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Temporal and spatial variations of allergenic pollen in cities

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Abstract

Background

Pollen allergies are widespread around the world, making the study of pollen levels in and around cities a task of high importance. This is also related to the fact that an increasing number of people is living in cities and urban areas are expanding. In addition, environmental changes in the context of climate change are expected to alter plant characteristics that affect the amount of pollen in the air. In order to assess the allergy risk, information on pollen that people are exposed to is needed. Monitoring of aeroallergens in cities is often conducted using a single trap. This trap can provide information on background pollen concentration, however, it does not reflect temporal and spatial variations of pollen at street level of the heterogeneous urban environment. The amount of pollen in the air is the result of a number of processes, one of which is pollen production. Pollen production is expected to be altered by climate change and is influenced by environmental factors such as temperature or air pollutants. To further investigate variations of allergenic pollen in cities, this thesis focuses on these central research questions:

- Are there differences in the amount of airborne pollen between urban and rural locations and do pollen loads vary at different spatial and temporal scales?
- What is the influence of land use and management on pollen loads?
- Is there a difference in pollen production of allergenic species between urban and rural locations and can the influence of environmental factors be detected?

Data and Methods

Data on airborne pollen were gathered in two study areas with various pollen traps. In Ingolstadt, Germany, background pollen concentrations of Poaceae were measured in 2019 and 2020. Additionally, a network consisting of twelve gravimetric pollen traps was set up during the grass pollen seasons of those years. For an additional sampling campaign, portable volumetric traps were used to measure pollen concentration at street level. Investigations were also conducted in Sydney, Australia, where concentrations of Poaceae, Myrtaceae and Cupressaceae pollen and *Alternaria* spores were measured

during three pollen seasons from 2017 to 2020. In the summer of 2019/2020, additional ten gravimetric traps were set up. For both study areas, pollen season characteristics and temporal and spatial variations of pollen concentrations and pollen deposition were analysed in context with land use, land management and meteorological parameters.

Furthermore, pollen production of the allergenic species *Betula pendula*, *Plantago lanceolata* and *Dactylis glomerata* was investigated in the Ingolstadt study area along an urbanisation gradient, which covered different types of land use. In total, 24 individuals of *B. pendula*, 82 individuals of *P. lanceolata* and 54 individuals of *D. glomerata* were analysed. Pollen production was compared between urban and rural locations and the influence of air temperature and the air pollutants nitrogen dioxide (NO₂) and ozone (O₃) was assessed. Air temperature was measured in the field and pollutant concentrations were computed using a land use regression model.

Results and Discussion

In Ingolstadt, grass pollen concentrations and deposition were generally higher at rural than at urban locations as documented in peak values and seasonal totals. Diurnal variations however, did not vary between urban and rural locations. Grass pollen levels were linked to local vegetation and land use but also land management, as grass cutting was reducing pollen levels in the surroundings.

In Sydney, pollen season characteristics varied between the seasons and we found significant correlations between daily pollen and spore concentrations and meteorological parameters. There were significant positive correlations with air temperature for all analysed pollen and spore types. Correlations with humidity (Myrtaceae, Cupressaceae, *Alternaria*) and precipitation (Myrtaceae, Cupressaceae) were negative. Spatial variations of pollen deposition were observed, but there were no correlations with land use. This could be attributable to the drought before and during this sampling campaign as well as the temporal setting of the sampling campaign.

The results from both study areas suggest that local vegetation is the main influence on the pollen amount at street level. However, when the season's intensity decreased, other influences such as resuspension or pollen transport become more important. Analyses of pollen production revealed statistically significant differences between urban and rural locations for *B. pendula* and *P. lanceolata*. Pollen production decreased with temperature

and urbanisation for all species, however, pollen production varied at small spatial distances within species. For increasing air pollutant levels, decreases were observed for *B. pendula* and *P. lanceolata*, but increases for *D. glomerata*. These results suggest that environmental influences seem to be species-specific.

The results from the studies contribute to the knowledge on temporal and spatial variations of airborne pollen in cities, as well as on pollen production of allergenic species. They highlight the importance of pollen monitoring at street level and at different locations in cities to assess the amount of pollen that people are exposed to. Further research in this area should investigate the influence of land management, which was identified to influence the amount of pollen in the air, and which could be a mitigation strategy for allergy-affected people. In addition, pollen production should be further examined with different kinds of experiments, as it is the major influence of ambient pollen concentration in the air.

Individual contributions

Three publications were prepared for this publication-based PhD thesis. They are included in this thesis as individual chapters (Chapters 3 to 5). All of the publications were submitted to different scientific journals, which ensure a peer-review process. The first and second publication have been published. The third publication has been submitted and is under review.

All publications were written at the professorship of Physical Geography/Landscape Ecology and Sustainable Ecosystem Development at the Catholic University of Eichstätt-Ingolstadt. In addition, the second publication (Chapter 4) was written in collaboration Paul Beggs and Jane Al Kouba of Macquarie University in Sydney, where I was on a research visit. The third publication (Chapter 5) was written in collaboration with Markus Fritsch of the University of Passau. The individual contributions to each publication are detailed below:

1. Jetschni J, Jochner-Oette S (2021). Spatial and temporal variations of airborne Poaceae pollen along an urbanization gradient assessed by different types of pollen traps. *Atmosphere*, 12, 974. doi: 10.3390/atmos12080974

Susanne Jochner-Oette contributed with suggestions for the study design, sampling, analyses and proofreading. I did most of the work (**90 %**). I scouted sampling locations, set up the sampling network, carried out field work and laboratory work, statistical analyses and wrote the manuscript.

2. Jetschni J, Al Kouba J, Beggs PJ, Jochner-Oette S (2023). Investigation of spatial and temporal variations of airborne Poaceae, Myrtaceae and Cupressaceae pollen and *Alternaria* spores in Sydney, Australia, 2017-2020. *Aerobiologia*, 39(1). doi: 10.1007/s10453-023-09783-w

Jane Al Kouba contributed with laboratory work and data curation of the 2017-2020 pollen and spore data. Paul Beggs supervised the 2017-2020 sampling and carried out the associated field work. He suggested and organised locations for the sampling campaign and contributed by proofreading. Susanne Jochner-Oette contributed with

suggestions for the study design, ideas for the analyses and by proofreading. I did most of the work (**80 %**), including field work, laboratory work, statistical analyses of all data, and manuscript writing.

3. Jetschni J, Fritsch M, Jochner-Oette S (2023). How does pollen production of allergenic species differ between urban and rural environments? Under review at International Journal of Biometeorology. Submitted on 19 January 2023

Susanne Jochner-Oette contributed with suggestions for the study design, statistical analyses and proofreading. Markus Fritsch provided the land use regression model to obtain air pollution data. He suggested statistical analyses, provided the code for some of the analyses, wrote the section about LUR and proofread the manuscript. I carried out most of the work (**80 %**), including field work, laboratory work, statistical analyses and writing.

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1 Introduction and research questions

Allergies are on the rise worldwide (Pawankar et al. 2013) and therefore the importance of aerobiological studies is increasing. Before delving into the details of the following chapters, it is important to develop a solid understanding of some terms and definitions that form the basis of this thesis titled “Temporal and spatial variations of allergenic pollen in cities”. This chapter provides some background information on aerobiology, an overview of aerobiological processes and pollen in cities.

Aerobiology. Aerobiology is an interdisciplinary research area (Agashe & Caulton 2009), in which many disciplines, from biology, ecology, meteorology, geography, to pathology and medicine, are involved. Aerobiological research focuses on organic particles in the air including their sources, release, dispersal and deposition and the effect of environmental conditions on each of these processes, as well as their impact on living organisms (Lacey 1994).

Pollen biology. In flowering seed plants, pollen are recognised as the male gamete and ensure gene flow and plant reproduction. Pollen develop in the anther and get released once they are mature and conditions are favourable (Shivanna & Rangaswamy 1992). Pollen grains vary in size and shape, depending on the plant species (Agashe & Caulton 2009). Their morphology can be classified and described by attributes such as size, apertures, sculpturing structure or wall morphology (Faegri & Iversen 1989). In general, wind-pollinated, anemophilous plants produce smaller and lighter pollen grains in large numbers compared to insect-pollinated, entomophilous plants. The large quantity of pollen from anemophilous plants ensures that the chances of successful fertilisation and reproduction are increased. In contrast, some entomophilous plants present pollen-kit, which makes pollen grains stick together and also to insects that visit the flower (Agashe & Caulton 2009).

Medical relevance. Studies on aerobiological topics are of medical and clinical relevance as aeroallergens can cause allergic respiratory reactions. Allergies are a global phenomenon, with 10 to 30 % of the adult population and 40 % of children worldwide being affected by allergic rhinitis. Depending on its severity and treatment, it affects quality of life and has psychological, physiological, social and financial consequences

(Pawankar et al. 2013; Vandenplas et al. 2018). The economic and financial impact, resulting from direct medical cost, has been estimated at approx. 3.4 billion USD (Meltzer & Bukstein 2011). For the EU, it has been estimated that costs between 55 and 151 billion € per year could be avoided through better care, treatment of allergies as well as a better communication of triggers and risks (Zuberbier et al. 2014). A phenomenon related to pollen allergy that has recently received more attention is thunderstorm asthma, that has led to increased hospital visits (D'Amato et al. 2007b; Beggs 2017; Silver et al. 2018; Thien et al. 2018; Damialis et al. 2020; Idrose et al. 2020; Straub et al. 2021). In addition, the medical relevance of pollen increased during the COVID-19 pandemic, as a positive relationship between pollen concentrations and SARS-CoV-2 infection rates was found (Damialis et al. 2021). The relevance is expected to increase in the future due to climate change (D'Amato et al. 2010; Reinmuth-Selzle et al. 2017; IPCC 2022a), which highlights the need for additional research and the development of mitigation and adaption strategies. Due to their medical relevance, aerobiological studies are particularly important in cities. Studies have shown that city dwellers are more affected by respiratory allergies than people living in more rural areas (Sedghy et al. 2018). Studies provided evidence that air pollutants such as nitrogen dioxide (NO₂), ozone (O₃) or diesel exhaust particles in cities can worsen symptoms in people with pollen allergies (D'Amato et al. 2010; Cakmak et al. 2012; Reinmuth-Selzle et al. 2017). More than 50% of the world's population live in cities (United Nations, Department of Economic and Social Affairs, Population Division 2019) and the number of people living in cities are increasing as is the size of metropolitan areas (OECD/European Union 2020), underlining the importance of monitoring aeroallergens in urban areas now and in the future.

Climate change. Anthropogenic activity has led to an increase in greenhouse gases in the atmosphere. These greenhouse gases include carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O), which are caused by activities such as the burning and use of fossil fuels, industrial activities, intensive agriculture, deforestation, drainage and the conversion of peat lands (IPCC 2022a). Increases in greenhouse gas concentrations have led to an increase in air temperature. For example, when comparing the period from 2011 to 2020 with the period from 1850 to 1900, the global surface temperature has increased by 1.1 °C (IPCC 2022a). Climate change has led to changes in aeroallergens, which have mostly been attributed to increasing air temperatures and atmospheric CO₂

concentrations. In relation to pollen, impacts have particularly been documented for pollen production, concentration, allergenicity, the onset and duration of the pollen season, and the distribution of plants and pollen (Beggs 2004, 2015; Menzel & Jochner 2016; Katelaris & Beggs 2018; Ziska et al. 2019; D'Amato et al. 2020; Zhang & Steiner 2022). The increasing relevance of aerobiological studies is also reflected in an increased amount of research on the topic of pollen and climate change in the last decades (Fig. 1).

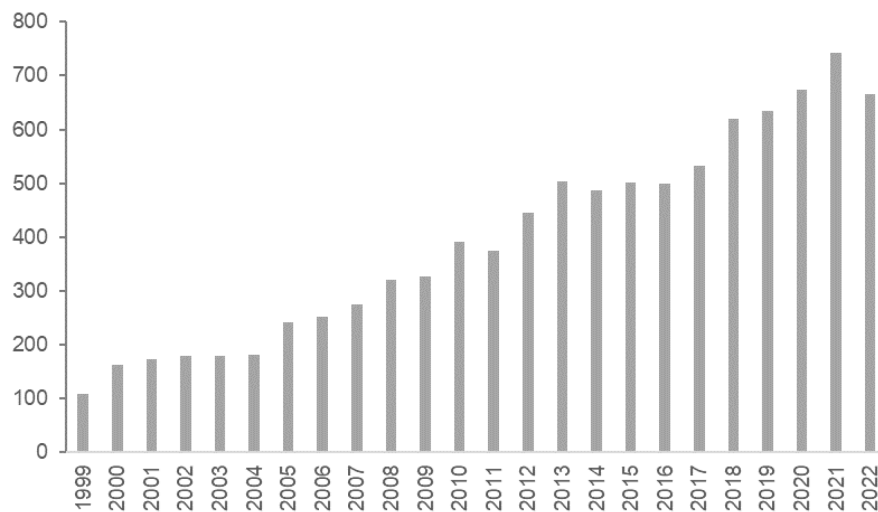


Fig. 1 Number of publications per year (1999 to 2022) based on the search for the topic "pollen climate change" (Web of Science; accessed on 04 May 2023).

Applications of aerobiological data. Aerobiological data can be used for a number of applications. Pollen data (of the past or present) provide information on vegetation composition that can be used to detect long- and short-term environmental changes. Furthermore, they can be used to predict crop yield or seed production (Westgate et al. 2003; Ribeiro et al. 2017) as well as for forecasting pollen load in the air (Sofiev et al. 2013a; Voukantsis et al. 2015; Muzalyova et al. 2021). By analysing the recorded pollen data, pollen calendars can be created (Šikoparija et al. 2018; Werchan et al. 2018), which can be a useful tool for those affected by pollen allergies. In addition, more specific risk maps (Dbouk et al. 2022) can help those susceptible to pollen allergies plan outdoor activities or travel.

1.1 Pollen in the air

Pollen in the air are the result of various processes, from pollen production at the source to aerobiological processes such as emission, release and dispersal, transport and deposition. All processes are controlled and influenced by factors associated with climate and the environment (Dahl et al. 2013) as illustrated in Fig. 2.

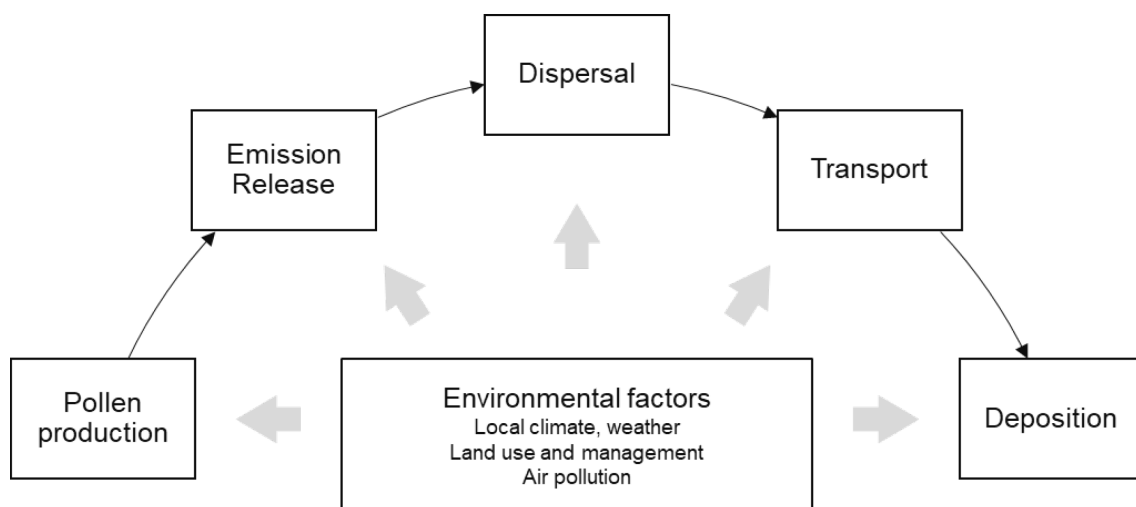


Fig. 2 Processes influencing the amount of pollen in the air (based on Dahl et al. 2013).

Pollen production. Pollen production describes the amount of pollen produced per reproductive unit. It can be measured per anther, flower or inflorescence or whole plant (Galán et al. 2017) and is the most important factor influencing the amount of pollen in the air (Agashe & Caulton 2009). The amount of pollen produced varies between species and individuals and is influenced by several factors. Environmental factors include site conditions (Faegri & Iversen 1989; González-Parrado et al. 2015; Allen et al. 2020; Pers-Kamczyc et al. 2020), air pollution (Jablonski et al. 2002; Darbah et al. 2008; Jochner et al. 2013; Zhao et al. 2017a; Kim et al. 2018) and air temperature (Jochner et al. 2013; Barnes 2018; Kolek 2021). Other factors include genetics (Ranpal et al. 2022), masting (Ranta et al. 2008; Crone & Rapp 2014) and climate change (Beggs 2004). Several studies have assessed pollen production of different plant species (e.g., Molina et al. 1996; Wayne et al. 2002; Piotrowska 2008; Damialis et al. 2011; Jochner et al. 2011; González-Parrado et al. 2015; Bogawski et al. 2016; Jung et al. 2018; Katz et al. 2020; Ranpal et al. 2022;

Severova et al. 2022), but most are limited to small sample sizes, especially in the case of non-woody species.

Pollen emission and release. Pollen grains are emitted from the plant as soon as pollen grains detach and are released into the air (Agashe & Caulton 2009). The first requirement for the actual release of pollen is the readiness to flower, which is determined by temperature, photoperiod and water stress. Once this phase is reached, pollen can be released if environmental conditions such as temperature, humidity, light conditions, evaporation and reabsorption are met (Dahl et al. 2013). However, the importance of these factors varies depending on the plant species and geographical location (Lacey and West 2006). The pollination period of the plant starts with the release of pollen grains into the air. Pollen calendars can indicate the timing of flowering based on data of previous years, which varies between countries and regions (Werchan et al. 2018).

Pollen dispersal. Once released from the anther, pollen grains are transported by the wind. Pollen dispersal is mainly dependent on air mass motion, turbulence and thermal convection (Lacey & West 2006), while aerodynamic characteristics, i.e., particle shape, size, weight and surface roughness of the particle, play a minor role. Pollen transport distances vary mainly with plant characteristics such as height and width but also with wind speed during the flowering season (Adams-Groom et al. 2017). In general, pollen concentrations decrease with increasing distance from the source (source-distance relationship; Bricchi et al. 2000). However, the height of release also influences this relationship: pollen released from lower pollen sources such as shrubs, herbaceous plants or grasses are higher concentrated close to the source, while pollen from higher sources, such as trees, reach highest concentrations further away (Skjøth et al. 2013).

Pollen transport. Once pollen are airborne, the only force keeping them in the air is air movement. Pollen transport can be distinguished in local, regional and long-distance transport (Sofiev et al. 2013b), depending on the distance transported away from the source. Pollen grains can travel long distances, for example, a study showed that birch and pine pollen released in Western Europe travelled several hundred kilometers to the North Pole (Rousseau et al. 2004). It has also been shown that the majority of pollen captured by a trap does not originate from local sources (Menzel et al. 2021). These factors lead to certain pollen being recorded in places where the corresponding species were not flowering or were not present at all.

Pollen deposition. Pollen grains descend by gravity. If they settle passively on a surface, the term *sedimentation* has been proposed (Sofiev et al. 2013b). This is the principle that passive pollen samplers are based on. In contrast, *impaction* occurs, when the particles stick to a surface after an active collision, which is how active pollen samplers collect airborne particles (Sofiev et al. 2013b). A more detailed description of the pollen samplers can be found in Chapter 2.1. Both processes are referred to as dry deposition, whereas wet deposition occurs when pollen are washed out of the atmosphere by precipitation (Sofiev et al. 2013b).

In summary, several factors interact and influence pollen in the air. These influences affect each process differently and should therefore be the subject of research. Several studies have focused on environmental factors influencing pollen in the air, such as local vegetation and land use (Cariñanos et al. 2002; Charalampopoulos et al. 2018; Cariñanos et al. 2019), urbanisation (Rodríguez-Rajo et al. 2010; Peel et al. 2014a; Katz et al. 2019; Katz & Batterman 2020) and meteorological parameters (Gassmann et al. 2002; Stennett and Beggs 2004b; Gassmann & Gardiol 2007; Puc 2012; Rojo et al. 2015; Tassan-Mazzocco et al. 2015; Vara et al. 2016; Oduber et al. 2019).

1.2 Pollen in cities

In urban areas, aerobiological processes are subject to urban-specific conditions. These conditions are particularly evident when urban areas are compared with more rural areas in their surroundings. Cities can also be a good study area to investigate the effects of climate change without long time series. Here, some conditions are already as they are expected to be due to climate change. Cities have therefore been described as “harbingers” of climate change (Ziska et al. 2003) that can be used as a proxy for future conditions.

The Urban Heat Island is described as the “relative warmth of a city compared with surrounding rural areas, associated with heat trapping due to land use, the configuration and design of the built environment, including street layout and building size, the heat-absorbing properties of urban building materials, reduced ventilation, reduced greenery and water features, and domestic and industrial heat emissions generated directly from

human activities“ (IPCC 2022b: 2926). On the basis of this definition, the characteristics of cities can be elaborated, especially in comparison with non-urban areas. Higher temperatures lead to earlier onset dates for the flowering of plants (Jochner & Menzel 2015) and thus the start of the pollen season. This and longer pollen seasons, e.g. for grass and birch trees, have been observed in urban areas (Rodríguez-Rajo et al. 2010; Anderegg et al. 2021; Kolek et al. 2021a). Higher temperatures leading to droughts, however, can shorten the pollen season for particular plant species (Gehrig 2006). In addition, artificial light at night has been found to influence the timing of phenological development in woody plants (Meng et al. 2022). Besides influencing flowering and season length, temperature can also influence pollen production (Jochner et al. 2013).

Compared to rural areas, urban areas are characterised by dense urban structures with buildings and infrastructure. Such structures increase surface roughness and influence wind circulation. For example, streets, bordered by tall buildings represent canyon-like structures that influence the dispersal of airborne pollen (Cariñanos et al. 2002; Peel et al. 2014a). A general heterogeneity of pollen levels in cities, leading to variations at small spatial scales, has been found in several studies (Emberlin & Norris-Hill 1991; Hjort et al. 2016; Werchan et al. 2017; Katz & Batterman 2020; Weger et al. 2020). In a recent study, Frisk et al. (2022) showed that grass pollen measured in urban locations originated from sources within 30 km, whereas in rural locations nearby sources (2-10 km) were more important.

In urban areas, atmospheric concentrations of most air pollutants are higher than in rural areas (Strosnider et al. 2017; Liu et al. 2019; Liu et al. 2021). Air pollutants can affect various plant characteristics and growth (Rivkin et al. 2020), and studies have reported the influence on pollen production, pollen allergen release and allergenicity (Beck et al. 2013; Frank & Ernst 2016; Sedghy et al. 2018; Galveias et al. 2020; Bernard-Verdier et al. 2022). Pollen production has been found to be affected by atmospheric concentrations of NO₂ (Jochner et al. 2013; Zhao et al. 2016; Zhao et al. 2017a), O₃ (Darbah et al. 2008; Albertine et al. 2014; Zhao et al. 2017b) and CO₂ (Ziska & Caulfield 2000; Stinson & Bazzaz 2005; Darbah et al. 2008). However, different results suggest that the effects of air pollutants on pollen production might vary between species.

Plant biodiversity is generally higher in urban areas, partly due to planting of non-native ornamental species or the import of exotic species for cultivation in parks and private gardens (Faeth et al. 2011). In addition, warmer conditions in cities favour the establishment of invasive species such as ragweed (*Ambrosia artemisiifolia*) or tree of heaven (*Ailanthus altissima*), as observed in Berlin, Germany (Werchan et al. 2022). Plant diversity can also be reflected in the pollen spectrum. In addition to the introduction of plants, the management and maintenance of green spaces in cities influences the pollen spectrum (Katz & Carey 2014; Jochner-Oette et al. 2017; Maya Manzano et al. 2017). In cities, green spaces are usually cultivated and subject to maintenance and management. Mowing grass areas or the cutting of trees and shrubs has a direct impact on the amount of pollen in the air, in particular if those measures are done before flowering. Jung et al. (2018) showed that the timing of grass-cutting can have a major impact on the amount of grass pollen in the air and on the severity of hay fever symptoms. The cutting and pruning of allergenic species have been included in recommendations for management practices with the goal to reduce allergenic pollen in the air (Ogren 2000; McInnes et al. 2017). However, observations of land management and maintenance practices are rarely included in aerobiological studies, even though they could provide information on their impact and therefore form the basis of recommendations for mitigation strategies.

In spring, the diurnal variations of allergenic pollen concentrations have been taken up by the media in Germany (e.g., Hexal AG 2023; Norddeutscher Rundfunk 2023; ntv.de 2023; tagesschau 2023) with recommendations for ventilation in urban and rural homes to help people with allergies. These recommendations suggest that urban homes should be ventilated in the morning and homes in the countryside in the evening. This advice is likely based on single studies and is therefore not suitable for making general recommendations. In fact, several aerobiological studies have investigated urban-rural differences in pollen production (Jochner et al. 2011; Kolek 2021) and pollen concentration (Mullins et al. 1986; Cariñanos et al. 2002; Gassmann et al. 2002; Kasprzyk 2006; Šikoparija et al. 2006; Bosch-Cano et al. 2011; Hugg et al. 2017; Kruczek et al. 2017; Lipiec et al. 2019; Frisk et al. 2022; Markey et al. 2022). In general, the results of these studies do not indicate a clear diurnal pattern of pollen concentration: for urban sites, some studies confirm comparatively low Poaceae pollen concentrations in the morning (Fuckerieder 1976; Mullins et al. 1986; Latałowa et al. 2005) but also higher

concentrations in the early evening (Mullins et al. 1986) or in the morning (Kasprzyk 2006) or the early afternoon (Markey et al. 2022). Two peaks, one in the morning and one in the afternoon, have also been observed (Latałowa et al. 2005). For rural sites, studies reported low Poaceae pollen concentrations in the evening (Fuckerieder 1976), higher concentrations at midday (Mullins et al. 1986), two peaks at midday and in the evening (Markey et al. 2022), or no distinct peak at all (Kasprzyk 2006). These different results indicate that no general diurnal pattern for Poaceae pollen concentrations exists, probably due to site-specific conditions. One of these drivers might be the different Poaceae species that flower at different times in the season with possibly different release and emission patterns during the day. In aerobiological studies, no differentiation is made between different species of the Poaceae family, because the grains share the same morphological characteristics and cannot be distinguished under the light microscope (Driessen et al. 1989; Jaeger 2008); this has to be considered when interpreting data. In addition, different meteorological conditions, which vary between years and regions (Camacho et al. 2017; Kmenta et al. 2017; Werchan et al. 2018), and distinct topo-climate (Jochner et al. 2012a; Wörl et al. 2022) influence variations of pollen loads.

In general, aerobiological data such as pollen concentration is mostly collected using volumetric traps of the Hirst design (Hirst 1952; Buters et al. 2018a). By installing the trap at roof level, background pollen concentrations are monitored that are representative of a surrounding area of approximately 30 km (Katelaris et al. 2004; Pashley et al. 2009). Studies have shown that pollen concentrations measured at rooftop level may not reflect pollen concentrations at street level, and may not reflect pollen exposure (Peel et al. 2014a; Werchan et al. 2017; Katz & Batterman 2020). Considering all this, it becomes clear that there is not enough knowledge on diurnal patterns at ground level, where people are actually exposed to allergenic pollen. Research is needed to clarify diurnal variations of grass pollen at street level in both urban and rural areas.

As mentioned at the start of this chapter, cities have been described as “harbingers” of climate change (Ziska et al. 2003). To put investigations on urban conditions into context, studies are readily conducted along urbanisation gradients that can be used to estimate the effect of increasing temperatures or, for example, increasing CO₂ concentrations (Pickett 1989; Ziska et al. 2003). In general, different environmental gradients have been used to conduct experiments in natural environments as they present, in contrast to

experiments in controlled environment, realistic environmental conditions (Tito et al. 2020). Moreover, along gradients, varying environmental factors can be observed over a relatively short distance (McDonnell & Pickett 1990; McDonnell & Hahs 2008). Urbanisation gradients have been used in studies for the investigation of pollen production of *B. pendula* (Jochner et al. 2011; Jochner et al. 2013; Kolek 2021), *Betula papyfera* (Darbah et al. 2008) or *Ambrosia artemisiifolia* (Ziska et al. 2003; Zhao et al. 2017a). Based on the investigation of the influence of urbanisation on pollen production, projections of pollen production under climate change conditions are made. However, the relationships that have been found do not follow a simple pattern that can be applied to all species and geographic locations.

1.3 Research questions and thesis outline

A survey of the existing literature in the previous sections indicates a need for more research on spatial and temporal variations of allergenic pollen. In particular, the influence of land use and land management on pollen levels, diurnal variations of pollen concentration as well as pollen production of allergenic species and their influencing factors need to be further investigated.

Therefore, the following central research questions will be addressed in this PhD thesis:

- (1) How do airborne pollen vary at different spatial and temporal scales?
- (2) Are there differences in the amount of airborne pollen between urban and rural areas?
- (3) What is the influence of land use and management on pollen loads?
- (4) Is there a difference in pollen production of allergenic species between urban and rural areas?
- (5) Do environmental factors such as air temperature or air pollutants influence the amount of pollen produced per plant?

This thesis is structured as follows:

The introductory part of this thesis (Chapter 1) provided an overview of the relevance of aerobiological studies and highlighted current research on aerobiological processes that influence the amount of pollen in the air. Special attention was given to airborne pollen

in urban areas. The chapter concluded with the defined research questions of this thesis. Next, Chapter 2 summarises the data and methods used to address the research questions.

This thesis contains three scientific publications in Chapters 3 to 5. The first two publications have been peer-reviewed and published (Chapters 3 and 4), whilst the third publication is under review (Chapter 5) (see Chapter “Individual Contributions”).

The first publication (Chapter 3), “**Spatial and temporal variations of airborne Poaceae pollen along an urbanization gradient assessed by different types of pollen traps**” explored airborne grass pollen levels in the city of Ingolstadt and its surroundings during the 2019 and 2020 grass pollen seasons. Three different types of traps were used, covering different temporal resolutions. Differences between measured pollen levels at roof level and at ground level were analysed. Furthermore, spatial and temporal patterns were also considered, taking into account influences such as land use, land management and meteorological data.

The second publication (Chapter 4), “**Investigation of spatial and temporal variations of airborne Poaceae, Myrtaceae and Cupressaceae pollen and *Alternaria* spores in Sydney, Australia, 2017-2020**”, examined the pollen and spore concentrations of selected pollen and spore types in Sydney and analysed them in relation to meteorological conditions. In addition, a sampling campaign was conducted to investigate differences in pollen deposition across the metropolitan area. The results are examined in the context of land use, meteorology and the large-scale weather situation.

The third publication (Chapter 5), “**How does pollen production of allergenic species differ between urban and rural environments?**”, examined the pollen production of the allergenic species silver birch *B. pendula*, ribwort plantain *P. lanceolata* and orchard grass *D. glomerata*. Pollen production was assessed quantitatively and compared between urban and rural sites. The influence of air temperature and atmospheric NO₂ and O₃ concentrations on pollen production was also investigated.

In the Discussion (Chapter 6), the main findings of the studies are discussed in the context of the current published literature and future research avenues are outlined. Chapter 7 presents the conclusion of this thesis. All references cited are listed in the References chapter, and in the Appendix is a list of all my publications, including those not included in this thesis.

2 Data and methods

2.1 Airborne pollen and spore data

2.1.1 Sampling methods for airborne pollen and spores

In this thesis, different methods were employed to collect data on airborne pollen and spores. They differ in their sampling principle (active vs. passive) and their temporal resolution of pollen concentration and pollen deposition. For the studies in this thesis, the following instruments were used: 7-day volumetric trap, portable volumetric trap (also referred to as personal volumetric air sampler), gravimetric pollen trap (also referred to as Durham sampler). The 7-day volumetric trap is the most common device to monitor aeroallergens (Buters et al. 2018a), while gravimetric traps and portable volumetric traps are not used as often. Advantages and disadvantages of each sampler are addressed in the Discussion in Chapter 6.1.

7-day volumetric trap. Volumetric pollen traps have been in use for several decades and have been the standard instruments for measuring atmospheric pollen concentrations. They are based on the Hirst-principle (Hirst 1952). A more detailed description of their mode of operation can be found in Chapter 3 and 4. The trap can be either mains or battery operated, allowing continuous monitoring. A temporal resolution of up to two hours can be achieved. Two traps of this type (Burkard Manufacturing Co Ltd, Rickmansworth, UK, Fig. 3) were used to record data in Ingolstadt and Kösching Germany, for the period from spring 2019 to autumn 2020 (Chapter 3). Daily and two-hourly Poaceae pollen concentration data were analysed for the corresponding study. Another trap of the same type and manufacturer was used to record data in Sydney, Australia, from 2017 to 2020 (Chapter 4). Data on daily pollen concentrations of Poaceae, Myrtaceae, Cupressaceae and the spore concentrations of *Alternaria* were analysed. These data were collected and analysed in collaboration with the Aerobiology Working Group of Macquarie University, Sydney.

Portable volumetric trap. Portable volumetric traps record pollen concentrations and work on the same principle as the 7-day volumetric traps, but are more compact and suitable for shorter measurement times (up to 60 minutes) due to their design (see Chapter

3 for details). Personal volumetric air samplers (PVAS, Burkard Manufacturing Co Ltd., Rickmansworth, England, Fig. 4) were used for the sampling campaign in Ingolstadt in 2019. They were mounted on tripods at a height of approximately 1.5 m above the ground to represent the average breathing height.

Gravimetric pollen trap. In contrast to the active traps described above, which rely on impaction, the gravimetric trap, or Durham sampler (Durham 1946), relies on sedimentation, i.e., the gravitational settling of pollen. As the volume of air sampled is not known, it is infeasible to measure pollen concentration with this trap. Instead, pollen deposition is described as pollen grains per surface area (pollen grains/cm²). Gravimetric traps were used in two studies (Chapters 3 and 4), and purpose-built after the same principle, only differing slightly in size (Fig. 5). There were 12 of these traps in use for the study in Ingolstadt, Germany, from June to August in 2019 and 2020. The traps were mounted on metal posts at a height of 2 m above the ground. A total of ten traps were used for a sampling in Sydney, over an eight-week period from November 2019 to January 2020. In this case, the traps were mounted to railings around the roof of air quality monitoring stations at a height of about 3 m above ground level.

Pollen samples were prepared in the laboratory for analysis and pollen and spores of interest in the respective studies were identified and counted using a light microscope. Poaceae, Cupressaceae, Myrtaceae and *Alternaria* were identified and counted to determine pollen concentration and pollen deposition in the studies in Chapters 3 and 4.



Fig. 3 7-day volumetric sampler.



Fig. 4 Portable volumetric trap, mounted on a tripod at a height of 1.5 m.



Fig. 5 Gravimetric pollen trap used in Germany (left) and Australia (right).

2.1.2 Measures for describing the amount of pollen in the air

Aerobiological data obtained from the 7-day volumetric trap (Chapters 3 and 4) were described following the recommended terminology by Galán et al. (2017). This included the start and end of the pollen or spore season, duration of the pollen or spore season, Seasonal Pollen Integral (SPIn), Annual Pollen Integral (APIn), Annual Spore Integral (ASIn), peak days with peak values, and number of days with pollen or spore concentrations above a certain threshold. Data from the portable volumetric traps (Chapter 3) were described using the proposed characteristics in a study by Jochner-Oette et al. (2017). This included the Campaign Pollen Integral (the sum of all measurements for each location) and the Daily Pollen Integral (the sum of all measurements of one day). Pollen deposition data obtained with gravimetric traps were described and analysed using a Campaign Pollen Integral and Weekly Pollen Integral (the sum of all measurements of the campaign and per week) (Chapters 3 and 4). A more detailed description of the terms can be found in the Methods sections of the corresponding studies.

2.2 Sampling and analyses for the estimation of pollen production

For the research conducted in Chapter 5, pollen production of the three allergenic species *B. pendula*, *P. lanceolata* and *D. glomerata* was quantitatively assessed. *Betula* pollen are considered to be the most allergenic tree pollen in northern, eastern and central Europe (D'Amato et al. 2007a) and is a major cause of allergic symptoms, with increasing sensitisation rates (Biedermann et al. 2019). In recent decades, sensitisation rates to *Plantago* pollen have also increased, almost doubling from 27 % (1998-2007) to 51 % (2008-2017) (Forkel et al. 2020). Grass pollen allergy is the most common allergy (García-Mozo 2017), and therefore it is important to study species belonging to the Poaceae family, such as *D. glomerata*. Plant material was collected, which was then further prepared and analysed in the laboratory. Values for pollen production were expressed per floral unit, i.e. per inflorescence (*B. pendula*, *P. lanceolata*), flower (*B. pendula*, *P. lanceolata*, *D. glomerata*), spikelet (*D. glomerata*) or centimeter (*D. glomerata*). The analyses for this study followed an approach proposed by Faegri & Iversen (1989) and Damialis et al. (2011), according to which the floral unit is placed in a solution consisting of potassium hydroxide and distilled water until some of the plant material has dissolved and pollen grains are released. Subsequently, samples are taken from this solution and the number of pollen grains is determined using light microscopy. The pollen production per floral unit is then calculated by extrapolating from the partial sample to the whole sample. While this method is based on the extraction and counting of pollen grains, and has been employed to allow comparison to recent studies on *Betula* pollen production (i.e, Ranpal et al. 2022, 2023; Kolek 2021), other methods that have been used to determine pollen production include the drying and weighing inflorescences and pollen grains (Jochner et al. 2011; Beck et al. 2013; Jung et al. 2018).

2.3 Land use and land cover data

For the study area in Germany (Chapters 3 and 5), data on land cover for 2019 were obtained from mundialis (2020) and data on grass cover for 2018 were obtained from Copernicus Land Monitoring Service (2020). For the study area in Australia (Chapter 4), land use and land cover data were obtained from Geoscience Australia (2017). Based on

these datasets for land use/cover, urban index according to Jochner et al. (2012b) was calculated in order to quantify the degree of urbanisation (Chapters 3, 4 and 5).

2.4 Land use regression model and pollution data

The land use regression model (LUR) by Fritsch & Behm (2021) was used in the study in Chapter 5 to obtain site-specific data on the atmospheric concentration of the pollutants NO₂ and O₃. The datasets used for the model were pollutant concentrations measured at the sites of the German air quality monitoring network in 2019 and 2020 (EEA 2022), land use based on the CORINE land cover classes (EEA 2020), topography (BKG 2021a), German administrative regions and population density (BKG 2021b), and the road traffic network (EuroGeographics 2022).

2.5 Meteorological data

For the period of airborne pollen sampling in Germany (Chapter 3), data on the meteorological parameters air temperature, wind speed and wind direction were obtained from the station “Ingolstadt (Flugplatz)” (located at 48.7112° N, 11.5362° E) that is operated by the German Meteorological Service. Precipitation data were obtained from the station in Ingolstadt operated by the Bavarian Environment Agency (LfU).

The temperature data used for the study on pollen production (Chapter 5) were derived from ten temperature loggers (Hobo Pro v2 U23-001, ONSET, Bourne, Massachusetts, USA) and two weather stations (Davis Vantage Pro2, Davis Instruments Corporation, Hayward, California, USA).

For the study in Sydney, Australia (Chapter 4), meteorological data, i.e., air temperature, precipitation, relative humidity, wind speed and wind direction, were obtained from ten stations operated by the New South Wales Department of Planning and Environment (DPE), which were equipped with the 7-day volumetric trap (continuously) at one location and gravimetric samplers (for the duration of the sampling campaign) at ten locations.

2.6 Statistical and spatial analyses and visualization

Statistical analyses were performed using R (4.2.2). Analyses included Spearman's correlation analyses, regression analyses, Wilcoxon rank-sum test and Kruskal-Wallis test. In order to analyse correlations in more detail, component plus residual plots were created. For spatial analyses ESRI ArcMap 10.6 was used, for the creation of maps QGIS 3.14 and for the heatmaps Microsoft Excel 2016 was used.

3 Spatial and temporal variations of airborne Poaceae pollen along an urbanization gradient assessed by different types of pollen traps

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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 semi-rural 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/m³ vs. 8.5 pollen grains/m³ in the morning and 10.4 pollen grains/m³ 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



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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].

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 × 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 × W × H = 5.5 cm × 9.5 cm × 3 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^3) 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^2).

SPATIAL AND TEMPORAL VARIATIONS OF AIRBORNE POACEAE POLLEN ALONG AN URBANIZATION GRADIENT ASSESSED BY DIFFERENT TYPES OF POLLEN TRAPS

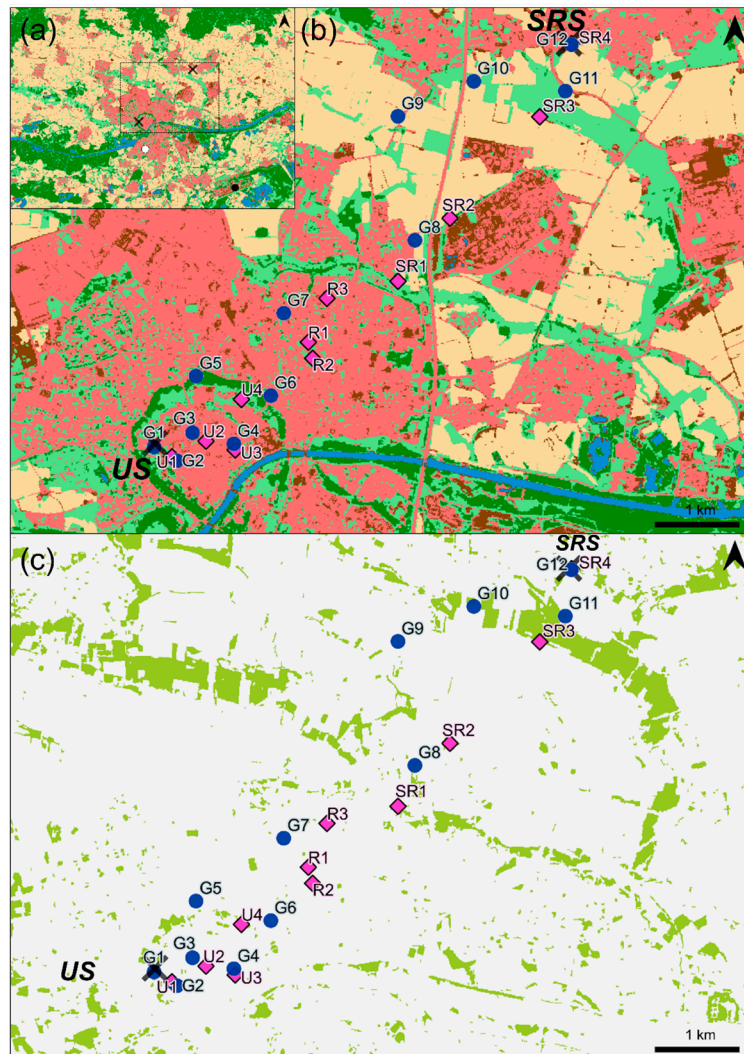


Figure 1. Locations of pollen traps: Blue circles—gravimetric traps; pink squares—PVAS; black crosses—volumetric pollen traps (roof level); (a) Study area (dashed box) and surrounding land use; white circle—station measuring precipitation (LfU); black circle—weather station (DWD); (b) Land use 2019: 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].

SPATIAL AND TEMPORAL VARIATIONS OF AIRBORNE POACEAE POLLEN ALONG AN URBANIZATION GRADIENT ASSESSED BY DIFFERENT TYPES OF POLLEN TRAPS

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 Code | Coordinates | Distance from Urban Station (US) [km] | Urban Index | Grass Cover [%] | Land Cover Classification [%] | | | Agriculture | Description of Immediate Surrounding and Management of Poaceae Area within a Radius of 100 m | |
|--------------|------------------------|---------------------------------------|-------------|-----------------|-------------------------------|----------------|----------|-------------|--|--|
| | | | | | Forest | Low Vegetation | Built-Up | | | Bare Soil |
| G1 | 48.7650° N, 11.4153° E | - | 0.51 | 6 | 8 | 29 | 62 | 1 | 0 | Green space of the Ingolstadt School of Management; irregular-cut Poaceae area, allowing it to flower for short 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 with small backyard; sealed inner-city areas with no 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 lawn and playground; sealed inner-city areas with no Poaceae area. |
| G5 | 48.7726° N, 11.4221° 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; irregular cut Poaceae area allowing flowering for short 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 next to a multilane street; residential and half-industrial 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 and agricultural land; 2019: field with Poaceae was cut occasionally allowing flowering for short periods; 2020: Field with Poaceae was cut once in August allowing 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 to the secondary school of Kösching and agricultural land; occasionally cut Poaceae area, allowing flowering for short periods. |

SPATIAL AND TEMPORAL VARIATIONS OF AIRBORNE POACEAE POLLEN ALONG AN URBANIZATION GRADIENT ASSESSED BY DIFFERENT TYPES OF POLLEN TRAPS

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.

| Station Code | Coordinates | Distance from US [km] | Urban Index | Grass Cover [%] | Land Cover Classification [%] | | | | Agriculture | Description of Immediate Surrounding and Management of Poaceae Area within a Radius of 100 m |
|--------------|------------------------|-----------------------|-------------|-----------------|-------------------------------|----------------|----------|-----------|-------------|--|
| | | | | | Forest | Low Vegetation | Built-Up | Bare Soil | | |
| 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; 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 maintained front and back yards; Poaceae areas/lawns 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 surrounded by houses; Poaceae areas/lawns cut 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; 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 neighbouring agricultural land; unmanaged and 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. Green area next to the secondary school of Kösching and agricultural land; occasionally cut Poaceae area, allowing flowering for short periods. |
| SR4 | 48.8081° N, 11.4831° E | 6.88 | 0.26 | 11 | 0 | 8 | 37 | 13 | 42 | |



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/m³) 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/m³ 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., CPIn_{grav} and CPIn_{PVAS} [51].
- Weekly Pollen Integral (WPIn)
 - The sum of all daily means of one week of background pollen concentration, i.e., WPIn_{US} for volumetric trap at US (roof level), and WPIn_{SRS} for volumetric trap at SRS (roof level).
 - The sum of all gravimetric measurements of all locations for one particular week, i.e., WPIn_{grav}. We refer to the week with the highest WPIn_{grav} as a peak week of the campaign.
- Daily Pollen Integral (DPIn)
 - The sum of all PVAS measurements of one day, i.e., DPIn_{PVAS}.

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_s).

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 × 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 × 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_s).

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/m³, 2020: 5725 pollen*day/m³ vs. US 2019: 933 pollen*day/m³, 2020: 2104 pollen*day/m³) 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).

SPATIAL AND TEMPORAL VARIATIONS OF AIRBORNE POACEAE POLLEN ALONG AN URBANIZATION GRADIENT ASSESSED BY DIFFERENT TYPES OF POLLEN TRAPS

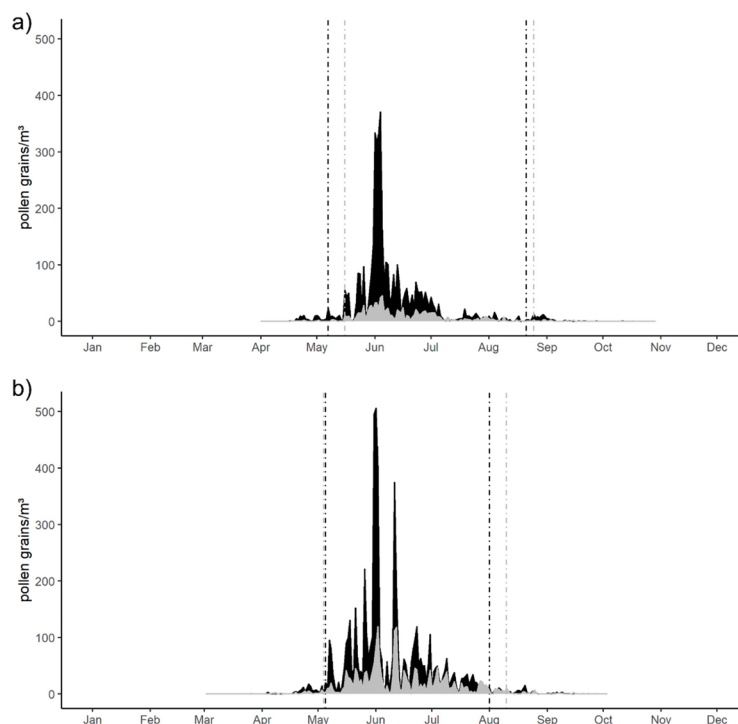


Figure 3. Poaceae pollen concentration (daily mean values) for the SRS (black) and US (grey) locations in the greater area of Ingolstadt. Dashed lines indicate start and end dates of the corresponding grass pollen season (a) 2019 and (b) 2020.

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.

| | US | | SRS | |
|---|-----------|-----------|-----------|-----------|
| | 2019 | 2020 | 2019 | 2020 |
| Seasonal Pollen Index (SPIn) (pollen*day/m ³) | 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/cm²) and CPIn_{grav} (max. 2299) were recorded at the

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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 $WPIn_{grav}$, was highest at G10 (semi-rural) with 1140 pollen grains/cm² and lowest at G4 (urban) with 39 pollen grains/cm² (approx. 3.5% of G10). The site with the highest $CPIn_{grav}$ was G10 ($CPIn_{grav} = 2299$ pollen grains/cm²), and the lowest $CPIn_{grav}$ was recorded for G5 (urban park; $CPIn_{grav} = 100$ pollen grains/cm²). Kruskal–Wallis test revealed no significant difference between sites (Kruskal–Wallis chi-squared = 7.301, p -value = 0.774).

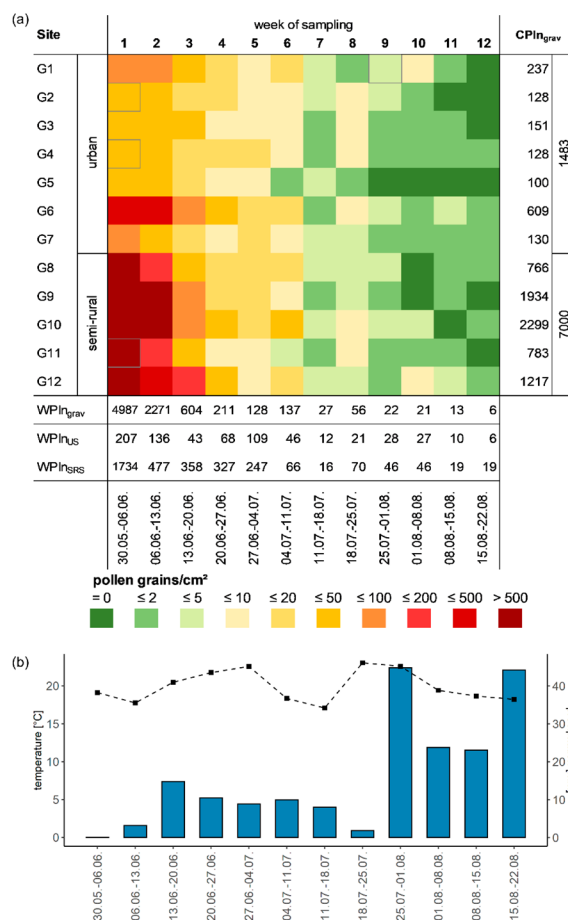


Figure 4. (a) Heat map showing weekly grass pollen deposition (pollen grains/cm²) in 2019 for the sampling campaign figure 30 May to 22 August 2019 and $WPIn_{US}$ and $WPIn_{SRS}$ for the respective weeks. Gravimetric traps are ordered by distance from the urban station (US). A grey outline indicates 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.

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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 $WPI_{n_{grav}}$, 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 week 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 $WPI_{n_{US}}$ and $WPI_{n_{RS}}$. Pollen deposition in the peak week (1 June–8 June) was highest at G8 (semi-rural), with 954 pollen grains/cm² and lowest at G4 (urban) with 27 pollen grains/cm² (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 |
| urban | G2 | 0.756 | - | - | - | - | - | - | - | - | - | - |
| | G3 | 0.985 | 0.969 | - | - | - | - | - | - | - | - | |
| | G4 | 0.937 | 0.400 | 0.837 | - | - | - | - | - | - | - | |
| | 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 | - | - | - | - | |
| | semi-rural | 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 | - | - | |
| 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 ($r_s = -0.726, p < 0.005$).

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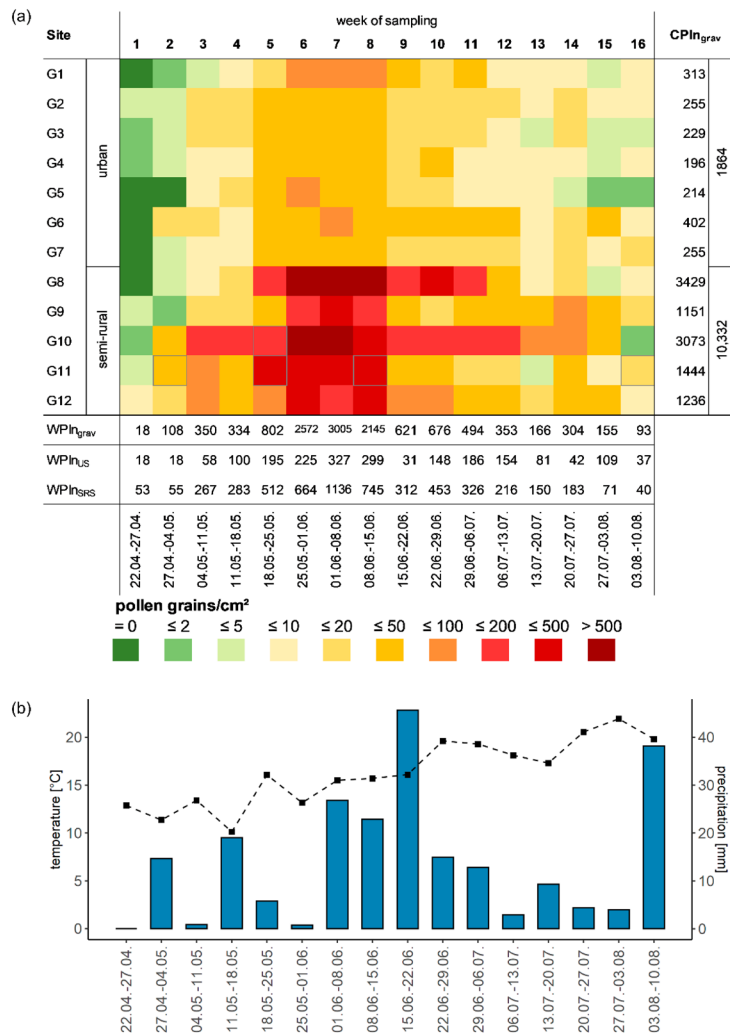


Figure 5. (a) Heatmap showing weekly grass pollen deposition (pollen grains/cm²) in 2020 for the sampling campaign from 22 April to 10 August 2020, WPin_{grav}, WPin_{US}, and WPin_{SRS} for the respective weeks. Gravimetric traps are ordered by distance from the urban station (US). A grey outline indicates 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.

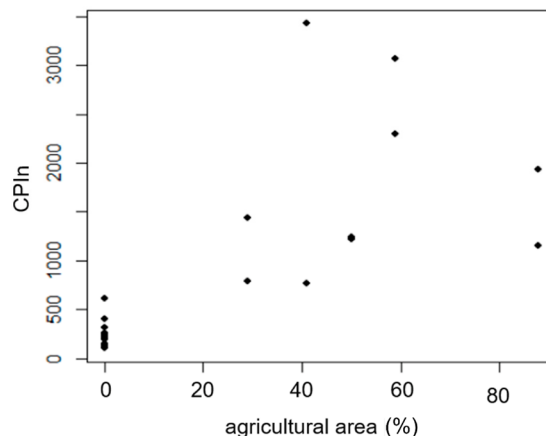


Figure 6. Scatterplot showing the relationship of CPIn of both years and the percentage of agricultural area within a 100 m radius of each sampling location.

3.3. PVAS Sampling Campaign 2019

3.3.1. Daily Variation

DPIn_{PVAS} 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/m³), SR4 (17.6 pollen grains/m³), and SR1 (15.8 pollen grains/m³) located in the countryside. The lowest means were associated with SR3 (3.6 pollen grains/m³) and R3 (7.6 pollen grains/m³). 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/m³, at noon 22.2 pollen grains/m³, and in the evening 10.4 pollen grains/m³. 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).

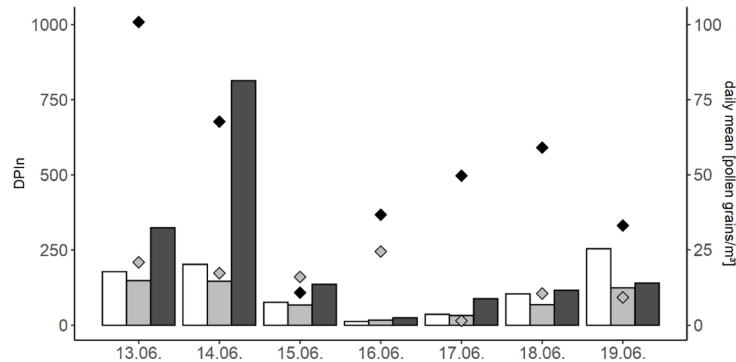


Figure 7. Sum of $DPIn_{PVAS}$ for locations in urban area/city centre (U; white), residential area (R; light grey), and semi-rural environment (SR; dark grey). Squares represent daily mean pollen concentration measured at rooftop level: black—SRS and grey—US.

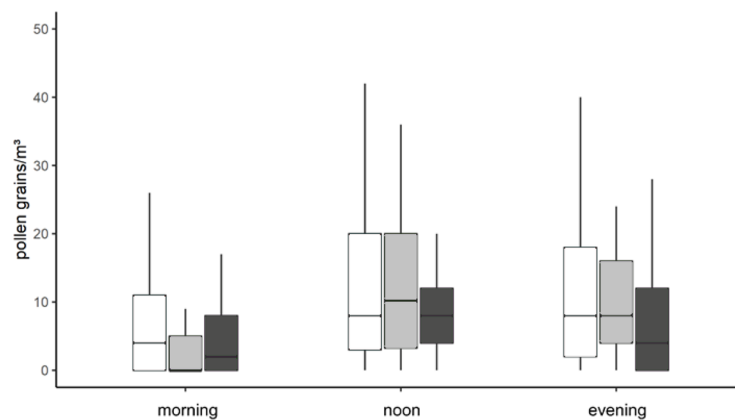


Figure 8. Boxplots showing distribution of pollen concentrations by measurement period. Horizontal line = median, boxes = interquartile range. Outliers not shown. Colours for sampling locations: white—city centre, light grey—residential area, dark grey—semi-rural area.

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.

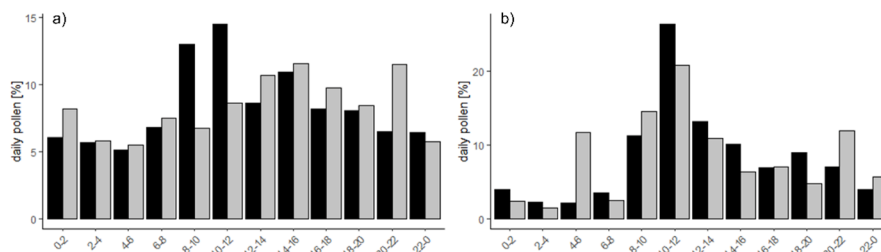


Figure 9. Diurnal pattern of Poaceae pollen concentration recorded by both 7-day volumetric traps, grey—US, black—SRS (a) during 2019; (b) during the week of PVAS sampling (13 June to 19 June 2019).

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 CPI_{PVAS} 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 SPln (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^3 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 $CPIn_{grav}$ in 2019 with a value above 1000 were measured at sites classified as semi-rural (Table 1). These high values of the $CPIn_{grav}$ 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, $CPIn_{grav}$ 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 $CPIn_{grav}$ (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 semi-rural 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 (WPI_n), Daily Pollen Integral (DPI_n), and Campaign Pollen Integral (CPI_n), should be defined and included in the recommend terminology for describing aerobiological data. Furthermore, we suggest the use of "pollen grains per cm²" 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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4 Investigation of spatial and temporal variations of airborne Poaceae, Myrtaceae and Cupressaceae pollen and *Alternaria* spores in Sydney, Australia, 2017-2020

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Investigation of spatial and temporal variations of airborne Poaceae, Myrtaceae and Cupressaceae pollen and *Alternaria* spores in Sydney, Australia, 2017–2020

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Abstract There is a need for information on pollen exposure to assess allergy risk. Monitoring of aeroallergens in a city is usually limited to the use of a single trap for the whole area. While a single trap provides enough information on background pollen concentration for the area, varying pollen exposure across different urban environments, however, is not considered. In this study, we analysed aerobiological data of three pollen seasons (2017–2020) recorded with a volumetric pollen trap in Sydney, Australia. In order to assess spatial differences in pollen exposure across the city, we installed ten gravimetric traps recording pollen deposition for eight weeks during the summer of 2019/2020. We considered the influence of meteorological variables, land use, urbanisation and distance to the sea. Our results showed differences in pollen season characteristics across the three analysed seasons and correlations with meteorological parameters. Considering all years, we found for Poaceae and *Alternaria* the strongest positive correlation with mean temperature and for

Myrtaceae and Cupressaceae with maximum temperature. Likewise, there were negative correlations with humidity (Myrtaceae, Cupressaceae, *Alternaria*) and precipitation (Myrtaceae, Cupressaceae). Days with medically relevant pollen and spore concentrations varied between years and we recorded the highest amount in 2017/2018 for Poaceae and *Alternaria* and in 2019/2020 for Myrtaceae. In addition, we found spatial and temporal variations of pollen deposition. However, we did not detect significant correlations between pollen deposition and land use, which can be attributable to drought conditions prior to the sampling campaign and the temporal setting in the pollen season. This study highlights the importance of continuous volumetric aerobiological monitoring as well as the assessment of pollen exposure at several locations across a large urban area.

Keywords Pollen concentration · Spatial and temporal variations · Gravimetric traps · Intra-urban variations

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1 Introduction

The analysis of pollen and spore seasons in a city or region is a necessity for assessing aeroallergen exposure to people living there. With an increasing number of urban inhabitants and with metropolitan areas expanding (OECD/European Union, 2020), the relevance of monitoring aeroallergens in cities will

even increase in the future. Information on pollen is crucial for human health, as between 10 and 30% of the population is affected by allergic rhinitis worldwide (Pawankar et al., 2013). In addition, the impact of climate change on aeroallergens could already be observed: increasing air temperature and atmospheric carbon dioxide concentration are potential drivers that lead to modifications in regard to pollen production/concentration, pollen allergenicity, start and duration of the pollen season as well as plant and pollen distribution (Beggs, 2015; Katelaris & Beggs, 2018; Menzel & Jochner, 2016; Ziska et al., 2019). These impacts are also relevant for spores such as the allergologically relevant *Alternaria* spore (D'Amato et al., 2020; Cecchi et al., 2010).

Scientists started studying pollen in the early twentieth century and the term “aerobiology” was used from the 1930s onwards (Agashe & Caulton, 2009). The first programme including aerobiological measurements was established in 1968 in the USA and the International Association of Aerobiology (IAA) was founded in 1974 in The Hague, the Netherlands (International Biological Program, 1970). However, the density of aerobiological measuring stations differs between continents: in 2016, there were only nine stations in Africa, 151 in America, 182 in Asia, 525 in Europe and 12 in Oceania (Buters et al., 2018). Compared to the Northern Hemisphere, there is only a small number of monitoring stations in Australia. This results in a lack of research on aeroallergens in Australia (Addison-Smith et al., 2021; Beggs, 2018; Davies et al., 2021), even though about 19% of Australia's citizens reported to have allergic rhinitis in 2017/2018 (Australian Institute of Health & Welfare, 2020).

In Sydney, the biggest city in Australia, air quality has been closely monitored for several years (Paton-Walsh et al., 2019; Simmons et al., 2019; Wadlow et al., 2019), with a network of monitoring stations run by the New South Wales (NSW) Department of Planning and Environment (DPE). However, pollen and spores are not included into the broad monitoring program. Nevertheless, several studies were conducted using volumetric traps analysing airborne pollen and spores (Bass & Morgan, 1997; Beggs et al., 2015; Davies et al., 2022; Katelaris et al., 2000, 2004; Medek et al., 2016; Stennett & Beggs, 2004b).

Grass pollen are one of the major aeroallergens in Australia and New Zealand (Davies et al., 2012;

Haberle et al., 2014), which, along with Cupressaceae pollen, accounts for over 50% of total airborne pollen throughout the year. These pollen types are followed by *Betula*, Myrtaceae, *Pinus*, Oleaceae, *Casuarina*, *Plantago* and *Rumex* (Haberle et al., 2014). Therefore, it is not surprising that some studies conducted in Sydney solely focus on grass pollen (Beggs et al., 2015; Medek et al., 2016), but some also consider pollen of other species such as Cupressaceae or Eucalypt species (Katelaris et al., 2000, 2004; Stennett & Beggs, 2004b) and spores such as *Alternaria* (Bass & Morgan, 1997; Stennett & Beggs, 2004a).

Between 2008 and 2013, 90% of grass pollen in Sydney was registered between late September and early April, with peak values recorded between October and December. In some years, a second peak around February could be observed, probably caused by different grass species with different flowering times (Beggs et al., 2015; Medek et al., 2016).

Cupressaceae pollen were recorded to have their peak between August and September (Katelaris et al., 2004). Myrtaceae pollen reach peak concentrations in the summer months December and January but can be detected with small quantities almost year-round (Bass & Morgan, 1997). *Alternaria* spores can also be found in the air of Sydney throughout the year, with peaks in the summer months (Bass & Morgan, 1997).

In order to evaluate spatial differences in pollen exposure across cities as well as to detect urban–rural differences, networks of gravimetric pollen traps have been used (Jetschni & Jochner-Oette, 2021; Katz & Carey, 2014; Stas et al., 2021; Werchan et al., 2017). The use of several traps can help to evaluate different influences on the amount of airborne pollen that should be considered: local vegetation and land use (Cariñanos et al., 2002, 2019; Charalampopoulos et al., 2018), urbanisation (Katz & Batterman, 2020; Katz et al., 2019; Peel et al., 2014; Rodríguez-Rajo et al., 2010) and meteorological parameters (Oduber et al., 2019; Puc, 2012; Rojo et al., 2015; Stennett & Beggs, 2004b; Tassan-Mazzocco et al., 2015; Vara et al., 2016) including the sea-land breeze circulation (Gassmann, Pérez and Gardiol, 2002; Gassmann & Gardiol, 2007).

The aim of this study was to investigate spatial and temporal aerobiological data from Sydney. We focussed on the pollen types Poaceae, Myrtaceae

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and Cupressaceae and the spore type *Alternaria*. We analysed seasonal characteristics and assessed the relationship with meteorological parameters during the period from 2017 to 2020.

2 Materials and methods

2.1 Study area

The pollen measurement campaigns were conducted in Sydney, Australia (33.85° S, 151.21° E). Sydney is located at the east coast of Australia with a mean elevation of 3 m a.s.l. The Sydney Metropolitan Area has more than 5.3 million inhabitants (Australian Bureau of Statistics, 2021). Its climate is classified as humid subtropical, with a mean annual minimum temperature of 14.7 °C, mean annual maximum temperature of 22.8 °C and an average precipitation of 1,150 mm (1991–2020) (Australian Bureau of Meteorology, 2021).

Due to the size of the Sydney Metropolitan Area (12,000 km²) that extends over 70 km to the west, a variety of land uses can be observed: in the north and

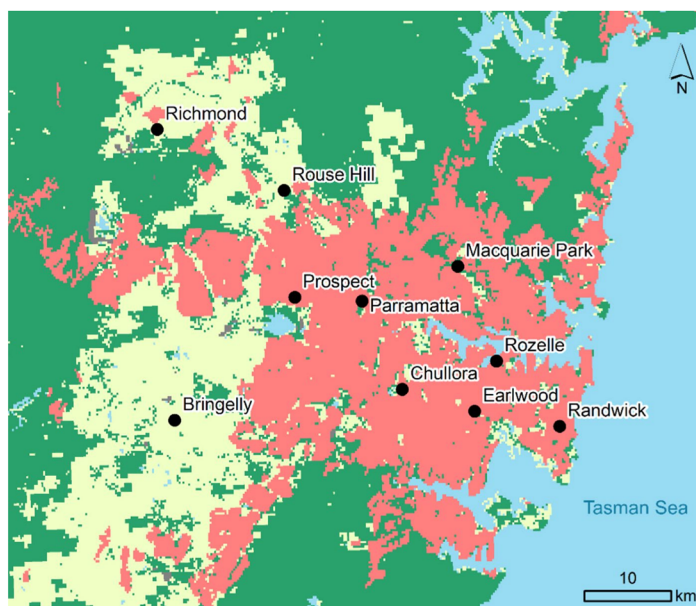
south, urban areas are adjacent to National Parks consisting of forests, while in the west, areas are covered by lower vegetation. Continuous airborne pollen and spore sampling was conducted at Richmond (October 2017 to February 2020) and we set up a network consisting of ten additional pollen traps across the study area in the summer of 2019/2020 (mid-November to mid-January) (Fig. 1).

2.2 Pollen monitoring

2.2.1 Volumetric pollen/spore trap

Airborne pollen and spores were monitored from 2017 to 2020 using a 7-day-recording volumetric pollen/spore trap (Burkard Manufacturing Co Ltd., Rickmansworth, UK) that is based on the Hirst principle (Hirst, 1952). This trap uses an integrated pump to aspirate 10 L of air per minute through an orifice (14×2 mm), behind which a rotating drum is mounted. A plastic strip (Melinex, DuPont Teijin Films, Luxembourg) attached to the drum is coated with a silicone solution (Silicone fluid mixed with Dimethylether; Lanzoni, Bologna, Italy) allowing

Fig. 1 Map of the Sydney Metropolitan Area with land cover and measurement locations. *Dark green*—woodland and forests, *yellow*—low vegetation and pastures; *red*—built-up area; *blue*—water; *grey*—mines and quarries. Land use data: Geoscience Australia (2017)



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airborne particles to adhere to the tape. The drum completes one revolution in seven days (2 mm per hour) and is therefore changed once a week. Daily samples were prepared in the laboratory.

The trap was set up in Richmond in north-western Sydney (33.6164° S, 150.7473° E) at an air quality monitoring station run by the New South Wales Department of Planning and Environment (DPE) (NSW Department of Planning & Environment, 2021). The trap was mounted to a railing around a platform on top of the station at a height of approx. 3 m above the ground (Fig. 2a). Sampling was performed according to the Australian Airborne Pollen and Spore Monitoring Network standards (Beggs et al., 2018).

The immediate vicinity of the trap is characterised by low grassland and a few isolated trees and shrubs. In the wider surroundings, built-up structures (buildings, roads) of the Hawkesbury Campus of the Western Sydney University can be found to the north and grassland and forest areas to the east, south and west (Fig. 2b).

2.2.2 Gravimetric pollen traps–sampling network

Ten purpose-built gravimetric traps (Durham-type; Durham, 1946) (Jetschni & Jochner-Oette, 2021), based on passive sampling, were used in this study. They were composed of two horizontal circular plastic discs with a diameter of 23 cm, which were held apart at a distance of 27 cm with four threaded rods. A wooden block ($L \times W \times H=9.5 \text{ cm} \times 5.5 \text{ cm} \times 4.0 \text{ cm}$) was attached to the lower disc and equipped with a clip to secure a microscope slide horizontally. The microscope slides

were coated with the silicone solution described in Sect. 2.2.1, allowing airborne particles to settle and adhere to them due to gravity. The traps were attached to the railing at about 3 m above the ground (Fig. 3), similar to the volumetric trap (Fig. 2).

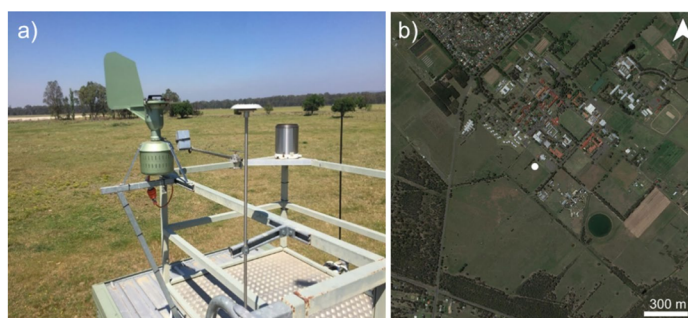
The traps were set up at ten locations throughout the Sydney Metropolitan Area (Fig. 1) where air quality monitoring stations (New South Wales Department of Planning and Environment) served as standardised sampling locations (e.g. same height above the ground). These stations can be found throughout the study area and provide easy accessibility and security. We selected the sampling locations based on representativeness of different environments, i.e. the distance to the sea, urbanisation and land use (Table 1).

Sampling started on 20 November 2019 and lasted for eight weeks until 14 January 2020. Microscope slides were changed weekly at the same time of the day (between 8 am and 2 pm), as the stations were



Fig. 3 Passive sampler mounted to the railing of an air quality monitoring station in Rouse Hill

Fig. 2 a) Pollen/spore trap mounted to the railing in Richmond b) Surroundings of the trap (white dot) (base map: CNES/Airbus, Maxar Technologies, Google 2022)



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Table 1 Sampling locations and surroundings of gravimetric pollen traps

| Station | Coordinates | Distance to the sea [km] | Urban index | Land cover [%] within 100 m | | | | Characteristics of the surroundings |
|----------------|-------------------------|--------------------------|-------------|-----------------------------|-----------------------------|----------------------|-------|---|
| | | | | Built-up area | Low vegetation and pastures | Woodland and forests | Water | |
| Randwick | 33.9318° S, 151.2428° E | 3.0 | 0.85 | 100 | 0 | 0 | 0 | Restricted military area; low cut and maintained grass area and built-up structures |
| Rozelle | 33.8644° S, 151.1639° E | 11.9 | 0.66 | 98 | 0 | 2 | 0 | Low cut maintained grass area, nearby shrubs and trees |
| Earlwood | 33.9162° S, 151.1358° E | 13.7 | 0.82 | 87 | 4 | 9 | 0 | Direct vicinity to residential area, playground, park land and football field; maintained and low cut grass |
| Macquarie Park | 33.7653° S, 151.1178° E | 18.2 | 0.65 | 50 | 2 | 48 | 0 | Sports area with football fields bordered by trees; nearby bushland |
| Chullora | 33.8916° S, 151.0461° E | 23.0 | 0.94 | 86 | 7 | 5 | 2 | Green space in an industrial area; grass area with nearby shrubs and trees |
| Parramatta | 33.7995° S, 150.9978° E | 29.4 | 0.77 | 89 | 2 | 9 | 0 | Park area with single buildings and low cut grass; nearby parking lot with trees |
| Prospect | 33.7942° S, 150.9142° E | 37.7 | 0.79 | 97 | 3 | 0 | 0 | Edge of recreational area with football field and run track; low cut grass, scattered trees and bushes |
| Rouse Hill | 33.6828° S, 150.9037° E | 40.8 | 0.39 | 0 | 65 | 35 | 0 | Area of low cut grass surrounded by trees and built structures |
| Bringelly | 33.9177° S, 150.7619° E | 50.7 | 0.50 | 0 | 100 | 0 | 0 | Rural environment, large areas of grassland with shrubs and trees; few houses and streets |
| Richmond | 33.6164° S, 150.7473° E | 57.3 | 0.80 | 11 | 79 | 10 | 0 | Grassland with scattered trees |

Land cover (Geoscience Australia, 2017) within 100 m of the traps (see Methods section). Stations ordered in ascending distance from the sea

visited one after the other in the same order every week. In total, we collected 80 slides, which were prepared in the laboratory.

2.2.3 Sample preparation

In the laboratory, we used glycerine jelly with fuchsine stain (Lanzoni, Bologna, Italy) to fix the daily lengths of plastic strip of the volumetric trap to microscope slides and to stain them. For the glass slides from the gravimetric trap, we used a mixture of distilled water, gelatine, glycerol, and fuchsine stain. The samples were analysed using light microscopy (AxioLab.A1 (Zeiss, Wetzlar, Germany) and Olympus CX23 (Olympus Scientific Solutions, Tokyo, Japan)) using 400× magnification.

Pollen of the taxa Poaceae, Myrtaceae and Cupressaceae as well as *Alternaria* spores were identified and counted along four longitudinal transects (resulting in approx. 10% of the whole slide). Counts were converted to pollen and spore concentration per cubic metre of air (pollen grains/m³, spores/m³). In the case of the gravimetric traps, Poaceae, Myrtaceae and Cupressaceae were identified and counted along four longitudinal transects and then converted to pollen grains per square centimetre of slide surface (pollen deposition in pollen grains/cm²) (Jetschni & Jochner-Oette, 2021).

2.3 Analyses

2.3.1 Aerobiological data

To describe aerobiological data of Poaceae, Myrtaceae, Cupressaceae and *Alternaria* collected by the volumetric trap, we used the recommended terminology by Galán et al. (2017). Because of the study's location in the Southern Hemisphere, pollen data were calculated and visualised with the course of 12 months starting on 1 July and ending on 30 June of the following year (Beggs et al., 2015; Medek et al., 2016). Gaps no bigger than five consecutive days were filled using interpolation (moving mean; Picornell et al., 2021).

Due to data gaps, we calculated neither start/end dates of pollen or spore seasons nor the Seasonal Pollen/Spore Integral. We did, however, calculate the Annual Pollen Integral (API_n; the sum of the daily mean pollen concentrations, pollen*day/m³)

during the course of a year (Galán et al., 2017) of the available data and the Annual Spore Integral (ASIn), respectively. In addition, we identified peak days with peak values, i.e. the days with the highest daily mean pollen/spore concentration of the season. In order to compare pollen and spore integrals between the years despite missing data, we calculated the mean of the daily pollen or spore concentrations by dividing the integrals by the number of observation days for each year. To account for days with medical relevance, the number of days above the threshold for “high pollen/spore days” was identified using following thresholds:

- Poaceae: > 50 grains/m³ (Galán et al., 2007; Bannister et al., 2021; Morton et al., 2011; Ong et al., 1995)
- Myrtaceae: > 50 grains/m³ (Galán et al., 2007)
- Cupressaceae: > 200 grains/m³ (Galán et al., 2007)
- *Alternaria*: > 10 spores/m³ (Aira et al., 2013)

Furthermore, we used the terminology used by Jetschni and Jochner-Oette (2021) to compare and describe data:

- Campaign Pollen Integral (CPI_n)
 - The sum of all measurements for each location during the sampling campaign, i.e. CPI_{n,grav} for the gravimetric and CPI_{n,vol} for the volumetric trap
- Weekly Pollen Integral (WPI_n)
 - The sum of all daily mean pollen concentrations of one week, i.e. WPI_{n,vol} for the volumetric trap at Richmond
 - The sum of the pollen deposition for all gravimetric sites for one week, i.e. WPI_{n,grav}

We compared CPI_ns, WPI_ns and mean values to assess spatial and temporal variations and tested pollen data from all sites from the sampling campaign for significant differences using Wilcoxon rank-sum test and Kruskal–Wallis test. We analysed the relationship between daily mean pollen concentrations and meteorological variables by calculating Spearman's correlation coefficients (r_s).

2.3.2 Land use/cover

Information on land use/cover was obtained from Geoscience Australia (2017). This dataset contains information on 35 land cover classifications at a

resolution of 250 m. For this study, we aggregated the classes to “urban area”, “low vegetation and pastures”, “woodland and forest”, “water” and “mines and quarries” (see Appendix A). We used these classes (except the latter since it was not observed) to analyse the surroundings of the sampling locations: we calculated the percentage of each class in a radius of 100 m. To quantify urbanisation, we calculated the urban index (Jochner et al., 2012) based on the same dataset. We presented Spearman’s correlation coefficients (r_s) to check for relationships between pollen deposition (CPIs) and land cover as well as pollen deposition and urban index.

2.3.3 Meteorological data

Hourly meteorological data for each sampling location were obtained from the monitoring network of DPE (see Fig. 1 and Table 1 for locations). For the pollen seasons from 2017 to 2020, we considered temperature (daily mean, minimum and maximum), precipitation (daily total), relative humidity (daily mean) and wind speed (daily mean) recorded at Richmond. The relationship between these variables and daily mean pollen concentrations was analysed by calculating Spearman’s correlation coefficients (r_s) on a seasonal basis. For the period of the sampling campaign (November 2019 to January 2020), we analysed hourly and weekly means of temperature, daily and weekly totals of precipitation as well as the most frequent wind direction and wind speed for all ten locations.

For statistical analyses and visualisation, we used RStudio (1.3.959), packages ggplot2 (Wickham, 2016), AeRobiology (Rojo, Picornell and Oteros, 2018) and OpenAir (Carslaw & Ropkins, 2012). We used ESRI ArcMap 10.6 and QGIS 3.14 for spatial analyses and visualisation.

3 Results

3.1 Volumetric aerobiological monitoring

Volumetric aerobiological monitoring resulted in data for three pollen seasons 2017/2018, 2018/2019 and 2019/2020, in which we observed different characteristics (Fig. 4).

Figure 4 also gives information on missing data, as the pollen trap at Richmond commenced operation for the first time on 7 October 2017 and therefore the 2017/2018 year is missing the first 99 days. In the years 2018/2019 and 2019/2020, periods of missing data after interpolation were 9 January to 29 April 2019 and 4 February to 30 June 2020, resulting in 111 and 148 days of missing data from these two years, respectively.

However, for all analysed pollen and spore types, there were differences in APIn, mean of daily pollen/spore concentration, number of peak days, peak values and number of days above a threshold with medical relevance. Figure 4 and Table 2 give an overview of the course of the pollen and spore seasons as well as aerobiological characteristics. When considering these, the missing data should be kept in mind, as annual totals, peak values and other metrics could be even higher.

3.1.1 2017/2018

In 2017/2018, there were missing aerobiological data from 1 July to 8 October, resulting in available data for a total of 266 days (Table 2). For this period and among the analysed pollen types, Poaceae had the highest APIn with 3,816 pollen grains*day/m³, which is almost twice the APIn of Myrtaceae (1,962 pollen grains*day/m³) and almost 3.5 times the APIn of Cupressaceae (1,113 pollen grain*day/m³). This was also reflected in the mean of the daily pollen concentration (Poaceae: 14 pollen grains/m³, Myrtaceae: 7 pollen grains/m³, Cupressaceae: 4 pollen grains/m³). The peak value for Poaceae was 154 pollen grains/m³ on 12 October, and there were 23 high pollen days in total.

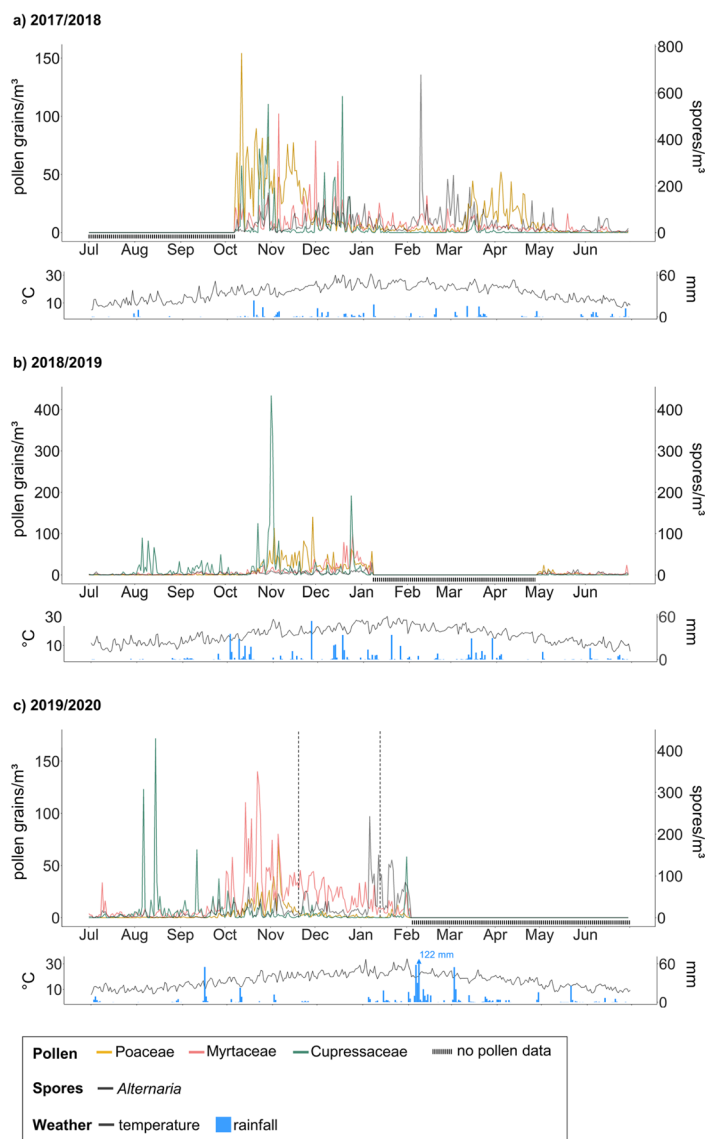
The peak value for Myrtaceae was recorded on 6 November (102 pollen grains/m³), and there were three high pollen days. The highest daily mean pollen concentration for Cupressaceae was 117 pollen grains/m³ on 19 December.

For *Alternaria* spores, the ASIn was 10,526 spores*day/m³, and the highest daily mean spore concentration was measured on 10 February (678 spores/m³). There were 200 high spore days. The mean of the daily spore concentrations was 40 spores/m³.

Considering the course of the Poaceae pollen concentration (Fig. 4a), two peaks could be identified: one peak was measured in October

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Fig. 4 Daily airborne pollen concentrations of Poaceae (yellow), Myrtaceae (red) and Cupressaceae (green), spore concentrations of *Alternaria* (black line; right axis) as well as temperature (black line) and rainfall (blue bars) in the bottom parts of the figures for the seasons **a** 2017/2018, **b** 2018/2019, **c** 2019/2020 with dashed lines indicating the start and end dates of the sampling campaign with gravimetric traps



(154 pollen grains/m³ on 12 October 2017). From the end of November onwards pollen concentrations decreased and remained on a lower level

until mid-March. Then, the pollen concentrations increased again with the highest value in April (52 pollen grains/m³ on 5 April).

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Table 2 Characteristics of the pollen (spore) seasons 2017/2018, 2018/2019 and 2019/2020 for Poaceae, Myrtaceae, Cupressaceae, *Alternaria* and the Annual Pollen Integral (APIn) or Annual Spore Integral (ASIn) measured at Richmond

| | Poaceae | Myrtaceae | Cupressaceae | <i>Alternaria</i> |
|--|---------|-----------|--------------|-------------------|
| <i>2017/2018^a</i> | | | | |
| APIn (pollen*day/m ³) | 3,816+ | 1,962+ | 1,113+ | 10,526+ |
| ASIn (spores*day/m ³) | | | | |
| Mean of daily pollen/spore concentration (pollen grains/m ³ ; spores/m ³) | 14 | 7 | 4 | 40 |
| Peak day | 12 Oct | 6 Nov | 19 Dec | 10 Feb |
| Peak value (pollen grains/m ³ ; spores/m ³) | 154 | 102 | 117 | 678 |
| High pollen/spore days | 23+ | 3+ | 0+ | 203+ |
| <i>2018/2019^b</i> | | | | |
| APIn (pollen*day/m ³) | 2,684+ | 1,795+ | 3,178+ | 4,718+ |
| ASIn (spores*day/m ³) | | | | |
| Mean of daily pollen/spore concentration (pollen grains/m ³ ; spores/m ³) | 11 | 7 | 13 | 19 |
| Peak day | 29 Nov | 26 Dec | 1 Nov | 8 Jan |
| Peak value (pollen grains/m ³ ; spores/m ³) | 140 | 99 | 434 | 148 |
| High pollen/spore days | 13+ | 4+ | 2+ | 125+ |
| <i>2019/2020^c</i> | | | | |
| APIn (pollen*day/m ³) | 846+ | 3,826+ | 1,158+ | 9,007+ |
| ASIn (spores*day/m ³) | | | | |
| Mean of daily pollen/spore concentration (pollen grains/m ³ ; spores/m ³) | 4 | 18 | 5 | 42 |
| Peak day | 6 Nov | 23 Oct | 15 Aug | 7 Jan |
| Peak value (pollen grains/m ³ ; spores/m ³) | 71 | 140 | 172 | 485 |
| High pollen/spore days | 1+ | 11+ | 0+ | 144+ |

Mean of daily pollen/spore concentration is calculated by dividing APIn/ASIn by the number of days with data

+ indicates the assumption of virtually higher values due to missing data

^a266 days with data; ^b254 days with data; ^c217 days with data

The course of the Myrtaceae pollen concentration was not characterised by isolated peaks. The highest concentrations were recorded in November and December 2017. Throughout almost the entire season, airborne Myrtaceae pollen were detected.

Most of the Cupressaceae pollen concentration was recorded from October to the end of December 2017. In 2018, smaller concentrations of airborne Cupressaceae pollen were identified. *Alternaria* spores were present in the air of Sydney on almost every day, and the highest concentrations were recorded from mid-February to March 2018.

3.1.2 2018/2019

In 2018/2019, there were missing aerobiological data from 9 January to 29 April 2019, resulting in data for a total of 254 days (Table 2). In this period, APIn for Cupressaceae was 3,178 pollen grains*day/

m³ and therefore higher than Poaceae (2,684 pollen grains*day/m³) or Myrtaceae (1,795 pollen grains*day/m³). The highest daily mean pollen concentration of Poaceae was registered on 29 November 2018 with 140 pollen grains/m³, of Myrtaceae on 26 December 2018 (99 pollen grains/m³) and of Cupressaceae on 1 November 2018 with a concentration of 434 pollen grains/m³. The mean of the daily pollen concentrations was for Poaceae 11 pollen grains/m³, Myrtaceae 7 pollen grains/m³, Cupressaceae 13 pollen grains/m³. Due to missing data, the course of the pollen and spore concentrations cannot be described completely (Fig. 4b).

For all pollen types, concentrations were higher in the period up to January than in the period May to July. Similar to the previous season, *Alternaria* spores were present in the air on almost all days, reaching the highest level on 8 January with a concentration of 148 spores/m³. The ASIn was 4,718+ spores*day/m³,

and the mean of the daily spore concentrations was 19 spores/m³.

3.1.3 2019/2020

In 2019/2020, there were missing aerobiological data from 4 February to 30 June 2020, resulting in data for a total of 217 days (Table 2). APIn of Poaceae was 846 pollen grains*day/m³, which is less than 25% of the APIn in 2017/2018 and about 30% of the APIn in 2018/2019. The mean of the daily grass pollen concentrations was 4 pollen grains/m³, which is 26% of 2017/2018 and 29% of 2018/2019. The highest value for Poaceae pollen concentration was measured on 6 November 2019 (71 pollen grains/m³), which was the only day with a mean pollen concentration above 50 pollen grains/m³. The course of Poaceae pollen concentrations can be described as a curve, increasing towards early November and decreasing after the peak on 6 November 2019 (Fig. 4c).

Myrtaceae APIn was 3,826 pollen grains*day/m³ with the peak day (140 pollen grains/m³ on 23 October 2019) occurring much earlier than in the previous season (-64 days). The mean of daily concentrations was 18 pollen grains/m³, which is more than twice than in the previous year. The course of Myrtaceae pollen was similar to the Poaceae course.

The APIn of Cupressaceae (1,159 pollen grain*day/m³) and the peak value (172 pollen grains/m³) were lower than in the previous sampling period and there was no high pollen day. The highest concentrations of Cupressaceae pollen were detected in August, with only low concentrations in the remaining season. The mean of daily Cupressaceae concentrations was 42 pollen grains/m³.

Alternaria spores had an ASIn of 9,007 spores*day/m³ with the peak day on 7 January 2020 (485 spores/m³) and 144 high spore days. The mean of the daily spore concentrations was 42 spores/m³. Highest concentrations of *Alternaria* spores were recorded in January 2020, while spores were present throughout most of the sampling period.

3.1.4 Meteorology

Daily data for temperature and rainfall are illustrated in Fig. 4. Mean, lowest and highest temperatures did not differ a lot between the years. However, rainfall

Table 3 Overview of the meteorological variables for the seasons 2017/2018, 2018/2019 and 2019/2020

| | Mean temperature [°C] | Minimum temperature [°C] | Maximum temperature [°C] | Rainfall sum [mm] |
|-----------|-----------------------|--------------------------|--------------------------|-------------------|
| 2017/2018 | 17.6 | -2.0 | 44.3 | 291.6 |
| 2018/2019 | 17.2 | -4.5 | 41.9 | 626.6 |
| 2019/2020 | 16.9 | -4.0 | 46.5 | 713.4 |

Every season starts on the 1 July and ends on 30 June of the following year

substantially varied: it was 291.6 mm in 2017/2018, 626.6 mm in 2018/2019 and 713.4 mm in 2019/2020 (Table 3). Extremely high rainfall at the start of February 2020 (Fig. 4) caused the pollen trap to break.

The correlation analyses between the three pollen types (Poaceae, Myrtaceae and Cupressaceae) as well as the spore *Alternaria* and the meteorological variables revealed significant relationships (Table 4). Correlations between temperature (mean, minimum, maximum) and each pollen type and spore were all positive for every year and significant apart from some cases paired with Cupressaceae. For this type, the pairing with minimum temperature (2017-2020) did not reveal a significant correlation, and in 2019/2020 a negative correlation with mean temperature ($r_s = -0.149$, $p < 0.05$) and maximum temperature ($r_s = -0.300$, $p < 0.01$) was observed. All significant correlations with humidity as well as precipitation were negative.

3.2 Gravimetric pollen sampling campaign

3.2.1 Pollen data

The temporal setting of the sampling campaign with gravimetric pollen traps in the 2019/2020 pollen season is shown in Fig. 4c. Sampling started after the season's peak days where the highest pollen concentrations were recorded (see Sect. 3.1.3; Table 2). We analysed the share of the Campaign Pollen Integral (CPI_{n,vol}) as part of APIn in order to evaluate the representativeness of this campaign for the whole season. For Poaceae (CPI_{n,vol}=98) it was 12%, for Myrtaceae (CPI_{n,vol}=1,208) 32% and for Cupressaceae (CPI_{n,vol}=80) 7% (Table 5).

These comparably low numbers were also reflected by pollen deposition, as illustrated in the

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Table 4 Spearman's rank correlation coefficients between daily meteorological parameters and pollen and spore concentrations for the whole period and single seasons (July–June)

| | Poaceae | Myrtaceae | Cupressaceae | <i>Alternaria</i> |
|---------------------|------------------|------------------|------------------|-------------------|
| <i>2017–2020</i> | | | | |
| Mean temperature | 0.609** | 0.625** | 0.147** | 0.690** |
| Minimum temperature | 0.520** | 0.475** | 0.010 | 0.583** |
| Maximum temperature | 0.584** | 0.664** | 0.254** | 0.678** |
| Mean humidity | −0.058 | − 0.251** | − 0.283** | − 0.118** |
| Precipitation sum | −0.034 | − 0.160** | − 0.102** | −0.048 |
| Mean wind speed | 0.083* | 0.172** | 0.066 | 0.179** |
| <i>2017/2018</i> | | | | |
| Mean temperature | 0.364** | 0.619** | 0.365** | 0.557** |
| Minimum temperature | 0.242** | 0.403** | 0.261** | 0.441** |
| Maximum temperature | 0.410** | 0.667** | 0.373** | 0.577** |
| Mean humidity | − 0.141* | − 0.388** | − 0.162** | − 0.248** |
| Precipitation sum | −0.067 | − 0.224** | 0.036 | −0.075 |
| Mean wind speed | 0.024 | 0.144* | 0.128* | 0.183** |
| <i>2018/2019</i> | | | | |
| Mean temperature | 0.746** | 0.697** | 0.501** | 0.617** |
| Minimum temperature | 0.699** | 0.601** | 0.309** | 0.470** |
| Maximum temperature | 0.671** | 0.663** | 0.553** | 0.684** |
| Mean humidity | 0.038 | −0.045 | − 0.357** | −0.042 |
| Precipitation sum | 0.008 | −0.009 | − 0.159* | −0.067 |
| Mean wind speed | 0.111 | 0.165** | 0.180** | 0.083 |
| <i>2019/2020</i> | | | | |
| Mean temperature | 0.545** | 0.604** | − 0.149* | 0.751** |
| Minimum temperature | 0.374** | 0.438** | − 0.300** | 0.679** |
| Maximum temperature | 0.579** | 0.646** | 0.044 | 0.665** |
| Mean humidity | − 0.251** | − 0.253** | − 0.225** | −0.098 |
| Precipitation sum | −0.122 | − 0.207** | − 0.191** | 0.025 |
| Mean wind speed | 0.155* | 0.145* | − 0.144* | 0.243* |

Bold values indicate significant correlations (* $p < 0.05$, ** $p < 0.01$)

Table 5 Characteristics of pollen/spore reference concentrations (volumetric trap at Richmond) during the gravimetric sampling campaign 20 November 2019 to 14 January 2020 for Poaceae, Myrtaceae and Cupressaceae

| | Poaceae | Myrtaceae | Cupressaceae |
|--|---------|-----------|--------------|
| CPI _{vol} | 98 | 1,208 | 80 |
| Share of 2019/2020 API _n | 12% | 32% | 7% |
| Peak day | 6 Dec | 21 Nov | 29 Nov |
| Peak value (pollen grains/m ³) | 6 | 45 | 12 |
| High pollen days | 0 | 0 | 0 |

heat maps (Fig. 5). The most abundant pollen types registered within our gravimetric sampling campaign belonged to the family Myrtaceae (CPI_{grav}:

1,330). Grass (Poaceae) and Cupressaceae pollen were also detected, but in much smaller quantities (CPI_{grav} 166 and 121, respectively).

We observed a temporal and spatial variation in Poaceae pollen deposition (Fig. 5a). The first week of sampling was characterised by the highest WPI_n of the campaign (62) and by the highest single value for deposition, which was recorded at Chullora (21 pollen grains/cm²). Values for WPI_n decreased after this week, showing a similar tendency compared to WPI_n recorded by the volumetric trap. The highest CPI_{grav} was recorded at Chullora (36) and the lowest CPI_{grav} at Rouse Hill (8).

Regarding Myrtaceae, the highest WPI_{grav} (271) was recorded in the third week of sampling. In this week, the highest value of 126 pollen grains/cm² was observed at Earlwood. Values for CPI_{grav}

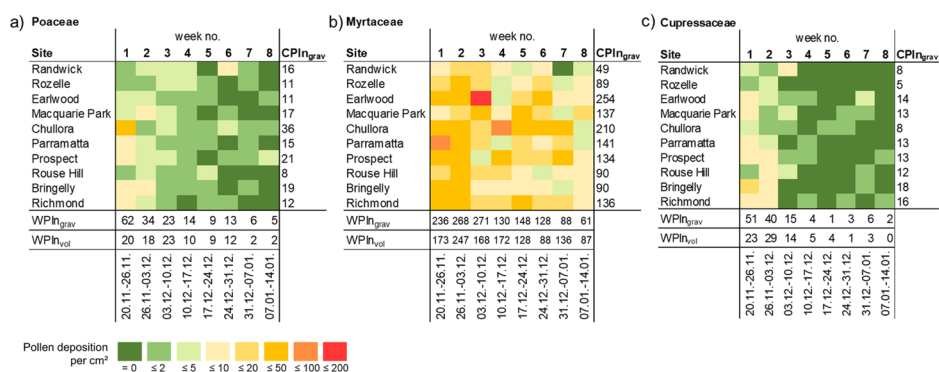


Fig. 5 Heat maps showing weekly **a** Poaceae, **b** Myrtaceae, and **c** Cupressaceae pollen deposition (pollen grains/cm²) for the sampling campaign 20.11.2019 to 14.01.2020 and WPin_{vol}

for the respective weeks. Gravimetric trap sites are ordered by distance from the sea

varied between 254 (Earlwood) and 49 (Randwick) (Fig. 5b).

Highest WPin of Cupressaceae pollen and highest single value for pollen deposition were recorded in the first week of sampling. WPin was 51 and the highest value for pollen deposition was recorded at Bringelly with 10 pollen grains/cm². CPI_n_{grav} values did not vary substantially (range 5 (Rozelle) to 18 (Bringelly)) (Fig. 5c). Kruskal–Wallis test revealed no significant differences in pollen deposition among sites (Poaceae: Kruskal–Wallis chi-squared=6.810, *p* value=0.657; Myrtaceae: Kruskal–Wallis chi-squared=16.793, *p* value=0.052; Cupressaceae: Kruskal–Wallis chi-squared=3.648, *p* value=0.933).

3.2.2 Land use

The land use surrounding the traps varied between the locations (Table 1 and Fig. 1). Areas classified as “built-up” were within 100 m of all but two of the locations (Rouse Hill, Bringelly). For Randwick, this was the only land cover class within 100 m of the trap. “Low vegetation and pastures” were surrounding eight traps.

We investigated the relationship of each CPI_n and each land cover class and urban index. We only detected one correlation of significance (*p*≤0.05), which was the relationship between CPI_n of Cupressaceae and the land use class “low vegetation and pastures” (*r*_s=0.663, *p* value=0.037).

3.2.3 Meteorology

Table 6 gives an overview of mean, minimum and maximum temperature and rainfall measured at the sampling locations. Mean temperature during sampling ranged between 21.2 °C (Randwick) and 22.6 °C (Rouse Hill). The lowest and highest temperature was both measured at Richmond (7.4 °C and 46.5 °C). During the sampling campaign, rainfall totals were 21.8 mm at Richmond, 18.6 mm at Rouse Hill and 20.8 mm at Rozelle. Most rain fell in the first and in the last two sampling weeks. The prevailing wind directions varied between locations (Table 6). Highest mean wind speed was registered at Randwick (3.0 m/s), which is the site closest to the sea.

4 Discussion

This study provides new data and insights into pollen in the ambient air of Sydney. Hereby, we add to previous studies, that published findings on pollen and spores from several years between 1993 and 2012 (i.e. Bass & Morgan, 1997; Beggs et al., 2015; Katelaris et al., 2004; Medek et al., 2016; Stennett & Beggs, 2004b). The novelty of our study was the assessment of spatial differences of airborne pollen in Sydney, which had not been done before to this extent. Until now, only Katelaris et al. (2000) and Katelaris et al. (2004) analysed spatial differences in airborne pollen

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Table 6 Overview of daily meteorological data measured at the sampling locations for the period of the sampling campaign (20 January 2019 to 14 January 2020)

| Location | Temperature [°C] | | | Rain [mm] | Main wind direction | Mean wind speed [m/s] |
|----------------|------------------|---------|---------|-----------|---------------------|-----------------------|
| | Mean | Minimum | Maximum | | | |
| Randwick | 21.2 | 12.2 | 38.8 | NA | NE | 3.0 |
| Rozelle | 21.6 | 12.4 | 40.3 | 20.8 | S | 2.3 |
| Earlwood | 22.1 | 10.5 | 44.5 | NA | S | 1.3 |
| Macquarie Park | 21.6 | 9.3 | 44.7 | NA | E | 1.5 |
| Chullora | 22.2 | 11.9 | 45.9 | NA | NE | 1.9 |
| Paramatta | 22.3 | 11.0 | 46.4 | NA | SW | 1.5 |
| Prospect | 22.5 | 12.0 | 45.9 | NA | SW | 2.0 |
| Rouse Hill | 22.6 | 11.6 | 45.7 | 18.6 | SW | 1.9 |
| Bringelly | 21.8 | 7.6 | 46.1 | NA | E | 1.7 |
| Richmond | 22.5 | 7.4 | 46.5 | 21.8 | E | 1.7 |

Precipitation data only available for three locations. See Table 1 for location characteristics

in Sydney using three volumetric pollen traps more than 20 years ago between 1997 and 1999.

We used a 7-day volumetric trap for volumetric aerobiological monitoring for three pollen seasons. Even though we had to deal with gaps in data, our data allowed us to examine the course and characteristics of pollen and spore seasons as well as the relationship with meteorological parameters. We found differences in the Annual Pollen Integral (API_n), Annual Spore Integral (ASI_n) and number of high pollen/spore days as well as peak values of daily mean pollen and spore concentrations and the mean of the daily concentrations. While the volumetric Hirst trap (Hirst, 1952) is used as a standard for continuous volumetric pollen monitoring (Beggs et al., 2018; Galán et al., 2014) as it is suitable to provide data over a longer period, sampling campaigns using gravimetric traps provide other advantages. These include cost-effectiveness and therefore the opportunity to set up numerous traps for one study. Furthermore, these traps are easy to operate, not very prone to breaking because of the absence of moving parts, and they do not require power. While pollen counts recorded by volumetric traps can be converted to pollen concentrations, gravimetric sampling is based on the sedimentation of pollen and can therefore only be expressed as pollen deposition, which does not allow precise comparison to pollen concentration (Agashe & Caulton, 2009; Jetschni & Jochner-Oette, 2021). In this study, we used ten gravimetric pollen traps to gather data on spatial variations of pollen deposition for eight weeks from late spring 2019 to mid-summer 2020. These traps have proven to be useful for comparing pollen deposition at a smaller spatial scale.

We used the category “high pollen days” and “high spore days” to describe the medical relevance of pollen and spore concentrations above specific thresholds. Such thresholds should ideally be specific to the region or country of the study, as they can vary across regions and are influenced by environmental conditions (Galán et al., 2007; Şimşek et al., 2019; Weger et al., 2013). We used thresholds that have been published in other studies (Aira et al., 2013; Bannister et al., 2021; Galán et al., 2007), which are, however, only in the case of Poaceae specific to Australia (Morton et al., 2011; Ong Singh and Knox, 1995). Future studies should additionally examine pollen and spore thresholds that exert allergic reactions and symptoms related to other species. A useful tool for this might be crowd-sourced symptom data. Recently, citizen science gained in popularity and crowd-sourced symptom data were increasingly used for analysing the relationship between pollen concentrations, pollen seasons and symptom severity in Europe (Bastl et al., 2021; Karatzas et al., 2018) as well as in Australia (Jones et al., 2020, 2021).

4.1 Poaceae

In the season 2017/2018, we observed two peaks of grass pollen concentration. A similar pattern was documented by Beggs et al. (2015) and Medek et al. (2016). The authors observed the peak date for grass pollen at the end of October/start of November in three out of five observed years (2008–2009, 2012–2013). There was a second peak around March, which could be explained by different flowering times of different grass species (Frenguelli et al., 2010), or

multiple flowering events of the same species (Medek et al., 2016). Grass can only be determined at the family level using light microscopy (Jaeger, 2008; Driesen, Willemse and Luyn, 1989); thus, phenological observation of different grass species might improve the interpretation of aerobiological data.

The peak values in our study were recorded on 12 October 2017 and 6 November 2019. In 2018, the highest value was recorded on 29 November. Despite of data gaps, we observed a varying intensity of the grass pollen seasons (APIs vary between $846 + \text{pollen} \cdot \text{day} / \text{m}^3$ and $3,816 + \text{pollen} \cdot \text{day} / \text{m}^3$, Table 2). Large variations in grass pollen concentrations might be attributable to different factors such as land use change or meteorology. In the case of the grass pollen season 2019/2020, the lower intensity might be caused by the prevailing drought until January 2020. In the period from 1 June to 1 January, 151.6 mm of rain was recorded, which is less than in previous years (2017: 653.8 mm, 2018: 344.4 mm).

Precipitation and temperature strongly influence pollen release and season duration in the air (Dahl & Standhede, 1996; Jato et al., 2002; Bonofiglio et al., 2008; García-Mozo et al., 2010; Oduber et al., 2019;). The effect of drought on the characteristics of grass pollen seasons has been observed in several studies: Gehrig (2006) observed an earlier onset of grass flowering during a heatwave in 2003 in Switzerland. Here, grass pollen production and dispersion were increased by warm conditions, but eventually led to a decrease as soon as water was not sufficiently available. García-Mozo et al. (2010) reported a relationship between pollen production and rainfall before flowering, pointing to the influence of water availability during the development of grasses. Correlation analyses conducted in our study revealed positive correlations between grass pollen concentration and mean, maximum and minimum temperature in all years. In each case, the correlation with mean temperature was the strongest. The correlations with the daily precipitation sum were negative in all seasons but 2018/2019, they were, however, not significant.

Grass pollen deposition during the sampling campaign 2019/2020 was strongly influenced by the drought in the second half of 2019 (Nolan et al., 2020) preceding this campaign. We observed small values of pollen deposition, which can be attributed to the drought and temporal setting of the campaign, which started in the post-peak period of a comparably

weak pollen season associated with a smaller number of days with medical relevance (> 50 pollen grains/ m^3). Our data showed spatial variations between the ten locations, but no relationship with land use (low vegetation/pastures and woodlands/forests) and meteorology could be found. This suggests the influence of other factors such as long-range pollen transport or resuspension of pollen. Other studies showed correlations between pollen deposition/concentration and land cover and land use in the surroundings of the traps. In Germany, Jetschni and Jochner-Oette (2021) detected correlations between pollen deposition and agricultural area, grass cover and urban index. Werchan et al. (2017) also observed highest grass pollen sedimentation in traps closest to agricultural land. The lack of correlation in this study might be due to the comparably small amount of pollen in the air. However, it can be also concluded that people affected by grass pollen allergies can plan their outdoor activities regardless of the surrounding land use, as pollen loads remain comparably low at this point in the season.

4.2 Myrtaceae

Myrtaceae pollen were present in the air almost year round. The trees belonging to the Myrtaceae family (Eucalypt trees) are native in Australia and pollinate throughout the year (Bass & Morgan, 1997). The presence of their pollen in the air was documented by Bass and Morgan (1997), who reported annual totals between 3,048 and 3,257 between 1993 and 1995, with lowest daily concentrations between June and August. In this study, we reported APIs between $1,795 + \text{pollen} \cdot \text{day} / \text{m}^3$ and $3,826 + \text{pollen} \cdot \text{day} / \text{m}^3$. Similar to the other pollen types investigated in our study, we detected positive correlations between daily mean Myrtaceae pollen concentration and temperature (mean, minimum, maximum), which was also observed in a study in China (Bishan et al., 2020). Considering precipitation, we found negative correlations, pointing towards the negative effect of rain on airborne pollen amounts (Oduber et al., 2019).

In the sampling campaign, Myrtaceae pollen were the most abundant pollen type registered. We did not detect correlations between Myrtaceae pollen deposition and land use. Overall, our weekly data reflect the seasonal course shown in the background pollen data, with decreasing amounts over the sampling time.

However, a comparably high amount of pollen deposition (126 pollen grains/cm²) was recorded in the third week at Earlwood. This indicates that even at a later time in the season, local conditions might lead to a temporary increase in pollen amount in the air or might point to long-distance transport of Eucalyptus pollen from other regions in Australia.

4.3 Cupressaceae

Cupressaceae pollen are, along with Poaceae, the most prevalent in urban areas in Australia (Haberle et al., 2014). There are multiple Cupressaceae genera and species (e.g. *Cupressus*, *Taxus*, *Juniperus*) and since the morphology of their pollen grains is very similar, they are only analysed at family level using light microscopy (Charpin et al., 2017). The predominant species of cypress pine in the studied region are *Cupressus sempervirens* and *Cupressus macrocarpa* with highest concentrations previously detected from August to November (Bass & Morgan, 1997). In this study, annual totals ranged between 1,307 and 11,788 pollen grains*day/m³, which is generally higher than the APInS that we recorded. These differences could be explained by changes of land cover, which was observed for some species and regions worldwide (García-Mozo et al., 2016; Ziello et al., 2012), as well as by the use of Cupressaceae species as ornamental plants in cities (Ziello et al., 2012). We found positive correlations between daily mean Cupressaceae concentration and temperature in the seasons 2017/2018 and 2018/2019, as it has also been found in other studies (Rojo et al., 2015). In 2019/2020, there was a negative correlation with temperature, which could be explained by the fact that peak concentrations were already reached in August, a month associated with lower temperatures compared to November and December, where peak values were recorded in the previous seasons. Similar to Myrtaceae, there was a negative correlation of daily precipitation and daily mean Cupressaceae pollen concentration in two seasons.

In our sampling campaign, we detected spatial differences in Cupressaceae pollen deposition, but overall low values, which is also reflected by the low intensity during the time of the gravimetric sampling campaign. Spatial variability across three different locations in Sydney was analysed by Katelaris et al. (2004), where differences in Cupressaceae pollen

concentration could be attributed to the vegetation species composition in the surroundings of the pollen traps. This was also documented by a study in Spain, where the abundance of cypress pines was reflected in the pollen spectrum (Rojo et al., 2015).

4.4 *Alternaria*

Alternaria is an important airborne allergen, regarded as the most prevalent (Burch & Levetin, 2002; Kasprzyk et al., 2021; Mitakakis et al., 2001a, 2001b), and is, like pollen, also influenced by climate change (D'Amato et al., 2020). These spores can be found on vegetation and the ground and cause high spore concentrations in the air, especially after crop harvesting (Mitakakis et al., 2001a, 2001b; Skjøth et al., 2012). *Alternaria* spores in the air of southwestern Sydney recorded in the years 1993 to 1995 were analysed by Bass and Morgan (1997). The authors found *Alternaria* to be present in the air year round, but with higher numbers in late spring and summer; annual totals ranged between 10,748 and 40,104 spores/m³. These numbers are higher than the ASIInS that we presented in this study, ranging between 4,718+ and 10,526+ spores/m³. These differences might be due to missing data, but also changes in land cover, i.e. crop land, that could have influenced the amount of airborne *Alternaria* spores (Olsen et al., 2020). Comparably high *Alternaria* spore concentrations in 2019/2020 with high numbers from January until end of the sampling in early February might be due to the drought in previous months. Eucalypt trees in Australia tend to drop leaves in response to drought, increasing litter on the ground, which promotes the growth of *Alternaria* spores (Bass & Morgan, 1997). In addition to leave fall, the dieback of annual grasses favours *Alternaria* growth (Mitakakis et al., 1997).

The influence of meteorological variables such as temperature on spore concentration was observed in several studies. Rodríguez-Rajo et al. (2005) identified a strong positive relationship between spore concentration and temperature, which was also reported by a study in Sydney analysing *Alternaria* spore concentration of the years 1992 to 1995 (Stennett & Beggs, 2004a). Similarly, we found significant strong positive relationships of daily spore concentration and mean temperature of the same day.

Temperature seems to increase spore concentration and prolong the spore season; however, only until a certain point, as it was observed that *Alternaria* concentrations decline after extreme heat (Picornell et al., 2022). Based on our data, we could not observe such a decline in 2019/2020.

5 Conclusion

Monitoring airborne pollen and spores is important as it generates knowledge on the seasonality and intensity of aeroallergens and therefore on people's exposure. Based on our data, it seems that land use has a limited impact on the amount of pollen in the air when the season's intensity decreases. In case of grass pollen, we observed two peaks in some seasons, which points to the importance of phenological observations, that are needed to evaluate the different timing of pollen released from different grass species. Compared to countries in the Northern Hemisphere, there is a lack of continuous monitoring in Australia, and therefore, data series are incomplete. In regards to climate change, longer data series would be needed to estimate its impact in the future in detail. We propose the implementation of continuous volumetric aerobiological monitoring at least at one location in the Sydney Metropolitan area. The installation of more than one trap would provide additional data on spatial variations. To facilitate monitoring, the use of automatic pollen monitors could be tested. Further studies should establish pollen and spore thresholds, which are medically relevant for allergic people specifically in Sydney/Australia. Therefore, we encourage the additional implementation of symptom data, skin prick test or molecular methods.

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Declarations

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

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Appendix A

See Table 7

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Table 7 Aggregated land cover classes (Geoscience Australia, 2017)

| Class | Aggregated class |
|-------------------------------------|-----------------------------|
| Extraction sites | Mines and quarries |
| Inland waterbodies | Water |
| Salt lakes | Water |
| Irrigated cropping | Low vegetation and pastures |
| Irrigated pasture | Low vegetation and pastures |
| Irrigated sugar | Low vegetation and pastures |
| Rainfed cropping | Low vegetation and pastures |
| Rainfed pasture | Low vegetation and pastures |
| Rainfed sugar | Low vegetation and pastures |
| Wetlands | Low vegetation and pastures |
| Tussock grasses—Closed | Low vegetation and pastures |
| Alpine grasses—Open | Low vegetation and pastures |
| Hummock grasses—Closed | Low vegetation and pastures |
| Tussock grasses—Open | Low vegetation and pastures |
| Shrubs and grasses—Sparse-scattered | Low vegetation and pastures |
| Hummock grasses—Open | Low vegetation and pastures |
| Shrubs—Closed | Low vegetation and pastures |
| Shrubs—Open | Low vegetation and pastures |
| Trees—Closed | Woodland and forests |
| Trees—Open | Woodland and forests |
| Trees—Scattered | Woodland and forests |
| Trees—Sparse | Woodland and forests |
| Built-up surface | Built-up area |

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5 How does pollen production of allergenic species differ between urban and rural environments?

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How does pollen production of allergenic species differ between urban and rural environments?

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Abstract

Pollen production is one plant characteristic that is considered to be altered by changes in environmental conditions. In this study, we investigated pollen production of the three anemophilous species *Betula pendula*, *Plantago lanceolata* and *Dactylis glomerata* along an urbanization gradient in Ingolstadt, Germany. We compared pollen production with the potential influencing factors urbanization, air temperature and the air pollutants nitrogen dioxide (NO₂) and ozone (O₃). While we measured air temperature in the field, we computed concentrations of NO₂ and O₃ from a land use regression model. The results showed that average pollen production (in million pollen grains) was 1.2 ± 1.0 per catkin of *Betula pendula*, 5.0 ± 2.4 per inflorescence of *Plantago lanceolata* and 0.7 ± 0.5 per spikelet of *Dactylis glomerata*. Pollen production was higher at rural compared to urban locations on average for *B. pendula* (+73%) and *P. lanceolata* (+31%), while the opposite was the case for *D. glomerata* (-14%). We found that there was substantial heterogeneity across the three species with respect to the association of pollen production and environmental influences. Pollen production decreased for all species with increasing temperature and urbanization, while for increasing pollutant concentrations, decreases were observed for *B. pendula*, *P. lanceolata* and increases for *D. glomerata*. Additionally, pollen production was found to be highly variable across species and within species – even at small spatial distances. Experiments should be conducted to further explore plant responses to altering environmental conditions.

Keywords (4-6): air pollution, *Betula pendula*, *Plantago lanceolata*, *Dactylis glomerata*, urbanization gradient, land use regression models

Statements and Declarations The authors report there are no competing interests to declare.

1 Introduction

Along urbanization gradients, a high variability of different environmental factors can be observed at short distances. The variability arises from varying degrees of anthropogenic influence, such as building density and traffic volume, which are related to the Urban Heat Island effect and emission of air pollutants (McDonnell and Pickett 1990; McDonnell and Hahs 2008). As such, those gradients are also suitable for estimating effects of increasing temperatures and pollutant concentrations using space-for-time substitution (Pickett 1989; Ziska et al. 2003). Increases in temperature have been observed in the context of climate change, e.g., the global surface temperature increased by 1.1 °C (2011-2020 compared to 1850-1900) (IPCC 2022). Anthropogenic activity and related changes in the surface of the earth contribute to changes in climatic conditions and are key drivers of air pollutant concentrations (Fritsch and Behm 2021a; Kovács and Haidu 2022). Therefore, urban areas can be regarded as “harbingers” of climate change (Ziska et al. 2003) and urban-rural gradients are useful for studying ecological changes.

Pollen production is among the plant characteristics that are expected to be influenced by climate change (Damialis et al. 2019; Beggs 2021). This pollen metric is the amount of pollen produced per reproductive unit, e.g., anther, flower or inflorescence of plant (Galán et al. 2017). It is one of the factors influencing the amount of pollen in the air, besides the abundance of plants, prevailing weather conditions and atmospheric transport (Agashe and Caulton 2009; Skjøth et al. 2013). In general, studies on aerobiology are highly relevant, as aeroallergens cause allergic respiratory reactions, which are increasing globally (Bergmann et al. 2016; Beggs 2021).

Pollen production has been studied for numerous species, such as the tree species *Betula* (Jato et al. 2007; Piotrowska 2008; Jochner et al. 2011; Katz et al. 2020; Kolek 2021; Ranpal et al. 2022), *Quercus* (Tormo Molina et al. 1996; Gómez-Casero et al. 2004; Charalampopoulos et al. 2013; Kim et al. 2018; Fernández-González et al. 2020; Katz et al. 2020), *Alnus* (Moe 1998), *Fraxinus* (Tormo Molina et al. 1996; Castiñeiras et al. 2019), *Acer* (Tormo Molina et al. 1996; Katz et al. 2020), *Corylus* (Damialis et al. 2011), *Cupressus* (Hidalgo et al. 1999; Damialis et al. 2011), *Olea* (Tormo Molina et al. 1996; Damialis et al. 2011), *Platanus* (Damialis et al. 2011; Katz et al. 2020), *Juniperus* (Pers-Kamczyc et al. 2020), *Pinus* (Sharma and Khanduri 2002; Charalampopoulos et al. 2013), and *Cedrus* (Khanduri and Sharma 2002) or for grass species (Subba Reddi and Reddi 1986; Prieto-Baena et al. 2003; Piotrowska 2008; Aboulaich et al. 2009; Tormo-Molina et al. 2015; Jung et al. 2018; Romero-Morte et al. 2018; Ali et al. 2022; Severova et al. 2022) and herbaceous plants such as *Artemisia* (Piotrowska 2008; Bogawski et al. 2016), *Rumex* (Piotrowska 2008), *Plantago* (Hyde and Williams 1946; Sharma et

al. 1999; Piotrowska 2008; González-Parrado et al. 2015), *Parietaria* (Fotiou et al. 2011) and *Ambrosia* (Ziska and Caulfield 2000; Wayne et al. 2002; Rogers et al. 2006).

Pollen production can be influenced by environmental factors (Ziska and Caulfield 2000; Rogers et al. 2006; Damialis et al. 2011). While Barnes (2018) reported an increase in pollen production under warmer conditions, another study found the opposite (Jochner et al. 2013). Furthermore, increased carbon dioxide (CO₂) concentrations have been reported to lead to an increase in pollen production (Ziska and Caulfield 2000; Jablonski et al. 2002; Wayne et al. 2002; Ziska et al. 2003; Rogers et al. 2006; Kim et al. 2018). For air pollutants, nitrogen dioxide (NO₂) might reduce pollen production (Jochner et al. 2013), but also the opposite has been documented (Zhao et al. 2017). In addition, a study demonstrated that O₃ affects plant reproduction (Darbah et al. 2008, Albertine et al. 2014). Other factors responsible for variations in pollen production include site characteristics such as stand density and exposure (Faegri and Iversen 1989), genetics (Ranpal et al. 2022) and masting including variations in resource allocation (Kelly 1994; Crone and Rapp 2014). These different findings on the effects of environmental factors on pollen production indicate that the effects may be species-specific.

In this study, we assessed the pollen production of 24 birch trees (*Betula pendula* ROTH), 82 individuals of ribwort plantain (*Plantago lanceolata* L.) and 54 individuals of orchard grass (*Dactylis glomerata* L.) along an urbanization gradient in Ingolstadt, Germany. We analyzed the relationship between pollen production and the potential influencing factors urbanization, air temperature, NO₂ and O₃, separately for the three species.

Materials and methods

2.1 Study area

The study area is the city Ingolstadt (48.7665° N, 11.4258° E, 374 m a.s.l.) and surrounding areas, located in southern Germany (Fig. 1a), which covers an area of approx. 13,335 ha and has roughly 140,000 inhabitants (Bayerisches Landesamt für Statistik 2020). The average annual temperature is 8.9 °C, and the average annual precipitation is 712 mm (1981–2010, DWD station “Ingolstadt Flugplatz (Airport)”). The surroundings of Ingolstadt are characterized by industrial and agricultural areas, forests in the north and riparian forests along the Danube River.

Sampling locations of the selected species *B. pendula*, *P. lanceolata* and *D. glomerata* were distributed along an urbanization gradient with a length of approx. 7.3 km (Fig. 1b, c, d). We focussed on these species due to their common occurrence in the study area and due to their allergological relevance (D'Amato et al. 2007; Forkel et al. 2020). The locations were characterized by different proportions of land use in their immediate surroundings.



Fig. 1 a) Location of the study area (black rectangle) in Germany (GeoBasis-DE/BKG 2018) and its surrounding land use b) sampling locations of *B. pendula*, c) *P. lanceolata* and d) *D. glomerata*. Land use: dark green – forest; light green – low vegetation; blue – water, red – built-up, dark brown – bare soil; light brown – agriculture (mundialis 2020).

2.2 Field work and laboratory analyses to assess pollen production

We selected 24 mature trees of *B. pendula* in the study area. For each tree, we collected a maximum of four male inflorescences (catkins) from different positions on the crown at approx. 2 m a.g.l. Catkins were harvested in February 2020, which ensured that anthesis had not started and no pollen had been released yet, following the recommendation of Damialis et al. (2011). We performed sampling and laboratory analyses according to Faegri and Iversen (1989), Damialis et al. (2011) and Ranpal et al. (2022): We measured the length and diameter of each catkin and noted the number of flowers. Catkins were put in a 10% KOH (potassium hydroxide) solution to break up plant tissue and to facilitate the release of pollen grains. We used a glass rod to

further crush the plant material. The solution was boiled for 10 minutes, and 70% Glycerol with staining Safranin was added to a volume of 20 ml to prevent pollen clumping. While stirring the solution, we used a micropipettor (Rotalibo, Carl Roth GmbH, Karlsruhe, Germany), took two samples of 10 μ l each, transferred them to microscope slides and covered them with cover slips.

For *P. lanceolata*, we sampled 82 individuals by collecting one inflorescence of each specimen in June 2020. The flowers buds were fully developed but had no opened yet. We measured diameter and length of the inflorescence and determined the number of flowers. The following laboratory analyses were based on Cruden (1977) and modified to enhance the workflow. Two closed flowers of each inflorescence were detached and each of them was placed in a reaction vial, to which 10% KOH solution was added. In a next step, we used a glass rod to break up the material and added one drop of Safranin and 60% Glycerol to a volume of 1 ml. The tube was shaken for 10 seconds using a test tube shaker (Rotalibo Mini Vortex, Carl Roth GmbH, Karlsruhe, Germany) in order to mix the solution. Subsequently, we used a micropipettor as described above.

We selected 54 individuals of *D. glomerata* and collected their inflorescences with fully developed closed flowers in June 2020 (similar to *P. lanceolata*). We determined the number of flowers of each spikelet and per cm. The extraction of pollen grains and the preparation of microscope slides were performed according to the procedure used for *P. lanceolata* inflorescences.

All microscope slides were stored horizontally and counted at 100x magnification using a light microscope (Axio Lab.A1; Zeiss, Wetzlar, Germany).

2.3 Estimation of pollen production

The count of each microscope slide was then used to determine pollen production at different levels, depending on the species (Table 1).

Table 1 Overview of the different analysis levels of pollen production.

| Abbreviation | Description | Species |
|-------------------------|-------------------------------------|--|
| <i>P_{ca}</i> | Pollen production per catkin | <i>B. pendula</i> |
| <i>P_{fl}</i> | Pollen production per flower | <i>B. pendula</i> , <i>P. lanceolata</i> , <i>D. glomerata</i> |
| <i>P_{infl}</i> | Pollen production per inflorescence | <i>P. lanceolata</i> |
| <i>P_{sp}</i> | Pollen production per spikelet | <i>D. glomerata</i> |
| <i>P_{cm}</i> | Pollen production per cm | <i>D. glomerata</i> |

B. pendula pollen production was estimated per catkin P_{ca} (Damialis et al. 2011). P_{ca} was calculated by multiplying the counted pollen grains with the volume of the whole suspension and dividing it by the volume of the sample. The pollen production per flower P_{fl} was calculated by dividing P_{ca} by the number of flowers of the catkin.

P. lanceolata pollen production per flower P_{fl} was estimated similarly by extrapolating from the number of pollen grains contained in the analyzed volume to the whole solution. Pollen production per inflorescence P_{infl} was calculated by multiplying P_{fl} with the number of flowers.

Pollen production of *D. glomerata* P_{fl} was estimated in the same way as P_{fl} of *P. lanceolata*. Multiplying the number of flowers per spikelet with P_{fl} results in P_{sp} . P_{cm} was calculated by multiplying P_{fl} with the number of flowers per cm.

2.4 Temperature data

In 2019, we established a network of ten temperature loggers (Hobo Pro v2 U23-001, ONSET, Bourne, Massachusetts, USA) and two weather stations (Davis Vantage Pro2, Davis Instruments Corporation, Hayward, California, USA) across the study area (Fig. 1). The locations were selected due to their accessibility, spatial setting in the study area and vicinity to birch trees. Temperature loggers recorded air temperature every 10 minutes, weather stations recorded hourly air temperature (mean, min, max). We focused on the period, which is likely to influence inflorescence and pollen formation and growth and calculated mean, minimum and maximum temperature. For *B. pendula*, we additionally considered the accumulated daily mean temperature (accTmean). The periods considered were June 01 to August 30, 2019 for *B. pendula* (Dahl and Strandhede 1996), and the time from the start of the vegetation period, April 02, 2020, until June 30, 2020 for *P. lanceolata* and *D. glomerata*. The start of the vegetation period was indicated when daily mean temperature was above 5 °C for seven consecutive days (Estrella and Menzel 2006). For this regard, we assessed the temperature data of the weather station in the city center of Ingolstadt, which resulted in a start on April 02, 2020.

2.5 Land use regression model

We used the land use regression (LUR) models of Fritsch and Behm (2021a) to obtain estimated mean annual pollutant concentrations of NO₂ and O₃ (µg/m³) for the locations where the plant material was sampled. The models are based on additive regression smoothers of spatial and structural explanatory variables and reflect the intra-urban variability (Jerrett et al. 2005) of background concentrations for the year 2019 (*B. pendula*) and 2020 (*P. lanceolata*, *D. glomerata*) at the different locations. The data used to fit the models were compiled following the description in Fritsch and Behm (2021b). We employed the most recent versions of the datasets

containing the pollutant concentration measured at the sites of the German air quality monitoring network in 2019 and 2020 (EEA 2022), land use based on the CORINE land cover classes (EEA 2020), topography (BKG 2021a), German administrative regions and population density (BKG 2021b), and the road traffic network (EuroGeographics 2022). Germany was separated into 1 x 1 km grid cells and one LUR model was estimated based on monitoring sites reflecting background concentrations for NO₂ and O₃. Overall, the models for NO₂ exhibited similar properties to the ones reported in Fritsch and Behm (2021a) and highlighted agglomeration and infrastructure effects – though concentrations were generally lower. In total, air pollutant concentrations were higher for NO₂ in more densely populated areas, while the opposite was the case for O₃. Each model was used to predict pollutant concentrations for the exact geographical locations at which pollen production was estimated.

2.6 Urbanization

We employed land use data including the classes forest, low vegetation, water, built-up, bare soil and agriculture, which are based on automatically processed Sentinel-2 satellite data and available at a resolution of 10 m (mundialis 2020). Urban index was calculated according to Jochner et al. (2012) for all sampling locations, which is defined as the share of built-up area within a 2 km radius surrounding the location. Sampling locations with an urban index of [0, 0.5] were defined as rural and those with]0.5, 1] as urban locations.

2.7 Statistical analyses

We analyzed the relationships between pollen production and temperature, urban index, and air pollutants by calculating Spearman's correlation coefficient (r_s) and investigated if there are differences in pollen production at urban and rural locations for the three plant species via a Wilcoxon rank sum test. We tested the null hypothesis that the correlation (difference) is zero for r_s (Wilcoxon rank sum test) and considered the correlations (differences) to be statistically significant when $p \leq 0.05$. We additionally investigated component plus residual plots for the different environmental influences urban index, temperature (Tmean, Tmin, Tmax, accTmean), NO₂, and O₃ on pollen production. The plots consider the effect of one explanatory variable X_j on pollen production P at once, while controlling for all other explanatory variables $X_{(j)}$ based on a linear regression. Each plot shows X_j (abscissa) and the partial residuals (ordinate). The latter are based on a linear regression of P on all environmental influences X and result from subtracting the partial effect of all environmental influences but the considered one $X_{(j)}\beta_{(j)}$ from P . The implied partial effect is illustrated by a regression line and a univariate spline function.

We used R (4.2.2) with the packages ggplot2 (Wickham 2016), dplyr (Wickham et al. 2022), mgcv (Wood 2017), terra (Hijmans et al. 2022), sf (Pebesma et al. 2022a), stars (Pebesma et al.

2022b), npreg (Helwig 2022) and car (Fox and Weisberg 2019) for statistical analyses and visualization. For spatial analyses, we used ESRI ArcMap 10.6 and for visualization QGIS 3.14.

3 Results

3.1 Pollen production of *B. pendula*, *P. lanceolata* and *D. glomerata*

P_{ca} of the studied *B. pendula* trees averaged at 1.2 ± 1.0 million pollen grains and varied between 83,000 and 3.7 million pollen grains. The number of flowers per catkin averaged at 134 ± 20 , mean length of catkins was 33.2 ± 6.4 mm and mean diameter 3.7 ± 0.3 mm. Mean P_{infl} of *P. lanceolata* was 5.0 ± 2.4 million pollen grains with a range of 1.5 to 12.1 million pollen grains. We counted 73 ± 29 flowers per inflorescence on average; the mean length of the inflorescences was 13.8 ± 3.6 mm and the diameter 6.2 ± 0.9 mm. For *D. glomerata*, average P_{sp} was 0.7 ± 0.5 million pollen grains and ranged from 79,450 to 2.4 million pollen grains. Mean number of flowers per spikelet was 16 ± 5 (Table 2).

Table 2 Descriptive statistics on pollen production at different analysis levels and summary statistics on investigated plant species *B. pendula*, *P. lanceolata*, and *D. glomerata*

| | Min | Median | Mean | Max | SD |
|---|-----------|-----------|-----------|------------|-----------|
| <i>Betula pendula</i> ($N_B = 24$) | | | | | |
| Flowers per catkin | 106 | 132 | 134 | 178 | 20 |
| P_{fl} | 703,4 | 6,560 | 9,212 | 32,349 | 8,110 |
| P_{ca} | 83,000 | 812,000 | 1,221,104 | 3,707,000 | 1,020,539 |
| Length of catkin (mm) | 22.5 | 32.6 | 33.2 | 49 | 6.4 |
| Diameter of catkin (mm) | 3.2 | 3.7 | 3.7 | 4.2 | 0.3 |
| <i>Plantago lanceolata</i> ($N_P = 82$) | | | | | |
| Flowers per inflorescence | 28 | 67 | 73 | 175 | 29 |
| P_{fl} | 40,950 | 65,000 | 68,716 | 129,950 | 17,755 |
| P_{infl} | 1,515,750 | 4,587,188 | 5,039,550 | 12,085,350 | 2,421,113 |
| Length of inflorescence (mm) | 6.6 | 13.2 | 13.8 | 27 | 3.6 |
| Diameter of inflorescence (mm) | 3.1 | 6.3 | 6.2 | 9.5 | 0.9 |
| <i>Dactylis glomerata</i> ($N_D = 54$) | | | | | |
| Flowers per spikelet | 7 | 16 | 16 | 32 | 5 |
| Flowers per cm | 3 | 12 | 13 | 24 | 5 |
| P_{fl} | 11,350 | 32,606 | 40,407 | 121,150 | 24,339 |
| P_{sp} | 79,450 | 487,088 | 660,272 | 2,423,000 | 503,273 |
| P_{cm} | 52,200 | 397,575 | 538,640 | 2,059,550 | 423,721 |

3.2 Pollen production and urbanization

Using the urban index to distinguish between urban and rural locations resulted in 10 rural and 14 urban locations for *B. pendula*, 46 rural and 36 urban locations for *P. lanceolata* and 26 rural and 28 urban locations for *D. glomerata*. Considering all locations, urban index varied between 0.30 and 0.72 (mean = 0.48).

Overall, daily mean temperature at urban locations was higher compared to rural locations, with statistically significant differences ($p < 0.001$). In 2019, during the considered period from June to August, mean temperature at rural location was 13.7 °C, at urban locations it was 2.1 °C higher with a mean of 15.9 °C. In the considered period in 2020 (April 02 to June 30), temperature at rural locations averaged at 13.7 °C, and at urban locations it was 1.2 °C higher with 14.9 °C.

We found different pollutant concentrations between the years and locations. While NO₂ concentrations ranged from 13.5 to 21.4 µg/m³ at the plant locations in 2019 with higher concentrations at urban (mean = 20.8 µg/m³) compared to rural locations (mean = 17.4 µg/m³). NO₂ concentrations in 2020 were about one third lower on average and ranged from 10.1 to 15.1 µg/m³ with higher concentrations at urban (mean = 13.5 µg/m³) compared to rural locations (mean = 12.2 µg/m³). In both years, the differences between the locations were statistically significant ($p < 0.001$). This also applies to O₃ concentrations, for which urban locations averaged at 45.9 µg/m³ in 2019 and 44.4 µg/m³ in 2020, while rural locations averaged at 47.7 µg/m³ in 2019 and 45.0 µg/m³ in 2020. The maps derived from the LUR model are included in the Appendix.

As illustrated in Fig. 2 and Table 3, pollen production varied between urban and rural locations for *B. pendula*: mean P_{ca} was 0.9 ± 0.9 (urban) vs. 1.6 ± 1.1 (rural) million pollen grains. This difference was statistically significant ($p = 0.016$). The mean number of flowers per catkin was 134 ± 24 (urban) vs. 135 ± 14 (rural) (no statistically significant difference, $p = 0.618$).

For *P. lanceolata*, mean P_{infl} was lower at urban locations (4.4 ± 2.4 million pollen grains) compared to rural locations (5.8 ± 2.2 million pollen grains). The mean number of flowers per inflorescence was higher at rural locations with 85 ± 31 flowers compared to 64 ± 24 flowers at urban locations. Both differences were statistically significant ($p \leq 0.003$).

For *D. glomerata*, mean P_{sp} were quite similar with an average of 0.7 ± 0.6 at urban and 0.6 ± 0.4 million pollen grains at rural locations. The mean number of flowers per spikelet was 17 ± 5 flowers at urban and 15 ± 6 flowers at rural sites ($p = 0.033$).

HOW DOES POLLEN PRODUCTION OF ALLERGENIC SPECIES DIFFER BETWEEN URBAN AND RURAL ENVIRONMENTS?

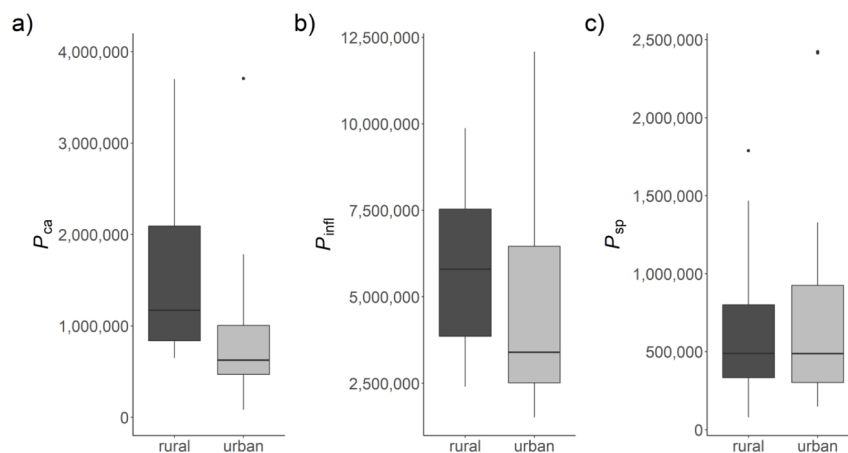


Fig. 2. Boxplots of pollen production at rural (dark grey) and urban (grey) locations. Pollen production **a)** of *B. pendula* (P_{ca}), **b)** of *P. lanceolata* (P_{infl}) and **c)** of *D. glomerata* (P_{sp}). Median indicated by horizontal line, interquartile range (IQR) by boxes, range of values within 1.5 times IQR by vertical lines; dots represent observations exceeding 1.5 times IQR.

Table 3 Descriptive statistics of pollen production at urban and rural locations. *B. pendula*: Pollen grains per catkin P_{ca} ; *P. lanceolata*: Pollen grains per inflorescence P_{infl} ; *D. glomerata*: pollen grains per spikelet P_{sp} .

| | Min | Median | Mean | Max | SD |
|---------------------------------------|-----------|-----------|-----------|------------|-----------|
| <i>Betula pendula</i> P_{ca} | | | | | |
| Urban | 83,000 | 624,250 | 936,679 | 3,707,000 | 909,646 |
| Rural | 649,000 | 1,171,167 | 1,619,300 | 3,699,000 | 1,079,064 |
| <i>Plantago lanceolata</i> P_{infl} | | | | | |
| Urban | 1,515,750 | 3,393,712 | 4,432,529 | 12,085,350 | 2,437,554 |
| Rural | 2,398,950 | 5,796,000 | 5,815,188 | 9,875,625 | 2,196,241 |
| <i>Dactylis glomerata</i> P_{sp} | | | | | |
| Urban | 148,225 | 487,088 | 707,434 | 2,423,000 | 590,016 |
| Rural | 79,450 | 489,050 | 609,482 | 1,788,800 | 394,738 |

3.3 Connection between pollen production and environmental influences

Table 4 shows the Spearman rank correlation coefficient r_s of pollen production of the three analyzed species with urban index, air temperature variables and air pollutants. While we observed negative correlations of pollen production with urban index and all temperature variables, the strength of correlations varied across the three species. For *B. pendula*, the correlations of Tmean, Tmin and accTmean were significantly different from zero. For *P. lanceolata*, this was the case for all considered correlations (for P_{infl}) and Tmean (for P_{fl}), while none of the correlations were statistically significant for *D. glomerata*. For the air pollutants, correlations were negative for NO₂ for all species besides P_{fl} for *P. lanceolata* and P_{sp} for *D. glomerata*. For O₃, negative correlations were obtained for *P. lanceolata* and positive ones for *B. pendula* and *D. glomerata*. None of the correlations was different from zero at the considered significance level.

Table 4 Spearman correlation (r_s) and p -values (p) for the Wilcoxon rank sum test that the correlation was zero between pollen production at different levels of the analyzed species and urban index, air temperature (Tmean, Tmin, Tmax, accTmean) and the air pollutants NO₂ and O₃. Bold letters indicate statistically significant correlations ($p \leq 0.05$).

| | | Urban index | Air temperature | | | | Air pollutants | |
|----------------------------|-------|---------------|-----------------|---------------|---------------|---------------|-----------------|----------------|
| | | | Tmean | Tmin | Tmax | accTmean | NO ₂ | O ₃ |
| <i>Betula pendula</i> | | | | | | | | |
| P_{ca} | r_s | -0.255 | -0.554 | -0.586 | -0.397 | -0.549 | -0.250 | 0.060 |
| | p | 0.229 | 0.005 | 0.003 | 0.055 | 0.005 | 0.239 | 0.780 |
| P_{fl} | r_s | -0.221 | -0.533 | -0.569 | -0.389 | -0.537 | -0.222 | 0.037 |
| | p | 0.298 | 0.007 | 0.004 | 0.060 | 0.007 | 0.296 | 0.866 |
| <i>Plantago lanceolata</i> | | | | | | | | |
| P_{infl} | r_s | -0.232 | -0.428 | -0.367 | -0.271 | - | -0.134 | -0.169 |
| | p | 0.036 | 0.000 | 0.001 | 0.014 | - | 0.231 | 0.128 |
| P_{fl} | r_s | -0.055 | -0.230 | -0.186 | -0.178 | - | 0.004 | -0.111 |
| | p | 0.621 | 0.038 | 0.094 | 0.109 | - | 0.973 | 0.321 |
| <i>Dactylis glomerata</i> | | | | | | | | |
| P_{sp} | r_s | -0.110 | -0.136 | -0.155 | -0.160 | - | 0.014 | 0.077 |
| | p | 0.430 | 0.325 | 0.262 | 0.249 | - | 0.918 | 0.579 |
| P_{fl} | r_s | -0.256 | -0.205 | -0.213 | -0.087 | - | -0.142 | 0.119 |
| | p | 0.062 | 0.137 | 0.123 | 0.534 | - | 0.305 | 0.391 |
| P_{cm} | r_s | -0.178 | -0.157 | -0.176 | -0.124 | - | -0.029 | 0.003 |
| | p | 0.197 | 0.258 | 0.202 | 0.370 | - | 0.833 | 0.981 |

As correlation coefficients are only suitable to assess bivariate relationships between variables, we also used the component plus residual plots displayed in Fig. 3 to investigate the connection between pollen production P and the j -th considered environmental influence, while controlling for all other influences via a linear regression. In the first line of the plot, for example, the effect of urban index on P was investigated for each species, while temperature (accTmean for *B. pendula*, Tmean for *P. lanceolata* and *D. glomerata*), NO₂ and O₃ are used as controls. Overall, the figure mostly confirms the findings reported in Table 4 and indicates that increasing temperature has a negative effect on pollen production. The degree of urbanization has a positive effect on pollen production of *B. pendula* and negative effects on *P. lanceolata* and *D. glomerata*. For the air pollutants, negative effects are indicated for *B. pendula* and *P. lanceolata* and positive ones for *D. glomerata*. When considering the other available temperature variables for the three plant species (Tmin, Tmax), the results remain qualitatively identical. The plots also indicate that a linear regression line approximates the relationship between pollen production and the environmental influences reasonably well, as the spline function and the regression line largely coincide.

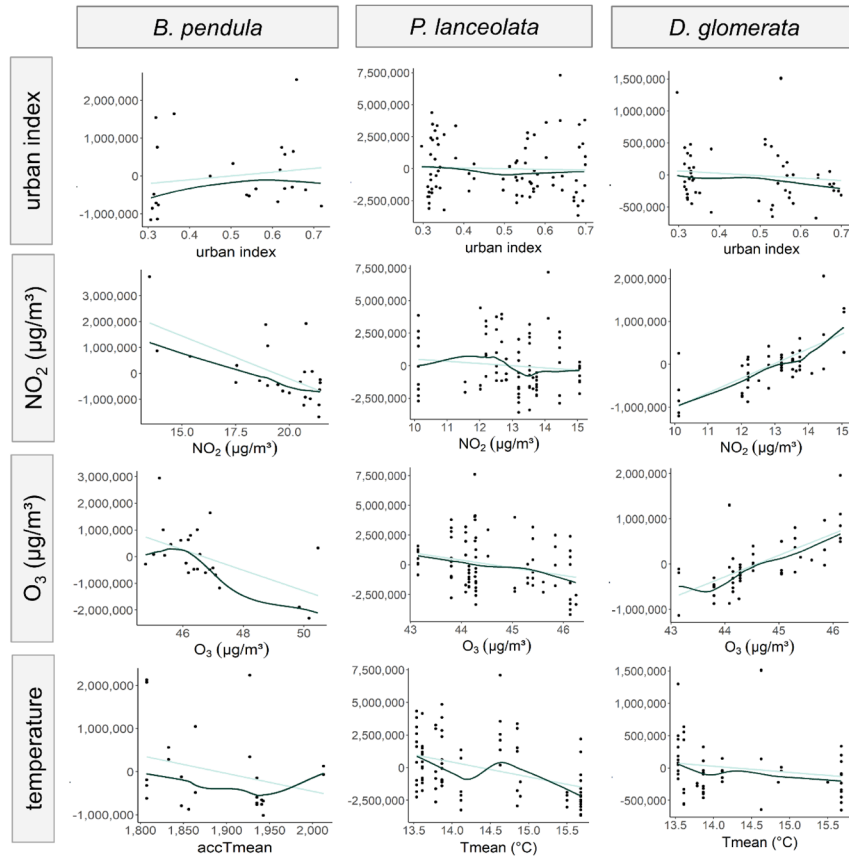


Fig 3. Component plus residual plots for species *B. pendula* (P_{ca}), *P. lanceolata* (P_{infl}) and *D. glomerata* (P_{sp}). Lines of plot illustrate effects of urban index, NO_2 and O_3 concentrations ($\mu\text{g}/\text{m}^3$) and temperature (abscissa) on pollen production, when controlling for all other environmental influences via a linear regression (ordinate). Regression line indicated by light line, spline function by dark line.

4 Discussion

4.1 Pollen production of *B. pendula*, *P. lanceolata* and *D. glomerata*

This study contributes to the knowledge on pollen production of the allergenic species *B. pendula*, *P. lanceolata* and *D. glomerata* and its connection with environmental influences. The chosen study area reflected urban-rural differences, as indicated by the differences between the sites regarding temperature and air pollutant concentrations. Urban-rural differences in temperature varied between 1.2 and 2.1 °C. NO_2 concentrations differed between the years, illustrating

the effect of COVID-19 related lockdowns on air pollution, which was documented for Germany (Balamurugan et al. 2021; Cao et al. 2022).

We investigated the pollen production of 24 *B. pendula* trees. Our results for P_{ca} averaged at 1.2 ± 1.0 million pollen grains (range from 83,000 to 3.7 million pollen grains). Other recent studies examining P_{ca} of *B. pendula* reported 1.7 ± 1.3 million pollen grains (Ranpal et al. 2022), 0.6 ± 0.6 million pollen grains (Kolek 2021), 10 million pollen grains (Piotrowska 2008), or between 4.8 and 8.2 million pollen grains (Jato et al. 2007). For other *Betula* species such as *B. papyrifera*, 24.3 million pollen grains per catkin were estimated (Katz et al. 2020).

Pollen production of *P. lanceolata* averaged at 5.0 ± 2.4 million pollen grains with a range of 1.5 to 12.1 million pollen grains per inflorescence in this study (sample size $N_P = 82$). González-Parrado et al. (2015) estimated a mean of 5.3 million pollen grains, with a minimum of 1.8 and a maximum of 9 million pollen grains. Piotrowska (2008) estimated P_{infl} of *P. major* in a similar range with 6.3 million pollen grains.

We documented a mean of $40,407 \pm 24,339$ pollen grains per flower of *D. glomerata* (sample size $N_D = 54$). Tormo-Molina et al. (2015) reported an average of 5,431 pollen grains per flower, ranging between 2,033 and 9,600, in Badajoz, Spain. The mentioned results vary greatly, possibly caused by the great difference of geographic location.

Several factors must be considered when comparing estimates of pollen production, one being the used sampling and laboratory method. The pollen produced by an inflorescence or flower can be determined by drying and weighing the flowers or inflorescence (Jochner et al. 2011; Beck et al. 2013; Jung et al. 2018) or the extraction and determination of the pollen amount using light microscopy (Cruden 1977; Damialis et al. 2011). The latter method is often applied in research (e.g., Damialis et al. 2011; Ranpal et al. 2022, 2023) and was also used in this study. However, when extrapolating from one analyzed level to others, e.g., from flower to the whole tree, small errors can alter the values to a great extent. In addition, the application of the method is easier for some species than for others. For example, single flowers in the correct phenological stage could easily be extracted from inflorescences of *P. lanceolata*. In case of *D. glomerata*, it turned out to be a bit more demanding due to the structure of the plant, spikelets and flowers. In addition, the estimation of pollen production is labor-intensive, which underlines the need for an automated procedure in pollen detection and counting, which has been in the focus of recent studies (e.g., Ali et al. 2022).

4.2 Pollen production, urbanity and environmental influences

We found a higher pollen production at rural locations for the two studied species, *B. pendula* (P_{ca}) and *P. lanceolata* (P_{infl}) compared to urban locations. In addition, we found a higher number of flowers per inflorescence of *P. lanceolata* and of flowers per spikelet of *D. glomerata*. All three described differences were statistically significant. There are only few studies that have analyzed differences in pollen production in regards to urbanization, while there are a few more that examined relationships between pollen production and environmental factors. Jochner et al. (2011) assessed pollen production of 26 *B. pendula* trees in urban and rural areas of Munich, Germany, in 2009. The authors detected higher pollen values in the rural area at the start of flowering (assessed using the weighing method). In Augsburg, Germany, pollen production of *B. pendula* was investigated by Kolek (2021) who reported higher pollen production with increasing urbanization. Ziska et al. (2003) assessed pollen production of common ragweed along an urbanization gradient in Maryland, USA. They found that ragweed produced more pollen in urban than in rural locations, which is different to our findings on the three studied species.

We analyzed the connection between environmental influences and pollen production by considering bivariate Spearman rank correlations and component plus residual plots. Our results indicate a negative effect of temperature on pollen production for *B. pendula* and *P. lanceolata* and that the functional relationship can be approximated by a linear regression. This indicates a decrease of pollen production with increasing temperatures (when leaving all other observable and unobservable influences constant). The relationship between the air pollutants NO_2 and O_3 and pollen production was found to be negative for *B. pendula* and *P. lanceolata*, while the opposite was the case for *D. glomerata*. This result implies that these air pollutants affect pollen production – even when accounting for the other considered environmental influences. In general, our results indicate that the effect of environmental influences on pollen production is species-specific. Jochner et al. (2013) reported negative correlations of birch pollen production with temperature, atmospheric NO_2 as well as foliar concentrations of the nutrients potassium and iron, but with temperature identified as the most important influencing factor. Furthermore, Kolek (2021) found no correlation between P_{ca} and cumulated minimum temperature of the summer months (June to August) of the previous year. In addition, there were no significant correlations between P_{ca} and O_3 or NO_2 . In our study, correlations between P_{ca} , P_{fl} and the cumulative temperature were, however, significant and negative. Darbah et al. (2008) studied the effect of elevated O_3 on *Betula papyfera* and reported a higher production of catkins along with a decrease in catkin length, diameter and mass. Studying *Ambrosia artemisiifolia*, Zhao et

al. (2017) found increased pollen and decreased seed production under elevated NO₂ concentrations.

We applied a land use regression (LUR) model to generate data on the air pollutants NO₂ and O₃ at a fine spatial scale. Our model took air quality data from the monitoring stations in Germany, land cover, topography, population density and road traffic into account. However, like all modeling approaches, LUR models have limitations (Hoek et al. 2008), i.e., the influence of individual pollutants is not considered separately and modeling accuracy strongly depends on the accuracy of the input variables. Despite these limitations, LUR models are a cost effective tool to obtain data on air quality, when the equipment of a large study area with air monitoring devices is too resourceful and when background concentrations of air pollutants are of interest. However, we encourage incorporating site-specific pollution data, as well as meteorological data, i.e., air temperature, precipitation, in further studies. These data cannot only be used for examining links to pollen production, but also for the comparison of data derived from LUR models.

By studying pollen production along an urbanization gradient, we made use of the space-for-time approach. However, investigations along other gradients should be considered for future studies. One approach was recommended by Tito et al. (2020), who suggested to use altitudinal gradients as ‘natural laboratories’ and to transplant and translocate species from different locations on the gradient to others. Such experiments could also consider all plant characteristics and the plant’s physiological performance, i.e., visual parameters of the plants such as the amount of flowers or foliage, but also other characteristics such as the allergenicity of pollen.

Besides temperature and air pollution, other site conditions might modify plant growth, which was also suggested by González-Parrado et al. (2015). These site conditions include the proximity to agricultural land to which fertilizers are presumably applied. In addition, better soil quality with higher soil moisture would be expected in more rural settings. When considering the immediate surroundings of our study sites, in some cases *P. lanceolata*, the species with higher pollen production at rural sites, was growing in areas close to agricultural land. In addition, some sites in urban areas did not seem favorable for plant growth due to the vicinity to roads and possible exposure to waste and high nitrogen input (Allen et al. 2020). In contrast, we did not observe higher pollen production of *D. glomerata* in rural areas. This might indicate that this grass species is not benefitting as much from the mentioned site conditions as *P. lanceolata*. Furthermore, a study examining *Juniperis communis* pollen reported that nutrient availability had an impact on the development of pollen grains (Pers-Kamczyc et al. 2020). Their

results on pollen production and pollen quality suggest that plants growing in nutrient-rich settings produce a higher amount of pollen to compensate for the lower quality of pollen grains.

Our results revealed differences in the number of flowers between urban and rural locations for *P. lanceolata* and *D. glomerata*. Similar results were found in the case of *Brassica rapa* (Rivkin et al. 2020), as there were significantly less flowers on plants in urban sites. Rivkin et al. (2020) suggested that the reason for this might be the exposure to exhaust fumes emitted by cars that might lead to foliar damage, affecting photosynthetic capacity and growth rates.

While our findings give new insights on the possible effects of changing temperatures and air pollution on pollen production, shortcomings have to be accounted for, such as the pollen production data that were based on one year. Conducting this investigation over more years would lead to more robust results, and in addition, influences on pollen production such as masting would be considered. Masting is the phenomenon of trees producing a high number of flowers and seeds in one year, which is then followed by a period of lower seed production (Herrera et al. 1998; Ranta et al. 2005; Ranta et al. 2008; Crone and Rapp 2014). The duration of this period can vary, for *Betula* it has been observed to be every two (Latałowa et al. 2002) or three years (Detandt and Nolard 2000). However, there have been no studies on the masting behavior of birch trees in cities, so it is not possible to assess the extent to which masting plays a role in our study. This phenomenon could explain the differences in pollen production estimates between our study and those from literature (e.g., Jato et al. 2007; Piotrowska 2008; Kolek 2021). Therefore, we strongly suggest the investigation of pollen production over several years in further studies.

5 Conclusion

In this study, we showed variations of pollen production of three allergenic species along an urbanization gradient. Pollen production for two species was overall higher in rural compared to urban locations and we found negative relationships with temperature for *B. pendula* and *P. lanceolata*, and positive relationships with NO₂ and O₃ for *D. glomerata*. Further studies should *inter alia* focus on the physiological performance of trees growing in urban areas, which might give a hint for explaining their behavior related to pollen production. Furthermore, additional locations representing semi-urban sites would support the continuous representation along the gradient. This is essential for drawing conclusions and may allow more profound predictions related to future effects of climate change on pollen production. In order to identify single influencing factors, a combination of experiments in a controlled environment with field research should be considered.

Author contributions

Conceptualization: J.J and S.J.-O.
Methodology: J.J, S.J.-O. and M.F.
Formal analysis and investigation: J.J and M.F.
Visualization: J.J., M.F.
Writing - original draft preparation: J.J., S.J.-O. and M.F.
Writing - review and editing: J.J, S.J.-O. and M.F.
Funding acquisition: S.J.-O.
Supervision: S.J.-O.

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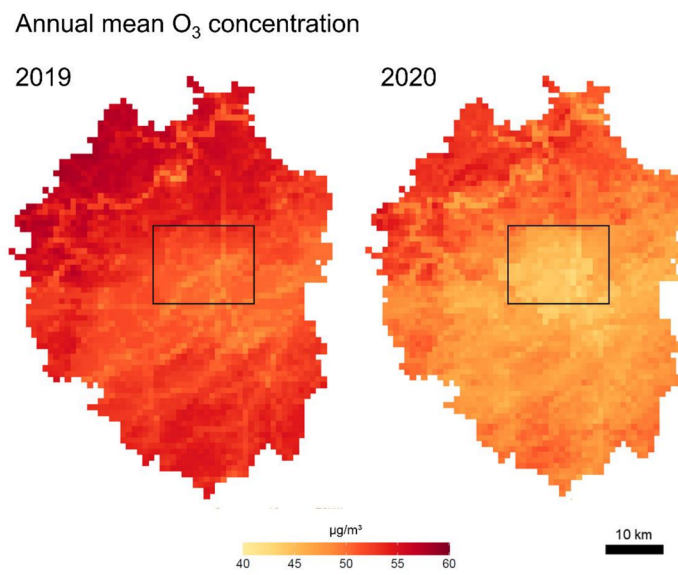
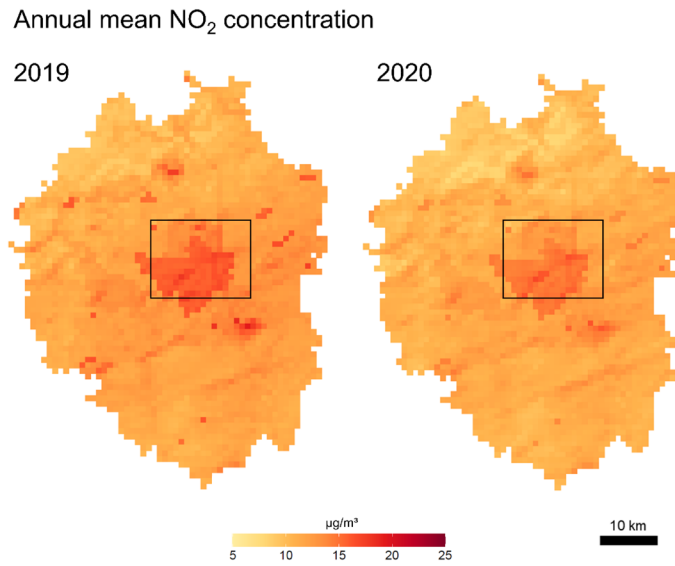
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Appendix

Maps illustrating the LUR-derived concentrations for NO₂ and O₃ for the years 2019 and 2020 for the study area (black rectangle represents the extent of Fig. 1a) and surrounding regions.



6 Discussion

The research described in the previous three chapters focused on pollen in cities and their spatial and temporal variation. While two chapters dealt with airborne pollen concentration and deposition, one chapter focused on pollen production. Pollen concentration and deposition were investigated at different spatial and temporal scales (Chapters 3 and 4). The results were analysed taking into account different influencing factors such as land use, land management and meteorology. Pollen production was investigated along an urbanisation gradient and the influence of air temperature and air pollutants was assessed (Chapter 5). Finally, these three studies have provided answers to the research questions outlined in section 1.3, which are discussed in this chapter and can be used as a basis for future research.

6.1 Spatial and temporal variations of airborne pollen

6.1.1 Spatial variations

Land use, local vegetation and pollen sources. In both studies on pollen load in the air (Chapters 3 and 4), spatial variations between the sampling locations were found. Background grass pollen concentrations were higher at the rural site Kösching than at the urban site Ingolstadt in 2019 and 2020 (Chapter 3), which was expressed by peak values, the number of high-pollen days and the Seasonal Pollen Integral. Spatial variations within the study area showed a similar tendency: higher amounts of grass pollen deposition and concentration were linked to lower urbanisation. Similar results were found in a study in Ireland that examined differences in grass pollen season characteristics between an urban and a rural site over two years (Markey et al. 2022). They measured overall higher Poaceae pollen concentrations at the rural location, similar to the Ingolstadt study. This was expressed in the Annual Pollen Integral and daily peak values and was probably related to the surrounding of the sampling site. The rural site was surrounded by rural grassland, whereas the other site was located in an urban environment. Antón et al. (2020) also reported overall lower values at the less urban site compared to the urban sampling locations, and identified the local surroundings as a major source of Poaceae pollen.

Data from the Ingolstadt and Sydney sampling campaigns provided a more detailed insight into the spatial variations of airborne pollen and the influence of land use and local vegetation on pollen at street level. Only a limited number of studies have sampled pollen in cities using a network of more than five sites (Katz & Batterman 2020). A study in Berlin assessed the spatial distribution of allergenic pollen across the whole city using 14 Durham-type traps (Werchan et al. 2017). In the case of Poaceae, they identified agricultural areas as the major source area, which led to increased Poaceae pollen deposition at the nearby trap. However, the study did not report a decrease in grass pollen deposition with proximity to the city centre and distance from the agricultural areas. The authors suggested that this may be due to urban grass pollen sources, which were reflected in grass pollen deposition across the city. Local vegetation has been identified as the driver for the spatial variation of pollen exposure by several other studies, such as by Charalampopoulos et al. (2018). They used portable volumetric traps to assess pollen exposure in the urban environment of Thessaloniki, Greece, and found spatial variations in pollen exposure even at small spatial distances, which was associated with local vegetation. Furthermore, a recent study found that grass pollen levels in the air are mainly influenced by sources nearby and that they vary at small spatial scales, within distances of up to 300 m (Frisk et al. 2023).

The data from the Ingolstadt sampling campaign (Chapter 3) supported these findings. Overall, higher grass pollen levels were recorded in the more rural areas, which were linked to the local vegetation, and correlation analyses showed their association with grassland and agricultural areas. Furthermore, the differences between urban and semi-rural sites were statistically significant. In contrast, the results of the Sydney study (Chapter 4) were somewhat different in terms of spatial variation. Spatial variations of pollen deposition were found for all analysed species in this study, but no notable relationships between pollen depositions and land use in the immediate surroundings. In the case of Poaceae, reason for this might have been the temporal setting of the sampling campaign, which only covered the second half of the pollen season. In addition, at the time of sampling, the effects of the drought were already visible, e.g., grass areas had dried out, which explained the low amounts of grass pollen in the air. This suggests that at this time of the season other processes, such as pollen transport or resuspension, are more important drivers than local vegetation. Furthermore, we investigated whether sea-

land breeze could have influenced pollen deposition, as it has been found to influence pollen loads (Gassmann et al. 2002; Gassmann & Gardiol 2007). Gassmann et al. (2002) found that sea breeze has the potential to locally increase the amount of airborne grass pollen in the late evening by stirring up pollen that have already been deposited. Based on our data, we could not identify an influence of the sea-land breeze on pollen deposition. Nevertheless, even the influence of sea breeze could be masked by local conditions, which seem to have a stronger influence on pollen concentrations as it has been documented by Bielory et al. (2021) for spore dispersion.

In addition to land use and local vegetation, city structures play a role in pollen dispersal and variations in pollen concentration measured at street level. They include heterogeneous structures, microclimate and development (Katz et al. 2019; Katz & Batterman 2020). Buildings and walls can act as obstacle that impede airflow and thus affect pollen dispersal (Peel et al. 2014a; Hugg et al. 2017). This effect was documented in the Ingolstadt study (Chapter 3), where the lowest levels of pollen deposition were recorded at urban sites that were partially enclosed, such as in courtyards. In addition, Werchan et al. (2017) suggested that surface roughness, which is increased in the city compared to the surrounding countryside, may have a further influence on pollen dispersal. The influence of buildings on pollen concentration at street level should be further investigated. For example, experimental pollen release combined with pollen and wind measurements could be used to track dispersal patterns. **Land management and maintenance.** The data gathered with gravimetric traps in two grass pollen seasons highlighted the influence of land management. At one site, pollen deposition was more than three times higher than in the previous year. This difference could be explained by the difference in management of the agricultural field next to the trap. In 2019, the field, which included Poaceae species, was cut several times during the pollen season, whereas in 2020, it was not cut until mid-August. The frequency of cutting in 2019 prevented plants from flowering at all or for a longer period. We also found that pollen concentrations in the urban area were about 1.4 times higher than in the residential area. Based on the land use data, it would be expected that more grass pollen would be recorded in the residential area due to larger grass areas in this area. However, these grass areas were well maintained throughout the sampling period. This indicates that even though it

was classified as grass area, a limited amount of grass species would be able to start flowering before it was cut again.

Jung et al. (2018) have elaborated on the importance of the timing of grass cutting. The local emission of grass pollen and allergens may be highly dependent on the timing of hay or silage cutting. In addition, it has been observed that the phenological development of grassland species is advancing, however hay-cutting events are not advancing at the same rate (Bock et al. 2013), which may result in extended flowering periods of grass species and consequently higher pollen loads. In order to identify grass pollen sources, it is important to base analyses not only on land cover data, but also on information on their management, both in urban and rural areas. Skjøth et al. (2013) classified grass areas as possible source areas based on the amount of cuts per year. Their study showed that most managed grassland did not release large amounts of pollen compared to unmanaged grass areas. This finding underlines that pollen exposure depends on local conditions. Charpin et al. (2011) investigated cypress trees and showed that trimming the hedges before flowering resulted in lower pollen concentrations in the vicinity compared to uncut hedges.

Such cutting and pruning of allergenic species seems to be an efficient way to reduce the amount of pollen in the air and have therefore been included in recommendations for management practices with the goal to reduce allergenic pollen in the air (Ogren 2000; McInnes et al. 2017). However, a recommendation to radically cut back allergenic plants before flowering in urban green spaces would be short-sighted. In urban green spaces such as parks, there are different interests regarding the park's vegetation, which collide, but all of which have to be considered. On one hand, they provide several ecosystem services such as the regulation of local climate and air quality, pollination, habitat for plants and animals, but also cultural services, recreation and mental and physical health (Millennium Ecosystem Assessment 2005; TEEB 2011), that have to be maintained. On the other hand, there are ecosystem disservices (Lyytimäki & Sipilä 2009) in the context with pollen allergies, which have been in the focus of research on the allergy-friendliness of urban green spaces and residential greenery (e.g., Cariñanos et al. 2014; Jochner-Oette et al. 2018; Battisti et al. 2019; Sabariego et al. 2021). This is the reason why such studies are particularly interesting, as a balance needs to be struck between allergy-friendly planting and management and *inter alia* biodiversity conservation. The challenge is to

maintain ecosystem services of a park while reducing its ecosystem disservice for allergy-affected people. Therefore, future studies on allergy-friendly gardening and management should follow an interdisciplinary approach to ensure that all interests and services of urban green spaces are considered. The monitoring of aeroallergens, e.g. via pollen sampling, would be an appropriate complement to assess the success of management strategies.

6.1.2 Temporal variations and influence of meteorology

Pollen season characteristics. Studies have shown that plants flower earlier in cities due to higher temperatures and therefore an earlier start of the pollen season is often documented (Rodríguez-Rajo et al. 2010; Jochner & Menzel 2015). This was not observed in the aerobiological data recorded in Ingolstadt (Chapter 3). In fact, the onset day of the grass pollen season was found to be nine days earlier at the rural site in 2019, and one day earlier in 2020. In addition to temperature, which is the main factor influencing the phenological onset of flowering (Menzel & Fabian 1999; Jochner & Menzel 2015), the process of pollen transport to the trap has to be considered. In addition, several studies have reported differences in pollen levels sampled at roof height and near ground level (e.g., Rojo et al. 2019; Kolek et al. 2021b). In particular, due to the low height of Poaceae species, wind conditions have to be favorable for pollen to be transported to the trap at roof level. Pollen from other taller species, such as trees, may reach the trap more easily. In addition, pollen transported over long distances are another input and could be recorded by the trap at roof level (Skjøth et al. 2013). With regard to Poaceae, it should be noted that the measured Poaceae pollen concentrations originate from different grass species (i.e., 12,000 species; García-Mozo 2017), whose composition and therefore flowering times may vary by location.

While increasing temperature can promote plant development and flowering, the effect of extreme temperatures, e.g., associated with drought, can inhibit plant development and flowering. This effect on the grass pollen season was reported in a study of the 2003 heat wave in Switzerland (Gehrig 2006). Jung et al. (2021) conducted a field experiment where different grass species were exposed to drought, which was simulated by excluding rain and increasing the temperature. They found varying responses depending on the grass

species and suggest that the effects of elevated air temperature and drought conditions on pollen production are highly dependent on the grass species. Another study drew attention to the influence of water availability during plant development (García-Mozo et al. 2010). The influence of drought on pollen season characteristics could be observed in the Sydney study (Chapter 4). Data from three pollen seasons showed that the 2019/2020 season was less intense than in previous years, likely related to the drought that prevailed until January 2020.

In regards to climate change, temperatures in cities are expected to increase more rapid than in rural areas (Liu et al. 2022), which makes studying pollen in cities even more interesting, as the effects of increased temperatures are observed sooner than in rural areas. In addition, the effect of increased temperature and limited water supply on plant growth and pollen production could be studied following an experimental study design.

Daily variations. In Chapter 4, we analysed daily pollen and spore data in regards to the influence of meteorological parameters. There is strong evidence from literature that there is an influence of precipitation and temperature on the timing of pollen release and pollen season (Dahl & Strandhede 1996; Jato et al. 2002; Gehrig 2006; Bonofiglio et al. 2008; García-Mozo et al. 2010; Oduber et al. 2019). Our results show positive correlations between mean, minimum and maximum temperature for all analysed species for every year. For humidity and precipitation, all statistically significant correlations with humidity and precipitation were found to be negative. Similar results have been found by a study on Myrtaceae pollen in China (Bishan et al. 2020), and for Cupressaceae (Rojo et al. 2015). For *Alternaria*, studies support these results as Stennett & Beggs (2004a) and Rodríguez-Rajo et al. (2005) found a strong positive relationship between *Alternaria* concentration and temperature.

Weekly variations. Weekly data of pollen deposition was able to reflect the course of the pollen season (Chapters 3 and 4). However, relationships between weekly deposition and weekly precipitation totals could not be identified in the Ingolstadt study (Chapter 3) because precipitation events were rather isolated events and therefore not reflected in the comparison of weekly totals. For the pollen deposition data in Sydney (Chapter 4), the temporal setting of the sampling and the strong influence of the drought could be observed, which was also documented in the daily pollen data and manifested itself in comparatively low deposition values.

Sub-daily variations. Sub-daily or diurnal variations of pollen concentrations have been in the focus of several studies (e.g., Mullins et al. 1986; Latałowa et al. 2005; Kasprzyk 2006; Peel et al. 2014c; Simoleit et al. 2017; Alcázar et al. 2019). However, results on patterns seem to vary depending on the sampling period or geographic location and setting of the city. Diurnal variations of Poaceae pollen concentration in Ingolstadt were examined in Chapter 3. Based on pollen data collected with portable pollen traps at street level over a week in June 2019, there were no indications for different diurnal variations of grass pollen exposure between urban and rural locations. For all locations, pollen concentrations were highest at noon. Background pollen data were also used to analyse diurnal variations: considering the whole grass pollen season of 2019, diurnal variations differed between the urban and the semi-rural location. At the urban location, most grass pollen were measured in the second half of the day, while at the other site, most grass pollen were recorded in the morning between 8 a.m. and 12 p.m. However, considering the data from the week of the sampling campaign with portable volumetric traps, this pattern cannot be confirmed. In this week, there were no variations between the locations, as for both most pollen were measured between 10 a.m. and 12 p.m. This suggests that diurnal variations vary depending on the time in the season, as different Poaceae species flower at different times and meteorological conditions vary. Based on these data and the literature, no general statements can be made about diurnal variations of Poaceae pollen at urban and rural sites. However, it has to be considered that our data on pollen concentration at street level were derived from measurements that were taken only three times a day. During the sampling campaign with portable pollen traps (Chapter 3), precipitation events could be linked to measured pollen concentrations due to the high temporal resolution of the pollen measurements. Here, the lowest Daily Pollen Integral during the sampling campaign occurred on the only day with rain that occurred in between our sampling times.

To gain further insights, continuous sub-daily pollen monitoring at street level would be required, in addition to systematic phenological observations of different Poaceae species in the source areas. To cover larger areas, remote sensing data can be employed to provide data on grass cover and seasonal greenness, which can be linked to the phenological development of grass (Devadas et al. 2018). In addition, environmental DNA analysis could be employed to allow a differentiation between different Poaceae species

(Campbell et al. 2023). An approach like this would help to identify the species that release pollen into the air. Such studies are particularly important for people allergic to pollen that aim to reduce their exposure to pollen, and because knowledge on diurnal patterns could provide strategies for allergy mitigation.

Furthermore, symptom data have the potential to build a bridge from pollen data to pollen exposure and medical relevance. A citizen science approach could help to generate data from people of different age groups and geographic locations. For example, the “patient’s hay-fever diary” has been implemented in aerobiological studies in Germany and Austria (e.g., Karatzas et al. 2014; Bastl et al. 2021) or the “AirRater” in Australia (Jones et al. 2020; Jones et al. 2021). The BAYSICS app has recently been developed (Batsaikhan et al. 2020; Divanis & Meng 2021) and one of its aims is to gather data on allergy symptoms and the location of allergenic species. By engaging enough citizen scientists, this app holds the potential to create risk maps that can be used by people to better plan outdoor activities, and by researchers to link symptom data to aerobiological data.

6.1.3 Sampling methods

Different types of traps have been used to sample airborne pollen and spores in the studies described in Chapters 3 and 4. While each type has demonstrated their potential, their inherent limitations also have to be considered.

7-day volumetric pollen trap. The 7-day volumetric trap was used in the studies described in Chapters 3 and 4. It is a standard for monitoring background pollen concentration in Europe and Australia (Galán et al. 2014; Beggs et al. 2018), and usually placed on the roof of buildings (Chapter 2). By installing the trap at this height, continuous monitoring can capture the pollen spectrum of an area of approximately 30 km around the trap (Katelaris et al. 2004; Pashley et al. 2009). When placed several metres above the ground, it is exposed to influences other than local conditions (Skjøth et al. 2013), such as regional winds or pollen transported from further away (long-range transport). They are suitable for continuous operation as they can be connected to a power outlet or a battery. However, they are comparably expensive and heavy.

Gravimetric pollen trap. The spatio-temporal variation of pollen loads in cities has been the subject to several studies. However, few have used more than five sampling locations

(see review in Katz & Batterman 2020). Networks of Durham-type gravimetric traps in urban environments have been installed by Bricchi et al. (2000), Ishibashi et al. (2008), Katz & Carey (2014), Werchan et al. (2017) and Stas et al. (2021). In addition, they have also been used to investigate pollen transport by adding vertically oriented glass slides in all four cardinal direction (Eisen et al. 2022; Wörl et al. 2022). The main advantage of this trap is its cost-effectiveness, as no special components are required and parts are easy to obtain. Other advantages are its ease of operation and maintenance, as there are no moving parts and no motor or electricity is required. This allows to equip a study area with a large number of traps on a small budget. However, there are also disadvantages to be considered. The main disadvantage is that the sampling efficiency cannot be determined, as the volume of air transporting pollen to the trap is not known. The catch is relatively low because sampling is by sedimentation rather than impaction, as is the case with volumetric traps. Despite the disadvantages, they are suitable for assessing and comparing pollen deposition. In two of the studies in this thesis, sampling networks with gravimetric traps were installed to collect data at different locations in the study areas (Chapters 3 and 4). Beyond the known drawbacks, the two studies included in this thesis provided data to better understand pollen deposition across cities, as local conditions could be linked to pollen deposition. In addition, it was shown that weekly data can reflect the course of the pollen season. The Sydney study was particularly important, as there has never been a study of this scale in Australia.

Portable volumetric trap. The portable volumetric trap allowed the assessment of temporal variations at a finer scale than the gravimetric traps (Chapter 3). As a volumetric trap, which relies on impaction, it was possible to calculate pollen concentrations, allowing comparison with the roof level volumetric trap. In the study area, they were placed on tripods about 1.5 m off the ground. With the portable traps, 11 locations could be covered, as the traps are compact and mobile. They have also been used for measurements taken on foot (Charalampopoulos et al. 2018). However, the setup with multiple portable traps can be labour-intensive, as each trap needs to be monitored and the microscope slide in the trap needs to be manually changed as well as started and stopped. Furthermore, the sampling time has to be limited to avoid overlay of pollen and particles. In addition, the sampling efficiency of the portable trap was found to be affected by wind speed (Peel et al. 2014b), as the orifice that draws air is facing upwards and is

not facing the wind as it is with the 7-day volumetric trap (see section 2.1.1). As the traps are also not equipped with a rain shield, they should preferably be used outdoors in dry conditions or for indoor studies (Jochner-Oette et al. 2022).

Continuous monitoring is highly important and needed to understand the seasonality and intensity of aeroallergens and therefore on people's exposure to pollen in the ambient air. Monitoring should be carried out with traps at rooftop level to reflect background pollen concentration, but also at street level to determine the amount of pollen people are exposed to. Setting up more than one trap would provide additional data on spatial variations. Ideally, automatic pollen monitors would be installed to provide real-time data (Buters et al. 2018b, Buters et al. 2022). Comprehensive aerobiological monitoring is not evenly distributed around the world (Buters et al. 2018a). Therefore, there is not only a lack of knowledge about the characteristics of the pollen spectrum in underrepresented areas, but also a lack of data on region-specific pollen and spore thresholds that exert allergic reactions. Future research projects should therefore consider conducting comprehensive aerobiological studies in those parts of the world, as well as medical studies to assess exposure, for example by employing exposure chambers (Pfaar & Zieglmayer 2020).

6.2 Pollen production

6.2.1 Pollen production of allergenic species

The pollen production of *B. pendula*, *P. lanceolata* and *D. glomerata* was assessed quantitatively, as described in Chapter 5. In total, 24 trees of *B. pendula*, 82 individuals of *P. lanceolata* and 54 individuals of *D. glomerata* were sampled. The sample size of more than 20 *Betula* trees is similar to other studies (Jochner et al. 2011; Ranpal et al. 2022) that assessed pollen production. However, such a large sample size of *P. lanceolata* or *D. glomerata* individuals within a study area has not been used to our knowledge. This highlights the importance of this current study as it provides new data on the pollen production of those species.

Pollen production of *B. pendula* averaged at 1.2 ± 1.0 pollen grains per catkin. Other studies on *B. pendula* have been carried out by Ranpal et al. (2022), Kolek (2021),

Piotrowska (2008) or Jato et al. (2007). Other birch species, such as *B. papyrifera*, have also been studied (Katz et al. 2020). The estimates of *P. lanceolata* pollen production averaged at 5.0 ± 2.4 million pollen grains per inflorescence. There was only one other study that determined pollen production at the same level of analysis, i.e., per flower and inflorescence (González-Parrado et al. 2015), while other studies estimated pollen production only per anther (Hyde & Williams 1946; Sharma et al. 1999). Pollen production of *D. glomerata* averaged at $40,407 \pm 24,339$ pollen grains per flower, which differs from a study conducted in Spain (Tormo-Molina et al. 2015). The large standard deviation of pollen production values for the analysed species is an indication of the high variability within each species. This could also be the reason for the different results reported in the mentioned studies. Other reasons for variations could be the sampling and laboratory techniques that were used, as well as naturally occurring variation. In addition, small sample sizes in pollen production studies may not be representative enough.

In order to further disentangle the observed variations in pollen production estimates, they were analysed in relation to urbanisation. Therefore, we calculated the urban index (Jochner et al. 2012b), a measure to describe the degree of urbanisation, for all sampling locations and compared the results based on the classification into urban and rural locations. For two of the analysed species, *B. pendula* and *P. lanceolata*, pollen production was significantly higher at rural sites. Differences in pollen production between urban and rural areas have only been investigated in a few studies. For example, for *A. artemisiifolia*, pollen production was found to be higher in urban areas (Ziska et al. 2003). There are two other studies (Jochner et al. 2011; Kolek 2021) conducted in Germany comparing pollen production of *B. pendula* between urban and rural locations. The study conducted in Munich found higher pollen amounts in rural locations, but only at the beginning of flowering (Jochner et al. 2011), while the study conducted in Augsburg reported higher pollen production with increasing urbanisation (Kolek 2021).

6.2.2 Environmental influences on pollen production

To explain the differences in pollen production between urban and rural locations, environmental factors that are known to influence pollen production were analysed. It has been documented in the literature that temperature has an effect on pollen production.

While some studies have reported increases due to warmer conditions (Barnes 2018), others have observed decreases (Jochner et al. 2013). In addition to temperature, elevated CO₂ concentrations have been associated with increases in pollen production in some studies of various plant species (Ziska & Caulfield 2000; Jablonski et al. 2002; Wayne et al. 2002; Ziska et al. 2003; Rogers et al. 2006; Kim et al. 2018). It is therefore generally assumed that pollen production, and in turn, pollen loads will increase in the course of climate change. However, with regard to the influence of atmospheric NO₂ concentrations, studies documented different effects: At elevated NO₂ concentrations, Jochner et al. (2013) reported reduced pollen production in *B. pendula*. Zhao et al. (2017a) reported the opposite effect in *A. artemisiifolia*. An experiment on the effect of elevated O₃ concentrations on *B. papyfera* documented its impact on plant reproduction, i.e., the number of catkins and catkin length, diameter and mass (Darbah et al. 2008).

Based on these studies, environmental factors seem to affect pollen production differently depending on the species. Such species-specific effects were also identified in our study (Chapter 5). We found a negative correlation between pollen production and temperature for *B. pendula* and *P. lanceolata*. The analyses of pollen production and atmospheric NO₂ and O₃ concentrations showed a negative relationship between them for *B. pendula* and *P. lanceolata*. For *D. glomerata*, however, the relationship was positive, as seen in the analyses after excluding the influence of the other variables. However, in nature, there are various influences that can also have a masking effect. Nonetheless, based on these results, findings in the literature on the effect of air pollutants NO₂ and O₃ on pollen production can be confirmed. These results also underline that this effect seems to be species-specific and might even vary within each species.

Regarding the study area, it has to be considered that the city of Ingolstadt presents a relatively small urban area with approx. 140,000 inhabitants (Bayerisches Landesamt für Statistik 2023) with the urban index of the study sites ranging from 0.30 to 0.72. Therefore, the results may not be applicable to much larger urban areas or to urban areas at higher or lower altitudes. Future studies should consider investigations along urbanisation gradients that cover a wider range of the urban index.

Our results indicate a decrease in pollen production with increasing temperature, which is contrary to the results of other studies. As this has implications for pollen loads in regards to climate change, this influence has to be further investigated: it needs to be

clarified how much influence temperature has on pollen production, whether it is the main driver of such variations, and whether all potentially masking effects have been taken into account. Moreover, future studies on the influence of air temperature and pollutants on plant characteristics such as pollen production should be assessed by a combination of experiments in a controlled environment and field research in order to give new indications on single and combined effects. Tito et al. (2020) recommended an approach using altitudinal gradients as ‘natural laboratories’ and proposed to transplant and translocate species from different locations on the gradient to others. Whether this could be conducted with larger trees is, however, questionable, and would require a different study design. Furthermore, it should be discussed how suitable urbanisation gradients are for aerobiological studies, and whether other gradients might be more suitable. However, such experiments should consider the whole plant and its physiological performance, i.e., visual parameters of the plants such as the amount of flowers or foliage and their condition should be assessed, but also characteristics such as the allergenicity of pollen. In addition, other species should be included in the investigation, such as other Poaceae species, to determine if the effect of temperature is different for other species of this family.

In order to better relate atmospheric air pollutant concentrations to pollen production, site-specific measurements should be made on each individual plant sampled, rather than deriving these data from a land use regression model. Nonetheless, land use regression models are highly useful when such data are not available.

For the studied species *B. pendula*, masting, which occurs in woody species, is another factor that plays a role in pollen production, as it might have the potential to mask the effect of other influences. The frequency of masting can vary and depends on the species. In the case of *Betula*, it has been documented to occur every two (Latałowa et al. 2002) or three years (Detandt & Nolard 2000). It is not entirely clear how large the areas are where synchronous masting takes place. It is usually a regional phenomenon (Ranta et al. 2008), but has also been found to appear at continent-scale (Koenig & Knops 1998, 2005). However, a large variation of pollen production can still be observed at smaller areas such as seed plantations (Ranpal et al. 2022). There have been no studies on the masting behavior of birch trees in cities, so it is not possible to assess the extent to which masting plays a role in our study. However, this phenomenon could explain the differences in pollen production estimates between the study in Chapter 5 and those from literature (e.g.,

Jato et al. 2007; Piotrowska 2008; Kolek 2021). In order to identify masting years, pollen production data over several years are needed. Thus, it is highly recommended to install pollen traps for long-term monitoring in cities in order to identify masting years retrospectively.

The estimation of pollen production is time-consuming as it is based on laboratory analyses. In order to efficiently determine the pollen production of several individuals, the laboratory technique for extracting and counting pollen grains should be automated. For example, Ali et al. (2022) counted pollen grains using software algorithms. In addition, there have been attempts to use proxies for pollen production, such as measures of biomass to indicate resource allocation in plants. This approach is based on the theory that inter-annual variation of leaf area influences the supply of assimilates, so that a year with rich foliage is associated with fewer flowers and the subsequent year with opposite characteristics (Dahl & Strandhede 1996). Therefore, remote sensing techniques have been employed to identify masting years by analysing NDVI (González-Naharro et al. 2019) or to infer the size of the pollen source by investigating tree characteristics using LiDAR (Light Detection and Ranging) (Bogawski et al. 2019). Ground-based investigations of LAI (Leaf Area Index) in relation to pollen production of hazel (*Corylus avellana*) have also been conducted by Jetschni & Jochner-Oette (2019), however, no clear relationship between those measures was found in the analysed years. This could be due to the fact that there is also a species-specific pattern of resource allocation, which needs to be studied for each tree species specifically.

Putting it all together: To get a holistic picture of the factors influencing pollen in cities, all aerobiological processes have to be considered. To link pollen concentration to pollen production, a detailed inventory of the relevant plant species and their phenological development should be established. In addition, pollen dispersal and transport models could link pollen sources to measured pollen levels. For this purpose, studies should include experimental pollen or spore release to model dispersal patterns in cities. For example, Katz et al. (2023) used pollen production and pollen emission to model airborne pollen concentrations within a city. They highlight that this approach has potential, but also further research is needed to cover larger areas and to apply it to different tree species.

7 Conclusion

This thesis presented an investigation on the temporal and spatial variations of allergenic pollen in cities. The focus was on pollen concentration, pollen deposition and pollen production. The most important findings highlight the importance of continuous pollen monitoring both at street and rooftop level, as pollen concentrations were found to vary with height. In addition, the importance of a network of several traps to collect data on spatial variations was highlighted. The studies showed that for the duration of the main pollen season, the spatial variation in grass pollen load was mainly linked to surrounding land use, but variations due to land management or meteorological factors were also identified. Outside of the peak season, other influences such as pollen transport contributed to the amount of pollen in the air and could only be detected by continued monitoring outside the main flowering period. In addition, only continuous monitoring can reflect diurnal variations, which were shown to vary within the season and between locations. For pollen production, the results showed variations within each species at small spatial distances. The fact that *B. pendula* and *P. lanceolata* were linked to a higher pollen production in rural, i.e., cooler, areas, shows that temperature alone is not sufficient to explain variations in pollen production. It still has to be clarified why certain species do not profit from elevated temperatures. In our study, we found that atmospheric NO₂ and O₃ concentrations tend to decrease pollen production in *B. pendula* and *P. lanceolata*, and increase pollen production in *D. glomerata*. This indicated that the influence of environmental factors is species-specific. However, this is of limited validity as more data and research would be needed to make more complete statements. Based on these findings, several promising research perspectives were outlined in Chapter 6. The relevance of aerobiological studies is ongoing, as the challenges posed by climate change need to be addressed.

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Appendix

Reviewed publications

1. Jochner-Oette S, Simmons M, **Jetschni J**, Menzel A (2017). Impacts of land clearance by fire on spatial variation of Mountain Cedar pollen concentrations in Texas. *Landscape and urban planning*, 162, 178-186.
doi: 10.1016/j.landurbplan.2017.02.013
2. Jochner-Oette S, Stitz T, **Jetschni J**, Cariñanos, P (2018). The influence of individual-specific plant parameters and species composition on the allergenic potential of urban green spaces. *Forests*, 9(6), 284. doi: 10.3390/f9060284
3. **Jetschni J**, Jochner-Oette S (2021). Spatial and temporal variations of airborne Poaceae pollen along an urbanization gradient assessed by different types of pollen traps. *Atmosphere*, 12, 974. doi: 10.3390/atmos12080974
4. Jochner-Oette S, **Jetschni J**, Liedl P, Menzel A (2022). Indoor pollen concentrations of Mountain Cedar (*Juniperus ashei*) during rainy episodes in Austin, Texas. *International Journal of Environmental Research and Public Health*, 19(3), 1541. doi: 10.3390/ijerph19031541
5. Wörl V, **Jetschni J**, Jochner-Oette S (2022). 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*, 13(12), 2007.
doi: 10.3390/atmos13122007
6. **Jetschni J**, Al Kouba J, Beggs PJ, Jochner-Oette S (2023). Investigation of spatial and temporal variations of airborne Poaceae, Myrtaceae and Cupressaceae pollen and *Alternaria* spores in Sydney, Australia, 2017-2020. *Aerobiologia*, 39(1).
doi: 10.1007/s10453-023-09783-w

Submitted publications

7. **Jetschni J**, Fritsch M, Jochner-Oette S (2023). How does pollen production of allergenic species differ between urban and rural environments? Under review at *International Journal of Biometeorology*. Submitted on 19 January 2023

Publications in preparation

8. **Jetschni J**, Rippert A, Jochner-Oette S (2023). Using phenology and aerobiology to evaluate the allergy risk in urban parks. In preparation for *Aerobiologia*.

Conferences and abstracts

Oral presentations

9. **Jetschni J**, Jochner-Oette S (2020). Spatial and temporal variations of Poaceae pollen concentration at rural and urban sites in Ingolstadt, Germany. 7th European Symposium on Aerobiology: Bioaerosols and Environmental Impacts, 16-20 November 2020, virtual edition.
10. **Jetschni J**, Al Kouba J, Beggs PJ, Jochner-Oette S (2021). Investigations on spatial and vertical variations of airborne pollen in Sydney, Australia. EGU General Assembly 2021, online, 19-30 April 2021, EGU21-9184.
doi: 10.5194/egusphere-egu21-9184
11. **Jetschni J**, Rippert A, Jochner-Oette S (2022). Using phenology and aerobiology to evaluate the allergy risk in urban parks. Phenology 2022: Phenology at the crossroads, 20-24 June 2022 in Avignon, France.

Poster presentations

12. **Jetschni J**, Kaffka S, Cattle D, Liedl P, Menzel A, Jochner-Oette S (2017) Quantification of indoor pollen concentrations (*Juniperus ashei*) during rainy episodes in Austin, Texas. PAAS 2017 - Palynology-Aerobiology-Allergy Symposium, 26-28 October 2017, Vienna, Austria.
13. **Jetschni J**, Kaffka S, Cattle D, Liedl P, Menzel A, Jochner-Oette S (2018) Quantification of indoor pollen concentrations (*Juniperus ashei*) during rainy episodes in Austin, Texas. EGU General Assembly 2018, 19-30 April 2021, Vienna, Austria
14. **Jetschni J**, Jochner-Oette S (2019) Investigations on the relationship between pollen production and Leaf Area Index of common hazel (*Corylus avellana*). Geophysical Research Abstracts 21, EGU General Assembly 2019, Vienna, Austria.