

Article

Birch Pollen Deposition and Transport along an Altitudinal Gradient in the Bavarian Alps—A Case Study Using Gravimetric Pollen Traps in the Pollen Season 2020

Verena Wörl, Johanna Jetschni and Susanne Jochner-Oette *

Physical Geography/Landscape Ecology and Sustainable Ecosystem Development, Faculty of Mathematics and Geography, Catholic University of Eichstätt-Ingolstadt, 85072 Eichstätt, Germany

* Correspondence: susanne.jochner@ku.de

Abstract: There is only little and partially contradicting knowledge on the variation of pollen abundance at different altitudes in mountainous regions. The aim of this work is to gain new insights on the influence of wind and surrounding trees on the deposition and transport of birch (*Betula spec.*) pollen in the Bavarian Alps, Germany. Data on birch pollen deposition were collected at five sites using gravimetric traps along an altitudinal gradient from Garmisch-Partenkirchen (720 m a.s.l.) to the Environmental Research Station Schneefernerhaus (2650 m a.s.l.) in the pollen season 2020. We compared these data with birch pollen concentration derived from a volumetric trap at Schneefernerhaus and with phenological data, i.e., flowering onset times observed at 21 birch trees at different altitudes. Wind data were gathered directly at or near each pollen trap and surrounding birch trees were mapped in the field. Whereas the pollen load was lowest at the highest location, substantially higher values were measured at medium altitudes (1300–1600 m a.s.l.). This can be explained by the pronounced mountain-valley wind system, which ensured the transport of pollen to the corresponding altitudes. We conclude that pollen levels are influenced by topography, local wind systems and the availability of pollen. Pollen levels in complex mountainous environments are therefore not substantially affected by the occurrence of birch trees in the immediate vicinity.

Keywords: aerobiology; *Betula*; elevation; gravimetric pollen traps; mountain-valley wind system; phenology

Citation: Wörl, V.; Jetschni, J.; Jochner-Oette, S. Birch Pollen Deposition and Transport along an Altitudinal Gradient in the Bavarian Alps—A Case Study Using Gravimetric Pollen Traps in the Pollen Season 2020. *Atmosphere* **2022**, *13*, 2007. <https://doi.org/10.3390/atmos13122007>

Academic Editor: László Makra

Received: 18 October 2022

Accepted: 25 November 2022

Published: 29 November 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 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/>).

1. Introduction

Plants produce pollen as part of their reproductive function [1]. Anemophilous species compensate for their low pollination performance by a high production of pollen to ensure fertilization [2]. In this context, birch (*Betula spec.*) is coming into focus because of its high allergenic potential, especially in Northern, Central and Eastern Europe [3]. The genus *Betula* originates from the Northern Hemisphere and comprises approx. 120 different species. In Europe, birches are particularly common in northern and central regions. In general, they grow in lowlands, but also at extreme sites, e.g., at high altitudes and latitudes [4]. In Germany, the genus is limited to an altitude of approx. 1800 m a.s.l. [5]. Most recent citizen-science-generated data indicate the occurrence of *Betula pubescence* in altitudes as high as 1840 m a.s.l. in the Bavarian Alps, and as high as 1754 m a.s.l. in the Wetterstein Mountains. Trees of *Betula pendula* were observed in altitudes up to 1610 m a.s.l. in the Bavarian Alps [6].

Its flowering is predominately observed between the months of March and May [7] and is associated with the release of a large number of pollen grains into the atmosphere [4]. Ranpal et al. [8] estimated the average pollen production of an inflorescence of *Betula pendula* (silver birch) at 1.7 million pollen grains. The highly allergenic birch pollen are a major source of allergic rhinitis in Europe [9,10]. In industrialized countries, allergic

rhinitis can affect more than 20% of the population [11]. Estimates suggest that 20% of hay fever patients in Central and Northern Europe suffer from birch pollen allergy [12]. Therefore, studying birch pollen is of major importance.

In general, warm and sunny days without precipitation favour a high number of pollen in the air [13]. Furthermore, solar radiation and temperature are most important for the growth and development of male catkins before flowering [14]. Wind speed and direction are mainly responsible for the dispersal of pollen of anemophilous species such as birch [9,14]. In principle, it is known that most of the pollen released in the air is deposited near its source [15,16]. Released pollen can be stirred up by turbulent vertical mixing and transported by the prevailing wind [9]. The distance that pollen grains can travel depends on many factors, including the velocity at which they sink downwards in still air caused by gravitational effects [17]. Pollen can also be transported downwards by (heavy) precipitation, resuspended after drying and involved in further airborne transport [18]. The release height of pollen from the source and the aerodynamic properties of different pollen species are also regarded as important influences on the transport of pollen [17,19]. Birch pollen are almost spherical, characterized by a size of approx. 20 μm in diameter, a low settling velocity (1 cm/s) and a release height of 5–25 m above ground [7]. Therefore, these pollen grains are among those that are furthest and most widely transported [20].

Wind as an influential environmental factor varies considerably in the mountains and generally leads to a high variability of pollen load at different altitudes [21]. In addition to wind, topography (e.g., steep slopes) plays a decisive role in the pollen dispersal and transport of pollen. Charalampopoulos et al. [21] found that the pollen of various anemophilous species could increase or decrease due to wind, depending on the mountain acting either as a source or as an obstacle to pollen transport.

In general, flowering of plants is delayed due to lower temperatures at higher sites [7]. With an increase of 100 m in altitude, air temperature decreases by approx. 0.6 $^{\circ}\text{C}$ [22]. Jochner et al. [7] reported a delay of birch flowering of 3 days/100 m along an altitudinal gradient in the Bavarian Alps, Germany, which corresponds to a phenological response rate to temperature of approx. $-7 \text{ days}/^{\circ}\text{C}$.

Airborne pollen concentration normally decreases with increasing altitude [23]. However, Frei [24], for example, found a larger amount of allergenic pollen including *Betula* at a treeless, high-altitude site at 2300 m a.s.l. in Switzerland. High pollen concentrations at higher locations can be partly explained by long- or medium-range transport of pollen by moving air masses. Menzel et al. [25] presented a climatology of pollen transport to Bavaria and documented that the start of the pollen season of different taxa (including *Betula*) at an alpine pollen station (Oberjoch) is primarily related to non-local pollen sources. Pauling et al. [26] found that the pollen load on mountain peaks can be similarly high as in adjacent valleys. Therefore, not only altitude (and its related vegetation abundance), but also actual weather conditions can have an influence on pollen concentrations.

In this study, we investigated pollen deposition and transport in 2020 in the area of Garmisch-Partenkirchen (Bavarian Alps, Germany) using five gravimetric pollen traps located at different altitudes ranging between 720 and 2657 m a.s.l. Our central aims were:

1. to detect differences in pollen deposition and transport at different altitudes;
2. to investigate the influence of wind patterns on the transport of pollen at the selected locations;
3. to identify the influence of the phenology and the proximity of birch trees in the vicinity of the pollen traps on registered pollen loads.

2. Study Area

The study area is located in Southern Bavaria (Germany) in the topographically complex region of the Zugspitze area in the Wetterstein Mountains (Figure 1a,b). The Zugspitze (2962 m a.s.l.) is the highest mountain in Germany and belongs to the Northern Limestone Alps [27]. The gravimetric pollen traps were placed at an altitudinal distance of approx. 300 m from 720 m a.s.l. in Garmisch-Partenkirchen at a building of the German

Meteorological Service [DWD] to 2657 m a.s.l. at the Environmental Research Station Schneefernerhaus [UFS] below the Zugspitze peak. Because of snow conditions, which commonly still prevail in early spring, traps were only installed up to 1619 m a.s.l. and again at 2657 m a.s.l. at the UFS, which could be reached with a cable car. Table 1 and Figure 2a–e give an overview of the site characteristics.

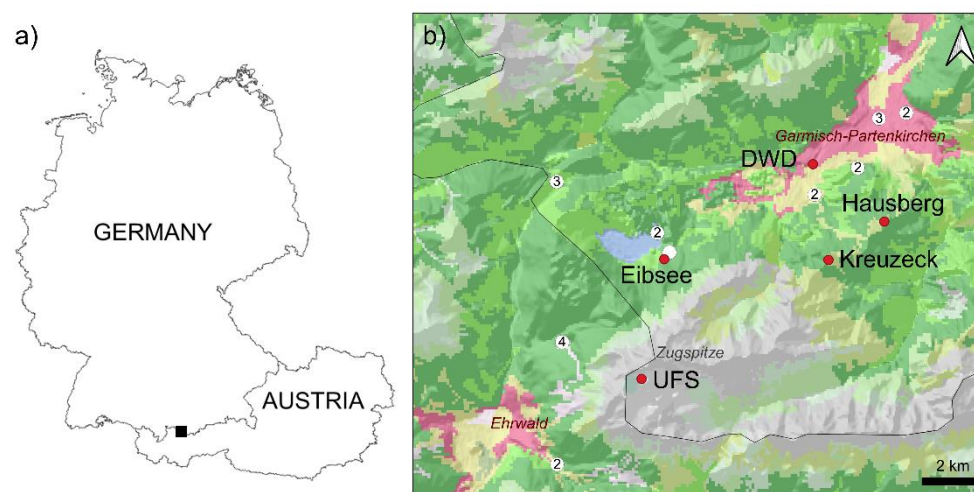


Figure 1. (a) Study area located in southern Germany (GeoBasis-DE/BKG 2018) and (b) locations of the gravimetric pollen traps (red dots) and locations of the examined birch trees (white circles; numbers indicate the amount of birch trees at this location when there was more than one). The line represents the border between Germany and Austria. Land cover: © European Union, Copernicus Land Monitoring Service 2018, European Environment Agency (EEA); Hillshade: EuroGraphics.

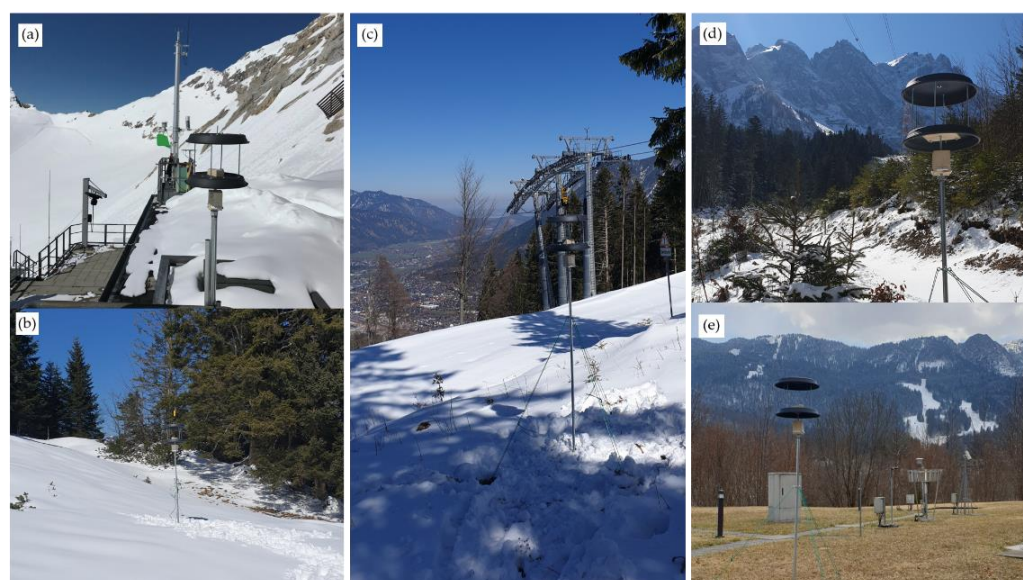


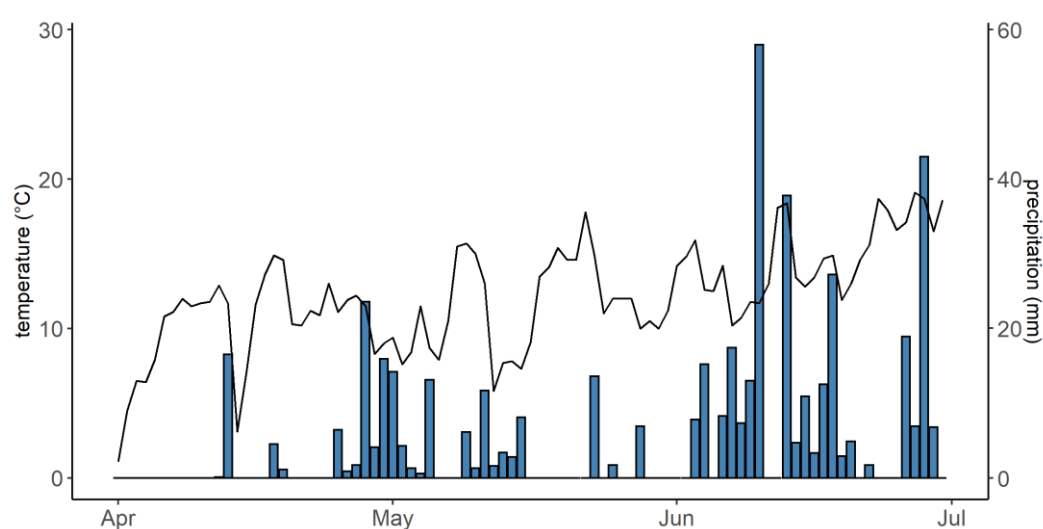
Figure 2. Gravimetric pollen traps at (a) UFS, (b) Kreuzeck, (c) Hausberg, (d) Eibsee and (e) DWD.

Table 1. Characteristics of the locations of gravimetric pollen traps.

Site	Coordinates	Elevation [m a.s.l.]	Number of <i>Betula</i> Trees within 400 m
UFS	47°24'59" N, 10°58'46" E	2657	0
Kreuzeck	47°27'09" N, 11°04'05" E	1619	0
Hausberg	47°27'51" N, 11°05'40" E	1329	0
Eibsee	47°27'14" N, 10°59'29" E	1016	10 (8 north and 2 east of the trap)
DWD	47°28'59" N, 11°03'43" E	720	8 (north, west and south of the trap)

A treeless alpine environment characterizes the highest site, the UFS. Here, the pollen trap was placed at the measurement platform of the research station. At the subalpine surrounding of Kreuzeck (1619 m a.s.l.), the forest mainly comprises spruce, fir and mountain pine. The pollen trap at Hausberg (1329 m a.s.l.) was located between a spruce forest and the infrastructure of the mountain railway and ski slopes. At lake Eibsee (1016 m a.s.l.), the pollen trap was placed near Eibsee Alm, underneath the infrastructure of the cable car and surrounded by forest in the east and west. The pollen trap located at DWD (720 m a.s.l.) presents the lowest elevation and is characterized by urban settlement and the river Loisach.

The weather in the region around the Zugspitze is characterized by seasonally varying large-scale weather situations. The topographical influence of the mountains leads to a clear difference in weather compared to the Alpine foothills [28]. The average annual temperature recorded at Zugspitze is $-4.3\text{ }^{\circ}\text{C}$ and the average precipitation sum amounts to 2084 mm (1981–2010). At Garmisch-Partenkirchen, the mean temperature is $7.2\text{ }^{\circ}\text{C}$ and precipitation sum is 1379 mm [29]. During the sampling campaign from April to the end of June 2020, the mean temperature was $12.2\text{ }^{\circ}\text{C}$ and the precipitation sum was 474 mm (Figure 3).

**Figure 3.** Daily meteorological data recorded at DWD station ‘Garmisch-Partenkirchen’ between 1 April and 30 June 2020 (solid black line—temperature, blue bars—precipitation). Data: DWD 2021.

3. Methods

3.1. Pollen Trapping and Preparation

In this study, self-constructed gravimetric pollen traps [30,31] based on the principles published by Durham [32] were used to determine pollen deposition (Figure 4). These traps passively collect airborne pollen by gravimetric deposition on a horizontally orientated microscope slide, which was coated with Vaseline. Vertical microscope slides orientated at the four cardinal directions (north, east, south and west) were used to capture information on pollen transport.

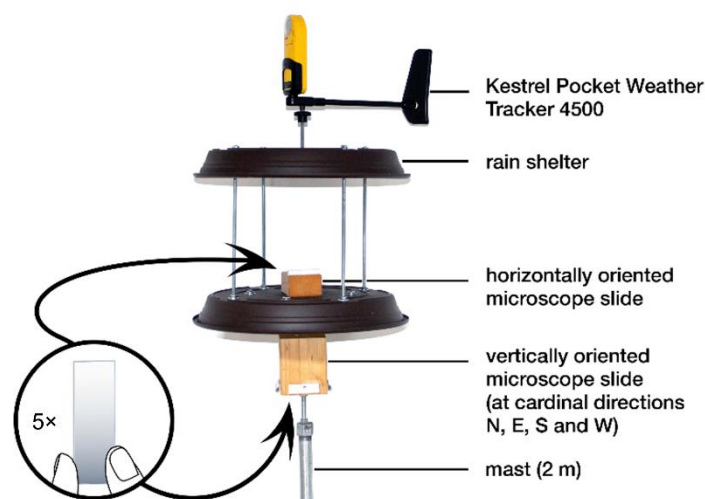


Figure 4. Gravimetric pollen trap used in this study.

Pollen measurements were conducted in spring/summer of 2020 (1 April to 3 June for Kreuzeck, Hausberg, Eibsee and DWD and until 1 July for UFS). The gravimetric pollen traps were installed at a height of two meters above the ground. The microscope slides were changed weekly around midday. Thus, the end date of a week is likewise the start date of the following week.

For comparison, we used data from a volumetric Hirst-type [33] pollen trap (Burkard Manufacturing Co Ltd., Rickmansworth, UK) at UFS, which is operated by the Technical University of Munich. This pollen trap is based on an integrated pump and aspirates 10 l of air per minute through an orifice. Airborne pollen sticks on a Vaseline-coated sample stripe adhered to a moving drum. The strip was cut into seven pieces, each presenting one sampling day, and fixed to microscope slides.

A mixture of distilled water, gelatin, glycerol and staining safranin was applied to the microscope slides. The slides were subsequently covered with slips and sealed with clear varnish after drying. Birch pollen were then counted under a light microscope (400x magnification, Zeiss Axio Lab A1; Wetzlar, Germany). We counted birch pollen along five equally distributed horizontal lines (approx. 10% of the area of the microscope slide). The number of pollen was converted to pollen grains per cm² (gravimetric trap) or pollen grains per m³ (volumetric trap).

3.2. Meteorological Data

On top of the gravimetric pollen traps at Kreuzeck, Hausberg and Eibsee, we installed a Kestrel Pocket Weather Tracker 4500 (Nielsen-Kellerman, PA, USA) with a moving arm for wind measurements. Due to a technical defect of the measurement device at Kreuzeck, we included meteorological data from a nearby meteorological station, which is operated by the Technical University of Munich [34] and measures meteorological parameters including wind speed and direction. Weather data for the location UFS were obtained from the meteorological station operated by the Environmental Research Station

Schneefernerhaus; data for the location DWD were downloaded from the CDC server [29]. All wind data therefore derive from the close or even closest proximity of the trap.

3.3. Phenological Data

We mapped all birch trees that were observed in a radius of 400 m around the pollen traps (see Table 1) and observed the flowering phenology of 21 birch trees at different altitudes, ranging from 696 m a.s.l. to 1222 m a.s.l. in Germany and Austria (Figure 1). Higher locations were not included due to aggravated accessibility. We noted the onset date of the beginning of flowering, i.e., the date of pollen release, for each birch tree.

3.4. Data Analyses

In order to make a comparison between the data derived from the gravimetric traps and the volumetric trap, the daily mean concentrations (pollen grains/m³) of birch pollen were summed up. This weekly pollen integral (WPI_n, [30]) was then compared with the weekly pollen deposition. The start and end date of the birch pollen season was calculated based on volumetric data and defined as the days where 1% and 99% of the Annual Pollen Integral (API_n) was reached, following the definition of the European Aeroallergen Network (EAN) [35]. We calculated the Campaign Pollen Integral (CPI_n), which is the sum of all measurements for each site [36]. We created heat maps to visualize the course of pollen deposition, transport, WPI_n and CPI_n throughout the sampling period.

The data on wind measurements were evaluated using wind roses. The week from 8 April to 15 April 2020 was a week with particularly high pollen loads at all locations and was examined more closely with regard to the influence of wind. In order to determine any local wind systems that may influence pollen transport, the data were categorized as daytime (7 am to 9 pm) or nighttime (9 pm to 7 am). At Hausberg, wind data were not available between 10 and 15 April because of a technical failure.

We calculated Spearman's rank correlation coefficient to analyse relationships between the start of flowering and elevation. Statistical analyses and visualizations were conducted using RStudio (version 1.3.5, Boston, MA, USA) with packages ggplot2 [37] and OpenAir [38]. We used QGIS (version 3.14, QGIS Association, <http://www.qgis.org>) to visualize spatial data and Microsoft Excel 2016 to create heat maps.

4. Results

4.1. Pollen Deposition and Phenology

In the first week of the measurement, from 1 April to 8 April, pollen deposition varied between 5 pollen grains/cm² at Eibsee and 18 pollen grains/cm² at DWD (Figure 5). The greatest value was registered at almost all sites in the second week of the study period, from 8 April to 15 April. This week was characterized by average maximum temperatures of 19.5 °C (DWD station "Garmisch-Partenkirchen") and hardly any precipitation (2.1 mm). Values between 53 pollen grains/cm² at DWD and 197 pollen grains/cm² at Kreuzeck were measured. The highest deposition at DWD, however, was measured in the third week (15 April to 22 April 2020) with 60 pollen grains/cm². This deposition value is also the highest of the third sampling week. The lowest pollen deposition of 19 pollen grains/cm² was recorded in the corresponding week at UFS. Overall, deposition values decreased from the second half of April onwards, and in May and June, very little pollen were occasionally observed at the sites.

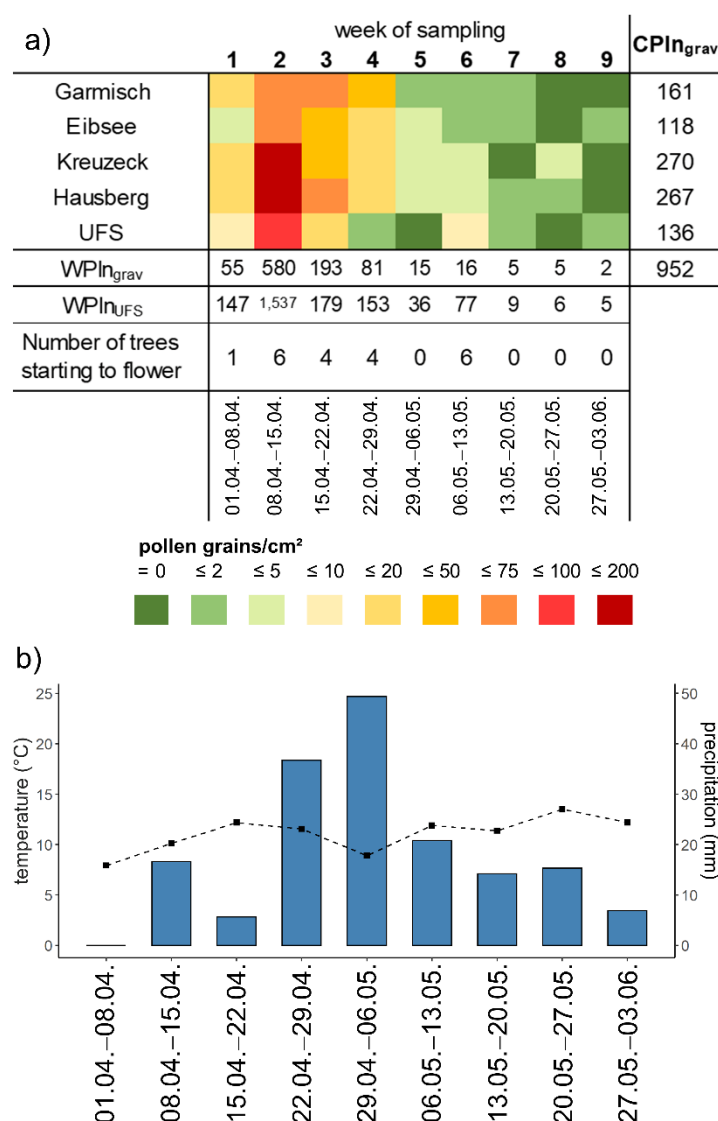


Figure 5. (a) Heat map showing weekly birch pollen deposition (pollen grains/cm²) on the horizontal slides during the flowering period 2020, WPIn_{UFS} and the number of birch trees starting to flower in the respective week. (b) Weekly mean air temperature (black points) and weekly sum of precipitation (blue bars) recorded at Garmisch-Partenkirchen between 1 April and 3 June 2020.

Over the entire period, most pollen were deposited on the microscope slides at Kreuzeck (270 pollen grains/cm²) and Hausberg (267 pollen grains/cm²). The birch pollen deposition at DWD, with a total of 163 pollen grains/cm², was substantially lower. Even lower values were measured at UFS (136 pollen grains/cm²) and Eibsee (118 pollen grains/cm²). At the sites above 1300 m a.s.l., at UFS, Kreuzeck and Hausberg, more than 70% of the total pollen were already deposited after the second week. This percentage was not reached at Eibsee and DWD until the third week of measurement.

The course of pollen deposition and concentration at UFS shows a similar temporal development. Since pollen data derived from the volumetric pollen trap provides daily mean values of pollen concentration, these can be added up to calculate the WPIn (Weekly Pollen Integral). The maximum was measured with both pollen traps in the period from 8 April to 15 April. Afterwards, a clear decrease was detected. In the week from 6 May to 13 May, a slight increase in pollen deposition was measured.

Based on volumetric data, we found that the birch pollen season at 2657 m a.s.l. started on 3 April reached its peak on 12 April (779 pollen grains/m³) and ended after 45

days on 18 May. The cumulated pollen concentrations within the measuring period of the volumetric pollen trap (17 March to 30 June) was 2161 pollen*day/m³.

The start of flowering ranged between the 7 April (DOY 98; 703 m a.s.l.) and 10 May (DOY 131; 974 m a.s.l.). Of the observed 21 birch trees, one tree located at 703 m a.s.l. started to flower in the first week of sampling, in the second week there were six trees (in an altitudinal range between 696 and 749 m. a.s.l.). This is temporally coincident with the highest measured WPIn_{grav} and WPIn_{UFS}. In the third and fourth week, we observed the flowering start of four birch trees each. These birches were located between 781 and 1105 m a.s.l. In the 5th week, no birch tree started to flower, which coincides with the weekly rainfall of 49.4 mm. In the following week, another six trees started to flower (altitudinal range 974 and 1222 m a.s.l.). In contrast to the second week, this was not reflected by WPIn_{grav}.

We observed a relationship between the start of flowering and the altitude of the observed birch tree (Figure 6), which is strong and significant ($r_s = 0.853$, $p < 0.001$). There are some points that deviate largely from the regression line; these locations are either westerly exposed (e.g., Ehrwalder Alm) and therefore have an earlier flowering onset date or are northerly exposed (e.g., Eibsee) and are therefore associated with later onset dates.

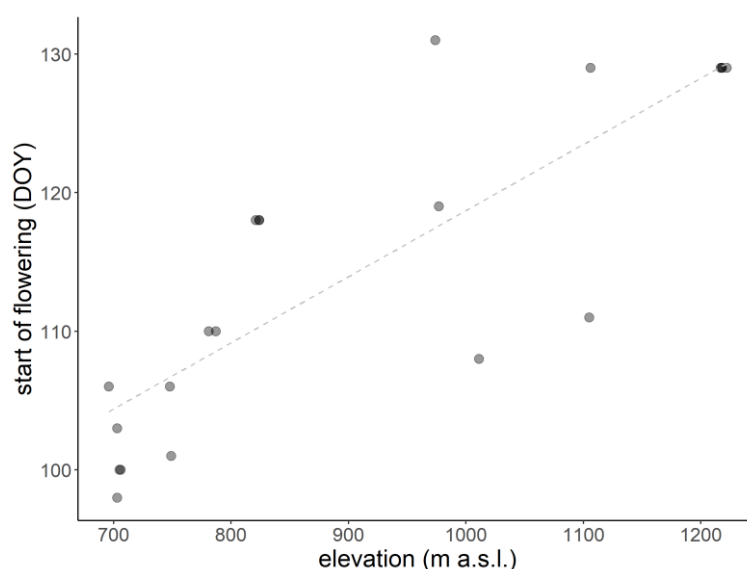


Figure 6. Scatterplot showing the relationship between the start of birch flowering (DOY) in 2020 and elevation in the region of Garmisch-Partenkirchen.

4.2. Pollen Transport

We analyzed the impacted pollen on each cardinal direction to assess the pollen transport. Similar to the pollen deposition on the horizontal slide, we also found highest values related to pollen impaction in the second week (Figure 7).

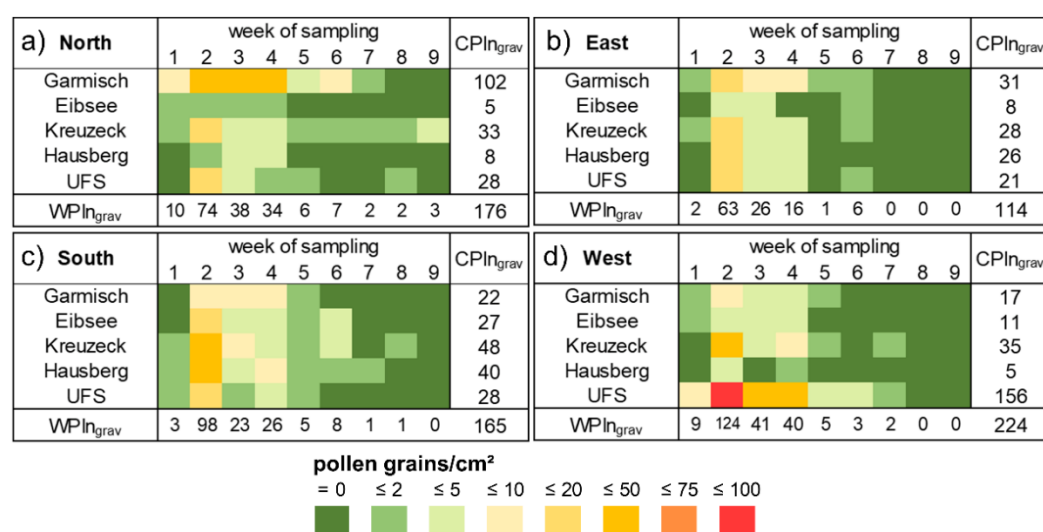


Figure 7. Weekly pollen (pollen grains/cm²) transported to the vertical orientated slides in the (a) north, (b) east, (c) south and (d) west between 1 April and 3 June.

At UFS, there was only one pollen grain/cm² at the southern slide and seven pollen grains/cm² at the western slide in the first week. In the following week, maximum pollen impaction was registered for all cardinal directions: 89 pollen grains/cm² were transported from the west, 20 pollen grains/cm² each from the north and south and 11 pollen grains/cm² from the east. In the third and fourth week, substantially larger amounts of birch pollen came from the west (28 and 25 pollen grains/cm², respectively), whereas only a few pollen were transported from other directions. The highest pollen impaction was recorded in the west over the entire period. The total value of 157 pollen grains/cm² sampled at the west at UFS is even higher than the total deposition (horizontally orientated slide) of 136 pollen/cm².

At Kreuzeck, a high pollen transport from the south and east was measured in the period during the peak of the pollen season from 8 April to 15 April: 26 pollen grains/cm² were counted in the south and 18 pollen grains/cm² in the east. Over the entire study period, most pollen were also transported from southern (40 pollen grains/cm²) and eastern directions (26 pollen grains/cm²), whereas from the north and west, the values were below 10 pollen grains/cm² each.

At the pollen trap at Hausberg, a higher pollen impaction was measured at all directions during the peak week. As for the pollen trap at Kreuzeck, which is 300 m of altitude higher than Hausberg, the highest pollen impaction over the entire period was registered in the south with 32 pollen grains/cm². In total, more pollen (144 pollen grains/cm²) were found on the four slides compared to Kreuzeck (79 pollen grains/cm²). Almost similar values were registered on the south and east slide along with greater deviations to the Kreuzeck location on the north and west slide.

The main transport of birch pollen at Eibsee could be observed at the southerly orientated slide. Overall and as already observed for the horizontally oriented slides representing deposition, there was a substantially less pollen transport compared to the four other trap locations. On all slides at Eibsee, a total impaction of 51 pollen grains/cm² could be determined. Among them was the highest total impaction from the south with 27 pollen grains/cm².

A total pollen impaction of 102 pollen/cm² at DWD was measured in the north over the entire study period. The peak of the pollen season could only be detected on the slides facing north and east. For this period (8 April to 15 April), an impaction of 36 pollen grains/cm² was registered in the north and 13 pollen grains/cm² in the east. In the west and south, we found seven pollen grains/cm² for the period 8 April to 15 April and five pollen grains/cm² for the following week.

In general, a peak was detected in the second week of the study at all sites. Afterwards, pollen transport decreased until mid-May, with increased levels that were registered again at Hausberg in the week from 6 May to 13 May.

4.3. Wind Data

The wind at UFS (Figure 8a) came predominately from the west with particularly high wind speeds and from the east with slightly lower wind speeds over the entire study period from the beginning of April to the beginning of July. In addition, partly higher wind speeds were measured in the south, but were substantially less frequent than from the west and east. The wind only rarely came from the north, attributable to the location of the UFS underneath the summit of the Zugspitze. Wind speeds at Kreuzeck (Figure 8b) were substantially weaker for the measurement period, which lasted to the 3 June and was up to approx. 2 m/s from the main directions south and east. At the pollen trap at Hausberg (Figure 8c), higher wind speeds (compared to Kreuzeck) up to 5 m/s were measured and associated with the main wind direction coming from the north. The second main wind direction at Hausberg was south and similar to that at Kreuzeck. The most frequent wind direction observed at Eibsee (Figure 8d) was south but was linked to very low wind speeds. This is similar to the location at Kreuzeck. A uniform weak wind was recorded from all other cardinal directions. A substantially stronger wind could be measured at the DWD (Figure 8e) from the north-east, which also represents the main wind direction. Similar distributions, but with lower wind speeds, were measured at DWD from the south-west.

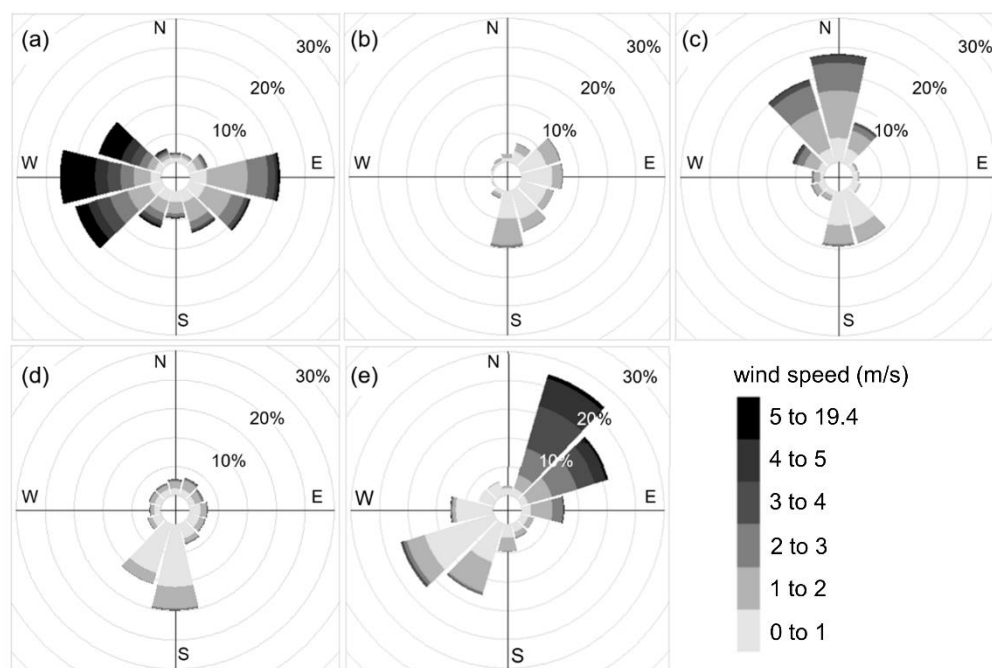


Figure 8. Overview of wind directions and wind speeds during the sampling campaign 2020 (1 April to 3 June or 1 July for UFS) at (a) UFS, (b) Kreuzeck, (c) Hausberg, (d) Eibsee and (e) DWD.

Figure 9 shows the prevailing wind directions for different times of the day (day: 7 am to 9 pm, night: 9 pm to 7 am) during the main birch pollen season, i.e., from 8 April to 15 April. This way, it was possible to identify and determine mountain-valley wind systems in the research area. During daytime, valley winds prevailed; at night, the local wind system reversed and valley downdrafts dominated. In our region of interest, these are winds from the north (during the day) or south (at night).

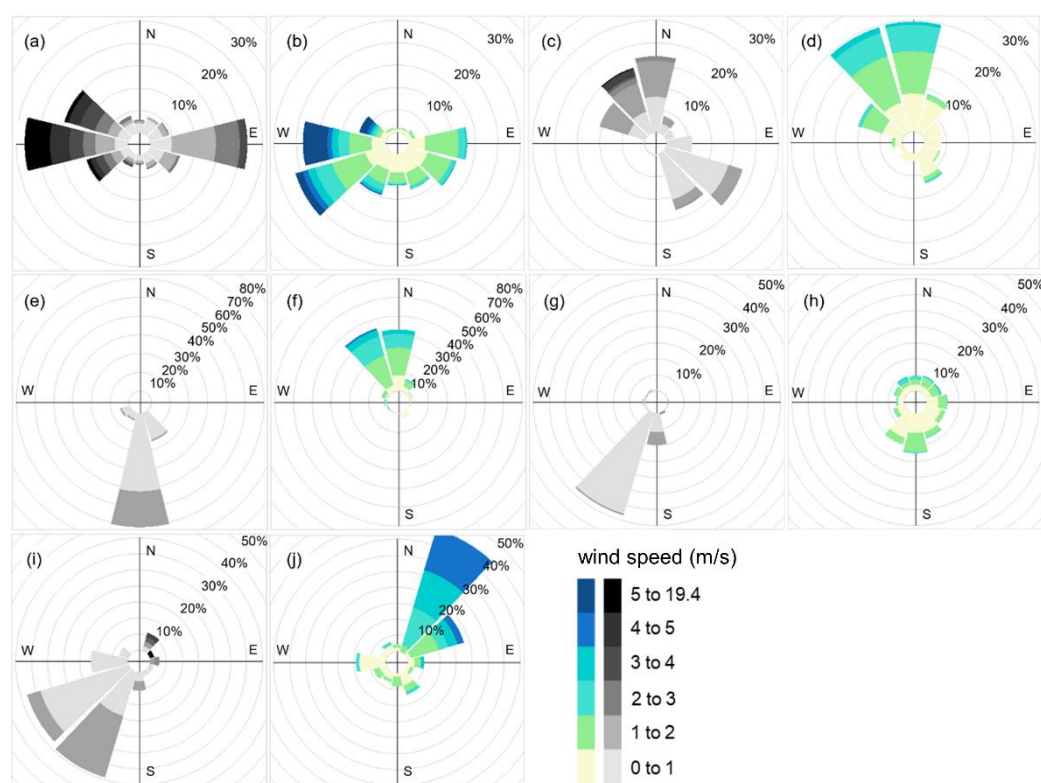


Figure 9. Overview of wind directions and wind speeds for the week from 8 April to 15 April 2020 divided into night from 9 pm to 7 am (grey) and day from 7 am to 9 pm (coloured) for (a,b) UFS, (c,d) Kreuzeck, (e,f) Hausberg, (g,h) Eibsee and (i,j) DWD.

At UFS (Figure 9a,b), situated approx. 300 m below the top of the mountain peak, the main wind direction from 9 pm to 7 am were westerly (W, WNW and WWS), which accounts for approx. 40% of the wind directions. However, winds from the east also corresponded to about 25% of the total wind during the night. During the day, winds from the west were distributed across the south and east with a larger share and stronger wind speeds from the west. For comparison (data not shown), wind at the Zugspitze summit came predominately (60%) from the north-west and south-west at night, and 50% during the day with a larger proportion from the south. In addition, the wind reached higher speeds at night than during the day. At Kreuzeck (Figure 9c,d), a clear difference between day- and nighttime can be seen. During the day, the wind from the north (NW, N and NE) reached a share of over 70%. At night, however, the share accounted only to 40%. The second most frequent wind direction was south-east with a share of approx. 30%.

Likewise, a clear difference between day- and nighttime was observed at the Hausberg site (Figure 9e,f). At night, about 75% of the wind came from the south, whereas during the day, about 75% of the wind came from the north. At this site, however, it should be mentioned that only data from two measurement days were included in the evaluation, because of a technical defect of the device. Likewise, at Eibsee (Figure 9g,h), a clear difference between the diurnal wind directions was recorded for the week with the highest pollen levels (8 April to 15 April). At night, the dominant wind direction was south. During the day, the wind came from all directions and a slightly increased proportion was attributed to the wind from the south. At DWD (Figure 9i,j), as at Hausberg, a similar behaviour of the wind was observed. The main wind direction during the day was north-east with a share of approx. 60%; during the night, it was south-west, with a share of approx. 75%.

5. Discussion

Warm and sunny days without precipitation favour a high number of pollen in the air [13]. These weather conditions prevailed in the Garmisch-Partenkirchen area, especially at the beginning of April 2020 (Figure 3) and probably led to a strong increase in airborne birch pollen concentrations.

Charalampopoulos et al. [21] found that pollen levels can increase or decrease due to the factor wind and this is dependent on the mountain acting either as a source or an obstacle to pollen transport. Similar findings are now provided by this study using gravimetric pollen traps at the five locations.

The gravimetric traps we used were a cost-effective way to cover pollen sampling at several locations and were successfully used in recent studies [30,31,39–41]. However, limitations of the method need to be considered when discussing and interpreting results. The limitations include the low sampling efficiency and the influence of wind speed, turbulence and direction and the unknown volume of sampled air [42].

The amount of deposited birch pollen increased strongly from 1016 m a.s.l. to 1619 m a.s.l. and then decreased again at 2657 m a.s.l. Whereas up to 1619 m a.s.l. the slopes are forested and moderately steep in our study area, from approx. 1800 m a.s.l., steep terrain with rock faces begins, which reach up to almost 3000 m a.s.l. (Figure 1). The comparison between the gravimetric pollen traps at UFS and DWD, the highest and lowest locations, showed that the pollen deposition was approx. 17% lower at the highest pollen trap than in the valley.

Jochner et al. [7] found a similar behavior in pollen decrease with altitude. At UFS, birch pollen concentrations in 2008 and 2009 were about 25% of the amount measured in the valley of Garmisch-Partenkirchen. In the aforementioned study, pollen concentrations were measured with volumetric pollen traps, whereas in this study, gravimetric pollen traps were used. Thus, a direct comparison is not possible. In this study, the comparably large amount of birch pollen at UFS was unexpected and requires a more detailed consideration of the influence of wind. Furthermore, it is remarkable that only at UFS the pollen amount impacted on the vertically orientated slides were greater than the deposition on the horizontal slide. This could also be explained by the influence of wind. The wind speed at UFS was much higher than at other sites as it reached maximum speeds up to 10 m/s.

Gehrig and Peeters [23] investigated pollen concentrations in the air at different altitudes and showed a decrease in pollen concentrations for some taxa (e.g., *Betula*, *Alnus glutinosa*-type) or an increase for some others (e.g., *Alnus viridis*) at high altitudes in Switzerland. For grass pollen, Charalampopoulos et al. [21] found no significant trend with altitude in the range from 300 m a.s.l. to 2100 m a.s.l. In contrast, Jochner et al. [6] reported a decrease with altitude in the range from 700 m a.s.l. to 1700 m a.s.l., whereas Gehring and Peeters [23] found no decrease up to 1500 m a.s.l. Due to these different results and the results from this study, it seems to be impossible to form a general rule for pollen distribution at different altitudes. The reason for this may be related to the complex topography of the Alps [23]. A lower pollen load is not only caused by altitude, but also by the respective exposure of the pollen measurement site [7]. The UFS is south-facing and located in a vegetation-free zone, 300 m below the Zugspitze summit. Pauling et al. [26] describe the site as not ideal, as it is difficult for pollen from the valley of Garmisch-Partenkirchen, the nearest larger pollen source area, to reach the site due to the complex topography. A lower pollen amount than in the valley is therefore not exceptional. The high pollen impaction and deposition in 2020 could originate from other and more remote sources that are not located in the valley, where the city of Garmisch-Partenkirchen is located. Jochner et al. [7] also detected large amounts of birch pollen, among others, on the UFS that did not originate from the immediate vicinity.

For the period with the highest pollen levels detected in our study (8 April to 15 April), predominantly westerly winds occurred at UFS and the majority of birch pollen were also transported from this direction. Since the start of the investigations, dry and warm weather prevailed over Central Europe [43] and several high-pressure areas

probably also ensured a high pollen load up to the high mountain areas in the corresponding week. Pollen grains are especially abundant when weather conditions provide low wind speeds and pollen are transported in the air at low velocities [44]. In the week with the highest pollen load, wind speeds were on average 1.89 m/s at UFS and 3.18 m/s at the Zugspitze summit. These rather weak wind speeds were probably responsible for the high levels of birch pollen measured at the vegetation-free UFS. The surroundings of UFS are steep and south-exposed terrain and wind speeds (that were somewhat higher than at the other locations) can explain the higher total impactation of pollen via transport compared to deposition.

The pollen trap at Kreuzeck was located in the forested zone; however, no birch trees grew within a radius of at least 400 m (Table 1). Likewise, no birch trees could be found within this radius of the site at Kreuzeck and Hausberg. However, it has to be noted that the number of birches is based on a terrestrial field survey and may be incomplete. These sites are located directly above Garmisch-Partenkirchen and are characterized by a mountain-valley wind system with daytime valley upwind (NE, E) and nighttime valley downwind (SW, WSW) [7]. Jochner et al. [7] assumed that this diurnal wind regime contributes to higher pollen levels at these two sites during the day, as pollen are transported from the valley up to the pollen traps. The wind required for this pollen transport came predominantly from a northerly direction during the day (7 am to 9 pm) at both sites in the week with the highest pollen deposition and impactation. At night (9 pm to 7 am), the nocturnal valley downdrafts from the south was only detectable at Hausberg. At Kreuzeck, the measured wind direction during the time between 9 pm to 7 am came mainly from the north, north-west, but also more rarely from the south-east and therefore cannot be clearly attributed to the nocturnal valley downdrafts. According to Jato et al. [13], turbulence leads to a higher pollen content in the air. Changing wind circulations during the day and night can cause the movement and further mixing of air masses and thus also of airborne pollen [21]. The mean wind speeds for the peak pollen week were 0.93 m/s for Kreuzeck and 1.22 m/s for Hausberg and were thus lower than measured at UFS. Pollen were transported mostly from the south at both sites in this week. In contrast, no increased pollen impactation was found on the slides facing north. The recorded transport was substantially lower compared to measured deposition. Since the calculated pollen sums correspond to one week, it is not possible to assign these aerobiological data exactly to the diurnal wind regime. Nevertheless, the prevailing ideal conditions with the influence of high-pressure (high temperatures, no precipitation and low wind speeds) at the beginning of April 2020 could be responsible for the increase of birch pollen impactation and deposition. The reason for this can be a pronounced mountain-valley wind system that probably transported high amounts of birch pollen to sites at 1329 m a.s.l. and 1619 m a.s.l., respectively.

At Eibsee, birch trees are located near the lake in the north of the pollen trap. One birch was located 100 m east of the pollen trap. Pollen was mostly transported from the south at this site, with a total impactation of 27 pollen grains/cm². Despite the fact that a birch tree was located 100 m to the east, only a total impactation of 8 pollen grains/cm² was measured. In addition, pollen deposition at Eibsee was associated with the lowest value of all pollen traps in the study area. Therefore, we conclude that local pollen sources might be less important than expected [45]. In addition, our phenological survey revealed that one of the birches observed at Eibsee was the latest flowering individual (10 May). Related to pollen data in the sixth week, this was only reflected at the southerly exposed microscope slide at which five pollen grains/cm² were captured. Therefore, not only birch tree abundance but also phenological onset dates are important for explaining pollen levels. Furthermore, local wind systems play a decisive role, but the deposition of airborne pollen can only be enhanced when local wind systems favor the transport of birch pollen from (remote) source areas. On the other hand, the prevailing large-scale weather situation with many successive high-pressure areas could also be responsible for stirring up pollen and transporting them further with the wind [20,44]. To confirm this assumption, it would be

necessary to conduct a more precise investigation with a smaller temporal resolution. The Eibsee is located further west of Garmisch-Partenkirchen and therefore not in the catchment area for pollen transport by the mountain-valley wind system that we have detected for Kreuzeck and Hausberg. This system, on the other hand, might be responsible for the high pollen quantities measured at Hausberg and Kreuzeck. The wind directions for the week with the highest pollen load also suggest a mountain-valley wind system, but the pollen trap at Eibsee was located directly at the foot of the Zugspitze in the aisle of the Bavarian Zugspitzbahn gondola line and thus no longer in an area with a potentially high pollen load.

It should be noted that the Eibsee is located at 1016 m a.s.l. and thus the birches flower later than in Garmisch-Partenkirchen (720 m a.s.l.). Our phenological data indicates that the beginning of flowering was generally delayed with increasing altitude. Similarly, Jochner et al. [7] found that flowering of birches is not only influenced by altitude (3 days/100 m), but also by the aspect of the respective site: For birches growing in a near proximity at similar altitudes at Eibsee (aspect: north) and Ehrwald (aspect: west), full flowering was delayed by 7 days at Eibsee due to lower temperatures. For this reason, it can also be assumed that substantially less birch pollen were present in the vicinity of the site during the main flowering phase in 2020.

Birch trees grew within a radius of 400 m at DWD; five birch trees were located north (NW to NE) and two south-west of the gravimetric pollen trap. Most of the birch pollen impacted during the study period was found on the microscope slides facing north. Pollen transport from the northern direction was distributed relatively evenly over the period from 8 April to 29 April. This could indicate a link between the proximity of the birch trees to the pollen trap and the amount of pollen on the slides. However, no increased pollen impaction was detected on the south-facing slides compared to the north-facing slides. Wind could play a crucial role here; the main wind direction during the entire study period from April to June was north-east with wind speeds ranging mostly between 3 and 5 m/s. In addition, somewhat weaker winds occurred that originated from the south-west. Although there were two birch trees located south-west of the trap, the amount of pollen was not pronouncedly higher than on the slides in the west and east. Based on these results and the results at Eibsee, it cannot be assumed that there is a clear influence on pollen deposition and transport from birch trees in the immediate vicinity.

As at Hausberg and Kreuzeck, the DWD site was influenced by a mountain-valley wind system. When considering the wind directions at daytime (7 am to 9 pm) and nighttime (9 pm to 7 am), wind came from the north-east (day) or south-west (night), accordingly. Jochner et al. [7] determined the same wind conditions for prior study years. Whereas the wind direction during the day was presented by mountain winds flowing uphill, the air masses flew downhill at night. These winds might be responsible for the corresponding pollen transport at DWD. The nightly south-western winds hardly brought any pollen from higher altitudes into the valley. The north-easterly winds, on the other hand, carried a large amount of birch pollen to higher altitudes during the day.

Jochner et al. [46] assumed large-scale air currents being responsible for increased pollen loads in 2010. In both 2010 [46] and 2020, high levels of birch pollen were transported from westerly directions at UFS. Small-scale wind systems probably dominated pollen transport at the other sites. An exact assignment of wind directions to aerobiological data is not possible with the selected temporal resolution of one week and does not allow an interpretation of the medium-term transport. For a detailed analysis of the influence of surrounding birch trees, a lower temporal resolution of aerobiological pollen data would be desired. In addition, volumetric Hirst-type pollen traps could be installed, since they provide bi-hourly data and a higher sampling efficiency at higher wind speeds [47], which cannot be provided by passive sampling with gravimetric pollen traps. Because of the special local conditions of the five gravimetric pollen traps, it is not possible to draw conclusions on other locations besides the Garmisch-Partenkirchen area. Likewise, a general statement according to Pauling et al. [27] is neither possible nor necessary.

6. Conclusions

The methodology used enabled us to obtain an estimate of the strength of the pollen load at many different altitudes at low cost. Whereas the pollen load was highest at the lowest location and lowest at the highest location, a substantially higher pollen load was measured at two sites at medium altitudes. This can be explained by the pronounced mountain-valley wind system that most likely ensured the transport of pollen to the corresponding altitudes. The assumption that birch trees growing near the pollen trap have an influence on the weekly pollen catch could not be confirmed based on our data. This finding emphasizes the vital need for incorporating meteorological data in any pollen forecasts. In general, pollen levels and temporal patterns that were observed in our study area cannot be compared with other alpine locations in a simple manner due to the complex and unique topography of the surrounding area, which forms its own topo-climate.

A detailed assessment of differences in pollen production, e.g., as previously conducted for birches growing in a seed plantation [8], at different altitudes would increase our knowledge of the relationship between altitude/temperature and pollen production—and would also increase our understanding of expected future pollen loads.

Author Contributions: Conceptualization, S.J.-O.; methodology, V.W.; software, V.W.; formal analysis, V.W. and J.J.; investigation, V.W.; resources, S.J.-O. and V.W.; data curation, V.W.; writing—original draft preparation, V.W. and S.J.-O.; writing—review and editing, V.W., S.J.-O., and J.J.; visualization, V.W. and J.J.; supervision, S.J.-O.; project administration, S.J.-O.; funding acquisition, S.J.-O. All authors have read and agreed to the published version of the manuscript.

Funding: This research is part of the project “pollenPALS—Biotic and abiotic effects on pollen production and allergenicity of birch and related health impacts (655850)” funded by the German Research Foundation (Deutsche Forschungsgemeinschaft) (DFG). The open access publication of this article was supported by the Open Access Fund of the Catholic University Eichstätt-Ingolstadt.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sharing not applicable.

Acknowledgments: We want to thank Till Rehm (UFS) and C. Benkert (Bayerische Staatsforsten) for technical assistance, and Bayerische Staatsforsten and Deutscher Wetterdienst for approval of sampling locations. We kindly acknowledge Annette Menzel (Technical University of Munich) and Jeroen Buters (Technical University of Munich) for the provision of meteorological data (Kreuzeck) and volumetric aerobiological data (UFS), respectively. We thank Miriam Sieverts for supporting our phenological observations, and Anna Eisen and Claudia Pietsch for the illustration of the pollen trap.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Lord, E.M.; Russell, S.D. The mechanisms of pollination and fertilization in plants. *Annu. Rev. Cell Dev. Biol.* **2002**, *18*, 81–105.
2. Damialis, A.; Fotiou, C.; Halley, J.M.; Vokou, D. Effects of environmental factors on pollen production in anemophilous woody species. *Trees* **2011**, *25*, 253–264.
3. World Health Organisation (WHO). Phenology and Human Health: Allergic Disorders: Report on a WHO Meeting, Rome, Italy. 16–17 January 2003. Available online: <https://apps.who.int/iris/handle/10665/107479> (accessed on 3 March 2021).
4. Emberlin, J.; Detandt, M.; Gehrig, R.; Jaeger, S.; Noland, N.; Rantio-Lehtimäki, A. Responses in the start of Betula (birch) pollen seasons to recent changes in spring temperatures across Europe. *Int. J. Biometeorol.* **2002**, *46*, 159–170.
5. Deutscher Wetterdienst. Anleitung für Die Phänologischen Beobachter des Deutschen Wetterdienstes; Deutscher Wetterdienst: Offenbach am Main, Germany, 1991.
6. BAYSICS Webportal. Tree Line Analyses. Available online: <https://www.portal.baysics.de/map/> (accessed on 17 November 2022).

7. Jochner, S.; Ziello, C.; Böck, A.; Estrella, N.; Buters, J.; Weichenmeier, I.; Menzel, A. Spatio-temporal investigation of flowering dates and pollen counts in the topographically complex Zugspitze area on the German–Austrian border. *Aerobiologia* **2012**, *28*, 541–556.
8. Ranpal, S.; Sieverts, M.; Wörl, V.; Kahlenberg, G.; Gilles, S.; Landgraf, M.; Köpke, K.; Kolek, F.; Luschkova, D.; Heckmann, T.; et al. Is pollen production of birch controlled by genetics and local conditions? *Int. J. Environ. Res. Public Health* **2022**, *19*, 8160.
9. D’Amato, G.; Cecchi, L.; Bonini, S.; Nunes, C.; Annesi-Maesano, I.; Behrendt, H.; Van Cauwenberge, P. Allergenic pollen and pollen allergy in Europe. *Allergy* **2007**, *62*, 976–990.
10. Biedermann, T.; Winther, L.; Till, S.J.; Panzner, P.; Knulst, A.; Valovirta, E. Birch pollen allergy in Europe. *Allergy* **2019**, *74*, 1237–1248.
11. Linneberg, A. The increase in allergy and extended challenges. *Allergy* **2011**, *66*, 1–3.
12. Bartra, J.; Montoro, J.; Jáuregui, I.; Dávila, I.; Ferrer, M.; Mullol, J.; Valero, A. From pollinosis to digestive allergy. *J. Invest. Allergol. Clin. Immunol.* **2009**, *19*, 3–10.
13. Jato, V.; Rodriguez-Rajo, J.F.; Aira, J.M. Use of phenological and pollen-production data for interpreting atmospheric birch pollen curves. *Ann. Agric. Environ. Med.* **2007**, *14*, 271–280.
14. Laaidi, M. Forecasting the start of the pollen season of Poaceae: Evaluation of some methods based on meteorological factors. *Int. J. Biometeorol.* **2001**, *45*, 1–7.
15. Bricchi, E.; Frenguelli, G.; Mincigrucci, G. Experimental results about Platanus pollen deposition. *Aerobiologia* **2000**, *16*, 347–352.
16. Adams-Groom, B.; Skjøth, C.A.; Baker, M.; Welch, T.E. Modelled and observed surface soil pollen deposition distance curves for isolated trees of *Carpinus betulus*, *Cedrus atlantica*, *Juglans nigra* and *Platanus acerifolia*. *Aerobiologia* **2017**, *33*, 407–416.
17. Skjøth, C.A.; Ørby, P.V.; Becker, T.; Geels, C.; Schlünssen, V.; Sigsgaard, T.; Bønløkke, J.H.; Sommer, J.; Søgaard, P.; Hertel, O. Identifying urban sources as cause of elevated grass pollen concentrations using GIS and remote sensing. *Biogeosciences* **2013**, *10*, 541–554.
18. Williams, C.G. Aerobiology of *Pinus taeda* pollen clouds. *Can. J. For. Res.* **2008**, *38*, 2177–2188.
19. Rapiejko, P. Pollen monitoring in Poland. In *Pollen and Pollinosis: Current Problems*, Spiewak, R., Ed.; Institute of Agricultural Medicine: Lublin, Poland, 1995.
20. Sofiev, M.; Siljamo, P.; Ranta, H.; Rantio-Lehtimäki, A. Towards numerical forecasting of long-range air transport of birch pollen: Theoretical considerations and a feasibility study. *Int. J. Biometeorol.* **2006**, *50*, 392–402.
21. Charalampopoulos, A.; Damialis, A.; Tsiropidis, I.; Mavrommatis, T.; Halley, J.M.; Vokou, D. Pollen production and circulation patterns along an elevation gradient in Mt Olympos (Greece) National Park. *Aerobiologia* **2013**, *29*, 455–472.
22. Barry, R.G. *Mountain Weather and Climate*; Methuen: London, UK; New York, NY, USA, 1981.
23. Gehring, R.; Peeters, A.G. Pollen distribution at elevations above 1000 m in Switzerland. *Aerobiologia* **2002**, *16*, 69–74.
24. Frei, T. Pollen distribution at high elevation in Switzerland: Evidence for medium range transport. *Grana* **1997**, *36*, 34–38.
25. Menzel, A.; Ghasemifard, H.; Yuan, Y.; Estrella, N. A first pre-season pollen transport climatology to Bavaria, Germany. *Front. Allergy* **2021**, *2*, 627863.
26. Pauling, A.; Clot, B.; Menzel, A.; Jung, S. Pollen forecasts in complex topography: Two case studies from the Alps using the numerical pollen forecast model COSMO-ART. *Aerobiologia* **2020**, *36*, 25–30.
27. Wetzel, K.F. On the hydrology of the Partnach area in the Wetterstein mountains (Bavarian Alps). *Erdkunde* **2004**, *58*, 172–186.
28. Hendl, M. *Klima*. In *Physische Geographie Deutschlands*; Liedtke, H., Marcinek, J., Eds.; Gotha: Stuttgart, Germany, 2002; pp. 23–120.
29. Deutscher Wetterdienst CDC (Climate Data Center). Available online: <https://cdc.dwd.de/portal/202007291339/searchview> (accessed on 4 January 2021).
30. Jetschni, J.; Jochner-Oette, S. Spatial and temporal variations of airborne Poaceae pollen along an urbanization gradient assessed by different types of pollen traps. *Atmosphere* **2021**, *12*, 974.
31. Eisen, A.-K.; Fussli, B.; Šikoparija, B.; Jochner-Oette, S. Aerobiological pollen deposition and transport of *Fraxinus excelsior* at a small spatial scale. *Forests* **2022**, *13*, 424.
32. Durham, O.C. Proposed standard method of gravity sampling. *J. Allergy* **1964**, *17*, 79.
33. Hirst, J.M. An automatic volumetric spore trap. *Ann. Appl. Biol.* **1952**, *39*, 257–265.
34. Schuster, C.; Estrella, N.; Menzel, A. Shifting and extension of phenological periods with increasing temperature along elevational transects in southern Bavaria. *Plant Biology* **2013**, *16*, 332–344.
35. Bastl, K.; Kmenta, M.; Berger, U.E. Defining Pollen Season: Background and Recommendations. *Curr. Allergy Asthma Rep.* **2018**, *18*, 73.
36. Jochner-Oette, S.; Jetschni, J.; Menzel, A.; Simmons, M. Impacts of land clearance by fire on spatial variation of mountain cedar pollen concentrations in Texas. *Landsc. Urban Plan.* **2017**, *162*, 178–186.
37. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*; Springer: Cham, Switzerland, 2016. ISBN 9783319242774.
38. Carslaw, D.C.; Ropkins, K. *openair—An R package for air quality data analysis*. *Environ. Model. Softw.* **2012**, *27–28*, 52–61. <https://doi.org/10.1016/j.envsoft.2011.09.008>.

39. Werchan, B.; Werchan, M.; Mücke, H.-G.; Gauger, U.; Simoleit, A.; Zuberbier, T.; Bergmann, K.-C. Spatial distribution of allergenic pollen through a large metropolitan area. *Environ. Monit. Assess.* **2017**, *189*, 169.
40. Stas, M.; Aerts, R.; Hendrickx, M.; Bruffaerts, N.; Dendoncker, N.; Hoebeke, L.; Linard, C.; Nawrot, T.; Van Nieuwenhuyse, A.; Aerts, J.-M.; et al. Association between local airborne tree pollen composition and surrounding land cover across different spatial scales in Northern Belgium. *Urban For. Urban Green.* **2021**, *61*, 127082.
41. Katz, D.S.; Carey, T.S. Heterogeneity in ragweed pollen exposure is determined by plant composition at small spatial scales. *Sci. Total Environ.* **2014**, *485–486*, 435–440.
42. Agashe, S.N.; Caulton, E. *Pollen and Spores: Applications with Special Emphasis on Aerobiology and Allergy*; Science Publishers: Enfield, NH, USA, 2009.
43. Deutscher Wetterdienst. Großwetterlage April 2020. Available online: <https://www.dwd.de/DE/leistungen/grosswetterlage/2020/grosswetterlage.html?nn=510076> (accessed on 16 February 2021).
44. Jato, V.; Rodríguez-Rajo, F.J.; Alcázar, P.; Nunttiis, P. de; Galán, C.; Mandrioli, P. May the definition of pollen season influence aerobiological results? *Aerobiologia* **2006**, *22*, 13–25.
45. Jochner-Oette, S.; Stitz, T.; Jetschni, J.; Cariñanos, P. The influence of individual-specific plant parameters and species composition on the allergenic potential of urban green spaces. *Forests* **2018**, *9*, 284.
46. Jochner, S.; Lüpke, M.; Laube, J.; Weichenmeier, I.; Pusch, G.; Traidl-Hoffmann, C.; Menzel, A. Seasonal variation of birch and grass pollen loads and allergen release at two sites in the German Alps. *Atmos. Environ.* **2015**, *122*, 83–93.
47. Peel, R.G.; Kennedy, R.; Smith, M.; Hertel, O. Relative efficiencies of the Burkard 7-Day, Rotorod and Burkard Personal samplers for Poaceae and Urticaceae pollen under field conditions. *Ann. Agric. Environ. Med.* **2014**, *21*, 745–752.