

Article



# Spatial and Temporal Variations of Airborne Poaceae Pollen along an Urbanization Gradient Assessed by Different Types of Pollen Traps

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Abstract: Grass pollen allergy is widespread all around the globe. With an increasing number of people living in cities, the examination of grass pollen levels within cities and their surroundings has increased in importance. The aim of this study was to examine different temporal and spatial scales of grass pollen concentration and deposition across urban and semi-rural environments in the years 2019 and 2020. We installed different types of pollen traps in the city of Ingolstadt (Bavaria, Germany) and its surroundings: volumetric pollen traps at roof level to assess background pollen concentration and gravimetric pollen traps and portable volumetric traps at street level. We considered grass pollen concentration and deposition in the context of land use and management. Our data showed that the grass pollen season in 2020 was longer and more intense than in 2019. Background grass pollen concentration was generally higher at the semi-rural site in both years: peak values were eight times (2019) and more than four times (2020) higher, and Seasonal Pollen Index was more than four times and almost three times higher in 2019 and 2020, respectively. Analyses of spatial variations measured at street level revealed higher numbers for pollen deposition and concentrations at semirural than at urban sites. Recorded values were linked to local vegetation and the management of grass areas surrounding the traps. Analyses of diurnal variations at street level in June 2019 showed that pollen concentration for all sites, independent of their degree of urbanization, were highest at noon (22.2 pollen grains/m<sup>3</sup> vs. 8.5 pollen grains/m<sup>3</sup> in the morning and 10.4 pollen grains/m<sup>3</sup> in the evening). Diurnal variations at roof level showed similarities for the same days but differed when considering the whole season. Our data suggest the importance of the management of grass areas as areas cut earlier have a decreased amount of emitted pollen.

**Keywords:** pollen concentration; Poaceae pollen; pollen deposition; urban-rural differences; spatial and temporal variations; personal volumetric air samplers; gravimetric traps; volumetric traps

## 1. Introduction

The most common pollen allergy in Europe and worldwide is grass-induced pollinosis [1,2]. In Germany, 14.8% of adults are affected by hay fever [3]. Various species contribute to the grass (Poaceae) pollen spectrum throughout the year (e.g., meadow foxtail *Alopecurus pratensis*, orchard grass *Dactylis glomerata*, annual bluegrass *Poa annua*, perennial ryegrass *Lolium perenne*, cultivated rye *Secale cereale*). Poaceae is amongst the most abundant pollen types in Germany [4] and represents the taxa with the longest pollination period [5]. Airborne Poaceae pollen in Germany can be present from mid-March to the end of October, with the main flowering period lasting from late May to mid-July [6]. Under climate change conditions, pollen allergies might intensify [7,8] and thus increase and prolong the burden for allergy-affected people. Thus, further and intensified monitoring of airborne grass pollen is crucial. This especially applies to urban areas since more than 50% of the world's population is living in cities [9].



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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). During spring, the media recommends that city dwellers air their rooms preferably in the morning and those living in the countryside do so in the evening, when pollen concentrations in the air are low. This advice is probably based on single studies and therefore not sufficient to formulate general recommendations. This is confirmed by the fact that many studies conducted in the last decades comparing circadian patterns of airborne Poaceae pollen between urban and rural sites indicate a less explicit pattern: many studies confirm comparatively low Poaceae pollen concentrations at urban sites during morning hours [10–14]. In addition, high pollen counts were detected in the early evening [11], but also in the morning [15] or with two peaks in the morning and afternoon [12]. For rural sites, low Poaceae pollen concentrations were detected in the evening [10] and high concentrations at midday [11] or not showing a distinct peak at all [15]. Even though no general pattern can be used to separate rural and urban pollen conditions, small-scale geographical and biological factors are important drivers for interdiurnal changes in pollen concentration [16] and have to be considered.

In aerobiological monitoring, a volumetric pollen trap of the Hirst design [17] is a widely used instrument [18]. It is usually installed at rooftop level to capture background pollen concentrations rather than pollen originating from plants in the immediate surroundings. The area within approx. 30 km of the pollen trap is considered to influence the pollen recorded by a rooftop level trap [19]. In cities, only one pollen trap is usually maintained, which is however related to some shortcomings, such as the following. Records of a single trap do not adequately account for locally occurring events linked to local pollen sources [20,21]. In addition, one trap cannot reflect pollen levels for a whole city as intra-urban pollen levels may vary [22,23] due to the city's heterogeneous features, development and microclimate. Moreover, temperature differences can affect the timing of pollen release [23], and urban features such as buildings and street canyons influence the trajectories of air masses transporting pollen [24]. In addition, land use, local vegetation distribution and management of green spaces, e.g., mowing [25], can affect local pollen levels and are considered to be relevant drivers of spatial variations of airborne pollen [26].

The above-mentioned studies conducted in urban areas mostly used data obtained from pollen traps installed at roof level several meters above the ground, as is the standard [27]. This height, however, is not the average breathing height for people. Pollen measured across a city at street level generally showed a considerable heterogeneity of pollen concentrations [22] and should therefore be considered when evaluating intra-urban pollen exposure. Nevertheless, Bastl et al. (2019) [28] found symptom data correlating more with pollen concentrations measured at roof level than with concentrations measured at ground level.

To measure airborne pollen at street level, gravimetric traps of the Durham type [29] have been used in several studies [30–32] as well as Rotorod samplers [22,33] or portable volumetric samplers [26]. A few studies combine different kinds of pollen traps, such as 7-day volumetric traps with gravimetric traps [31,34], Rotorod samplers [24,35], nasal air samplers [36,37], or portable volumetric samplers [38,39]. There are very few studies that combine more than two pollen samplers: nasal air samplers, portable volumetric samplers, and a 7-day volumetric trap were used by Mitakakis et al. (2000) [40]. Peel et al. (2014) [41] compared the efficiencies of 7-day volumetric traps, Rotorod samplers, and portable volumetric pollen traps.

To build on findings from the mentioned literature and to gain new insights, this study presents a novel approach using three types of pollen traps across different temporal and spatial scales of grass pollen concentration and deposition across the city of Ingolstadt, Germany, and its surroundings. We sampled airborne grass pollen using volumetric pollen traps (7-day) at roof level at two locations for a duration of two years, gravimetric pollen traps at ground level at twelve locations, and portable volumetric pollen traps at ground level at eleven locations. Our central aim was to evaluate temporal variations of Poaceae pollen exposure between urban and semi-rural areas taking surrounding land use and management into account.

### 2. Materials and Methods

# 2.1. Study Area

The study site was the city of Ingolstadt (48.7665° N, 11.4258° E, 374 m.a.s.l.) and its surroundings. Ingolstadt is located in the southern part of Germany, in Bavaria at the Danube, has a population of roughly 140,000 inhabitants, and covers an area of 13,335 ha [42]. The main land uses in the area surrounding Ingolstadt are industrial and agricultural (Figure 1a). There are no distinct differences in altitude within the study area. The average annual temperature is 8.9 °C, and the average annual precipitation is 712 mm (see Figure 1a for location of DWD station "Ingolstadt Flugplatz (Airport)", 1981–2010). Airborne pollen sampling was conducted at various locations along an urbanisation gradient and across different land uses (Figure 1b,c). Ending points of the gradient were the old town in the city centre of Ingolstadt (urban environment) and the southern edge of the town of Kösching (semi-rural environment).

# 2.2. Pollen Monitoring

## 2.2.1. Volumetric Pollen Traps (7-Day)

To gather data on background pollen concentration, we used two 7-day-recording volumetric pollen traps (Burkard Manufacturing Co Ltd., Rickmansworth, UK) that are based on the Hirst principle [17]. Using an integrated pump, this pollen trap aspirates 10 L of air per minute through a  $14 \times 2$  mm orifice. A rotating drum is mounted behind this orifice, on which a plastic strip (Melinex, DuPont Teijin Films, Luxembourg), coated with a thin layer of pharmaceutical Vaseline, is attached. Aspirated pollen and other airborne particles adhere to the Melinex tape. The drum moves with a speed of 2 mm per hour and completes one revolution in seven days. The drum was changed once per week, and daily samples were prepared in the laboratory. One trap was set up in the city centre of Ingolstadt, on the roof of Ingolstadt School of Management of the University of Eichstätt-Ingolstadt (48.7658° N, 11.4156° E) at a height of 13 m a.g.l., representing urban conditions (urban station (US)). The other trap was located northeast of this location at a straight-line distance of approx. 7 km on the roof of the secondary school of Kösching (48.8084° N, 11.483° E) at a height of 15 m a.g.l., representing semi-rural conditions and therefore referred to as semi-rural station (SRS) (Figure 1b). In order to cover the main flowering period (late-May to mid-July [6]), the traps operated from 1 April to 21 October (semi-rural station) and 24 April to 30 October (urban station) in 2019. In 2020, the traps operated from 30 January to 27 September 2020 (semi-rural station) and 11 February to 3 October 2020 (urban station). The sampling periods differed slightly due to the accessibility of the buildings. Sampling was performed according to the standard methods proposed by the European Aerobiology Society [27].

#### 2.2.2. Gravimetric Pollen Traps—Sampling Network

During spring and summer, a network of twelve gravimetric pollen traps was established. These traps were self-constructed, and their design was based on Durham samplers [29]. Our traps consisted of two horizontal circular disks with a diameter of 34 cm and a downward sloping rim. The disks were made of plastic and fitted at a distance of 20 cm from each other with four threaded rods holding them in place. In the centre of the lower disk, a wooden block ( $L \times W \times H = 5.5 \text{ cm} \times 9.5 \text{ cm} \times 3 \text{ cm}$ ) was bolted and equipped with a clip on top to secure the microscope slide horizontally (see Figure 2). The microscope slides were coated with Vaseline to adhere to airborne particles.

The traps were set up at and between the urban station (US) and semi-rural station (SRS) (Figure 1), mounted on a metal pipe at a height of 1.8 m a.g.l., reflecting the breathing height of people. Locations were selected based on representativeness of different environments, i.e., the rate of urbanisation and land use (Table 1), but also on accessibility and security. The stations were numbered (G1-G12) according to the distance from the urban station (US). Furthermore, we noted management of adjacent Poaceae area and land use in the surroundings. Sampling lasted from 30 May to 22 August (2019) and from 22 April to

10 August (2020) in order to cover the majority of the main flowering period (ca. late-May to mid-July [6]). Sites were equipped with pollen traps later in 2019 due to organizational issues, e.g., related to permissions. Microscope slides were changed every seven days between 8 a.m. and 10 a.m., with the exception of the first week of sampling in 2020, where the sampling period was only 5 days. In total, we collected 140 slides in 2019 and 187 slides in 2020. Due to heavy winds and the damage of a pollen trap, data of nine slides (four in 2019, five in 2020) were missing.

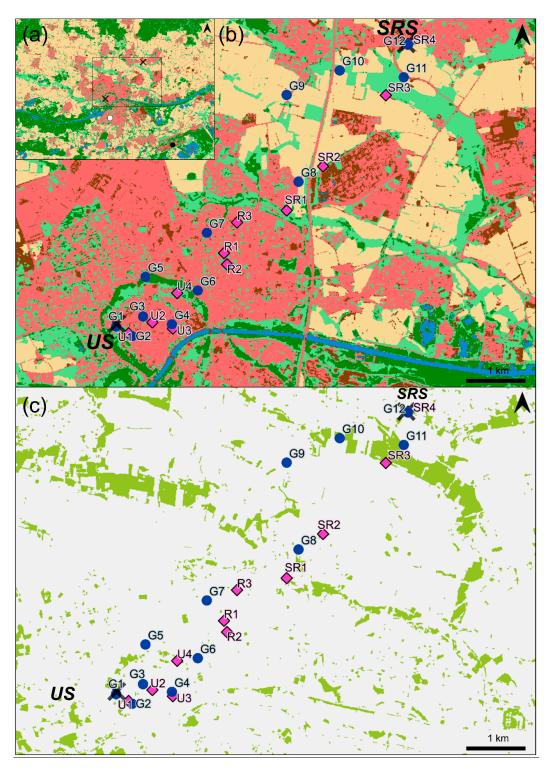
#### 2.2.3. Personal Volumetric Air Samplers—Sampling Campaign

We used personal volumetric air samplers (PVAS; Burkard Manufacturing Co Ltd., Rickmansworth, England) that are based on the Hirst principle [17] for a sampling campaign in 2019. PVAS were mounted on tripods at a height of ca. 1.5 m and set to aspirate 10 L of air per minute. Air aspirates through a horizontally oriented orifice, and the airborne particles contained in It adhere to a microscope slide coated with Vaseline.

Sampling was conducted during the Poaceae pollen season on seven consecutive days, 13 June 2019–19 June 2019, and at eleven locations. Four sites were located in a semi-rural setting ("SR"), three sites in a residential area ("R") and four sites in the city centre of Ingolstadt ("U") (see Figure 1 for locations and Table 2 for location characteristics). Pollen was sampled for 25 min at each site three times a day: 6 a.m. to 8 a.m., 12 p.m. to 2 p.m., and 9 p.m. to 11 p.m.

#### 2.2.4. Sample Preparation

After exposure, the Melinex tape and microscope slides were fixed with a mixture of distilled water, gelatine, glycerol, and staining safranin. The samples were analysed under a light microscope (Axio Lab.A1; Zeiss, Wetzlar, Germany) using  $\times 400$  magnification. No distinction between individual genera or species of the Poaceae family were made, as Poaceae pollen grains share the same characteristics using light microscopy [43,44]. Grass pollen were counted along three longitudinal transects in the case of the 7-day volumetric traps (resulting in a screening of 10.3% of the whole slide) and along four longitudinal transects in the case of the gravimetric traps. In the case of the PVAS samples, the whole slide was examined. For further analyses, pollen counts were converted to pollen grains per cubic meter of air (pollen grains/m<sup>3</sup>) for volumetric traps as is the standard [27]. Pollen counts for the gravimetric traps were converted to pollen grains per square centimetre of slide surface (pollen grains/cm<sup>2</sup>).



**Figure 1.** Locations of pollen traps: Blue circles—gravimetric traps; pink squares—PVAS; black crosses—volumetric pollen traps (roof level); (a) Study area (dashed box) and surrounding land use; white circle—station measuring precipitation (LfU); black circle—weather station (DWD); (b) Land use 2019: dark green—forest; light green—low vegetation; blue—water; red—built-up; dark brown—bare soil; light brown—agriculture [45]; (c) Grassland 2018: green—grass area [46].

| Station | Coordinates                              | Distance from              | Urban | Grass        |        | Land Cov          | er Classificat | ion [%]      |             | Description of Immediate Surrounding and   |  |
|---------|--|----------------------------|-------|--------------|--------|-------------------|----------------|--------------|-------------|--|--|
| Code    |  | Urban Station<br>(US) [km] | Index | Cover<br>[%] | Forest | Low<br>Vegetation | Built-Up       | Bare<br>Soil | Agriculture | Management of Poaceae Area within a Radius of 100 m  |  |
| G1      | 48.7650° N, 11.4153° E                   | -                          | 0.51  | 6            | 8      | 29                | 62             | 1            | 0           | Green space of the Ingolstadt School of Management;<br>irregular-cut Poaceae area, allowing it to flower for short<br>periods.   |  |
| G2      | 48.7635° N, 11.4190° E                   | 0.32                       | 0.53  | 0            | 0      | 1                 | 98             | 1            | 0           | Fully secluded courtyard of two-story houses with ornamental plants; sealed inner-city areas with no Poaceae area.   |  |
| G3      | 48.7665° N, 11.4216° E                   | 0.49                       | 0.58  | 0            | 0      | 0                 | 99             | 1            | 0           | Half-secluded driveway to apartment building complex<br>with small backyard; sealed inner-city areas with no<br>Poaceae area.  |  |
| G4      | 48.7653° N, 11.4283° E                   | 0.95                       | 0.62  | 0            | 0      | 1                 | 93             | 6            | 0           | Courtyard of three-story apartment complex with cut<br>lawn and playground; sealed inner-city areas with no<br>Poaceae area.   |  |
| G5      | $48.7726^{\circ}$ N, $11.4221^{\circ}$ E | 0.98                       | 0.64  | 0            | 60     | 16                | 24             | 0            | 0           | City park area; unmanaged Poaceae area.  |  |
| G6      | 48.7705° N, 11.4343° E                   | 1.52                       | 0.64  | 0            | 3      | 7                 | 89             | 1            | 0           | Small green area at an intersection of a busy street;<br>irregular cut Poaceae area allowing flowering for short<br>periods.   |  |
| G7      | 48.7793° N, 11.4364° E                   | 2.22                       | 0.61  | 0            | 1      | 16                | 82             | 1            | 0           | Green area with short cut grass of apartment buildings<br>next to a multilane street; residential and half-industrial<br>area; regularly cut lawn.   |  |
| G8      | 48.7871° N, 11.4577° E                   | 3.97                       | 0.38  | 22           | 0      | 19                | 40             | 0            | 41          | Residential area with freestanding houses with gardens<br>and agricultural land; 2019: field with Poaceae was cut<br>occasionally allowing flowering for short periods; 2020:<br>Field with Poaceae was cut once in August allowing<br>flowering for longer periods. |  |
| G9      | 48.8003° N, 11.4549° E                   | 4.89                       | 0.26  | 0            | 0      | 2                 | 9              | 1            | 88          | Agricultural land; unmanaged Poaceae area.   |  |
| G10     | 48.8041° N, 11.4672° E                   | 5.79                       | 0.27  | 25           | 4      | 34                | 2              | 1            | 59          | Agricultural land; unmanaged Poaceae area.   |  |
| G11     | 48.8030° N, 11.4821° E                   | 6.48                       | 0.26  | 39           | 0      | 52                | 16             | 3            | 29          | Agricultural land; unmanaged Poaceae area.   |  |
| G12     | 48.8080° N, 11.4831° E                   | 6.91                       | 0.26  | 11           | 0      | 4                 | 30             | 15           | 50          | Green area next the secondary school of Kösching and<br>agricultural land; occasionally cut Poaceae area, allowing<br>flowering for short periods.   |  |

**Table 1.** Sampling locations and surroundings of gravimetric pollen traps. Grass cover [46] and land cover [45] within 100 m of the traps (see methodology section for calculation). Land cover class "water" is not listed as its values equalled 0 for all sampling locations. Station codes G1 to G12 (G = gravimetric) ascending with distance from the urban station (US).

| Station | Coordinates            | Distance from | Urban | Gras         |        | Land Cov          | er Classificat | ion [%]      |             | - Description of Immediate Surrounding and  |
|---------|------------------------|---------------|-------|--------------|--------|-------------------|----------------|--------------|-------------|---|
| Code    |                        | US [km]       | Index | Cover<br>[%] | Forest | Low<br>Vegetation | Built-Up       | Bare<br>Soil | Agriculture |   |
| U1      | 48.7639° N, 11.4182° E | 0.26          | 0.53  | 0            | 2      | 13                | 82             | 3            | 0           | Courtyard of an apartment building. Almost fully enclosed; no Poaceae area.   |
| U2      | 48.7656° N, 11.4238° E | 0.61          | 0.59  | 1            | 0      | 3                 | 96             | 1            | 0           | Open public space and streets, completely sealed area; no Poaceae area.   |
| U3      | 48.7646° N, 11.4285° E | 0.96          | 0.61  | 0            | 0      | 1                 | 95             | 4            | 0           | Wide street in pedestrian zone, completely sealed area;<br>no Poaceae area.   |
| U4      | 48.7700° N, 11.4295° E | 1.16          | 0.64  | 15           | 16     | 55                | 26             | 2            | 0           | Sidewalk of a main street, little park area with unmanaged Poaceae area.  |
| R1      | 48.7762° N, 11.4404° E | 2.19          | 0.59  | 0            | 0      | 12                | 88             | 0            | 0           | Residential area with single-family houses with<br>maintained front and back yards; Poaceae areas/lawns<br>cut regularly preventing flowering.        |
| R2      | 48.7745° N, 11.4411° E | 2.14          | 0.60  | 0            | 0      | 33                | 66             | 0            | 0           | Residential area, maintained public playground<br>surrounded by houses; Poaceae areas/lawns cut<br>regularly preventing flowering.                    |
| R3      | 48.7809° N, 11.4434° E | 2.68          | 0.53  | 6            | 0      | 13                | 81             | 6            | 0           | Residential area with multi-story apartment buildings;<br>Poaceae areas/lawns cut regularly preventing flowering.                                     |
| SR1     | 48.7827° N, 11.4549° E | 3.48          | 0.47  | 10           | 18     | 45                | 17             | 0            | 20          | Edge of a residential area with allotment gardens<br>neighbouring agricultural land; unmanaged and<br>occasionally cut Poaceae area.                  |
| SR2     | 48.7894° N, 11.4634° E | 4.42          | 0.32  | 15           | 5      | 28                | 12             | 7            | 47          | Agricultural land; between country road, highway and industrial area; unmanaged Poaceae area.   |
| SR3     | 48.8003° N, 11.4779° E | 6.01          | 0.27  | 48           | 0      | 46                | 2              | 6            | 46          | Agricultural land; unmanaged Poaceae area.  |
| SR4     | 48.8081° N, 11.4831° E | 6.88          | 0.26  | 11           | 0      | 8                 | 37             | 13           | 42          | Green area next to the secondary school of Kösching and<br>agricultural land; occasionally cut Poaceae area, allowing<br>flowering for short periods. |

**Table 2.** Sampling locations and surroundings of PVAS. Grass cover [46] and land cover [45] within 100 m of the traps (see methodology section for calculation). Land cover class "water" is not listed as its values equalled 0 for all sampling locations. Classification of station code: U = urban, R = residential, SR = semi-rural.



Figure 2. Self-constructed gravimetric pollen trap used for this study.

## 2.3. Analyses

2.3.1. Aerobiological Data

We used the recommended terminology by Galán et al. (2017) [47] to describe the aerobiological data recorded by the 7-day volumetric traps. Start and end dates of the Poaceae pollen seasons were calculated using the 95% method [48]: the pollen season starts when 2.5% of the annual pollen amount is collected and ends when 97.5% of total pollen is collected. We calculated the Seasonal Pollen Integral (SPIn), i.e., the sum of the daily mean pollen concentrations (pollen\*day/m<sup>3</sup>) during the pollen season [47]. Furthermore, we determined the lengths, start and end days of the seasons, and the days with the highest daily mean concentration. Days with a daily mean pollen concentration of at least 50 pollen grains/m<sup>3</sup> were classified as high pollen days, as suggested by Pfaar et al. (2019) [49]. Missing data (2019: 17 days, 2020: 8 days) were interpolated by calculating the moving mean of daily pollen concentrations (7-day volumetric traps) using the method included in the R-package [50]. We analysed diurnal patterns by examining two-hourly variations in grass pollen. Missing data for gravimetric traps (2019: 4 out of 144 entries; 2020: 5 out of 192 entries) were interpolated by calculating the mean of previous and following values of the respective and the two closest traps.

In addition to the standard terminology, we used the following terms to describe and compare data:

- Campaign Pollen Integral (CPIn)
  - The sum of all measurements for each location during one sampling campaign, i.e., CPIngrav and CPIn<sub>PVAS</sub> [51].
- Weekly Pollen Integral (WPIn)
  - The sum of all daily means of one week of background pollen concentration, i.e., WPIn<sub>US</sub> for volumetric trap at US (roof level), and WPIn<sub>SRS</sub> for volumetric trap at SRS (roof level).
  - The sum of all gravimetric measurements of all locations for one particular week, i.e., WPIngrav We refer to the week with the highest WPIngrav as a peak week of the campaign.
- Daily Pollen Integral (DPIn)
  - The sum of all PVAS measurements of one day, i.e., DPIn<sub>PVAS</sub>.

We analysed data in regard to spatial and temporal variations and diurnal patterns by comparing CPIn, WPIn, and DPIn, as well as means and medians. Sites (gravimetric traps, PVAS) and measurement times (PVAS) were tested for significant differences using Wilcoxon rank-sum test and Kruskal–Wallis test. The relationships between WPIns or DPIns of different traps were analysed by calculating Spearman's correlation coefficient (r<sub>s</sub>).

# 2.3.2. Land Cover and Grass Cover

Information on land cover for the year 2019 was obtained from [45]. This dataset provides the land cover classifications "forest", "low vegetation", "water", "built-up", "bare soil", "water", and "agriculture" for Germany with a spatial resolution of 10 m  $\times$  10 m. We calculated the percentage of each class in a radius of 100 m from all sampling locations. In addition, we used the Grassland 2018 dataset [46] with a spatial resolution of 10 m  $\times$  10 m to calculate the percentage of grassland in a radius of 100 m. We calculated the Urban Index as a measure of urbanisation [52] based on the 2019 land cover data for all sampling locations. The relationships between these variables and pollen concentrations and pollen deposition (CPIns) were analysed by calculating Spearman's correlation coefficients (r<sub>s</sub>).

### 2.3.3. Meteorological Data

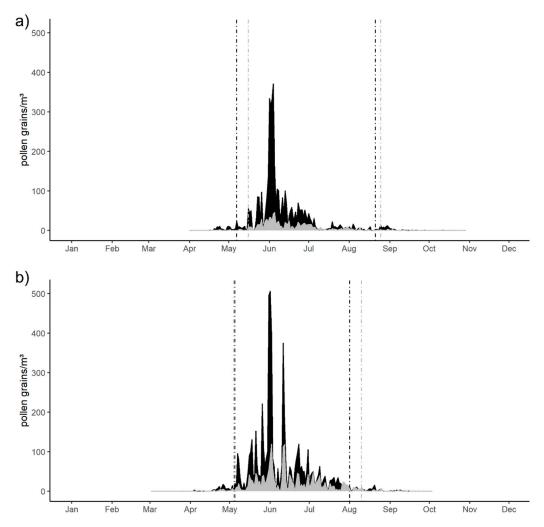
Weather data, i.e., air temperature, wind speed, and wind direction, for the period of the sampling campaigns were obtained from the DWD station "Ingolstadt (Flugplatz)" (48.7112° N, 11.5362° E, see. Figure 1a). Precipitation data were obtained from a station operated by the Bavarian Environment Agency (LfU) (48.74286° N, 11.42355° E, Figure 1a). For the duration of the sampling campaigns with gravimetric traps, we calculated weekly means for temperature, weekly sums for precipitation and the frequency of counts by wind direction for each sampling week starting at 8 a.m. For the duration of the PVAS sampling campaign, we calculated hourly means (temperature), hourly sums (precipitation), and the frequency of counts by wind direction for each day of sampling, from the start of the first measurement (6 a.m.) to the end of the last measurement (11 p.m.).

We used RStudio (1.3.959), packages AeRobiology 2.0.1 [50], OpenAir 2.8-1 [53], ggplot2 [54] for statistical analyses and visualisation. For spatial analyses, we used ESRI ArcMap 10.6 and for visualisation QGIS 3.14 and Microsoft Excel 2016.

### 3. Results

#### 3.1. Background Pollen Concentration: Characteristics of the Grass Pollen Seasons 2019 and 2020

The pollen seasons of both years and locations (urban station (US), semi-rural station SRS) showed different characteristics (Figure 3). We observed differences in SPIn, length of the pollen seasons, peak values, peak dates, and number of high-pollen days (Table 3). In both years, grass pollen concentration was generally higher at SRS, as shown by SPIn (2019: 4071 pollen\*day/m<sup>3</sup>, 2020: 5725 pollen\*day/m<sup>3</sup> vs. US 2019: 933 pollen\*day/m<sup>3</sup>, 2020:  $2104 \text{ pollen*day/m}^3$ ) but also by peak values and number of high-pollen days. In 2019, there were no high-pollen days recorded at the urban station but 25 at the SRS. However, in 2020, we detected six high-pollen days at the US and 33 at the SRS. Peak values at the SRS were eight times (2019) and more than four times (2020) higher than the ones recorded at the US. This is also reflected by SPIn (more than four times higher at the SRS in 2019, almost three times higher in 2020). Grass pollen season always started in May; in 2020, 12 days earlier at the US than in 2019. At the SR, the difference was smaller, as grass pollen season only started two days earlier in 2019. The pollen season ended in August, and the length of the season was almost identical for the US (-3 days in 2020) but 18 days longer in 2020 for the SRS. Thus, the year 2020, was (partly) a longer and more intense Poaceae pollen season. The start of the season was 9 days later at the US in 2019 but 1 day earlier in 2020 compared to the SRS. Instead, the end of the season was always recorded later at the US (2019: 4 days, 2020: 9 days), resulting in a shorter period in 2019 (-5 days) and a longer period in 2020 (+10 days).



**Figure 3.** Poaceae pollen concentration (daily mean values) for the SRS (black) and US (grey) locations in the greater area of Ingolstadt. Dashed lines indicate start and end dates of the corresponding grass pollen season (**a**) 2019 and (**b**) 2020.

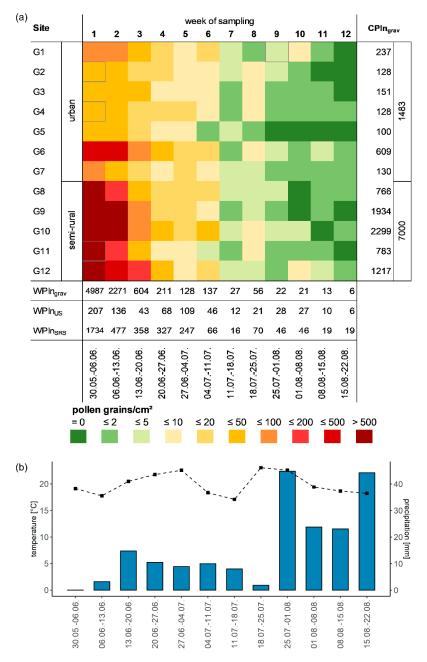
Table 3. Characteristics of the grass pollen season 2019 and 2020 for US (urban station) and SRS (semi-rural station) in the greater area of Ingolstadt.

|   | Ŭ         | <b>S</b>  | SI        | S         |  |
|---|-----------|-----------|-----------|-----------|--|
|   | 2019      | 2020      | 2019      | 2020      |  |
| Seasonal Pollen Index (SPIn) (pollen*day/m <sup>3</sup> ) | 933       | 2104      | 4071      | 5725      |  |
| Length of season (days)                                   | 102       | 99        | 107       | 89        |  |
| Start day/day of year                                     | 16.05/136 | 04.05/125 | 07.05/127 | 05.05/126 |  |
| End day/day of year                                       | 25.08/237 | 10.08/223 | 21.08/233 | 01.08/214 |  |
| Peak day/day of year                                      | 05.06/156 | 02.06/154 | 04.06/155 | 01.06/153 |  |
| Peak value (pollen grains/ $m^3$ )                        | 46        | 120       | 371       | 506       |  |
| High pollen days  | 0         | 6         | 25        | 33        |  |

## 3.2. Gravimetric Pollen Traps

## 3.2.1. Sampling Campaign 2019

In general, semi-rural locations were linked to higher values for pollen deposition and urban locations to lower values, as illustrated in the heat map in Figure 4a. The course of grass pollen deposition cannot be described for the whole season, as sampling started when the season had already begun. In the first week of measurement, however, peak values at most sites were reached. The highest weekly pollen deposition during sampling (max. 1440 pollen grains/cm<sup>2</sup>) and CPIn<sub>grav</sub> (max. 2299) were recorded at the sites G9, G10, and G12, which are situated in the semi-rural environment. The lowest values (i.e.,  $CPIn_{grav} = 128$ ) were measured at G2 and G4, located in the inner city, and were partially secluded by buildings, e.g., in courtyards (see Table 1 for details). Pollen deposition in the peak week (30.5. to 6.6.), the week with the highest WPIn<sub>grav</sub>, was highest at G10 (semi-rural) with 1140 pollen grains/cm<sup>2</sup> and lowest at G4 (urban) with 39 pollen grains/cm<sup>2</sup> (approx. 3.5% of G10). The site with the highest CPIn<sub>grav</sub> was G10 (CPIn<sub>grav</sub> = 2299 pollen grains/cm<sup>2</sup>), and the lowest CPIn<sub>grav</sub> was recorded for G5 (urban park; CPIn<sub>grav</sub> = 100 pollen grains/cm<sup>2</sup>). Kruskal–Wallis test revealed no significant difference between sites (Kruskal–Wallis chi-squared = 7.301, *p*-value = 0.774).



**Figure 4.** (a) Heat map showing weekly grass pollen deposition (pollen grains/cm<sup>2</sup>) in 2019 for the sampling campaign figure 30 May to 22 August 2019 and WPIn<sub>US</sub> and WPIn<sub>SRS</sub> for the respective weeks. Gravimetric traps are ordered by distance from the urban station (US). A grey outline indicates interpolated values. (b) Weekly mean temperature (black squares/dotted line) and weekly sum of precipitation (blue bars) during the sampling campaign with gravimetric traps from 30 May to 22 August 2019.

During sampling, mean temperature was 19.9 °C, and precipitation sum was 193 mm (Figure 4b). There was no week without precipitation. In the week with the highest WPIn<sub>grav</sub>, we recorded 1 mm of rain. During sampling, the wind came predominantly from the west, the southwest, and the northwest. The mean wind speed was 1.3 m/s. In the first weak of sampling, there was mostly westerly wind with a mean wind speed of 2.5 m/s.

## 3.2.2. Sampling Campaign 2020

In 2020, semi-rural sites were again linked to higher values of pollen deposition (Figure 5a). The course of the grass pollen season can be seen in the heat map (Figure 5a). The peak around the first week of June (week 7) can also be found for both WPIn<sub>US</sub> and WPIn<sub>SRS</sub>. Pollen deposition in the peak week (1 June–8 June) was highest at G8 (semi-rural), with 954 pollen grains/cm<sup>2</sup> and lowest at G4 (urban) with 27 pollen grains/cm<sup>2</sup> (approx. 2.8% of G10). Furthermore, the highest single and total values were recorded at G10 and G12, both situated in the semi-rural environment. Kruskal–Wallis test and Wilcoxon rank -sum test revealed significant differences between sites: all significant differences occurred between semi-rural and urban sites; this means pollen deposition of every urban site was significantly different from at least the values of one semi-rural site (Table 4).

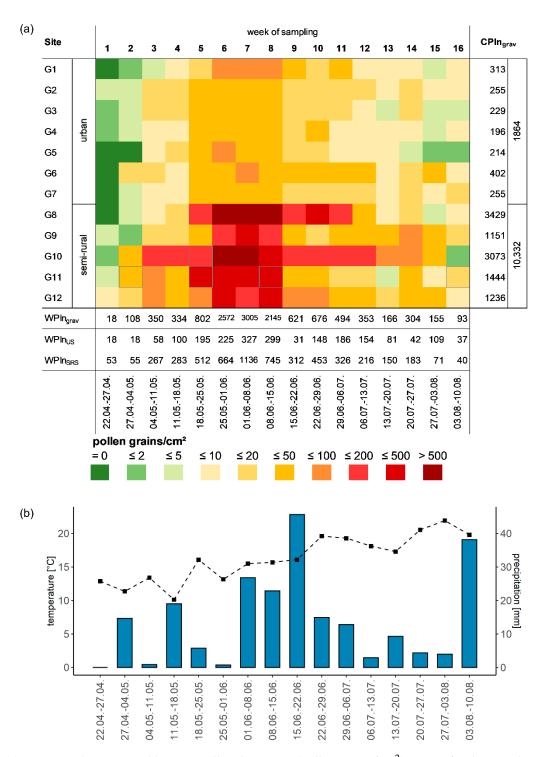
**Table 4.** Pairwise comparisons of pollen deposition 2020 between all sites using Wilcoxon rank sum test, bold values indicate significant differences (p < 0.05).

|            |     | Urban |       |       |       |       |       |       |       | Semi-Rural |       |       |  |
|------------|-----|-------|-------|-------|-------|-------|-------|-------|-------|------------|-------|-------|--|
|            |     | G1    | G2    | G3    | G4    | G5    | G6    | G7    | G8    | G9         | G10   | G11   |  |
|            | G2  | 0.756 | -     | -     | -     | -     | -     | -     | -     | -          | -     | -     |  |
|            | G3  | 0.985 | 0.969 | -     | -     | -     | -     | -     | -     | -          | -     | -     |  |
| ,          | G4  | 0.937 | 0.400 | 0.837 | -     | -     | -     | -     | -     | -          | -     | -     |  |
| urban      | G5  | 0.758 | 0.473 | 0.754 | 0.855 | -     | -     | -     | -     | -          | -     | -     |  |
|            | G6  | 0.235 | 0.273 | 0.168 | 0.055 | 0.110 | -     | -     | -     | -          | -     | -     |  |
|            | G7  | 0.760 | 1     | 0.762 | 0.415 | 0.492 | 0.235 | -     | -     | -          | -     | -     |  |
|            | G8  | 0.235 | 0.489 | 0.400 | 0.220 | 0.201 | 0.836 | 0.534 | -     | -          | -     | -     |  |
|            | G9  | 0.117 | 0.095 | 0.082 | 0.033 | 0.042 | 0.534 | 0.087 | 0.985 | -          | -     | -     |  |
| semi-rural | G10 | 0.010 | 0.007 | 0.006 | 0.006 | 0.006 | 0.010 | 0.006 | 0.510 | 0.105      | -     | -     |  |
|            | G11 | 0.087 | 0.087 | 0.078 | 0.029 | 0.042 | 0.471 | 0.140 | 0.888 | 0.834      | 0.220 | -     |  |
|            | G12 | 0.028 | 0.029 | 0.022 | 0.010 | 0.011 | 0.147 | 0.029 | 0.816 | 0.534      | 0.201 | 0.770 |  |

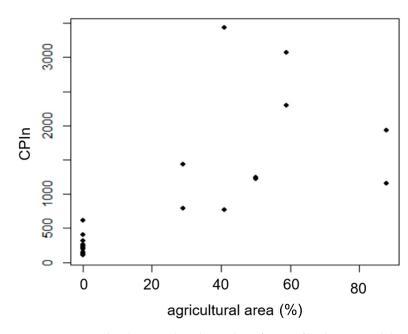
During sampling, the mean temperature was 16.4 °C and the precipitation sum was 222.9 mm. There was no rain in the first week of sampling, and less than 1 mm in the 6th week. In the peak week, we recorded 26.8 mm of rain (Figure 5b). During sampling, the wind came predominantly from the west, east and northeast. The mean wind speed was 2.3 m/s. In the peak week, the predominant wind direction was western; the mean wind speed was 2.5 m/s.

## 3.2.3. Land Use

Table 2 gives an overview of the land use surrounding the traps. Based on the Grassland 2018 dataset [46], locations with a grass area in a radius of 100 m surrounding the traps were only documented for five sites: G11 (39%), G10 (25%), G8 (22%), G12 (11%), and G1 (6%). We investigated the relationship of CPIn and grass cover, land cover and urban index in the surrounding 100 m (Tables 1 and 2) of the respective trap locations. The highest correlation coefficient was revealed for CPIn and the percentage of agricultural area ( $r_s = 0.836$ , p < 0.005) (Figure 6), the lowest for the percentage of grass cover ( $r_s = 0.692$ , p < 0.005), and a negative correlation for Urban Index ( $r_s = -0.726$ , p < 0.005).



**Figure 5.** (a) Heatmap showing weekly grass pollen deposition (pollen grains/cm<sup>2</sup>) in 2020 for the sampling campaign from 22 April to 10 August 2020, WPIn<sub>grav</sub>, WPIn<sub>US</sub>, and WPIn<sub>SRS</sub> for the respective weeks. Gravimetric traps are ordered by distance from the urban station (US). A grey outline indicates interpolated values; (b) weekly mean temperature (black squares/dotted line) and weekly sum of precipitation (blue bars) during the sampling campaign with gravimetric traps from 22 April to 10 August 2020.



**Figure 6.** Scatterplot showing the relationship of CPIn of both years and the percentage of agricultural area within a 100 m radius of each sampling location.

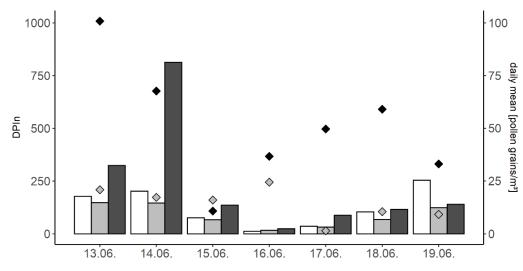
# 3.3. PVAS Sampling Campaign 2019

#### 3.3.1. Daily Variation

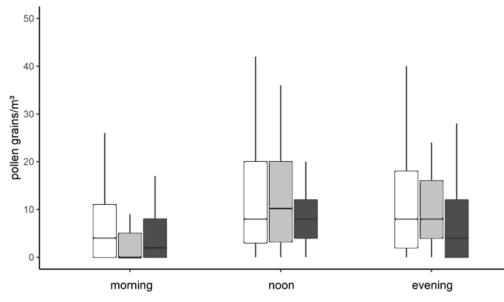
DPIn<sub>PVAS</sub> for locations in semi-rural (SR), urban (U), or residential (R) environments (see classification in Table 2) differed considerably during the seven days of sampling (Figure 7). The highest values were measured at sites located in SR, and the lower values in U. The highest means were associated with the sites SR2 (42 pollen grains/ $m^3$ ), SR4 (17.6 pollen grains/ $m^3$ ), and SR1 (15.8 pollen grains/ $m^3$ ) located in the countryside. The lowest means were associated with SR3 (3.6 pollen grains/ $m^3$ ) and R3 (7.6 pollen grains/ $m^3$ ). There were no significant differences between all eleven sampling locations (Kruskal–Wallis chi-squared = 11.937, *p*-value = 0.289). On the first two days, the highest DPIns for SR and R were recorded. DPIns were lowest on 16 June and 17 June, days, which were affected by precipitation. The rainy period started in the evening of 15 June, just after the end of the evening measurements, and lasted until 3 a.m On the morning of 16 June, there was light rain between the first and second measurements of the day. The mean temperature for the sampling period was 20.8 °C, and total precipitation was 14.8 mm, which mostly fell between measurements. Wind directions and wind speed varied between the days: on 13, 14, 17, and 18 June, wind predominately came from northeast and east; on 15 and 16 June from north, northwest, and west; and on 19 June from south, southeast, and southwest. The highest wind speed was recorded on 15 June (7.6 m/s).

#### 3.3.2. Diurnal Variation

Analyses of diurnal variation showed that pollen concentrations for all sites, independent from their degree of urbanisation, were highest at noon (Figure 8). Mean pollen concentrations for measurements in the morning were 8.4 pollen grains/m<sup>3</sup>, at noon 22.2 pollen grains/m<sup>3</sup>, and in the evening 10.4 pollen grains/m<sup>3</sup>. The differences between the three daily measurements were significant (Kruskal-Wallis chi-squared = 14.471, *p*-value = 0.001). Wilcoxon rank-sum test revealed significant differences between morning and noon measurements (*p* = 0.001) and between noon and evening measurements (*p* = 0.006). Instead, the concentrations did not differ significantly between noon and evening measurements (*p* = 0.327).

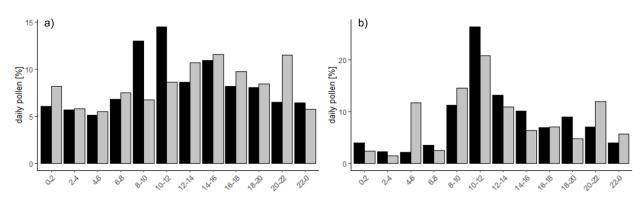


**Figure 7.** Sum of DPIn<sub>PVAS</sub> for locations in urban area/city centre (U; white), residential area (R; light grey), and semi-rural environment (SR; dark grey). Squares represent daily mean pollen concentration measured at rooftop level: black—SRS and grey—US.



**Figure 8.** Boxplots showing distribution of pollen concentrations by measurement period. Horizontal line = median, boxes = interquartile range. Outliers not shown. Colours for sampling locations: white—city centre, light grey—residential area, dark grey—semi-rural area.

We compared our data on diurnal variation with data measured by the two 7-day volumetric samplers at roof level during PVAS sampling and during the whole season (Figure 9). Considering the whole season, most grass pollen was measured between 2 and 4 p.m. and between 8 and 10 p.m. at the US. At the SRS, most grass pollen was measured between 8 a.m. and 12 p.m. (Figure 9a). Just considering the week of the PVAS sampling campaign, peaks for both SRS and US were between 10 a.m. and 12 p.m. (Figure 9b). These results show similarities to PVAS measurements, where the mean pollen concentration was highest at noon.



**Figure 9.** Diurnal pattern of Poaceae pollen concentration recorded by both 7-day volumetric traps, grey—US, black—SRS (a) during 2019; (b) during the week of PVAS sampling (13 June to 19 June 2019).

#### 3.3.3. Land Use

Land use surrounding the traps varied depending on locations (Table 2). Based on grassland data (European Union 2020), areas covered with grass within 100 m were found in 7 out of 11 locations: U2 (1%), U4 (15%), R3 (6%), SR1 (10%), SR2 (15%), SR3 (48%), and SR4 (11%). Based on land cover classification 2019 [43], areas defined as low vegetation could be found in the surroundings of at all sites except U3; agricultural areas were present at sites SR1 (20%), SR2 (47%), SR3 (46%), and SR4 (42%). We found no statistically significant relationship between CPIn<sub>PVAS</sub> and the percentage of agricultural area, grass cover, low vegetation, or Urban Index.

## 4. Discussion

With this study, we are adding to the few studies that combine different types of pollen traps (e.g., [31,39,41]). We used two 7-day volumetric pollen traps to gather data on background pollen concentration, twelve gravimetric pollen traps to assess spatial variations of pollen deposition for two grass pollen seasons, and portable volumetric traps to assess spatial and diurnal variations of pollen concentration at eleven locations. The volumetric trap at roof level is suitable to measure background pollen concentration for a larger region. With the portable volumetric pollen traps that we used (PVAS), differences at smaller spatial and temporal scales can be addressed. With the placement of this trap at street level, the results are probably more relevant for people allergic to pollen, since sampling is conducted at breathing height. Gravimetric pollen traps can be used to compare cumulated pollen deposition for a longer interval, which cannot be done with PVAS. Our results indicate that the differences between pollen amounts recorded at urban and semi-rural locations mainly originated from different land use (more pollen was linked to agricultural land and grass areas nearby) and land management (i.e., grass cutting).

#### 4.1. Spatial Variations

# 4.1.1. Background Pollen Concentration

Volumetric pollen traps set up at roof level are not only useful to receive data on background grass pollen concentration; these data are also appropriate to place data obtained by sampling campaigns (conducted at street level and at certain periods or intervals) into a broader context. Analyses of background pollen concentrations at the two stations revealed different characteristics of the pollen seasons of both studied years. We found that the more rural site was linked to higher peak values in both years, a higher number of high pollen days, and SPIn (see Table 3).

This finding was also confirmed in other studies: Antón et al. (2020) [55] reported that total pollen, peak values, and days with high levels were higher and that peak days were one day earlier at the less urban site. Bosch-Cano et al. (2011) [56] counted a higher number of days with more than 10 pollen grains/m<sup>3</sup> at more rural sites. In general, the urban heat island effect is associated with an earlier flowering, which is most pronounced

for plants flowering in spring [57]. In our study, we could not confirm an earlier onset of the flowering period in 2019 using aerobiological data since the start was recorded nine days later. In 2020, the difference between the US and the SRS was only one day. This varying pattern might be attributable to different land use and management, i.e., the abundance of grass or different species with different flowering periods. The placement of a trap several meters above the ground implies the exposure to other influences such as the dominance of regional winds, the additional input of long-range transported pollen, and other differences in meteorological parameters compared to the ground. Therefore, these different conditions of trap exposure should be kept in mind when comparing data of pollen traps set up at different heights above the ground [28,58]. A wind sensor attached to the top of the pollen trap could give detailed information on the wind direction and therefore on the possible pollen source.

#### 4.1.2. Gravimetric Sampling Campaigns

Using gravimetric samplers, we observed differences in pollen deposition across sampling locations in both years. Despite different grass species contributing to the Poaceae pollen spectrum, the seasonal pattern was rather uniform in 2019 and 2020, with the main peak in the first week of June as also confirmed using the results of the volumetric rooftop trap. The highest CPIn<sub>grav</sub> in 2019 with a value above 1000 were measured at sites classified as semi-rural (Table 1). These high values of the CPIn<sub>grav</sub> can be attributable to a higher abundance of grass areas, as well as fewer obstructions such as buildings or courtyard walls that might hinder airflow and transport of pollen to the traps [33]. Surprisingly, in 2020, CPIn<sub>grav</sub> at G8 was much higher compared to the previous year (considering the same sampling period of 10 weeks, more than three times higher). This can be explained by the different management of an agricultural field adjacent to the trap (Table 1). In 2019, the field consisting of mostly *Plantago* sp., *Rumex* sp., Poaceae, and *Trifolium* sp. was mowed more than once during the sampling period, which hindered long-lasting flowering of grasses. In 2020, the field was not mowed until mid-August, allowing grass species to flower for a much longer period and therefore contributing to a higher pollen deposition.

The pairwise comparison of pollen deposition in 2020 (Table 4) revealed significant differences between urban and semi-rural sites, e.g., between G1 and G10 and G12, G2 and G10 and G12, G3 and G10, and G12 and G4. There were no significant differences between different urban sites or between different semi-rural sites. Thus, inner-urban exposure can be regarded as identical to each other but different from those in semi-rural conditions. A study conducted by Werchan et al. (2017) [30] tested for spatial autocorrelation and found a positive correlation between nearby as well as farther traps in most cases. In our other year of sampling, 2019, however, no significant differences could be detected. Factors responsible for differences in grass pollen concentration comprise land management/crop cultivation [59] but also meteorology before, during, and after anthesis, including long-range transport and resuspension [60]. Long-term monitoring at these sites would allow a more profound conclusion about the reasons for diminished differences in certain years.

The positive and strong correlation of  $\text{CPIn}_{\text{grav}}$  (year 2020) with the coverage of agricultural land ( $r_s = 0.836$ , p < 0.005; Figure 6) and weaker correlation with grass cover ( $r_s = 0.692$ , p < 0.005) suggests that agricultural areas are a more suitable indicator for the amount of pollen deposition in semi-rural environments. However, the basis of the data set has to be considered. Even with a comparatively good spatial resolution of 10 m, the data set does not completely reflect current conditions, and smaller areas might not be considered. As various different grass species are distributed within the city and the countryside at many locations, the real conditions of pollen emissions cannot be depicted using these data. Similar findings were reported by Cariñanos et al. (2019) [25] and Charalampopoulos et al. (2018) [26]. Besides reasons related to the data source and the associated resolution, grass areas might often be grazed or mowed and therefore do not necessarily present areas with high pollen emission. The PVAS sampling campaign, instead, did not reveal any significant correlations, pointing to the temporal effect of land management. In addition, an even

higher resolution of land cover data may be appropriate to identify small grass patches. Studies focusing on these data or on an extended vegetation survey might reveal even stronger correlations between pollen concentration and land use characteristics.

The negative correlation with Urban Index ( $r_s = -0.726$ , p < 0.005) is similar to observations in the study by Hugg et al. (2017) [33], who demonstrated that pollen amounts decreased with increasing urbanisation. Reference [30] recorded for the study area of Berlin the highest grass pollen deposition at a site close to agricultural land with permanent pastures and grassland. The great influence of local pollen sources has also been pointed out by Ciani et al. (2020) [61].

Since mean wind speed during the peak week in 2019 and 2020 was both 2.5 m/s, these conditions, combined with low precipitation and high temperatures, have probably also contributed to the high pollen catch (see Figures 4 and 5).

The use of gravimetric pollen traps is a cost-effective way to measure pollen deposition at various locations. While advantages include easy operation, low cost, the absence of moving parts, and the requirement for power; disadvantages are the method itself, i.e., the passive sampling. This method is solely based on the sedimentation of pollen, and the volume of air that is sampled is unknown [62]. Therefore, neither a concentration (particles per volume) nor the sampling efficiency can be determined, which leads to the fact that pollen deposition cannot directly be compared to traps recording pollen concentration. Furthermore, the catch is relatively low and influenced by wind speed, direction, and turbulence [62].

## 4.1.3. PVAS Sampling Campaigns

Our results from the PVAS sampling campaign showed that sites located in the semirural environment (SR) were predominately linked to higher Poaceae pollen concentrations, except for SR3. Although this site was located next to agricultural fields, we recorded low pollen concentrations there. Instead, we recorded much higher values at SR2, located at a distance of approx. 1.5 km with similar surroundings. The reason for this is not entirely clear—it could originate from a sampling or operational error or a gust of wind transporting more grass pollen. Our results indicate a great variance in pollen concentrations measured throughout the sampling period (Figure 8). This variance is also shown by the daily mean pollen concentration measured each day at rooftop level at the SRS and US. This might be due to changing meteorological parameters (during day and night or influenced by the general weather situation) such as wind direction, wind speed, temperature, and humidity. Especially the latter factors are responsible for anther dehiscence and thus influence the process of pollen emission. These factors might have a greater influence on near-ground measurements with a short sampling duration (in our study, 25 min). Other aerobiological studies were conducted with similar or even shorter sampling durations, e.g., 15 min [39] or 30 min [38]. In Section 2.1, we included references with similar findings of our observed spatial variation.

The sampling campaign was strongly influenced by precipitation on the third and fourth days, as well as lower temperatures on the fourth and fifth days. Wind speeds were highest on the third day, just before sampling in the morning. These weather conditions seemed to reduce pollen concentrations at all locations, which was also observed by Laaidi et al. (2013) [63], who showed that *Betula* pollen dispersal was promoted by windy conditions and low precipitation (less than 2 mm). A study has shown the negative correlation between pollen concentration and precipitation [55] and the positive correlation with wind speed close to the pollen trap [36]. Peel et al. (2014) [41], on the other hand, reported significant negative correlations of pollen concentration measured with PVAS and wind speed, which can be attributable to the horizontally oriented orifice and the absence of a wind vane, which leads to a reduced sampling efficiency. Nevertheless, PVAS are useful and easy-to-handle devices for measuring pollen concentration at street level, especially during calm conditions due to the reported shortcomings related to strong winds. When aiming for measurements at multiple locations, operating them, however, can be

labour-intensive, because each trap needs to be supervised for changing microscope slides manually. In addition, these slides need to be changed after a short sampling period in order to avoid overlay of pollen and particles.

# 4.1.4. Land Use Data

Remotely sensed land use and grass cover data are a good source of standardized data, especially since field assessment is time-consuming. However, land management, i.e., mowing of grass, cannot be detected solely with land cover data, and the resolution of the dataset might influence its accuracy. Therefore, it is suggested to note land management for the duration of pollen sampling since this factor might strongly influence local pollen abundance. For example, the severity of hay fever symptoms can be strongly influenced by the timing of hay cutting [59]. Skjøth et al. (2013) [64] compared measured pollen concentrations with NDVI based maps of grass pollen sources and highlighted that only a part of grass areas is able to reach maturity and produce flowers because of the strong influence by management. They suggest a combination of NDVI maps and management maps to identify grass pollen sources successfully. Furthermore, they state that grass pollen concentrations reflect the source distribution and is therefore a local-scale phenomenon, which is also supported by the results of our PVAS sampling campaign (Figure 7), and of one of our main findings: the importance of small-scale land management in addition to land use data. Meteorological data, especially wind direction [65], recorded at every sampling location could provide further insights into the transport of pollen from the surroundings.

## 4.2. Diurnal Variations

Results obtained from outdoor volumetric traps at roof level revealed that grass pollen concentration was lowest in the morning hours between 2 a.m. and 6 a.m. in urban areas. This was also observed by Latałowa et al. (2005) [12], who observed the lowest concentrations of Poaceae pollen at an urban site in Gdańsk, Poland, between 1 a.m. to 7 a.m. For the semi-rural site, we recorded the lowest grass pollen concentration between 1 a.m. and 6 a.m. as well. In our study, at the semi-rural site, most pollen was present between 8 a.m. and 12 p.m., and at the urban site between 2 p.m. and 4 p.m. and 8 a.m. and 10 p.m.

A similar pattern with a distinct and partly earlier peak has been observed by Latałowa et al. (2005) [12]: The authors examined urban areas in Gdańsk, Poland, and detected high concentrations between 9 a.m. and 1 p.m. and in the late evening and low concentrations between 1 a.m. and 7 a.m In addition, Kasprzyk (2006) [15] found high grass pollen concentrations between 8 a.m. and 10 a.m. at an urban site in Rzeszów, Poland. At the rural site (at a distance of about 10 km), highest concentrations were detected between 6 a.m. and 6 p.m. with no distinct peak. Reference [10] observed lower grass pollen concentrations in the urban area in the morning and in the rural area in the evening. The same pattern with high concentrations in the early evening at urban sites and low concentrations in the morning were also found by Mullins et al. (1986) [11]. In rural areas, high concentrations were found at midday; four hours earlier than at the urban site.

Based on PVAS data, which was based on three daily measurements, a clear diurnal pattern could be observed: grass pollen concentrations differed significantly between morning and noon measurements and noon and evening measurements, regardless of the degree of urbanisation. When comparing background pollen concentrations, this holds true for the month of June, when our PVAS sampling campaign took place, but not for the whole grass pollen season. Therefore, a sampling campaign with a duration of one week might not be sufficient to draw conclusions about the pattern of the whole year. This implies that the pattern detected by a short measurement campaign does not necessarily reflect the whole season and should not be used for universal recommendations. We also believe that the pattern of a single pollination period cannot be transferred to other periods or regions, as shown by the findings from the literature.

We propose correlating PVAS data with symptom data generated by Citizen Science (e.g., Pollen Diary/Patient's Hayfever Diary [66]; BAYSICS app [67]) as these data were collected at street level at the average breathing height of 1.5 m above ground level and covered heterogeneous environments. These further studies could add to the findings of previous studies [28,68]. In addition, gravimetric pollen traps, which allow the assessment of weekly cumulated pollen deposition, could be used in agriculture, to assess pollination intensity, or in landscape ecology, where data on pollen deposition could help to understand the fragmentation of, for example, whole plant populations.

In addition to different sampling efficiencies, different temporal resolutions (week vs. two hours) make comparisons within pollen monitoring challenging. There is no standard to compare pollen averaged or cumulated over a period other than a year or pollen season, for example over a week, day (e.g., with a small number of measuring times), or the duration of a sampling campaign. Indices, such as a Weekly Pollen Integral (WPIn), Daily Pollen Integral (DPIn), and Campaign Pollen Integral (CPIn), should be defined and included in the recommend terminology for describing aerobiological data. Furthermore, we suggest the use of "pollen grains per cm<sup>2</sup>" as a unit when dealing with gravimetric traps.

## 5. Conclusions

This study highlights the importance of considering local vegetation, land management and meteorology when measuring airborne pollen at street level. Furthermore, we presented a novel approach using three different types of pollen traps that were operating at different spatial and temporal resolutions. Due to the fact that we measured pollen not only on roof but also on ground level (PVAS and gravimetric traps), our results give detailed information on the grass pollen exposure, which is especially important for allergenic individuals. With the use of different pollen traps, we were able to show differences and similarities based on our results. We conclude that PVAS, which performed measurements for a short duration and only three times a day, are appropriate to reflect the general conditions (i.e., background pollen concentration obtained by volumetric trap at roof level). However, they cannot be used to characterize the mean diurnal conditions of the whole pollen season. Gravimetric traps are appropriate to show differences in cumulated pollen loads, but disadvantages resulting from the coarse temporal resolution have to be considered; i.e., connecting these results with meteorological data is challenging. For further research, our findings could be tested in a more controlled environment, e.g., using experimental pollen or spore release, which facilitates the assessment of distribution patterns in complex and heterogeneous urban environments. In order to draw conclusions about the effects of pollen concentrations on health, pollen data should be assessed in light of symptom data.

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