa, depending also on the regional vegetation, pollen production levels and meteorological factors per study area (García-Mozo, 2017). To signify temporal but also abundance patterns, a simple but also accurate visualisation has to be established, common between monitoring stations across the globe, so as to be easily comprehensive to the wider public. Towards this approach, Galán et al. (2014) have provided a list of minimum requirements while monitoring airborne pollen in aerobiological stations. Our own monitoring stations have also followed these ‘gold-standard’ instructions. Therefore, here we thoroughly examined the 13 most abundant pollen types (>0.5% relative abundance), which overall accounted for 91.8% of the total. As in most temperate environments, pollen from *Betula* and Poaceae were among the most representative of the regional airborne flora, but in Augsburg Urticaceae was the leading taxon consistently in all years.

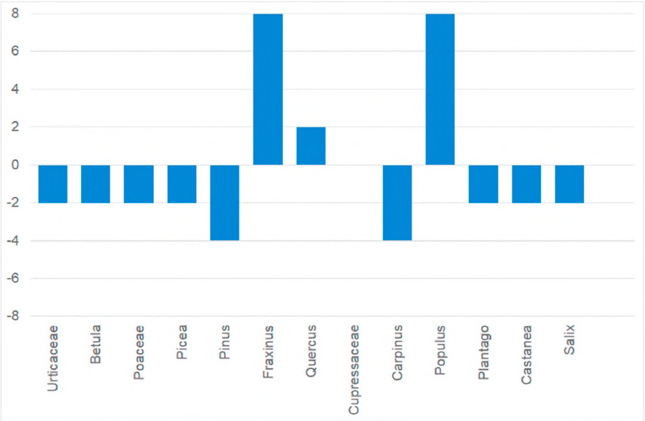

Fig. 4. Temporal differences of airborne pollen occurrence of the diurnal pollen peaks between the rooftop-vs. the ground-level station. The *X*-axis shows the pollen type of the 13 most abundant taxa in Augsburg during 2015–2017 (March–October). The *Y*-axis indicates the number of hours lagging between the diurnal pollen peaks. Minus signs refer to earlier pollen peak occurrences at the rooftop-level station.

As far as airborne pollen concentrations are concerned, we noticed that pollen was not more abundant at ground-level, as it is often assumed. In fact, the Seasonal Pollen Integrals seem to be of similar magnitude between the gold-standard rooftop-level measurements and the ground-level ones. However, when we zoom in to the per-taxon pollen concentrations, it is evident that certainly differences exist, but actually towards the opposite direction. At rooftop-level, concentrations tended to be higher, especially for woody taxa, either with smaller-sized grains as in *Betula*, or aerodynamic grains as in *Picea*; the cases which clearly show a higher representation at ground-level are mostly herbaceous or entomophilous taxa or with larger grains, as in *Plantago*, *Salix* and *Carpinus*.

Regarding pollen seasonality, as has been documented before in temperate climates, also in Germany (for the period 2011–2016; Werchan et al., 2019), most airborne pollen circulate from early spring to early autumn. Our findings, likewise, showed that the main pollen season extended mainly from March to October, with the highest peak occurring between April and May. What was additionally revealed, though, was that at the gold-standard rooftop-level station, the pollen seasons of most taxa were observed earlier and the overall seasons were longer. Also, it was unveiled that every year there have been repeated incidents of late-occurring pollen amounts in autumn. Consequently, pollen seasons based at rooftop-level measurements are longer and starting earlier, with main exceptions herbaceous taxa, like Poaceae and Urticaceae, whose pollen seasons are shifted earlier but not elongated, compared to the ground-level measurements.

When we examine occurrence and abundances at the finest level, every 2 h, it was found that pollen concentrations were observed either at midday to early afternoon (Urticaceae, Poaceae, *Plantago*, and mostly taxa at ground-level), or night to early morning, frequently with multi-modal diurnal patterns (*Betula*, *Fraxinus*, and mostly taxa at rooftop-level). Airborne pollen occurrence was earlier at rooftop height approximately for 2 h (median and average).

The salient finding of our study actually was by the integration of the seasonal and diurnal pollen distribution information, which made evident that the spatiotemporal pollen distribution patterns are more complex than previously regarded and in a 3-dimensional perspective. Also, long-range pollen transport incidents are not only underestimated but rather consistent, at least as proven for some taxa in the area of Augsburg (Ghasemifard et al., 2020). Our findings here might seem as if opposing to previously published results, i.e. that ground-level pollen abundances are higher compared to those at higher heights, for instance, reported by Charalampopoulos et al. (2018) and Rojo et al. (2019a). In the case of Charalampopoulos et al. (2018), as this study refers to a totally different ecosystem of Mediterranean type, with not the same biodiversity and abundance, any differences might be attributed to the different vegetation, as well as different micro- and macro-meteorology because of variable topography and lower altitude. In another Mediterranean-type ecosystem, Badajoz, in southwest Spain, findings from three years during spring-summer revealed results similar to ours by showing slightly elevated pollen concentrations at the higher sampling site (Fernández-Rodríguez et al., 2014). In this same study, though, it is interesting that the differences found in pollen abudance were also detected for Pinacaeae, like in ours here, but not in *Plantago* and *Quercus*. Likewise, Fernández-Rodríguez et al. (2014) did not identify any temporal variations in the pollen season occurrence between the two examined sampling heights. This is partly attributed to the microclimatic and regional vegetation differences, as also stated by Charalampopoulos et al. (2018). Finally, other studies, e.g. Damialis et al. (2017), have shown that at higher elevations, even near the atmospheric boundary layer, airborne pollen may be significantly more abundant compared to the ground-level ones. The conclusion is that all studies point towards the same truth: scientists are missing some factors or their interaction, or both.

Interestingly, our results are different to an extent from those by Rojo et al. (2019a, 2020). The study of Rojo et al. (2019a) aimed mainly to identify differences in pollen abundances comparing two different heights in a wide variety of monitoring stations. Their salient finding is that no major differences were observed between ground- and rooftop-level measurements because of high statistical background noise. However, the approach used there was very different, as they did not conduct a hypothesis-based experiment but collected data that were available from around the world, pairing low and high stations, but with pairs differing in the exact height of the low and the high station and being up to 10 km apart. Given the nature of data in their dataset, a lot of uncertainty is naturally expected that could have even masked any height effect, a fact that has been actually highlighted by the authors themselves in their study. In our study, we chose a ground-level station against a rooftop-level with distance between them less than 50 m. In this way, we kept as low as possible the variability due to the position of stations and examined the genuine vertical variability, eliminating differences because of micrometeorology and local vegetation. The more recent work of Rojo et al. (2020) refers to an experiment similar to our own in the present work, noticeably in the neighbouring city of Munich, Germany. Rojo et al. (2020) obtained robust results, but, interestingly, sometimes to the opposite direction than those from our study: they found the most intense differences in the abundances of pollen, rather than in the temporal occurrence. There are two major differences in that study and our own: first, the rooftop level lies at 35 m above ground level in the study of Rojo et al. (2020), in contrast to 12m in ours. Second, the study by Rojo et al. (2020) refers to two monitoring sites with a distance between them a bit more than 4 km and while data processing was conclusive analytically, questions are raised regarding the effects of microclimate, urbanity and local vegetation.

A major factor that could determine such differences is regional vegetation. As Augsburg is located in southeast Germany, and relatively close to the alpine region, and belonging to the continental climatic zone (Trnka et al., 2011), cold winters limit the seasonal distribution of the different pollen types to spring and summer, because of the increase in temperatures during this period (Grinn-Gofroń and Mika, 2008). Moreover, Augsburg is surrounded by a rich and diverse nature. The rivers Lech and Wertach, as well as a canal system, together with alluvial forests and a large urban nature reserve, the Siebentischwald, provide valuable and species-rich habitats, a meeting place for elements of continental, subalpine and sub-Mediterranean flora. Continental plant species (dealpine) carried along the course of the river from the Alps grow together with sub-Mediterranean species. Typical tree species are pine (*Pinus sylvestris*), oak (*Quercus robur*), linden (*Tilia cordata*), hornbeam (*Carpinus betulus*), common ash (*Fraxinus excelsior*) and mountain ash (*Sorbus aucuparia*) (Bayerisches Landesamt für Umwelt, 2020), although beech (*Fagus sylvatica*) would be the leading tree species of the potentially natural forest communities of the region (Waldfunktionsplan für die Region Augsburg, 2013). Due to these characteristics, the Augsburg region is special compared to other temperate forests that do not have this series of species from different climatic regions.

Second major factor is the integration of the double periodicity pollen release and transport by rule display: seasonal within each year and diurnal within each day. In our study area, Augsburg, the combination of rich regional vegetation, the location near the unique alpine region and particularities in micro and macro-climatic conditions, lead to peculiarities in airborne pollen seasonality, circadian periodicity and abundances, repeatedly every year. Ščevková et al. (2015) published similar results in Bratislava, Slovakia, over a period of nine successive years (2002–2010), as well as Perez-Badia et al. (2011) in Spain, and Puc (2011) in Poland, where pollen concentrations sometimes displayed lowered values in the afternoon. Other studies, as by Grewling et al. (2016) highlighted even more the probability and intensity of the nightly pollen circulation and the potential effects of long-distance transport. In terms of actual pollen exposure but also translated into clinical practice, it is concluded that there is no definite safe time for pollen allergic patients, seasonally or diurnally. This is particularly highlighted by the prolonged pollen season as measured at higher sampling heights.

The integration of all the spatiotemporal distribution information of airborne pollen, for the majority of taxa, and at a 3-dimensional rationale, builds up the knowledge regarding the actual pollen exposure intensity and duration and the associated symptomatology risk, particularly under continuously and dramatically changing climate and the associated impacts on allergic respiratory diseases (Beggs, 2004; Bielory et al., 2012). While our relatively short time-series (three years) does not allow for conclusive evidence on pollen distribution patterns over time in Augsburg (Galan et al., 2017), our study managed to efficiently integrate pollen information at variable temporal resolutions and equally examine biodiversity, abundance and occurrence of pollen. Therefore, we showed that there could still be increased allergy risk either because of prolonged seasons or of isolated, unexpected pollen incidents. Pollen may be transported from distant sources too, as reflected in rooftop-level measurements rather than ground-level concentrations, and display higher pollen peaks at unpredictable diurnal intervals, as in midnight or early morning. In contrast to the general belief that the bulk of pollen would be detected at midday when air temperatures are higher and that we can assess our actual pollen exposure at ground-level where we live and commute, it appears that the exposure process and the associated allergy risk is completely different. Such phenomena have been documented repeatedly by other researchers too, as for example by Grewling et al. (2016) in Poland (2016) and Damialis et al. (2005) in Greece. Other reasons could be contributing to these processes as well, though, as for instance, resuspension phenomena particularly after high daily peaks in the main season (Sehmel, 1980).

As suggested by Damialis et al. (2017), it is highly probable that the formation of ‘pollen clouds’ may comprise an additional health risk and also relevant to clinical practice. Rooftop-level measurements, as anyway suggested by the European Aerobiology Society (Galan et al., 2014) is proven to be indeed the most accurate technique and elevation to monitor airborne pollen. Nevertheless, as airborne pollen can be frequently distributed far away from the main emission sources, horizontally and vertically, up to the boundary layer (Damialis et al., 2017), it is still not completely clarified what the allergy risk is, even at previously thought as harmless environments and at an even more diverse range of environments. Even though (Rojo et al., 2019a)) reported that pollen abundance variances are not expected to be significantly large at a similar sampling height range and they revealed a higher abundance at ground-level measurements, compared sites often were rather distant from each other and, moreover, they did not check for the temporal occurrence at a finer resolution. Through our present study, it is comprehended that far-transported pollen has a more pronounced and clinically relevant effect and questions are raised regarding the health risk complications.

On the other hand, microclimate, spatially and temporally, may alter pollen and spore exposure (Aguilera and Valenzuela, 2012). Even within the same city and using different monitoring areas, considerable heterogeneity in pollen levels was shown on a small scale (Charalampopoulos et al., 2018; Katz and Batterman, 2020). Our results go further and the heterogeneity that occurs in the same place but at different heights is observed, which is important since the population is not only highly exposed at ground level, but also inside buildings when the windows are open. This suggests that the use of a single monitoring site will not reflect pollen exposure in an urban region and can lead to significant measurement errors in epidemiological studies, mainly when limited to a daily only timeframe (Katz and Batterman, 2020). In future, multiple monitoring stations may be necessary, especially when the regional topography and prevailing meteorological conditions favour unusual and unpredictable or long-distance transport of pollen and fungal spores (Damialis et al., 2017; Mohanty et al., 2017).

This study evaluates pollen distribution at different scales (diurnal and seasonal) and furthermore, diversity and abundance at vertical level. Many studies evaluate the pattern on pollen season with a few taxa at a single site; analyses of multiple taxa are required at stations within the same region to elucidate microclimatic impacts on allergenic pollen and potential public health consequences. Moreover, it is necessary to regularly examine the whole pollen diversity per region, as climate change may eventually cause alterations in species' distribution or introduction of new species, thus, posing a potentially dire impact on human health (Rasmussen et al., 2017; Sikoparija et al., 2017). In addition, the diurnal pollen (and fungal spore) abundance information has to be integrated in the current allergy risk information services worldwide, as this may be the most relevant in everyday clinical practice. For that reason, as future prospect, we expect to combine pollen and fungal spore measurements with panel study symptoms for checking the clinically relevant pollen exposure and co-exposure of pollen/spores and cumulative symptoms. Alongside, integration and evaluation of modern, automatic, real-time monitoring methods should be included in future studies (Oteros et al., 2015, 2020; Crouzy et al., 2016; Schiele et al., 2019; Sauvageat et al., 2020).

5. Conclusions

Based on a multi-resolution approach at two different sampling heights and a biodiversity of more than 40 pollen taxa, we found that pollen is present from early spring until end of autumn and throughout each day. Diurnal peaks occur either at midday to early afternoon (mostly for herbaceous taxa or at ground-level measurements), or at night or early morning (mostly for woody taxa and at rooftop measurements). Pollen abundances differ between ground- and rooftop-level, however, these are taxon-specific and related to the regional vegetation and pollen transport patterns. For this, measurements at ground-level occur with a delay effect of approximately 2 h, compared to the ones at rooftop-level. Cases of multi-modal diurnal pollen distribution, or out-of-season pollen occurrence within each calendar year, signify long-distance transport of pollen, as potentially for the cases of *Betula* and *Fraxinus* pollen in Augsburg. In contrast to what is widely hypothesised, there can be a great amount of pollen even in autumn, also at around midnight, and with biodiversity and abundance highly varying at vertical scale even at such a small range of less than 15 m. The conclusion of this study is that the length and temporal occurrence of the pollen exposure is potentially the foremost and most relevant clinically, rather than the overall abundance alone.

The acquired information here strongly suggests that there is urgent need for:

- Finer-resolution pollen measurements (per 2 h).
- Determination of the vertical diversity and abundance of pollen per different taxon.
- Operation of aerobiological networks continuously throughout each calendar year.
- Personalised pollen measurements to eliminate or interpret the high spatial, 3-dimensional statistical noise.

Credit author statement

FK: pollen classification, data curation and analysis, visualization, reviewing and approving final draft; MPP: pollen classification, data curation and analysis, visualization, writing original draft, reviewing and approving final draft; AC: pollen classification, data curation, reviewing and approving final draft; CTH: provision of aerobiological data, funding acquisition, reviewing and approving final draft; AD: Conceptualization, project supervision, pollen classification, data curation and analysis, visualization, writing original draft, reviewing and approving final draft.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atmosenv.2021.118774.

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