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Drivers of the release of the allergens Bet v 1 and Phl p 5 from birch and grass pollen

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ABSTRACT

The drivers affecting the Pollen Allergen Potency (PAP, amount of allergen released per pollen) are sparsely known. *Betula* and Poaceae airborne pollen are the two main allergenic pollen in the World. Airborne pollen and their allergens Bet v 1 and Phl p 5 were simultaneously measured from 2010 to 2015 in Davos (Switzerland) and Munich (Germany) by using volumetric traps and ChemVol cascade impactors. Daily variations in PAP were analysed in $PM_{>10}$ and $PM_{2.5\text{-}10}$ air fractions and generalized additive models were created to explain which factors determine PAP, including meteorological parameters and inorganic pollutants.

87.1 ± 13.9% of Bet v 1 and 88.8 ± 15.5% of Phl p 5 was detected in the fraction $PM_{>10}$ where most pollen grains were collected. Significantly higher PAP for grasses (3.5 ± 1.9 pg Phl p 5/pollen grain) were observed in Munich than in Davos (2.4 ± 1.5 pg/pollen grain, $p < 0.001$), but not for *Betula* (2.5 ± 1.6 pg Bet v 1/pollen grain in Munich and 2.3 ± 1.7 in Davos, N.S.). PAP varied between days, years and location, and increased along the pollen season for Poaceae, but remaining constant for *Betula.* Free allergens (allergens observed in the fraction with limited pollen, $PM_{2.5\text{-}10}$) were recorded mostly at the beginning or at the end of the pollen season, being linked to higher humidity and rainy days. Also, PAP was higher when the airborne pollen concentrations increased rapidly after one day of low/moderate levels. Our findings show that pollen exposure explains allergen exposure only to a limited extend, and that day in the season, geographic location and some weather conditions need to be considered also to explain symptoms of allergic individuals.

Contributions

J.M. experimental design, data collection, data analysis and leading the redaction of manuscript; J.O. and J.R. experimental design, data collection, data analysis and redaction of manuscript; C. T-H and C S–W supervision and redaction of manuscript; J.B. experimental design, data collection, supervision, redaction of manuscript and management.

1. Introduction

The lack of agreement between pollen concentrations and allergy symptoms was already indicated by Feinberg and Steinberg (1933), and more recent studies corroborated that those symptoms in sensitized people do not occur exactly at the same pollen concentrations during the whole season (Bastl et al., 2016; Buters et al., 2010). For decades, airborne pollen monitoring was considered as the main (and often only) way of providing information to allergic patients about their potential pollen allergens exposure (Maya-Manzano et al., 2020; Scheifinger et al., 2013). Although pollen monitoring alone is valuable as a good proxy for allergen exposure, it might not be sufficient (Cecchi, 2013). Monitoring the airborne allergens themselves should be preferred but remains absent in many pollen monitoring stations, probably due to

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resources limitations (Beggs, 2010; Buters et al., 2018). Pollen grains from *Betula* and Poaceae species are the ones affecting people in Europe most (Bastl et al., 2016; Biedermann et al., 2019; D'Amato et al., 2007). Therefore, these pollen types are commonly forecasted in many studies (Maya-Manzano et al., 2020; Scheifinger et al., 2013). Bet v 1 (Ipsen and Lewenstein, 1983) and Phl p 5 (Hrabina et al., 2008) are major allergen for *Betula* and Poaceae pollen, respectively.

An important limitation for patients when pollen concentrations are reported is that there is not always an evident association between pollen grains and allergens, as highlighted by *HIALINE (Health impacts of Airborne Allergen Information Network)* working group (Buters et al., 2012, 2015) and other authors (Plaza et al., 2016). The allergen release from pollen was different for each geographical area (Buters et al., 2015; Galan et al., 2013), and varied along the pollen season (Buters et al., 2012, 2015). In addition, the same pollen type can stem from different species (especially important for Poaceae) that could release different amount of allergen (García-Mozo, 2017). All these factors together make the task of linking allergens and pollen to symptoms difficult.

The idea of pollen potency (Buters et al., 2010), afterwards renamed as pollen allergen potency, PAP (Galán et al., 2017), was in the early days linked with the amount of "toxic fraction" of the pollen, removable by certain solvents (Feinberg and Steinberg, 1933). It was only recent that we obtained a better understanding that pollen can induce different symptoms depending on the exposure conditions and its surrounding environment (De Weger et al., 2013). Already Blackley (1873) observed that the same amount of pollen could produce different symptoms depending on the weather or whether the pollen was fresh or stored. An extreme case is thunderstorm asthma, when free allergens from ruptured pollen are thought to result in dramatic symptoms (Thien et al., 2018). In addition to pollen rupture, other different factors could increase the PAP, such as the temperature and its role triggering allergen synthesis due to abiotic stress (Ahlholm et al. 1998).

Inorganic pollution and the degree of maturity for pollen grains have been reported as other factors to be considered, as pollen grains from polluted areas and from longer matured pollen grains showed a higher allergenicity (Buters et al., 2010), even changing their shape, colour and composition for tectum (Chehregani et al., 2004; Majd et al., 2004). The fragility of the exine when pollen is exposed to pollutants (Sénéchal et al., 2015) and the consequent release of intracellular content could influence pollen allergenicity. Some authors reported that high levels of ozone and PM_{10} leads to higher levels of Bet v 1 (Beck et al., 2013; Ziemianin et al., 2021) and Phl p 5 (Ščevková et al., 2020). According to Gilles et al. (2018), when plants react to biotic and abiotic stressors, some allergenic proteins and secondary metabolites such as lipid mediators can be produced on pollen. Besides, pollen grains may act as carriers for inorganic pollutants and other particulate matter adhered in the exine of pollen (Galveias et al., 2022). This might be a cause that explains why more sensitized individuals are living in cities with high environmental pollution than in less polluted areas (Cortegano et al., 2004; Morgenstern et al., 2012).

The variations in PAP could also be provoked by genetic differences between species or varieties, resulting in different quantities of the same (cross-reacting) allergen. This would be more pronounced in species such as grasses (Jung et al., 2018) and other herbs as mugwort (Grewling et al., 2020), due to the higher species richness in herbs and grasses compared to trees such as birch. Taken together, because of the variable nature of the PAP from pollen, PAP could be a piece in the puzzle of the link between pollen concentrations, allergens and allergy symptoms. The main aim of this study is to understand the role of environmental parameters on the amount of pollen/allergens (PAP) in the air, focusing on *Betula* and Poaceae. A second objective of this study is to determine the relevance of free airborne allergens by quantifying their proportion and the factors behind their release. A third objective is to characterize the aerobiological spectrum in two different environments: at high altitudes in Davos (Switzerland) and in lowlands at Munich (Germany). Understanding the different parameters that influence PAP could help to avoid episodes of exposure to high-allergenic pollen for allergy patients.

2. Material and methods

2.1. Areas of study: climatic, geographical and biotic characteristics

The study was carried out in the cities of Davos, located in the Northern and central Grisons region (southern Switzerland) at 46° 47′ 45″ N, 9° 49′ 26″ E, and Munich, located in Bavaria region (southern Germany) at 48° 08′ 13″ N, 11° 34′ 32″ E. Their altitudes are 1560 m.a.s.l (meters above sea level, the highest city in Europe) for Davos and 519 m.a.s.l for Munich (Fig. S1). The Köppen Climate Classification for Davos can be defined as Dfc (cool continental climate/subartic with cold summer), whilst for Munich is determined as Cfb (continental with warm summer) (Kottek et al., 2006). Because Davos is placed at the bottom of a valley, the predominant wind direction for this city is N-NE (0–45°) and S-SW (180–225°), reaching an annual precipitation of 1022 mm and having an average mean temperature of 3.5 °C (MeteoSwiss, 2021a, 2021b). In Munich, the annual rainfall along the same period was 944 mm, having an annual mean temperature of 9.7 °C, with the predominant wind direction blowing from W-SW to NE (DWD, 2021). Fig. S2 shows the relative abundance (% of the total ring surface) and distribution of the main land uses in buffers of 5, 10, 25 and 50 km radius surrounding the pollen traps for both cities. This map is the result of a reclassification for the classes of land uses considered by the Corine Land Cover project (Corine Land Cover, 2018). For Davos, the dominant land uses are shrublands – grasslands and coniferous (Pine, Spruce and Fir) forests, with less abundant surfaces occupied by broad-leaved and mixed forests like birch trees. For Munich, agricultural areas, agro-forestry areas and coniferous trees are the majority, second urban areas and mixed forests.

The percentage of occupation for the two taxa per km^2 can be seen in Supplementary material (Figs. S3a and S3b for *Betula* and Poaceae). This material was elaborated considering the abundance map from *European Atlas of forest tree species* for birch (*Betula* genus), showing the percentage of abundance per km^2 (based on the relative probability of presence derived from the harmonization of the different mapping forest sources (De Rigo et al., 2016). For grasses, a database for grasslands generated by remote sensing techniques (Copernicus, 2018) was used instead, due to its higher resolution (10 m) compared to other databases. The spatial dataset of grasses was resampled getting the percentage of pixels with grass presence per km^2.

2.2. Meteorological and pollution data

The meteorological data (2010–2015) for Davos were obtained from the Agency for Nature and Environment (ANU, station Seehorn), whilst for Munich data during the same period came from the websites of the national meteorological services of Germany (DWD, 2021). The meteorological data that were obtained were: air temperature (mean, maximum and minimum, in °C), relative humidity (%), pressure (hPa), rainfall (mm) and wind speed (m/s). The data regarding inorganic pollutants from Munich (background levels) were obtained from the website of (Bayerisches Landesamt für Umwelt, 2021) and for Davos from the Agency for Nature and Environment (ANU, station Davos Bubenbrunnenplatz or Promenade). The pollutants were: particulate matter (smaller than 10 μm, PM_{10} and smaller than 2.5 μm, $PM_{2.5}$), nitrogen oxides (NO, NO_2 and NO_x) and Ozone (O_3). All of them were expressed in daily μg/m^3.

2.3. Pollen and allergen sampling

Pollen Allergen Potency (PAP) is calculated by the ratio between airborne Phl p 5 and Bet v 1 and airborne Poaceae and *Betula* pollen, expressed in our case in pg allergen/Pollen grain (this represents the allergen content per pollen grain). Airborne pollen data from 2010 to

2015 were obtained from the pollen station of Davos-Wolfgang (DPS), 46° 49′ 44″ N and 9° 51′ 22″ E, on a flat roof 15 m above ground. For Munich, the pollen trap was located at 2 m' height in the gardens of the Center of Allergy and Environment (ZAUM), near the city center (48° 9′ 52″ N and 11° 35′ 34″ E). The pollen traps were Hirst-type volumetric traps (Hirst, 1952). Pollen was counted according to the International Association for Aerobiology (Galán et al., 2014), counting four longitudinal scans across the slide, and data were comprehended from midday to midday. The results were expressed as daily average values (pollen grains/m^3).

Aeroallergens were daily sampled at the same locations with the same time interval as for pollen. A ChemVol 2400 high-volume cascade impactor (Albany, NY, USA) was used, with a volume of 800 L/min, which can split the sampled particles of the air (one sample every day) into 3 stages for particulate matter: $PM_{>10}$, $PM_{2.5\text{-}10}$ and $PM_{2.5}$ μm (Buters et al., 2010).

The impacting substrate was prewashed polyurethane filters. After collection, samples were stored separately in polypropylene tubes at −80 °C until further extraction, as described before in Buters et al. (2010). The quantification of allergens was done with specific ELISA for Bet v 1 or Phl p 5 (Buters et al., 2010, 2015).

2.4. Statistical analysis

All the statistical analysis were carried out by using the R programming language (R Core Team, 2021), using the package *Aerobiology* for pollen data visualization (Rojo et al., 2019). The MPS for the period 2010–2015 was calculated with the 95% method (Andersen, 1991), taking as start date the moment when the concentration for one pollen type reached 2.5% and taking as end date the moment when the concentration reached the 97.5% (Fig. S4). Also, the number of days with pollen concentrations higher than 100 pollen grains/m^3 (*Betula*) and 50 pollen grains/m^3 (Poaceae) were quantified, according to the thresholds specified by Pfaar et al. (2017). DOY (Day Of the Year) refers to the period of the pollen season (Table S1).

Due to the non-linear relationship between the environmental parameters (inorganic pollutants, pollen grains and meteorological parameters) and the PAP (Figs. S6 and S7), Generalized Additive Models (GAM) (Wood, 2011) were established to explain the influencing parameters in PAP using the package *mgcv* (Wood, 2017) with the expansion *itsadug*, containing the function *fvisgam* (van Rij et al., 2020) that allows exclusion of random effects. Their residuals were previously checked, finding a normal distribution.

Pollen concentrations from different time periods were tested and included in the modelling of the PAP. They represented the time spent in the anther before the pollen are released (Buters et al., 2010) and the meteorological conditions in previous days.

Also, days with free allergens were checked separately (Figs. S8 and S9), being classified as "*only free allergens*" days when allergen only appeared in the fraction of $PM_{2.5\text{-}10}$. On these days allergens were not attached to bigger particles (e.g., pollen grains, $PM_{10} < 0.01$ μg/m^3). Only values above the detection limit of 0.01 μg/m^3 of the ELISA were analysed. On the other hand, there were days with allergens only in the fraction of $PM_{>10}$. These were the "*No free allergens*" days defined as allergen $PM_{2.5\text{-}10}$ <0.01 pg/m^3 and $PM_{>10}$ is > 10 pg/m^3 10 pg/m^3 was conservative chosen to avoid false labelling. "*Normal*" was when none of the previous descriptions applied, which represents the most common situation of having pollen and allergens simultaneously. Fig. S9 shows how the allergens in the groups "*normal*" and "*free allergens*" are distributed regarding different environmental conditions.

To check for statistical differences between the PAP for Davos and Munich and calculations for Fig. S9, a Mann-Whitney *U* test was applied, as the data did not follow normal distribution according to the Shapiro Wilk test for normality.

Differences were reported for p-value ≤ 0.05. Data for days with concentrations lower than 10 pollen grains/m^3 were removed for analysis (Adamov et al., 2021; Buters et al., 2015).

3. Results

3.1. Distribution for birch and grasses in the surroundings of Davos and Munich

Fig. S3 and S4 (*Betula*) and S3b (Poaceae) show the percentage of abundance/km^2 for taxa belonging to each pollen types in both cities within a 50 km-radius. Few birch trees are present in Davos, and the most abundant sources are in the south (35–40% occupation) and with lower percentage of occupation (5–15%) in the west of the city. Munich has two patches with percentages of 35–40% in the southwest of the city and one closer in the northeast (25–40%), but is surrounded by several sources, especially in the southwest and the east, and less in the north. For grasses, the more rural environment in Davos can be noticed (as it is also evident in Fig. S2), since a great area surrounding the city is occupied by grasslands. For Munich, we see a circle comprehending the metropolitan area with little percentage of grass occupation (0–5%), due to urban/residential areas and urban parks mostly covered by trees (Fig. S2), and areas further away from the trap are those that are mostly covered by grasses/agricultural lands, especially in the south.

3.2. Mean pollen season characteristics

The MPS′ characteristics for Poaceae and *Betula* during the studied period (2010–2015) are shown in supplementary material (Fig. S5 and Table S1). *Betula* and Poaceae airborne pollen concentrations were higher in Munich than in Davos, whilst for *Betula* there were remarkable differences amongst years. In Davos only two days during the six years reached a concentration of 100 pollen grains/m^3, whilst for Munich the number of days achieving such a concentration ranged from 9 days in 2011 to 18 in 2012. The Seasonal Pollen Integral (SPIn) for *Betula* also was higher in Munich (7741 ± 1682 pollen * day/m^3 on average) than in Davos (573 ± 268 pollen * day/m^3). For Munich, the highest concentration was reached in the year 2013 (2216 pollen grains/m^3, April 24th), whereas the highest concentration in Davos was also in 2013, but one week earlier and much lower (248 pollen grains/m^3, April 18th). The start dates are quite similar for both places, although having in average a longer MPS in Davos (42 against 25 days in Munich).

For Poaceae the MPS between cities were quite similar, having a slightly shorter MPS in Davos (100 against 104 days in Munich). The peak-day concentrations for Davos were obtained for mid-June and early July (the highest recorded on June 22nd 2015, 210 pollen grains/m^3) and earlier in Munich, with peaks in late May and early June (June 3rd 2015, 306 pollen grains/m^3). The SPIn was in average, 1725 ± 408 pollen * day/m^3 in Davos and 2435 ± 789 pollen * day/m^3 for Munich. There were less days with more than 50 pollen grains/m^3 in Davos (37 days during the six years) than in Munich (64 days).

3.3. Allergens and PAP

Allergen concentrations were higher for both, Bet v 1 and Phl p 5 in Munich (Fig. 1). The amount of allergens per pollen grains changed every year. For Bet v 1, higher concentrations varied between the years. The higher allergen concentrations were recorded for 2010 and 2011 for Munich, and for Davos, in 2010 and 2013. The average concentrations of Phl p 5 were especially high in Munich during 2010 and 2014, whilst for Davos this was in 2015.

Significantly higher PAP for grasses (3.5 ± 1.9 pg Phl p 5/pollen grain) were observed in Munich than in Davos (2.4 ± 1.5 pg/pollen grain, $p < 0.001$), but not for *Betula* (2.5 ± 1.6 pg Bet v 1/pollen grain in Munich and 2.3 ± 1.7 in Davos, N.S.), (Fig. 2).

Phl p 5 and Bet v 1 were approximately 9-fold higher (87.1 ± 13.9% of Bet v 1 and 88.8 ± 15.5% of Phl p 5) in the fraction $PM_{>10}$ (the fraction containing the pollen, Fig. 3) compared to $PM_{2.5\text{-}10}$.

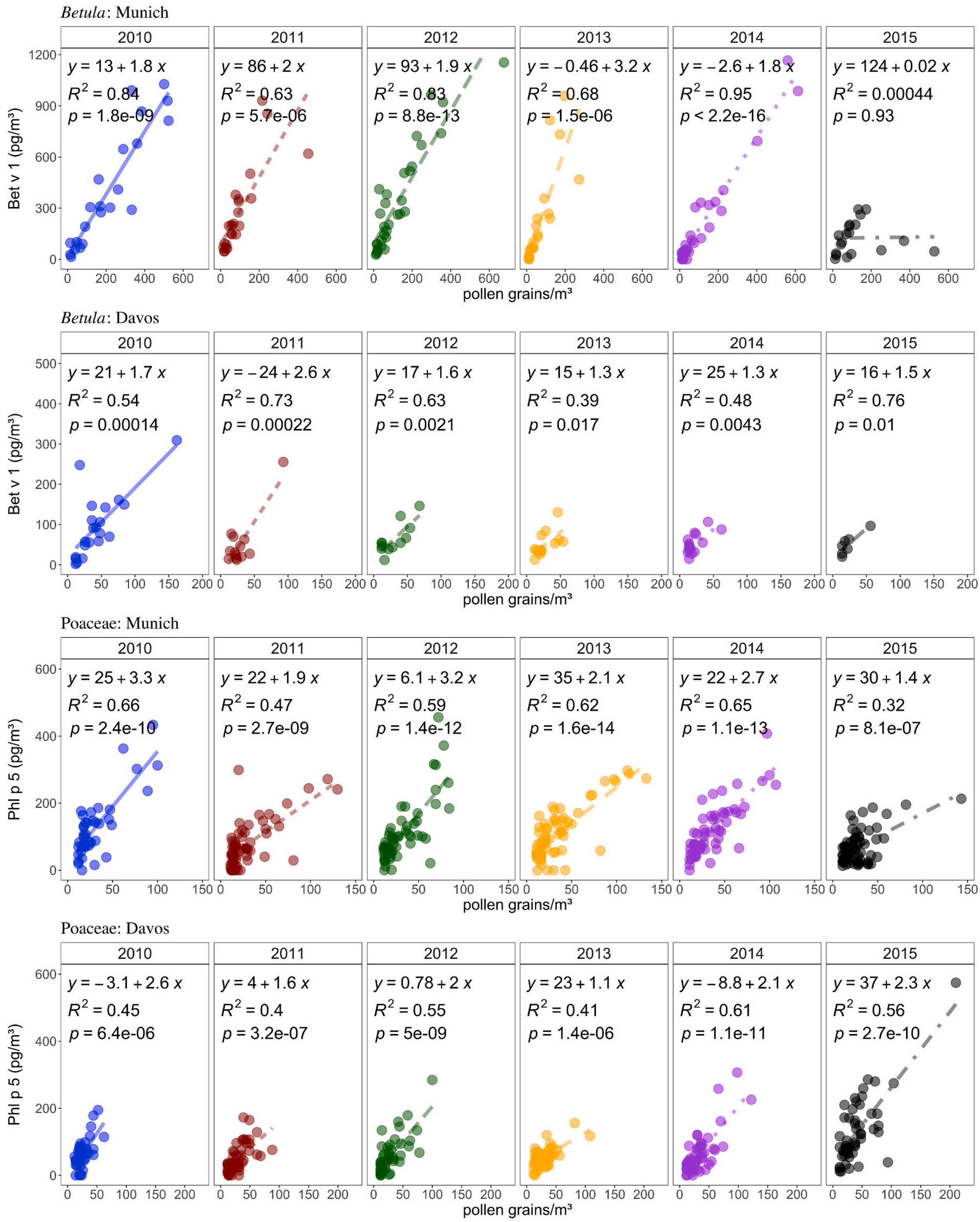

Fig. 1. Annual variations in PAP for Munich and Davos (relationship between airborne pollen grains and allergens). The position for each point regarding the fit line shows the concentration of allergen that pollen grains are carrying each day. Thus, everything placed above the line shows that those pollen grains are having higher Bet v 1 and Phl p 5 concentrations than could be the expected, meaning that during those days, pollen with higher allergenicity can be found in the air. Please note the differences in the x and y axis.

We also found days with Phl p 5 only in fraction $PM_{>10}$ (concentrations around 100 pg/m^3). This means that for some days whole pollen grains are present, without free airborne allergens. On the contrary, there are days with allergens in the fraction $PM_{2.5\text{-}10}$ but not in PM_{10}. Those days represent days with only free allergens. Bet v 1 and Phl p 5 are present in more days without free allergens, and less present in days with only free allergens. Moreover, the relationship between allergens in the different fractions also varied per year. The dates along the MPS with a higher likelihood to find free allergens were at the beginning and at the end of the season, specially for Phl p 5 (Fig. S8, in red colour), whilst the rest of time allergens are found almost always together with the pollen grains (blue colour). The appeareance of free allergens on rainy or with highly humid days was found for both locations and for both pollen types, although more often and only significant for Poaceae pollen in

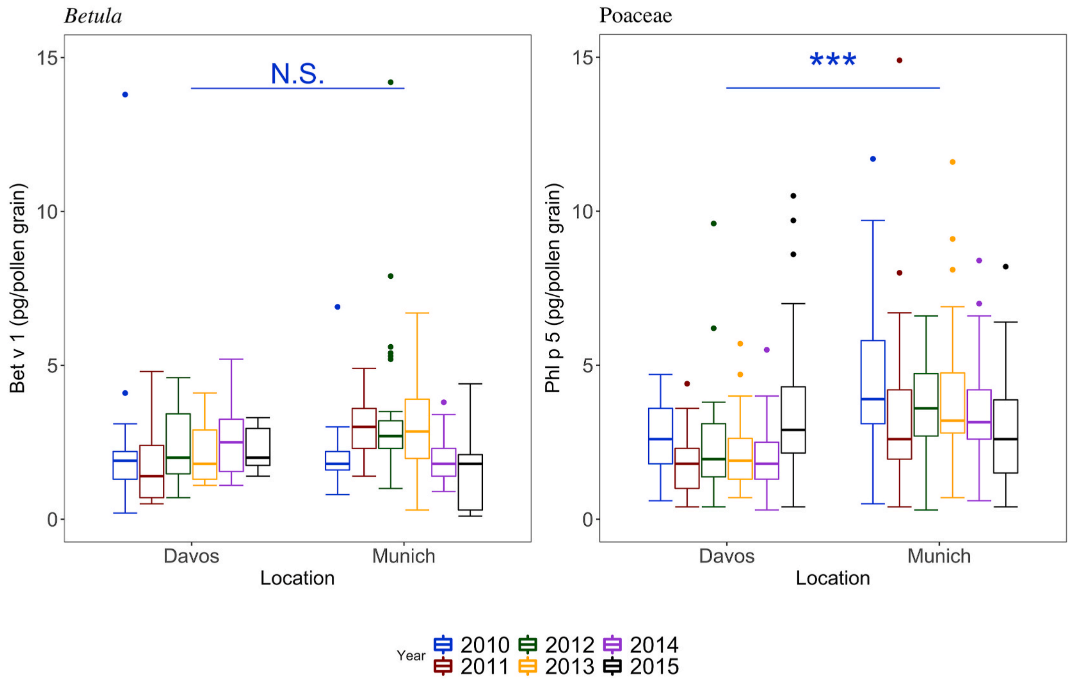

Fig. 2. Differences in PAP between study locations. N.S, *, ** and *** means p-value > 0.05, p-value ≤ 0.05, p-value ≤ 0.01 and p-value ≤ 0.001, respectively.

Munich ($p = 0.011$). In addition, days with higher maximum temperatures decreased the likelihood of finding free Bet v 1 allergens in both cities (Fig. S9).

Other factors that can also increase the PAP is the moment within the pollen season or DOY (Fig. 4). This seemed to be more important in the case of Poaceae, and not as relevant for *Betula*.

3.4. Explanatory model for PAP

Table 1 shows the significance of the parameters involved on each model, where "smooth" means that the impact of the variable depends on the range of values and "interaction" means that the impact of both terms depends on each other.

The time spent in the anther before releasing the pollen grains was found to be significant according to the GAM model (Fig. 5), being an important factor working to load the pollen with more allergens. This period was shorter for Munich, only needing one day (lags with 1 day for Poaceae and *Betula*) but longer for Davos in the case of *Betula* (three days of lag). Moreover, we observe that the importance of lag decreases when the pollen season progressed.

The additive models explained that for Poaceae the PAP was higher at the beginning and at the end of the pollen season (lower and higher DOY, respectively) whilst for *Betula* the PAP increased at the end of the pollen season (higher DOY), having less importance during the rest of the season. Agreeing with Fig. 4, the DOY was statistically significant for grasses (both with $p < 0.001$) and less important for *Betula* ($p>0.05$ for Munich and $p < 0.01$ for Davos). Other important meteorological factors for PAP of grasses were temperature (maximum for Munich and minimum for Davos), pressure and rainfall for Munich, and the humidity for Davos. For Davos the concentration for Ozone recorded two days earlier than pollen release was found to be significant correlated to PAP ($p < 0.001$). Ozone can be considered a proxy for good weather, and it could be that the pollen was produced and stored until the next occasion suitable to release such pollen.

In the GAM models, the explained deviance was 53.0% ($R^2 = 0.504$, $n = 301$) for Poaceae in Munich, whilst it was 47.9% ($R^2 = 0.457$, $n = 292$) for Davos. For *Betula*, in addition to the commented DOY and lag for concentrations, the minimum temperature and the rainfall in the previous day were important. For birch, the GAM model explained 74.5% ($R^2 = 0.761$, $n = 147$) of the PAP for Munich and 66.1% ($R^2 = 0.713$, $n = 95$) for Davos. The GAM models for *Betula* fitted better than those created for Poaceae (Fig. S10). Also, the goodness of fit was higher for Munich in both pollen types.

4. Discussion

Davos had 13-fold less birch pollen than Munich (573 ± 268 vs 7741 ± 1682 Pollen * day/m^3, $p < 0.001$) and the average release of allergen was also different between Munich and Davos (326.1 ± 414.6 vs. 76.2 ± 118.4 pg/m^3, $p < 0.001$), showing the fewer sources of birch trees surrounding Davos. However, Davos had about the same amount of grass pollen compared to Munich (70% of yearly Seasonal Pollen Integral, $p = 0.12$) and land use analysis showed many grasslands present in Davos. Thus, due to grass pollen releasing less allergen (pg/m^3) in Davos (71.0 ± 66.3) than Munich (102.9 ± 82.3, $p < 0.001$), we can argue that Davos grass pollen is being exposed to a different climate than Munich, allowing the modeling of parameters influencing PAP.

Year-to-year variations for allergen concentrations were found for both cities and pollen types. Other authors explained the variations by differences in genotype as proposed by Schäppi et al. (1996), and by medium or long-range transport (Moreno-Grau et al., 2016), but in our case these differences are more likely due to different environmental conditions i.e. ripening (Buters et al., 2008) between Davos (1500 m a.s.l.) and Munich (520 a.s.l.), allowing the study of pollen allergen drivers.

Most of days with high pollen allergenicity were days with low pollen counts (concentrations below 50 pollen grains/m^3, Fig. 1). This inverse association between pollen concentrations and PAP agrees with previous evidence found during the *HIALINE* project (Buters et al., 2012, 2015) and reports by other authors (Jochner et al., 2015; Moreno-Grau et al., 2016; Ščevková et al., 2020).

We found statistically significant differences for PAP of grass pollen between Munich and Davos ($p \leq 0.001$), with Munich having higher PAP. Assuming that the pollen from Poaceae is mostly local (Rojo et al., 2020; Romero-Morte et al., 2018), PAP could also differ because the species of grasses contributing to the pollen flight in these two cities are different, with different genetics, which could result in pollen grains

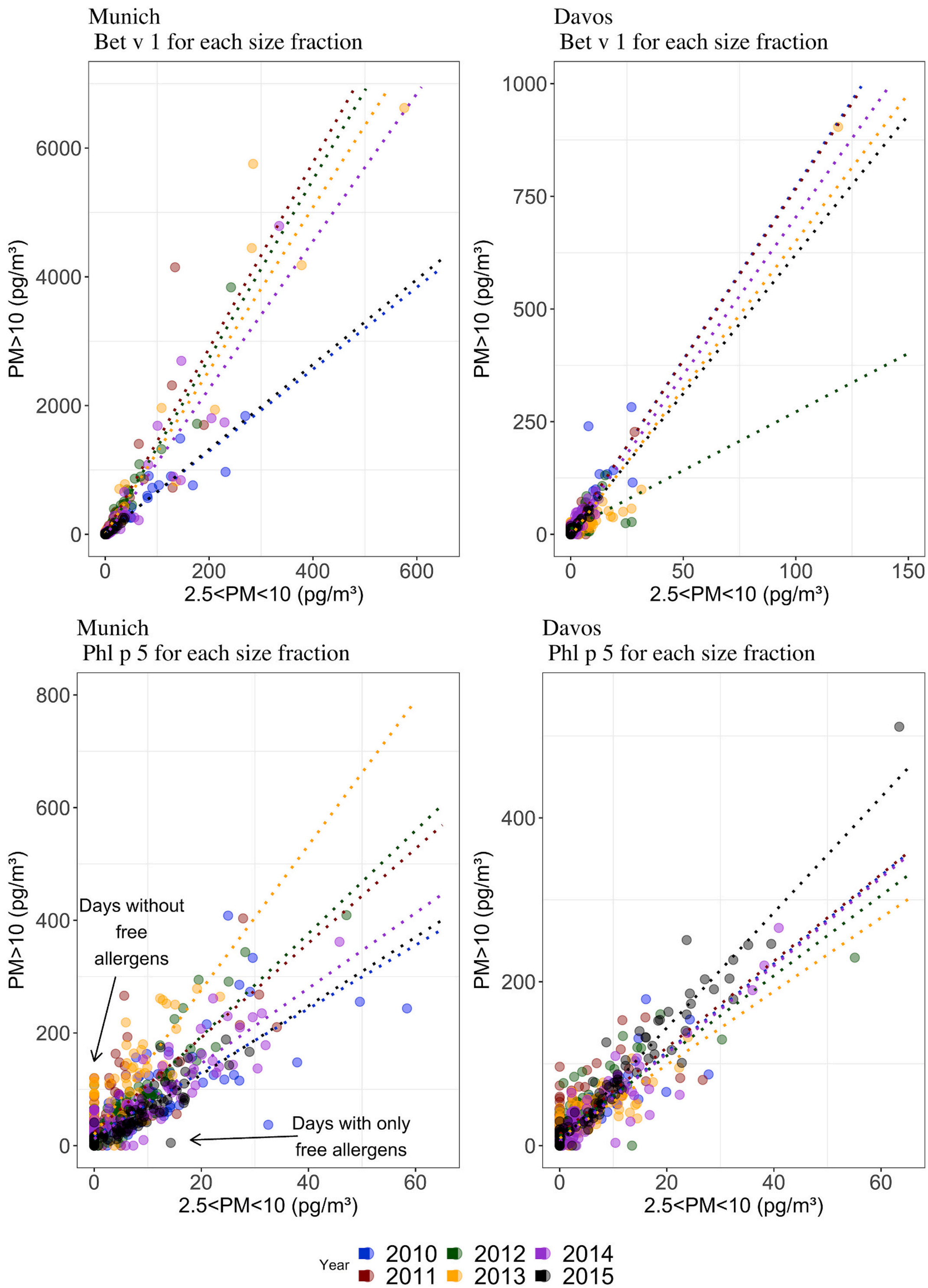

Fig. 3. Relationship between airborne allergens (Phl p 5 and Bet v 1) in two size fractions of the air: $PM_{2.5\text{-}10}$ (2.5 μm < PM < 10 μm) and $PM_{>10}$ (PM>10 μm). Allergens observed in the $PM_{2.5\text{ -}10}$ fraction are supposed to be 100% free (without being adhered to pollen grains). Lines represent the linear regression line. Please note the different scale for x and y axis.

with different allergen content (Cecchi, 2013; García-Mozo, 2017). The species composition and their abundance reflect differences in environmental parameters (including altitude) (de Bello et al., 2013) and differ between low lands such as Munich versus alpine landscapes such as Davos (Byars et al., 2007, 2009). While Munich climate is characterized by a continental climate with warm summers, Davos has a cool continental - subarctic climatic condition with cold summers (Peel et al., 2007). Those conditions lead to completely different distribution of genus for grasses (Leuschner et al., 2017; Pfadenhauer and Klötzli, 2020) with the richness of species being higher in lower altitudes (Grabherr et al., 1994; Kazakis et al., 2021). The amount of allergens was linked to stress response (PR 10,- *Patoghenesis Related*-proteins for Bet v 1 (Chen et al., 2016; Songnuan, 2013). Their function was linked to different metabolic events, including cell wall loosening and germination for grasses (Choi et al., 2006). If we consider that role for allergens is linked to diverse actions that favor the possibilities for being successful during

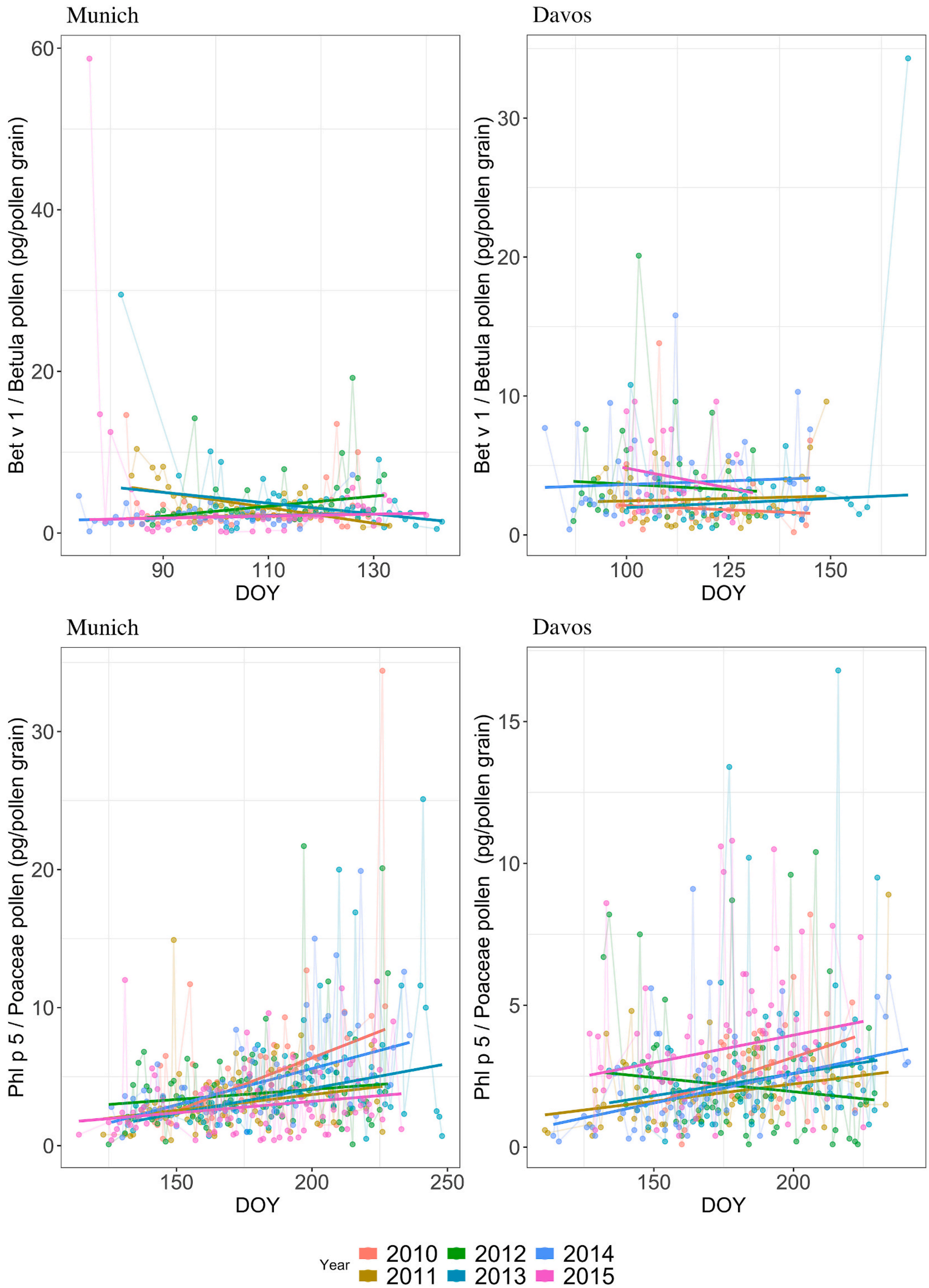

Fig. 4. Relationship between the day of the year (DOY) and the PAP. Lines represent the linear regression that better are fitted to their points. Only PAP higher than 0.01 pg/Pollen grain are shown.

pollination and fertilization (reproductive competition), the fact of Munich having higher PAP makes sense, due to the higher competition reported for low lands, where the abiotic stress is lower (Callaway et al., 2002).

For *Betula* PAP, no differences were found between Davos and Munich, see Fig. 2. In the models, *Betula* PAP for Davos was correlated with the concentrations in the previous three days, which could be indicative of some episodes of long-range transport of pollen originated in lower-lying lands. Due to the small size for *Betula* pollen grains, they can be dispersed over long distances (Hjelmroos, 1992; Maya-Manzano et al., 2021; Skjøth et al. 2007, 2008), and birch pollen is less dependent on local dispersal (Bastl et al., 2019; Rojo et al., 2020). In Davos, *Betula* airborne pollen are thus likely to originate largely from outside the area, also as few sources surround the city (Fig. S3). Our pollen observations show a clear lack in reproductive phenology between both sites (Table S1).

The difference in PAP based on the geographical origin for pollen grains has been highlighted by Buters et al. (2008), who showed a

Table 1
Terms involved on GAM models for Betula and Poaceae, in Munich and Davos.

Variable	*Betula*		Poaceae	
	Munich	Davos	Munich	Davos
Intercept	0.0308 *	0.0381 *	0.0007 ***	0.0023 ***
Atmospheric pressure	0.0188 *	0.0258 *	0.0011 ***	0.0037 **
Relative Humidity	Non significant	Non significant	0.0274 **	5.39e-09 ***
Smooth (**Day of the Year**)	Non significant	0.00993 **	<2.00e-16 ***	Non significant
Interaction (**pollen concentration** on day 0 with previous days)	4.51–05 *** (with day −1)	0.00607 ** (with day −3)	2.24e-13 *** (with day −1)	0.0002 *** (with day −2)
Interaction (**air temperature** on day 0 with previous days)	2.96e-05 *** (with day −3)	0.02823 * (with day −1)	8.24e-07 *** (with day −1)	0.0425 * (with day −2)
Interaction (**rainfall** on day 0 with previous days)	0.00297 ** (with day −3)	3.41e-05 *** (with day −1)	Non significant	Non significant
Interaction (**O$_3$** on day 0 with previous days)	Non significant	Non significant	Non significant	7.71e-05 *** (with day −2)
Interaction (**relative humidity** on day 0 with previous days)	Non significant	Non significant	Non significant	2.22e-05 *** (with day −1)

three-fold variation in PAP of the same species from two 600-km-apart regions (Buters et al., 2015). Galan et al. (2013) also reported a lack of correlation between the allergens and the pollen counts for *Olea*, and reporting pollen 7.6 times higher allergen per pollen for Córdoba (Spain) than in Évora (Portugal). It is possible that PAP variations detected within the same area are provoked by having pollen from different origins (Jochner et al., 2015).

Allergens Phl p 5 and Bet v 1 were approximately 9-fold higher in the fraction $PM_{>10}$ (carried by pollen) than the recorded for $PM_{2.5\text{-}10}$, which agrees with previous works (Buters et al., 2012, 2015; Jochner et al., 2015). It is no surprise that birch pollen (22 μm) and grass pollen (45 μm) are predominantly found in the $PM_{>10}$ μm fraction. However, 12.9 ± 13.9% of Bet v 1 and 11.2 ± 15.5% of Phl p 5 was detected in the fraction $PM_{2.5\text{-}10}$. We expect this to be mostly due to the imperfect separation of particles by impactors. Nevertheless, if allergen detected in this fraction goes up (e.g., in the extreme case none in the biggest fraction), this must be due to an increase of smaller than pollen allergen containing particles, as described before (Buters et al., 2015). No allergen was found in the even smaller fraction $PM_{2.5}$ (Buters et al., 2010). We hypothesize that this observation could also be due to irreversible absorption by diesel particles that also land in this fraction, as absorption properties of diesel particles are known (Bergstrom et al., 2007).

In our study we found that for Phl p 5, most free allergens was recorded on days at the beginning or at the end of MPS (Fig. S8, spring and late summer), those periods with lower pollen concentrations as was also mentioned by Jochner et al. (2015).

The presence of free allergens during rainy or wet days (days with little or no pollen) was higher than recorded for days with normal behaviour (days having pollen and allergens, Fig. S9), although only significant in the case of rainfall for Poaceae in Munich. This association between rainy days and the release of higher concentration of allergens was also reported by other authors (Fernández-González et al., 2011; Rodríguez-Rajo et al., 2011). This phenomenon might be relevant to explain thunderstom ashma. Allergens were reported to be released in the air during episodes of strong and unstable atmospheric conditions, such as thunderstorms (D'Amato et al., 2008, Thien et al., 2018). Authors suggested that the electric charges can facilitate the rupture of the pollen grains (Emmerson et al., 2021; Taylor and Jonsson, 2004; Visez et al., 2015) and the release of the allergens (Horváth et al., 2011; Miguel et al., 2006), enabling fragments to reach the lower respiratory pathway. Sudden exposure to relative humidity facilitates the release of allergens contained in cytoplasm (Aloisi et al., 2018; Stewart et al., 2014). These relationships were also highlighted by our models, where rainfall (3 out of 4) and humidity (in the remaining case) were statistically significant to explain the rise in PAP (Fig. S9). Other authors (Jochner et al., 2015) found that the PAP was correlated with low humidity and rainfall. The presence of free allergens has been associated to long distance transport episodes (Moreno-Grau et al., 2016), which are more frequent at the beginning and at the end of the MPS (Fernández-Rodríguez et al., 2020). Also, some pollen resuspension under

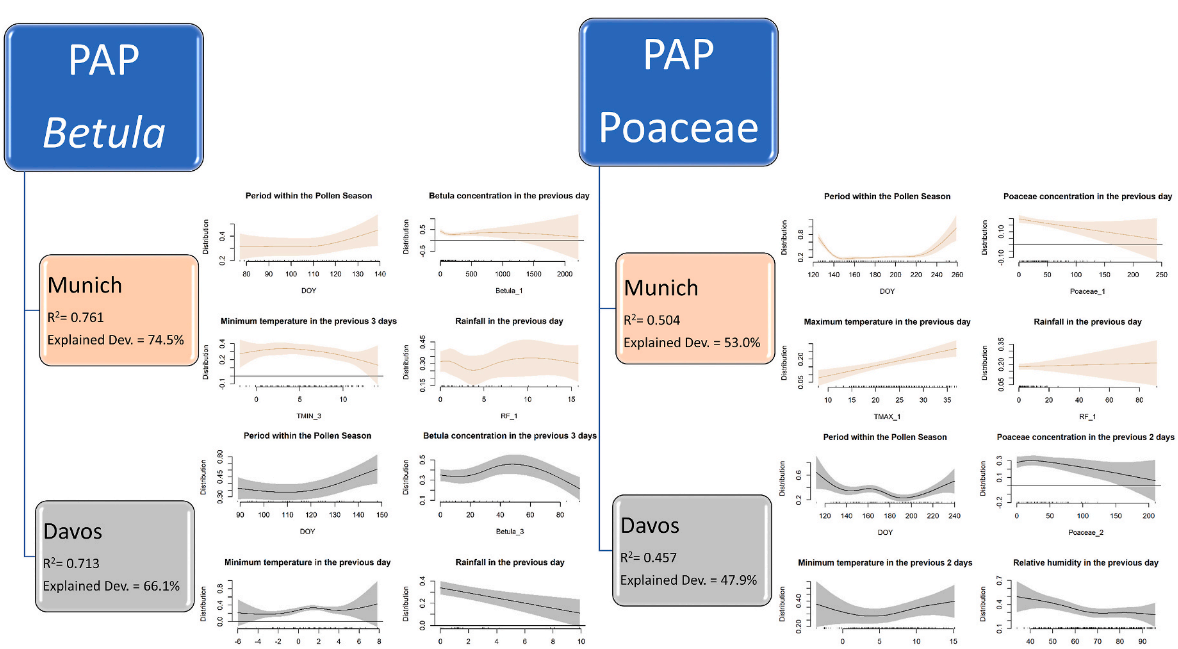

Fig. 5. Overview for the GAM models created to explain the most influencing factors for PAP in *Betula* (Bet v 1) and Poaceae (Phl p 5) in Munich and Davos.

turbulence phenomena conditions (Sehmel, 1980) could explain this late presence of free allergens at the end of the pollen season. However, old pollen is likely to have released the allergens after long exposure to atmosphere and thus leading to lower allergen concentrations (Sofiev et al., 2013).

Our study shows that other factors can also increase the PAP, such as the moment of the pollen season (Fig. 4, see Table S1). This is a very interesting result which would corroborate the interspecific dependence of the allergen load of the pollen grains. That seemed to be important in the case of Poaceae, possibly because of the higher number of species integrated in the Poaceae family, having different flowering timings (Romero-Morte et al., 2018), pollen production and allergen content (due to different genetic load) along the pollen season (Brennan et al., 2019; Jung et al., 2018). According to Rowney et al. (2021), some species such as *Cynosurus cristatus* and *Phleum pratense* are associated with higher amount of asthma-related hospital admissions, which could indicate different allergen load (Jung et al., 2018) and thus different PAP. We find that at the end of the season other plants with higher PAP (Fig. S7, Munich). Our study shows that in the case of *Betula,* the importance of DOY on PAP is limited, probably due to less different species in the *Betula* genus in both Munich and Davos. The main tree species belonging to the genus *Betula* in the studied area are *Betula pendula* and *Betula pubescens* (Rojo et al., 2021), with *B. pendula* more abundant in this area of Europe (Beck et al., 2016).

The lag with the pollen was found to be significant according to the GAM model. An explanation is that more time spent in the anther allows pollen to synthesize more allergens. In fact, no Bet v 1 allergen was detected in birch pollen until the ripening of the inflorescences started (Swoboda et al., 1995), i.e in the last week before pollination (Buters et al., 2010). For *Betula*, the models show that days with higher pollen concentrations compared to the previous day led to high PAP. Thus, a delay in pollen release, provoked by high humidity, rain or low temperature allows pollen to mature longer inside the anther, leading to a higher PAP. The contrary is possible too. If the pollen was released in the previous day with high PAP, is likely that PAP in the current date will be lower (less time to generate allergens and to load the pollen with these allergens). Ripening of pollen while being airborne is unlikely, as pollen dry within minutes after release from the anthers (Pacini and Dolferus, 2019). Biological reactions need water and indeed, the potency of pollen did not change after 600 km being airborne (Antunes et al., 2013).

Environmental drivers explaining the PAP are still barely studied and the evidence is inconclusive (Tegart et al., 2021). These authors concluded that the resulting PAP is determined by a complex combination of factors interacting amongst them, as the models presented here show. Data obtained in this work showed that PAP for each location has its own characteristics to be considered. By creating GAM models, we explained part of the complex relationships between seasonality and environmental parameters, that are not sufficiently explained by traditional linear relationships (Fig. S6). GAM models fitted better for *Betula* (Munich, $R^2 = 0.76$; Davos, $R^2 = 0.71$) than for grasses (Munich, $R^2 = 0.50$; Davos, $R^2 = 0.46$) and in both cases fitted better for Munich (Fig. 5, Fig. S7).

In addition to the effects of rainfall and humidity on allergenicity, temperature is an important factor, because some plants can trigger allergens as a response to abiotic stress (Ahlholm et al. 1998). We found that for grasses, temperatures (maximum temperature for Munich and minimum for Davos) correlated well with PAP, the same as reported by Plaza et al., (2020). Moreover, as they found, we also found positive correlations with humidity (Poaceae in Davos) but also for rainfall for both locations regarding *Betula*. Regarding inorganic pollutants no clear relationships with PAP were found except for ozone in Davos for grasses, as reported by others (Eckl-Dorna et al., 2010; Masuch et al., 1997; Ščevková et al., 2020). The contrary was described by Rogerieux et al. (2007), who found that exposure to ozone decreased pollen allergenicity.

This work shows the changing nature of PAP depending on external factors such as meteorology, year or location, and how they change in their importance along the pollen season (Fig. 5). The diversity of factors influencing the PAP was quite site-specific, and demonstrated inter-specific, and therefore can not be easily extrapolated to other locations (see Fig. 5 each city a model for birch and one for grasses). We also show that the representativeness of pollen counts as a proxy for allergen exposure is limited. We suggest adding aeroallergen sampling to routine pollen count to understand pollen exposure.

5. Conclusions

PAP was different between the two cities studied for grasses, but not for birch. Moreover, the pollen potency changed every year (i.e., in Munich for grasses PAP ranged from 2.8 ± 1.7 to 4.4 ± 2.3 pg Phl p 5/pollen grains) but was always higher in Munich. Most allergens (9-fold more) were trapped in the fraction $PM_{>10}$, the fraction where most pollen are collected. Factors like ripening for pollen in the case of *Betula* and the DOY for Poaceae influenced the PAP. In the case of Poaceae, the highest PAP at the end of the MPS could be due to other species varying in genetics and consequently allergenicity. This effect was not so strong for *Betula*, a genus with a more homogeneous pollination. Free allergens were recorded at the beginning and at the end of the MPS, especially for days with rain or high relative humidity. No clear influence of inorganic pollutants (O_3, PM_{10}, $PM_{2.5}$, NO_x) was seen except for ozone in Davos for grasses. Our results demonstrate that the airborne pollen monitoring, although essential, should be complemented with allergen analysis.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.113987

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