

## Spatial and temporal monitoring of *Betula* pollen in the region of Augsburg, Bavaria, Germany

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**Spatial and temporal monitoring  
of *Betula* pollen  
in the region of Augsburg,  
Bavaria, Germany**

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*Science, my lad, is made up of mistakes, but  
they are mistakes which it is useful to make,  
because they lead little by little to the truth.*

Jules Verne - A Journey to the Center of the Earth

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All tables were based on own data unless otherwise stated.

# Abbreviations

a.g.l.	Above ground level
APE	Aqueous pollen extract
a.s.l.	Above sea level
BBCH	Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundessortenamt und Chemische Industrie (biological federal institute for agriculture and forestry, federal plant variety office and chemical industry)
BSA	Bovine serum albumin
DPBS	Dulbecco's Phosphate Buffered Saline
DWD	Deutscher Wetterdienst (German Weather Service)
EAACI	European Academy of Allergy and Clinical Immunology
EAS	European Aerobiology Society
ELISA	Enzyme-linked Immunosorbent Assay
KOH	potassium hydroxide
LTB 4	Leukotriene B4
n.a.	not available
n.s.	not significant
NO	Nitrogen monoxide
NO <sub>2</sub>	Nitrogen dioxide
O <sub>3</sub>	Ozone
PALM	pollen-associated lipid mediator
PGE 2	Prostaglandine 2
PID	Stiftung deutscher Polleninformationsdienst (German Pollen Information Service foundation)
SD	Standard deviation
UI	Urbanity Index
WGS	World Geodetic System
WHO	World Health Organisation

**A**

**Aim, research background and  
structure**

Allergic diseases comprise a major health problem, affecting up to 40 % of the population of Europe (D'Amato et al. 2007a; Pawankar et al. 2013; Bieber et al. 2016). Also they are increasing in prevalence and are expected to further increase in the future, notably up to 50 % of the European population (European Academy of Allergy and Clinical Immunology 2015). This rise is shown especially for allergies caused by airborne particles like pollen and fungal spores. The sensitisation rate in Germany in 1998 was 29.8% and rose to 33.6% in 2008-2011 (Haftenberger et al. 2013).

Worldwide, 9 out of 10 people are affected by air pollution (World Health Organisation 2018). It is known that air pollutants can have negative effects on human health and can cause or promote the development of respiratory diseases (Krzyzanowski and Cohen 2008). This effect can be seen especially in children (Morgenstern et al. 2007). Exposure to air pollutants also increases the risk of developing an allergy or of worsening allergic symptoms (Westgate et al. 2003; Brauer et al. 2007).

Allergic patients can overall have a significantly reduced life quality due to their allergy symptoms (Baiardini et al. 2006). This reduction of life quality can be caused by a negative impact on social activities, reduced quality of sleep or reduced overall performance (Blaiss et al. 2018; Jernelöv et al. 2013; Muzalyova et al. 2019). And not just the personal life of people is affected by the allergy, it also has a considerable economic effect due to reduced productivity, being a burden for the health system and because of school and work absenteeism (Bhattacharyya 2012; Bousquet et al. 2013; Bieber et al. 2016; Linneberg et al. 2016; Traidl-Hoffmann 2017; Muzalyova et al. 2019).

The reasons for the rising number of allergic patients are not completely understood yet but it is already shown that the westernised lifestyle and air pollution are affecting the prevalence and severity of the symptoms (Behrendt et al. 1997). And plants, being themselves a sensitive indicator for climate change, are also affected by changes in the environment (their pollen included) (Beck et al. 2013; Ziello et al. 2012b; Lake et al. 2017; Emberlin et al. 2002).

For this reason, the here presented work attempts to explain the temporal and spatial patterns of the whole reproductive cycle of the production of male inflorescences and pollen, the flowering phenology, the allergenicity of pollen and, ultimately, pollen release from inflorescences and airborne pollen transport, as well as the connection between all

these processes and their interaction with the ambient environment. As *Betula* is a widespread genus in temperate climates (Atkinson 1992; Beck et al. 2016), and *Betula* pollen is one of the major allergy triggers in Europe, this research has a main focus on *Betula*.

The influence of environmental factors (e.g. meteorology, air pollution, biodiversity, urbanity) on plants and pollen can be seen in changes in the patterns of pollen seasons (Latałowa et al. 2002; Stach et al. 2008; Jahn-Schmid et al. 2005; Prescott 2020) and can give a hint on future conditions due to climate variability (Ziska et al. 2003; Jochner et al. 2013). Therefore, one part of the dissertation, the aerobiological monitoring in Augsburg, addresses the occurrence (presence), abundance (concentration), and timing (phenology) of airborne pollen in Augsburg. This research gives an overview of the spectrum of anemophilous plants in the study regions and patterns of their occurrence on different temporal scales.

Moreover, plant phenology has been often reported to be connected with changing environmental conditions. Factors like precipitation, soil humidity, air pollutants and competition are known to influence the timing of phenology (Andersen 1980; Fenner 1998; Menzel and Fabian 1999; Menzel 1999; Wielgolaski 1999). This topic is therefore addressed in the section about phenological observations of the prevalent in temperate climates *Betula pendula*. Here, the observation of the development of male inflorescences and especially the flowering period of different individuals of *B. pendula* in the study region is thematised. Temporal and spatial patterns influencing the timing of phenological observations will be analysed.

The same environmental factors also influence the amount of pollen that is produced by a plant (Jablonski et al. 2002; Ladeau and Clark 2006; Rogers et al. 2006; Wan et al. 2002; Wayne et al. 2002; Ziska and Caulfield 2000; Damialis et al. 2011). The variable of pollen production is examined in the section about the production of pollen, flowers and male inflorescences of *Betula pendula*. It reveals the actual quantity of pollen produced, along with flowers and inflorescences, for different individuals of *B. pendula* and the influencing factors for the observed variability.

The allergen content likewise exhibits appears to be sensitive to various environmental regimes (Beck et al. 2013). The allergenicity of *Betula pendula* pollen is, hence, assessed

by laboratory investigations in this dissertation. The allergen content is examined for different individuals of *B. pendula*. Also, for this aspect, spatial and temporal patterns are laboured.

The overall aim of this dissertation, rather than examining the isolated effects of several parameters on the final atmospheric abundance and timing of produced pollen, was to investigate the above-mentioned factors in a holistic approach and integrate the obtained information to obtain knowledge about patterns at all spatiotemporal levels and, potentially, on the underlying mechanisms. Towards the ‘One Health’ approach (Pali-Schöll et al. 2021), the variable missing from comprehending the genuine exposure of human to airborne pollen is the entirety of exposome. In this dissertation, the pursuit is to unveil the additive impact of a wide combination of environmental and biological parameters, which contribute to the real-life, genuine exposure, as undoubtedly, multiple exposures are certainly not the exception but the rule under natural conditions. Such a high-resolution, multi-factorial knowledge will contribute to the most accurate and timely evaluation of climate change effects on the observed biological system, and also to the most efficient allergy management via personalised predictive models in the future.

**B**

# **Introduction**

## **B.1. Aerobiological monitoring**

### **B.1.1 Aerobiology**

Aerobiology is the scientific field “study[ing] airborne particles of biological origin and is concerned with their sources, liberation, dispersal, deposition and impact on other living organisms and of the effects of environmental conditions on each of these processes” (Holgate and Busse 2000). Airborne particles of biological origin are the so called bioaerosols and they include viruses, bacteria, actinomycetes, myxomycetes, fungi (fungal spores and hyphae), lichens, algae, bryophytes, pteridophytes, pollen grains, plant fragments, invertebrates (insects, arachnids), fragments of invertebrates, aerosols with urinary proteins and skin particles (Gregory 1961).

Aerobiology as a scientific discipline was established in the 1860s as “micrography” with the rejection of the spontaneous generation theory and experiments that proved the existence of particles in the air (Ariatti and Comtois 1993). In the 1930s, the research field was first called Aerobiology by F. C. Meier (Lacey and West 2006).

Today, Aerobiology is a multi- and inter- disciplinary scientific field that connects environmental sciences, like plant and microbial ecology and biology, meteorology, and geography, with health sciences.

### **B.1.2 Biological background**

#### **B.1.2.1 Pollen formation**

Pollen grains contain the male gametes and therefore are necessary to enable the reproduction of plants. Pollen grains are formed in the anther (in Angiosperms) or the microsporangia (in Gymnosperms), the male apparatus of the flower. For reproduction, the pollen is transferred from the male apparatus to the stigma (Angiosperms) or the megasporangium (Gymnosperms). (Solomon 1978)

Pollen from all Angiosperms and Gymnosperms consists of a two-layered wall with an outer layer (exine) and an inner layer (intine). The inner part of the pollen is filled with cytoplasm that contains the vegetative nucleus as well as the generative nucleus. (Shi et al. 2015)

The allergenic protein, found in the protoplast of the pollen, is released during the hydration process while the pollination (Songnuan 2013; Grote 1999). Pollen allergens, e.g. Bet v 1 and Bet v 2 in the species of *Betula*, belong to the group of profilins and are located in the pollen cytoplasm and released through the apertures within minutes during the hydration process and afterwards found on the whole surface of the pollen (Grote et al. 1993; Chen et al. 2016)

### **B.1.2.2 Pollen structure**

Pollen grains of different taxa can be distinguished by different features: the polarity, the shape, the apertures, the structure of the exine and the size of the pollen grain.

Before the meiosis, the pollen grains are arranged in tetrads. For this reason, pollen grains can have a certain polarity. This polarity can often be seen with the help of the apertures and the general shape of the pollen.

The shape is described as the ratio between the length of the polar axis (P) and the length of the equatorial axis (E). For isodiametric pollen, the P/E ratio is +/- 1, pollen grains with a longer polar axis are prolate, pollen grains with an equatorial axis longer than the polar axis are oblate. Another description of the shape refers to the three dimensional shape of the pollen grains and can be for example spheroid-, cup-, cube- or triangular prism-shaped. (Halbritter et al. 2018; Erdtman 1943)

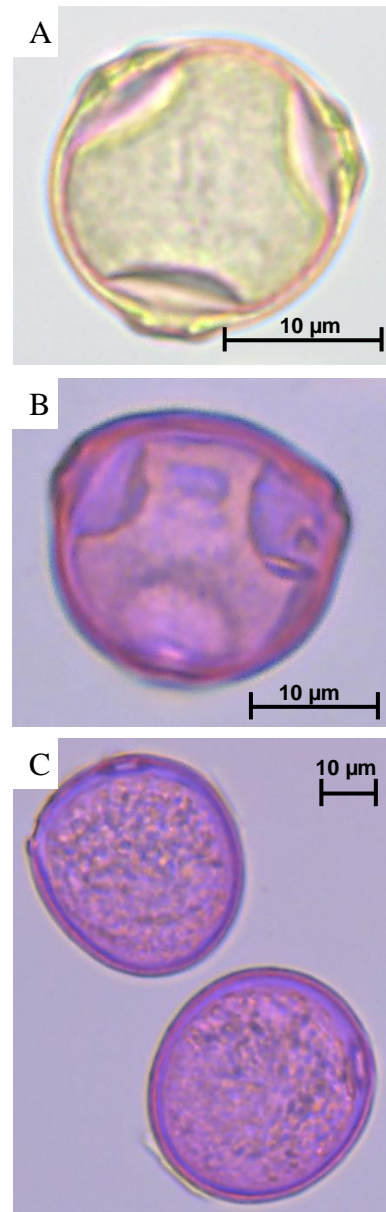
Apertures are areas in the pollen wall that differ significantly in morphology from the surrounding pollen wall. There are two kinds of apertures in pollen grains that can be distinguished: pori and colpi. Pori (sg.: porus) are round apertures, whereas elongated apertures are called colpi (sg.: colpus). A combination of a colpus and a porus, viz. a colpus with a porus in the widest area, is called a colpore (pl.: colpore). Pollen grains can have no, one or more apertures. Pollen grains with no apertures are called inaperturate, pollen grains with one aperture monoaperturate, pollen grains with three (four, five) apertures triaperturate (tetra-, penta-aperturate). Pollen with more than 6 apertures are

polyaperturate. This terminology can be also used with –porate or –colpate instead of –aperturate to specify the kind of apertures. Also, the positions of the apertures on the surface of the pollen grain is important. Apertures can be distributed around the equator (zono- or stephano-aperturate) or on the whole surface (pantoaperturate) of a pollen grain. (Halbritter et al. 2018; Punt et al. 2007; Solomon 1978; Erdtman 1943)

So, a *Betula* pollen grain (**Figure 1A**) or a *Corylus* pollen grain (**Figure 1B**) can be described as tri-zonoporate while a Poaceae pollen grain (**Figure 1C**) is monoporate.

The structure of the surface of the pollen surface is determined by the exine. The exine consists of two layers, the inner endexine or nexine and the outer ektexine or sexine. The endexine is unsculptured while the ektexine can be sculptured and influences the appearance of the pollen wall. The pollen wall can among others appear psilate, reticulate, striate, reticulate or echinate. (Halbritter et al. 2018; Punt et al. 2007; Erdtman 1943; Solomon 1978)

The size of airborne pollen grains ranges from less than 10 µm and more than 100 µm (see **Table 1**). Larger pollen are mostly produced by entomophilous plants.



**Figure 1:** pollen of *Betula pendula* (A), *Corylus avellana* (B) Poaceae (C) under a light microscope (400x) (photo by Franziska Kolek)

**Table 1:** sizes of airborne pollen (Halbritter et al. 2018)

Size (diameter)	category	Examples
< 10 µm	very small	<i>Mimosa pudica</i> , <i>Myosotis</i> spp., <i>Peperomia</i> spp.
10 µm – 25 µm	small	<i>Betula</i> spp., <i>Fraxinus</i> spp., <i>Salix</i> spp.
26 µm – 50 µm	medium	<i>Allium</i> spp., <i>Carpinus betulus</i> , <i>Oxalis</i> spp.
51 µm – 100 µm	large	<i>Lilium</i> spp., <i>Pinus</i> spp., <i>Salvia</i> spp.
> 100 µm	very large	<i>Abies</i> spp., <i>Hibiscus</i> spp., <i>Picea</i> spp.,

### **B.1.2.3 Pollination**

Pollen can be dispersed in different ways. The main ways of distribution are abiotic, as by wind (anemophily) or water (hydrophily) or biotic, for example through self-pollination or by animals (zoophily).

Most gymnosperms but also various angiosperms, like *Betula* that is studied in this dissertation, are anemophilous plants. A main characteristic of anemophilous plants are the missing or underdeveloped floral traits to attract insects (prominent floral parts, scent, nectar production) and high amounts of pollen due to the untargeted pollination. The pollen grains are small and light to be transported by air. The stamen of the plants are well exposed to trap pollen from the air (Faegri and van der Pijl 1980)

### **B.1.3 Timeliness and usefulness**

Anemophilous pollen can, after being released from the plant, be measured in air samples, and give information about the flowering of different taxa.

There are several reasons to monitor bioaerosols. First, the observations enable a general overview over their biodiversity and abundance in the air and allow for detection of temporal patterns on different scales. This helps to understand the vegetation in the observed area and makes it possible to provide information to allergic people about the timing of the appearance of allergenic pollen on both daily and seasonal scale. For example, it is shown that the widely accepted belief that pollen are mainly distributed during daylight (Alcázar et al. 1999; Dahl et al. 2013) is not true for all pollen types at all places (Grewling et al. 2016). With respect to allergy sufferers, the monitoring of aeroallergens in combination with the severity of their symptoms can lead to a better knowledge about allergic reactions (Damialis et al. 2019a).

With a long-term dataset of aeroallergens, it is also possible to detect changes in diurnal distribution patterns, seasonality, yearly and daily abundance and in the biodiversity spectrum of the observed particles per examined plant taxon. This can help to identify short-term variations but also long-term trends. With this knowledge, predictive models

of airborne pollen concentrations are possible (Helbig et al. 2004; Damialis et al. 2007; Sofiev and Bergmann 2013; Oteros et al. 2015a).

To fully understand the spatiotemporal patterns, also influencing co-factors must be investigated. The influences of meteorological factors on the pollen season are already described (Galán et al. 1991; Emberlin et al. 2002; Latałowa et al. 2002) but still not fully understood. The influences of environmental factors on the pollen emitting plants are described in Chapter B.3.

As it is known that the duration of exposure and the amount of pollen influence the severity of allergic symptoms (Cecchi et al. 2010; Raulf et al. 2014), one can utilise this knowledge so as to elaborate on patterns in the pollen season as a helpful tool for allergy sufferers to manage their disease in a more effective way (Spieksma et al. 1995). Pollen exposure can exhibit dire health effects even on non-allergic people, as airborne pollen can weaken the immune defence against respiratory viruses (Gilles et al. 2020). Such information becomes even more valuable and timely given the COVID-19 pandemic initiated in March 2020: recent evidence (Damialis et al. 2021) proves the above relationship in an international context, during an unprecedented pandemic, which highlights the importance of and need for airborne pollen monitoring and the neglected role of co-exposure. While it is known that the risk of developing an allergy or worsening of allergic symptoms is increased by exposure to air pollutants (Westgate et al. 2003; Brauer et al. 2007), little is known about the co-exposure to different types of pollen, fungi, viruses or the influence of different meteorological conditions (e.g. thunderstorm asthma (D'Amato et al. 2007b)). Therefore, information about pollen levels in the air is obviously considered crucial, both spatially and temporally, to enable a better management of pollen-induced health issues for patients and medical professionals.

Aerobiological data are also an important indicator of environmental changes like altering meteorological factors (Stach et al. 2008; Jahn-Schmid et al. 2005; Anderegg et al. 2021), biodiversity (Haahtela et al. 2013; Prescott 2020; Marselle et al. 2021), pollutants (Beck et al. 2013) or other environmental parameters like viruses (Gilles et al. 2018). Consequently, airborne pollen measurements have been defined also as a sensitive indicator for climate change; the seasons and concentrations of pollen of different taxa can give information about changes in the vegetation and adaptation of plants to changed environmental conditions (Spieksma et al. 1995; Beggs 2004; Beggs 2010; Ziello et al.

2012b; Beck et al. 2013; Ziska et al. 2019; Anderegg et al. 2021). Also on spatial scale, aerobiological data can identify environmental influences like aspects of urbanity (e.g. temperature, pollutants) on sources of aeroallergens and with these altered conditions give a hint on future conditions due to climate variabilities. (Ziska et al. 2003; Jochner et al. 2013)

As pollen and fungal spores are the most prominent research fields in Aerobiology and pollen are a sensitive indicator for changes in the environment (Lake et al. 2017; Emberlin et al. 2002), the here presented research will focus on pollen.

### **B.1.4 Aerobiological records of pollen**

Aerobiological measurements in a systematic way have been performed since the 19<sup>th</sup> century. In 1859, Pierre Miquel developed the first air sampler, the Aeroscope, to analyse particles in the air (Ainsworth 2002). Also other devices were developed, like the gravity slide sampler by O. C. Durham in 1946, the rotating-arm sampler by W. A. Perkins in 1957 or the Cour Girouette sampler by P. Cour in 1974 (Agashe and Caulton 2008). The most successful development was the Hirst-type sampler (Hirst 1952) that has been established in aerobiological measurements all over the world until today (Buters et al. 2018).

Currently, there are over 800 active pollen monitoring sites around the world of which over 600 are equipped with a Hirst-type sampler. Over 500 of these sites are situated in Europe (Buters et al. 2018).

In Germany, pollen monitoring has been performed mainly by *the Stiftung deutscher Polleninformationsdienst* (PID, German Pollen Information Service foundation). This foundation runs about 40 pollen samplers in Germany and collects the data of all of them. In Bavaria, there is only a limited amount of publications about recent airborne pollen (Richter et al. 2013; Höflich et al. 2016; Damialis et al. 2019a; Oteros et al. 2019a; Oteros et al. 2019b; Picornell et al. 2019).

### **B.1.5 Aerobiological monitoring of *Betula* pollen in Augsburg**

The plant genus of *Betula* consists of anemophilous species that produce airborne pollen, which are released in spring in central Europe (Werchan et al. 2013). *Betula* pollen grains (**Figure 1A**) are tri-zono-porate spheroid pollen grains with a diameter of approximately 20  $\mu\text{m}$ .

Until very recently, there has been no pollen information available in Augsburg, and only after 2015 airborne pollen have been monitored in Augsburg (Kolek et al. in review\_a). The same was true also for the whole of Bavaria, with also no pollen calendars available before (Kolek et al. in review\_a), just for north and central Germany, more investigations are available (Werchan et al. 2018).

Considering the fast changing environmental conditions today, it is important to record pollen continuously to be able to observe changes in the biodiversity and patterns in the abundance of different pollen. Especially in Augsburg, close to the German Alps, this will be important as high mountain regions are especially influenced by rising temperatures and changes in precipitation (Hock et al. 2019) what will lead to a change in biodiversity. And also in the city of Augsburg, during 1961 and 2017, the yearly temperature has been increasing significantly while yearly precipitation decreased (Deutscher Wetterdienst).

Together with the knowledge about the high allergenic potential of *Betula* (Gioulekas et al. 2004), this makes the present work valuable for the citizens and visitors of this area, as it is the first to show the biodiversity and abundance of airborne pollen in Augsburg, Bavaria, Germany.

## **B.2. Phenological observations**

### **B.2.1 General**

“Phenology refers to recurring plant and animal life cycle stages, such as leafing and flowering, maturation of agricultural plants, emergence of insects, and migration of birds. It is also the study of these recurring events, especially their timing and relationships with weather and climate.” (Schwartz 2013)

In combination with aerobiological monitoring, phenological observations help to characterise the timing and intensity of flowering of anemophilous plants and add a spatial component to the aerobiological observations, as plant individuals prefer specific conditions to grow and therefore show spatial patterns in abundance.

Plant phenology is examined since millenniums. The oldest records that are still known nowadays, were found in China for the cherry blossom season, and dated back in the 11<sup>th</sup> century BC. From the 17<sup>th</sup> century, records of Konrad Gessner are published about the vegetation in a systematic way. In the 18<sup>th</sup> century, more people, among others Carl von Linné, Karl Theodor, Elector Palatine of the Rhine, Adolphe Jaques Quételet and Charles François Antoine Morren, studied the phenology of plants. (Puppi 2007)

Plant phenology is influenced by different environmental factors. Temperature is the most prominent factor and it is well known that higher spring temperatures cause an earlier onset of phenological development (Andersen 1980; Menzel and Fabian 1999; Wielgolaski 1999; Lee et al. 2020). This may be explained by the fact that the start of the growing season, indicated by the start of mitosis in tissues neighbouring to shoot apical meristems, is influenced by a specific sum of days above a certain temperature. This threshold is often assumed as 5°C as this is the threshold for the possibility of respiration (Worrall 1999). This temperature is considered as one of the most decisive factors to determine the onset of flowering, leafing and fruiting, usually referred to as ‘growing degree days’ (Nuttonson 1957; Gilmore and Rogers 1958; Grigorieva et al. 2010).

Water influences phenology too, mainly in two ways; while dry weather conditions trigger flowering (Andersen 1980), drought can delay the development (Aikens et al. 2020). Also competition between plants can influence phenology, causing either an earlier or later development, dependent on the kind of competition (Fenner 1998). Pollutants are reported to influence phenology as well; Ozone (O<sub>3</sub>) is reported to delay spring phenology (Jochner et al. 2015), while a combination of O<sub>3</sub>, Nitrogen Dioxide (NO<sub>2</sub>) and soil nitrate accelerated the development (Eller et al. 2020).

NO<sub>2</sub> is elevated in urban areas since its main source are fossil fuels, released by industrial factories, power stations and motor vehicles (Lee et al. 2013).

O<sub>3</sub> is interacting with different substances in the atmosphere, including nitrogen oxides. Under the influence of temperature and UV radiation, this leads to complex interactions. This is relevant especially for tropospheric ozone.

In urban areas and near streets, O<sub>3</sub> levels are lower due to elevated NO<sub>2</sub> concentrations and the reaction of ozone with nitrogen oxide under the influence of UV radiation (Last et al. 1994; Sillman 1999).

The threshold for the concentration of NO<sub>2</sub> in the ambient air is defined by the World Health Organisation (WHO) as 200 µg/m<sup>3</sup> of air in a 1-hour-mean and 40 µg/m<sup>3</sup> in a yearly mean. For O<sub>3</sub>, the 8h-threshold, also defined by the WHO, is 100 µg/m<sup>3</sup> (World Health Organisation 2005).

The sensitivity of the interactions between NO<sub>2</sub> and O<sub>3</sub> to changing temperatures (Sillman 1999), it is also important in the topic of climate change.

For 200 years, it is known that urban areas have different environmental conditions from rural areas. Especially temperatures can differ between a city and the surroundings (Howard 1818). These differences can also result in an altered phenology what makes it important to observe phenology not just temporally but also spatially. This can also be used as a surrogate for climate change (Ziska et al. 2003). Urbanity is also reflected in altered pollutant levels like NO<sub>2</sub> and O<sub>3</sub>.

Knowledge of the flowering season and its sensitivity to different environmental factors can contribute to the prediction of crop, fruit, or seed production levels (Galán et al. 2008). Also estimating the flowering season of plants releasing allergenic pollen, may improve

forecasts of allergic symptoms that are caused by these pollen (Chuine et al. 1999; Estrella et al. 2006).

This dependence on environmental factors makes flowering a sensitive indicator for changes in these parameters. (Sparks and Carey, P. D., Combes, J. 1997; Peñuelas et al. 2009; Piao et al. 2019; Lee et al. 2020). This is also recognised by the Intergovernmental Panel on Climate Change (IPCC) as they list phenology as a bioindicator of climate change (IPCC 2007, 2015).

Due to changing climatic conditions, the length of the growing season in Europe extended about two weeks from the 1980s to the 1990s with an earlier start in spring and a later end in autumn (Menzel and Fabian 1999) and the IPCC predicts an earlier onset of spring of 2.3 to 5.2 days per decade in the future (IPCC 2007). This increases the importance of understanding the underlying mechanisms for an improved prediction of the phenology.

## **B.2.2 Phenological observations of *Betula pendula***

The genus *Betula* was chosen for this dissertation because of its species' frequent prevalence in the study region. The species *Betula pendula* represents the majority of individuals of *Betula* in the study region (San-Miguel-Ayanz et al. 2016). This was confirmed by the mapping of all *Betula* individuals in Augsburg.

Another reason, why this dissertation focusses on *Betula*, is the flowering in the beginning of the year. The male inflorescences of *Betula* are formed in the summer of the previous year (Piotrowska 2008) so in the beginning of the flowering season they do not have to be formed and are able to develop to the stage of flowering fast and so be triggered by and highly dependent on the timing of rising spring temperatures. It is reported, that the start of the yearly growing season of *Betula pendula* is limited by low night temperatures (Atkinson 1992). This means that the timing of the *Betula* flowering season is an indicator for changes in spring temperatures that can currently be observed in Europe (Emberlin et al. 2002).

Taking into account the allergenicity of *Betula* pollen, it is important to acquire more knowledge about the timing of the flowering of *Betula*.

## **B.3. Production of flowers, and inflorescences and pollen**

### **B.3.1 General**

Anemophilous plants, like *Betula pendula*, produce large amounts of pollen to ensure successful pollination (Reddi and Reddi 1986; Molina et al. 1996; Piotrowska 2008). These amounts are varying, what leads to different concentrations of pollen in the air that can be seen with aerobiological monitoring.

It is shown that pollen production is sensitive to environmental variability (Jablonski et al. 2002; Ladeau and Clark 2006; Rogers et al. 2006; Wan et al. 2002; Wayne et al. 2002; Ziska and Caulfield 2000; Damialis et al. 2011). This makes it a good indicator for changes in environmental conditions like meteorological factors (Faegri et al. 1989; Moore et al. 1991; Ziska et al. 2019). Nonetheless, studies on pollen production are only limited and the pollen load is often estimated by aerobiological monitoring. Due to this situation, it is still not known how many pollen grains are released per plant to quantify pollen-related distribution models. As trees (and also shrubs) can be of different sizes, it is for this work also relevant to estimate the pollen production per tree like done from Moore et al. 1991 and Rogers 1993. This lack of research may be due to the missing awareness for the importance of this parameter but also due to methodological issues as different plants develop their flowers in different forms and so the technique used to count the contained pollen, need to be modified from species to species.

As pollen are causing allergic symptoms (La Diaz de Guardia et al. 2006; Gioulekas et al. 2004) and symptoms get more severe with a higher amount of inhaled pollen (Karli et al. 2013), knowledge about the pollen production can help to forecast the severity of the symptoms. *Betula* pollen have a high allergenic potential and make up between 17% and 19% of the airborne pollen in the air of the study area what makes them the second most abundant pollen type after Urticaceae and gives the research a high relevance (Kolek et al. in review\_a).

### **B.3.2 Production of flowers, inflorescences and pollen of *Betula pendula***

There are only a few existing studies about pollen production of woody species (Reddi and Reddi 1986; Allison 1990; Molina et al. 1996; Moe 1998; Mondal and Mandal 1998; Hidalgo et al. 1999; Cuevas and Polito 2004; Gómez-Casero et al. 2004; Ferrara et al. 2007; Jato et al. 2007a; Khanduri and Sharma 2009; Damialis et al. 2011), and just one for *Betula* (Jato et al. 2007b). Most studies on pollen production focus on the production per inflorescence, flower and / or anther and do not consider the production per individual (Beri and Anand S.C. 1971; Vries 1971; Cruden 1977; Reddi and Reddi 1986; Allison 1990; Bera 1990; Spalik and Woodell 1994; Moe 1998; Mondal and Mandal 1998).

## **B.4. Allergenicity**

### **B.4.1 General**

Allergic diseases are a major health problem with a rising prevalence over the last decades, already affecting up to 40 % of the population of Europe (D'Amato et al. 2007a; Pawankar et al. 2013; Bieber et al. 2016; European Academy of Allergy and Clinical Immunology 2015). For aeroallergens, the sensitisation rate in Germany rose from 29.8% in 1998 to 33.6% in 2008-2011 (Haftenberger et al. 2013).

People with allergies to bioaerosols suffer from symptoms like sneezing, rhinorrhoea, nasal congestion, coughing, wheezing or even asthma (Averbeck et al. 2007; Bantz et al. 2014; Valero et al. 2017; Damialis et al. 2019b).

Allergic symptoms are caused after exposure to the associated allergen by the production of IgE antibodies. They bind to IgE-specific receptors on immune cells. If the person is exposed to the allergen again, the allergen can activate these immune cells by binding to them. Due to this process, the immune cells release histamine what causes vasodilation, nerve stimulation and mucous secretion. (Beck 2014; Averbeck et al. 2007; Damialis et al. 2019b)

### **B.4.2 Allergenicity of *Betula pendula* pollen**

As the allergen structure of *Betula* is similar, cross-reactions with other pollen from the Betulaceae family like *Alnus*, *Carpinus* and *Corylus* as well as pollen from the order of Fagales, like Corylaceae or Fagaceae, are quite common (Mothes and Valenta 2004). In addition to respiratory symptoms and immune responses, also cross-reactions to food like fruit, vegetables and spices can be developed (Ebo et al. 2010; Tolkki et al. 2013; Treudler and Simon 2017; Damialis et al. 2019b).

Another reason for *Betula* as a study subject is the allergenic potential of their pollen (Gioulekas et al. 2004) and their importance of this allergen in the study region, having the second highest prevalence for allergies in Germany (Haftenberger et al. 2013).

Pollutants as NO<sub>2</sub> and O<sub>3</sub> are influencing the allergenicity of pollen (Beck et al. 2013). They seem altering in urban areas (Sillman 1999; HEI Panel on the Health Effects of Traffic-Related Air Pollution 2010; Beck et al. 2013) and are also affecting the health of allergic people (Behrendt et al. 1997; Darbah et al. 2007).

This increases the importance of the seasonality of flowering and pollen atmospheric distribution, as well as the allergenicity of *Betula*.

In this study, the allergen content is expressed by the concentration of the major allergen in *Betula*, Bet v 1. The majority of *Betula*-pollen allergic people are sensitised to Bet v 1 (100% in Finland, 98% in Sweden and Austria, 90% in France, 65% in Switzerland and 62% in Italy), fewer people are sensitised to minor allergens but not to Bet v 1 (Biedermann et al. 2019).

# C

## Materials and methods

## C.1. Study area

The study area of the presented work consists of the region in and around the city of Augsburg (48°36'N, 10°89'E, 494 m above sea level), which is situated in Bavaria, in the south of Germany. The area lies north of the Alps at the confluence of the rivers Lech and Wertach on quaternary river terraces. (Figure 2, Figure 4)

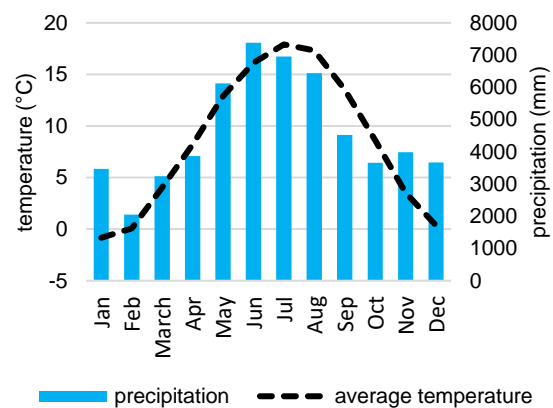
The climate is temperate oceanic (Köppen climate classification Cfb; Temperate, without dry season, warm summer) (Figure 3) with a yearly mean temperature of 13.2°C and a mean total precipitation of 766 mm (30 years averages, 1981-2017) (Deutscher Wetterdienst). It belongs to the biogeographical region “continental” (European Environment Agency). The highest precipitation is measured in July (99.7 mm) and the lowest in February (36.6 mm).

The potential natural vegetation in the region of Augsburg consists mainly of oak-hornbeam forests (Galio-Carpinetum) that are typical for the gravel areas of the foothills of the alps but the actual vegetation is, besides agricultural and urban areas, dominated by pine-oak forests due to cultivation and species-rich limestone lawns (Hiemeyer 1978; Suck and Bushart 2012).

Due to the Alpine river Lech, the flora of Augsburg shows a high diversity including plants from higher altitudes and non-native plants (Hiemeyer 1978).



**Figure 2:** location of Augsburg in Europe (map based on: OpenStreetMap 2020)



**Figure 3:** climate chart of Augsburg  
Data (1950 – 2018): Deutscher Wetterdienst

The city of Augsburg has 296,582 inhabitants (Statistisches Bundesamt 2020) on an area of 146.84 km<sup>2</sup>. The study area extends also to the surroundings of the city of Augsburg, district Augsburg and district Aichach-Friedberg. The population of the district Augsburg in the west of the city of Augsburg, is 251,534 on an area of 1,071.13 km<sup>2</sup>, district Aichach-Friedberg in the east of the city of Augsburg has an area of 780.33 km<sup>2</sup> and 133,596 inhabitants. (Bayerisches Landesamt für Statistik 2019)

That sums up to a total population of 681,712 on an area of 1,998.30 km<sup>2</sup> for the greater region of Augsburg.

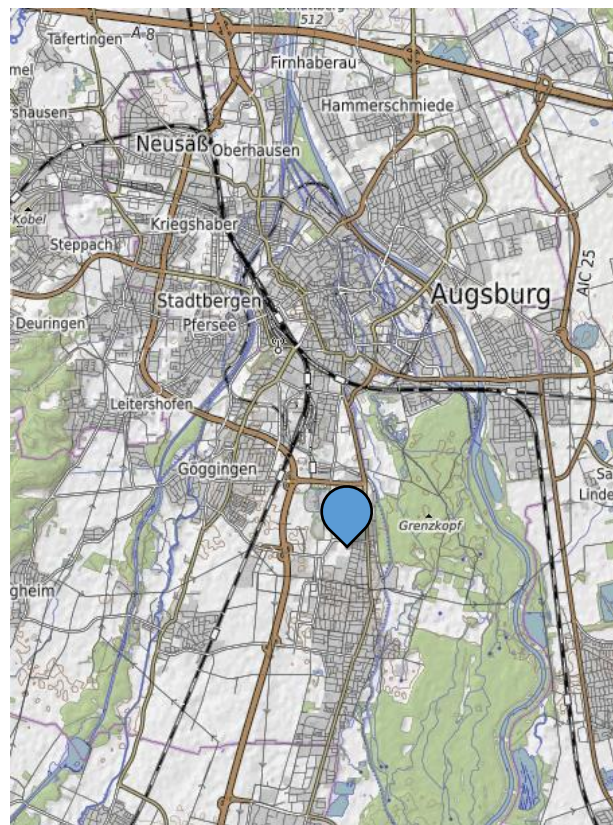
### C.1.1 Sampling sites

The sampling site for the concentration of airborne pollen is located in the southern part of the city of Augsburg at 48.326078 N, 10.903089 E, 496 m above sea level (**Figure 4**).

The sampler is located on ground level, so the sample is taken from a height of 1.6m what resembles the position of the human upper airway to reflect the exposition of a human to the collected particles.

The international standard, as defined by the European Aerobiology Society (EAS), recommends to measure pollen on a higher level like on rooftops in a height of approximately 12 m a.g.l.

(Jäger et al. 1995; Galán et al. 2014). This should guarantee a representative value for the whole region around the sampling point. In Augsburg, a super-site has been operating since 2015, therefore the sampling area actually consists of multiple sampling sites with



**Figure 4:** location of pollen sampling site in Augsburg (map based on: OpenStreetMap 2020)

three samplers to measure pollen in the ambient air. Two conventional, ‘gold standard’ Hirst-type volumetric traps (Burkard Manufacturing Co. Ltd <sup>1</sup>) (Hirst 1952) (**Figure 4**); one on ground level and one on rooftop level (approx. 12 m above ground-level) and a state-of-the-art, real-time, automated pollen sampler (Hund BAA 500<sup>2</sup>). According to recent findings (Kolek et al. in review\_a), pollen diversity between the different sampling heights does not significantly alter, but this stands true for the relative abundance too, as *Betula* spp. pollen seems to be highly abundant in both heights and of similar magnitude.

The here presented research is performed with the Hirst-type volumetric traps. Even though the above-mentioned automated sampler has been reported as highly reliable (Oteros et al. 2015b), we are aware that all automated samplers are still under ongoing development and it is not proven yet to have reliable results for all pollen types, as recently documented also by Schiele et al (2019). Compared, though, to the ‘gold-standard’ device, the automated pollen sampler is developed to improve the disadvantages of the manual pollen sampling, like the need for well-trained personnel to manually classify the sampled air particles and the time-intensive work on the taxonomic identification of pollen. But still there is room for much improvement and, hence, all airborne pollen measurements relied exclusively on the Hirst-type device.

To study the flowering phenology, but also for the pollen-, flower- and inflorescence-production and the estimation of allergenicity, individuals were selected in a radius of 25 km around the city of Augsburg.

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<sup>1</sup> Burkard Manufacturing Co. Ltd, Uxbridge, United Kingdom

<sup>2</sup> Helmut Hund GmbH, Wetzlar, Germany

### C.1.2 Studied species

The study was performed on different individuals of *Betula pendula* (**Figure 5A**).

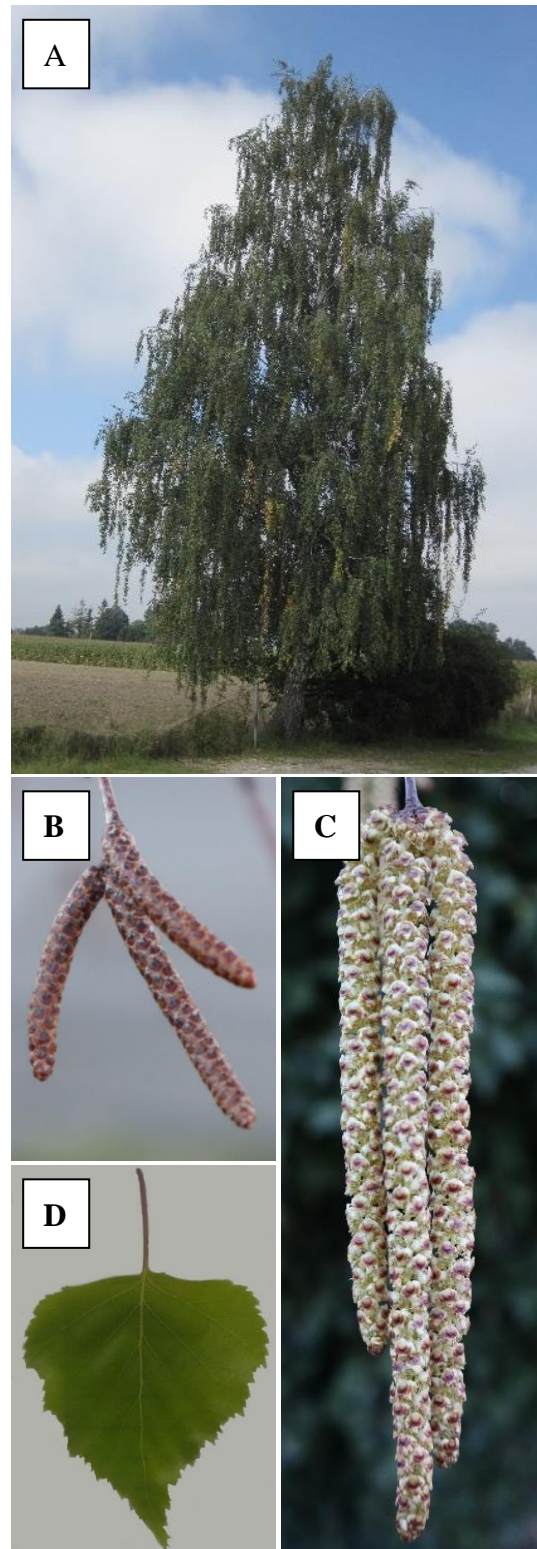
*Betula* is a genus of deciduous trees and shrubs that is wide spread on the northern hemisphere, a pioneer taxon with a high demand on light but a high tolerance to high and low temperatures, and different climatic and soil conditions. (Puc et al. 2015)

*Betula pendula* is an important pioneer species that colonises wetlands and brown lands. *B. pendula* prefers light and well-drained soils but is tolerant to different pH conditions (Beck et al. 2016). It is a middle-sized tree of 15 – 25 m that is characterised by pendulous twigs that are eponymous.

The roots of *Betula pendula* are reported to reach depths of up to 120 cm with most roots in the first 50 cm of depth and a horizontal reach of up to 6 m (Köstler et al. 1968; Mauer and Palátová 2012)

The canopy of *Betula pendula* is open and the leaves are triangular with double serrated margins (**Figure 5D**). The bark is white and becomes rugged in older individuals.

*Betula pendula* are anemophilous trees with flowers in male inflorescences arranged in catkins (**Figure 5B, C**). The seeds are also arranged in catkins, winged and small to be



**Figure 5:** *Betula pendula*

A) tree

B) male inflorescence in winter

C) male inflorescences after flowering

D) leaf

(photos by Franziska Kolek)

distributed by the wind. The male catkins are developed in the summer to flower in the next spring. The female catkins are built in spring for the pollination in spring and they release the seeds in summer. *Betula* are monoecious, so both, male and female catkins, can be found on one individual.

### **C.1.3 Selection of sampling individuals**

For the phenological observations, the analysis of allergenicity and pollen- and flower production, it was scheduled that 60 individual trees would be sampled and observed. For phenological observations and allergenicity, this was performed from 2013 until 2018, pollen- and flower-production was analysed from 2015 to 2018. For data consistency, the here presented work focusses on three continuous years, 2015 to 2017.

Over the years, some of the trees were logged, lower branches were cut or access to the tree was lost, so the study design had to be adjusted by adding new trees. This leads to a total sum of 112 trees from 2013 until 2018 and for the here presented work, 78 trees were observed and sampled in total. Of these, in this dissertation, results just from all 43 trees that could be observed and sampled in all three years are analysed, to ensure comparability among years and higher robustness of the results obtained.

The selection criteria of the trees were as follows:

- The tree had to be healthy, with no visible sign of any disease, and sexually mature.
- The owner of the tree (the urban green space planning office in case of public property) had to give approval for the observations and measurements.
- The tree had to be accessible for the observations every day from mid of March to end of May.
- The lower branches of the tree had to be in a height that can be observed by eye without any tools; the lowest branches should not be higher than 2m above the ground.
- The tree should have produced a considerable number of male inflorescences by March to ensure the observations and the sampling. As for measuring the

allergenicity, a sample of 100 male inflorescences was collected, so trees that produced significantly fewer male inflorescences were not included in the study.

- Influences by fertilisation, human-induced watering, pruning, and use of phytoprotective substances should be excepted.

The exact location of each tree was assessed with a Garmin Dakota 10 GPS device<sup>3</sup> in the World Geodetic System WGS 84.

The morphometric traits of the trees were also assessed, namely tree height, crown height and diameter, and trunk perimeter, for every observed tree. The tree height was measured from the ground to the highest point of the crown, using an optical hand clinometer by Breithaupt<sup>4</sup>. The crown height was measured with the same technique from the lowest branch of the tree to the highest point of the crown. The trunk perimeter was measured in a height of 1.00 m – 1.20 m at a height of the trunk without branches or excrescences with a measuring tape. For the crown diameter, the distance from the trunk to the most distant point of the crown was measured in three directions with a measuring tape. The mean of these three values was calculated. The final selection and characteristics of trees can be seen in Appendix G.5.

## **C.1.4 Environmental factors**

### **C.1.4.1 Urbanity index**

To estimate the urbanity of each tree, an index of the urbanity of the surroundings of the tree is calculated. The so called urbanity index (UI) used in this study was assessed for every observed tree (Appendix G.5) according to Jochner et al. 2012. The index is based on the CORINE 2006 land cover data (European Environment Agency 2013). The urbanity index describes the part of urban areas in an area of 2 km around each tree. When the index is 0, this refers to a rural environment with no urban areas, an index of 1 describes an urban environment without any non-urban areas. The radius of 2 km was

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






<sup>3</sup> Garmin Deutschland GmbH, Garching Germany

<sup>4</sup> F. W. Breithaupt & Sohn, Kassel, Germany

chosen to “ensure that mesoclimatic effects are covered by the index and extensive/thinly urbanised areas are identified as such” (Jochner et al. 2012).

The index was assessed using QGIS Version 2.4.0<sup>5</sup>. For the calculation, a radius of 2 km was displayed around each tree in the GIS program, overlaying the CORINE land cover data (European Environment Agency 2013). The proportions of different land cover types within this radius are extracted from the CORINE data and labelled in two categories to distinguished “urban” and “rural” land cover. The urban and rural land cover categories were classified as in Jochner et al. (2012) with considering areas with continuous and discontinuous urban fabric, industrial or commercial units, road and rail networks and associated land, airports, dump sites and construction sites as urban (**Table 2**).

**Table 2:** CORINE Land Cover categories for the region of Augsburg urban land uses marked in bold.

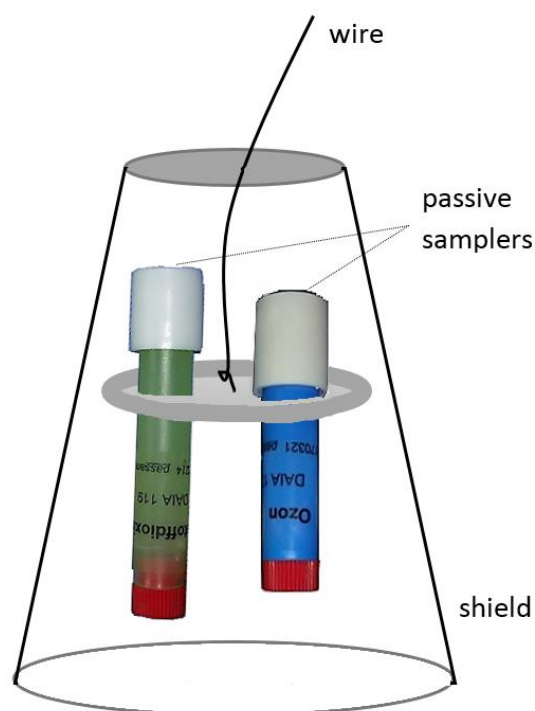
	<b>Continuous urban fabric</b>		Land principally occupied by agricultures, with significant areas of natural vegetation
	<b>Discontinuous urban fabric</b>		Broad-leaved forest
	<b>Industrial or commercial units</b>		Coniferous forest
	<b>Road and rail networks and associated land</b>		Mixed forest
	<b>Airports</b>		Natural grasslands
	<b>Dump sites</b>		Moors and heathland
	<b>Construction sites</b>		Transitional woodland-shrub
	Green urban areas		Bare rocks
	Sport and leisure facilities		Sparsely vegetated areas
	Non-irrigated arable land		Water courses
	Pastures		Water bodies
	Complex cultivation patterns		no data

<sup>5</sup> QGIS Development Team (2018). QGIS Geographic Information System. Open Source Geospatial Foundation Project. <http://qgis.osgeo.org>

#### C.1.4.2 Air quality measurements

Air quality measurements were performed in all three study years at all observed trees (Appendix G.5) for four weeks in end of March to end of April, in parallel to the phenological observations and the sampling of pollen. The measurements were done with passive samplers for two periods of two weeks each; the analysis of the passive samplers was performed by passam ag, Zurich, Switzerland.

The samplers were placed under a plastic shield to be protected against precipitation. The shield is open on the bottom to ensure an undisturbed air exchange. The passive samplers are placed under the plastic shield with the opening on the bottom. (Figure 6).



**Figure 6:** scheme of setup of one set of passive samplers under the rain-shield (outline by Franziska Kolek)

In some cases, small insects settle in the samplers and block or reduce the air flow. That can strongly affect the exposure of the sampler to the measured substance, hence, all measurements are done with two samplers per pollutant for every period to ensure reliable values. This construction is fixed on each of the studied trees. After fixing it thoroughly with a wire and if necessary, with additional rope and adhesive tape to ensure that it is in a stable position for the exposure time, the passive samplers were opened so the reagent is exposed to the air. After two weeks of exposure, the samplers are collected from the trees, sealed, and sent to the provider<sup>6</sup> for analysis. A second set of samplers is placed on the tree for a second 2-week-period of observation with the same setup as the first period.

#### C.1.4.3 Meteorological parameters

As mentioned before, meteorological parameters can influence pollen flight (Galán et al. 1991; Emberlin et al. 2002; Latałowa et al. 2002), phenology (Andersen 1980; Menzel

<sup>6</sup> passam ag, Zurich, Switzerland

and Fabian 1999; Wielgolaski 1999; Lee et al. 2020; Damialis et al. 2020), and the production of flowering attributes (Wan et al. 2002; Damialis et al. 2011). Therefore, hourly data for temperature and precipitation was assessed via the German Weather Service (Deutscher Wetterdienst (DWD))<sup>7</sup>. The weather station (DWD ID: 232) is situated at Augsburg Airport (48.4254; 10.9420) in the north of the city of Augsburg, on an elevation of 461 m above sea level.

In contrast to the actual pollen flight itself, when current wind vectors and presence and amount of precipitation are the decisive factors (e.g. Damialis et al. 2005), for the assessment of pollen production and allergenicity, as well as for phenological observations, cumulative values of both temperature and precipitation have to be considered.

For Aerobiological and phenological observations as well as allergenicity, cumulative temperature from January 1<sup>st</sup> is calculated as these parameters are influenced by the environmental conditions just before flowering (Emberlin et al. 1997). As the development is limited by minimum temperatures (Worrall 1999), the cumulative temperatures are also calculated by the daily minimum temperatures. For the production of flowers, inflorescences and pollen, cumulative temperatures from the previous summer (June – August) are taken into account as *Betula* inflorescences are built in this time. Also here, the values are calculated, based on the daily minimum temperature.

#### **C.1.4.4 Pedological and geological parameters**

An important factor to characterise the environment of a tree is to identify characteristics in the soil and the underground of the soil that are responsible for providing the tree with water and nutrients.

For this reason, pedological and geological aspects for all observed sites (Appendix G.5) were assessed from the BayernAtlas, a service for geographical maps in Bavaria, provided by the Bayerische Vermessungsverwaltung.

For pedology, the soil type (Food and Agriculture Organization of the United Nations 2014) was extracted from the “Übersichtsbodenkarte” (overview soil map) (Bayerische

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<sup>7</sup> Deutscher Wetterdienst, Offenbach, Germany 2019

Vermessungsverwaltung 2019) and characterized afterwards by main grain size and potential nutrient availability. The main grain size is an indicator for potential water availability as it is known that a higher amount of medium to coarse material in the soil reduced the soil water availability for plants (Kramer and Boyer 1983).

The information about the geology is extracted from the from the geological map (Bayerische Vermessungsverwaltung 2019) and categorised afterwards by the main grain size as an indicator of potential water availability.

The variables were pooled in different categories. For the soil, grain sizes are categorised in fine (silt and clay; grain size  $< 0.036$  mm), medium (sand; grain size  $0.036$  mm –  $2$  mm) and coarse (gravel, cobble; grain size  $> 2$  mm) (ISO 14688). Mixed grain sizes with similar proportions of different grain sizes are categorised mixed. Areas in the city with no pedological data are categorised unknown. The potential water and nutrient availability is categorised in high, middle, low, following the description in the pedological map (Bayerische Vermessungsverwaltung 2019). Soil types with inconsistent features are marked as diverse.

For the parent material, the grain sizes are categorised as for the soil in fine (silt and clay; grain size  $< 0.036$  mm), medium (sand; grain size  $0.036$  mm –  $2$  mm) and coarse (gravel, cobble; grain size  $> 2$  mm) (ISO 14688).

#### **C.1.4.5 Surroundings**

Additionally to the above-mentioned environmental factors, the surrounding environment of the tree also plays an important role. Not just the soil and geology, but also the surface of the soil, the vegetation close to the tree and the shading caused by natural or artificial elements. The surface of the soil can influence the water availability, for example by being sealed, compressed, or covered with different kinds of vegetation. The vegetation close to the tree can influence the availability of water and nutrients, whereas shading can result in a lower energy input and lower temperatures for the tree. The above refer to competition for more sunlight, more water, and more nutrients.

To investigate the surroundings of the trees, information on the surroundings in a radius of 5 meters was manually acquired. This radius was chosen due to the fact that the roots of *Betula pendula* are reported to reach a diameter of up to 6 meters (Köstler et al. 1968).

This area was characterised with regards to the sealing of the surface, the type of the ground cover and the appearance and type of different plants. Therefore, the following parameters were investigated (**Table 3**):

**Table 3:** parameters to characterise the surroundings of the trees

<b>Parameter</b>	<b>categorisation</b>
open water bodies (e.g. lake, river)	no / yes
non-biological sealing	no / gravel / concrete / wall / mixed
street	no / yes
sealed areas	no / low (<50%) / high (>50%)
diversity of plants in the herbaceous layer	no / low diversity (agricultural fields) / medium div. (meadow, garden) / high div. (multiple environments)
diversity of trees and shrubs	no / <i>Betula</i> / mixed incl. <i>Betula</i> / mixed, other / shrubs
shading of the tree	no / low / medium / high
positions of buildings	cardinal directions from the tree

## C.2. Aerobiological monitoring in Augsburg

### C.2.1 Airborne pollen monitoring with Hirst-type pollen sampler

Airborne pollen measurements were performed using a 7-day recording Hirst-type volumetric sampler (Hirst 1952) (**Figure 8**) by Burkard Manufacturing Co. Ltd<sup>8</sup>, according to the guidelines from the British Aerobiology Federation (Caulton and Lacey 1995). A sampler was situated on ground level so the sample was taken in a height of 1.5 m a.g.l.. A second sampler was situated additionally to a height of approximately 13 m a.g.l. with a horizontal distance of 50 m, as reported by Kolek et al. (2021), whose results are closely in agreement with the one at ground-level in terms of biodiversity, and therefore not studied in the frame of this dissertation.

The Hirst-type sampler generates a constant and stable airflow with a volume of 10 litres per minute. This corresponds to the average air volume, a human inhales in the same time.

This airflow is conducted through a thin orifice (2mm x 14 mm) behind which, a clockwork-driven mechanism is installed to rotate a cylindrical drum for 2 mm per hour. Within one week, the drum performs one



**Figure 8:** Hirst-type pollen sampler by Burkard Manufacturing Co. Ltd (photo by Franziska Kolek)



**Figure 7:** drum of a Hirst-type pollen sampler with Melinex<sup>®</sup> tape (photo by Franziska Kolek)

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<sup>8</sup> Burkard Manufacturing Co. Ltd, Uxbridge, United Kingdom

revolution so the whole length of the tape was exposed to the air current. A transparent polyester tape (Melinex ®<sup>9</sup>) is placed and fixed on the drum (**Figure 7**) and coated with a thin layer of petroleum jelly to trap particles from the air that impinge on the tape. After seven days of sampling, the tape is replaced.

After seven days of sampling, the tape is prepared in the laboratory to prevent contamination. It is carefully removed from the drum without touching the sampled surface. Afterwards, it is cut into seven equal parts with a length of 48 mm each, corresponding to one daily sample. Each part of the tape is prepared on a microscope slide and covered by a cover slip, using a heated (~ 40°C) solution of glycerol, distilled water, gelatine (Glycerol<sup>10</sup> : distilled water : gelatine<sup>11</sup> = 7 : 6 : 1) and small amount of safranin<sup>12</sup> that sets when cooled down to room temperature. With the solution, each one of the seven parts of the weekly tape is fixed and stained at the same time and the sample is protected and conserved. After the sample is prepared, it is sealed with a varnish (Roti®-Seal<sup>13</sup>) to conserve it from humidity and damage.

The pollen monitoring was performed in the following periods:

26/03/2015 – 26/10/2015  
21/03/2016 – 16/11/2016  
29/03/2017 – 24/10/2017.

For the sampling methods, as well as the optical microscopy taxonomic classification techniques below, the minimum requirements for pollen monitoring, according to the European Aerobiological Society (EAS) (Galán et al. 2014) were considered.

## **C.2.2 Taxonomic identification and counting of pollen**

The air samples were analysed with a Leica DM750 light microscope<sup>14</sup>. With a 400x magnification, pollen of 45 different taxa (**Table 7**) were identified and counted. For the

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<sup>9</sup> Burkard Manufacturing Co. Ltd, Uxbridge, United Kingdom

<sup>10</sup> Merck KGaA, Darmstadt, Germany

<sup>11</sup> Merck KGaA, Darmstadt, Germany

<sup>12</sup> Merck KGaA, Darmstadt, Germany

<sup>13</sup> Carl Roth GmbH + Co. KG, Karlsruhe, Germany

<sup>14</sup> Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany

identification, different morphological features of the pollen (see B.1.2.2) were considered. The identification and counting were performed on a bihourly basis. For every bihourly sample that corresponds to 4 mm of the length of the tape, particles on a traverse that corresponds to an area of 8.4 mm<sup>2</sup> were identified and counted. The examined area referred to 14.9% of the total area sampled, so it fulfils the requirements for obtaining a statistically representative area for each sampling interval (Galán et al. 2014).

### C.2.2.1 Atmospheric load of pollen

All pollen grains are counted in a representative per two hours transverse traverse. To obtain homogenous and comparable numbers of pollen, pollen counts are converted into concentrations and expressed as pollen grains per m<sup>3</sup> of air. To assess these, the following calculations are applied:

Knowing that the sampler has an intake of 10 litres per minute, the air volume the bi-hourly sample refers to equals to:

$$0.001 \frac{m^3}{minute} 60 \text{ min} \times 2 \text{ hours} = 1.2 \text{ m}^3$$

The examined area is:

$$\textit{field diameter} \times \textit{length of line} \times \textit{number of lines}$$

For this work, the field diameter (Leica DM750 light microscope with Hi PLAN 40x/0.65 objective <sup>15</sup>) is 0.60. The length of one counted line is 14 mm and one line is counted. That gives an examined area of 8.4 mm<sup>2</sup>.

The part of the examined area equals to:

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<sup>15</sup> Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany

$$\frac{1}{\frac{\text{total sampled area}}{\text{examined area}}}$$

The total area for a 2-hourly interval is 56 mm<sup>2</sup>. The examined area (see above) is 8.4 mm<sup>2</sup>. So, the proportion of examined area of the total sample area is 6.7%.

Conversion factor to refer to 1 m<sup>3</sup> of air:

$$\frac{\text{Portion of examined area}}{\text{air volume}}$$

The conversion factor is 5.58.

Pollen grains per m<sup>3</sup> of air:

$$\frac{\text{conversion factor}}{\text{number of counted pollen}}$$

So, one pollen per 2-hourly interval refers to 5.58 pollen per m<sup>3</sup> of air, given the microscope and objectives that have been used and the counting techniques that has been followed, according also to the international specifications as per Galán et al. 2014.

### **C.2.2.2 Seasonal pollen circulation**

To define the exact timing of airborne pollen per year, one has to decide how a ‘pollen season’ is defined. Pollen timing has a distinct seasonality and there are various approaches to define this.

An approach that focuses more on the definition of a season in terms of continuous appearance of pollen and clinical relevance to associated symptoms was developed by Pfarr et al. (2017). They, therefore, defined as onset of the main pollen season when five consecutive days of pollen were observed and the pollen sum in the same period was above a certain threshold, which varies per taxon. Also a definition that considers too allergic symptoms for each season was developed by (Bastl et al. 2015). The above definitions, while they may be more relevant for the manifestation of pollen-related

symptoms, have certain limitations, the main of which is the availability of reliable day-to-day registries of allergic symptoms, and that of the lack of homogenised and generalised pollen thresholds for symptoms. Karatzas et al. (Karatzas et al. 2018a; Karatzas et al. 2018b), have recently proven that the pre-defined thresholds and the consequent pollen season definition of Pfaar et al. (2017) are not valid for another study area, for the same pollen taxa and even though belonging to the same bioclimatic region.

For this reason, as many approaches to define the pollen season are either not proven for the whole world but just specific regions (Nilsson and Persson 1981; Sánchez Mesa et al. 2003) or still under discussion (Jato et al. 2006; Hoffmann et al. 2020; Karatzas et al. 2018a), the main pollen season in this dissertation was defined according to Nilsson and Persson (1981) , as the 5% (onset) and 95% (end) of the cumulative sum of the annual pollen season.

### **C.2.2.3 Daily pollen circulation**

It is known that flowers open and close at specific times of the day (Linnaeus 1751). Therefore, diurnal patterns of pollen distribution are expected as they are already published in the past (*Betula*: Mahura et al. (2009); Poaceae: Reddi et al. (1988), Norris-Hill (1999), Peel et al. (2014); various pollen: Lipiec et al. (2019) Grewling et al. (2016) Kolek et al. (in review\_a)).

To display daily pollen distribution patterns, the pollen data is analysed on a bi-hourly scale. For a better understanding of the patterns, not just the sums of pollen in the respective interval is calculated but also the frequency of cases during which there were pollen grains. Comparing the sums and the frequency, it is possible to differentiate between consistent patterns and patterns that are caused by single or rare events of high pollen. (Kolek et al. in review\_a)

To calculate the pollen sum, all pollen of one taxon observed in a certain time interval over all days of all observed seasons were summed up. For the frequency of pollen counts, cases with pollen for one taxon higher than 0 for a certain time interval was determined.




### C.3. Phenological flowering observations of *Betula pendula*



The BBCH (Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundessortenamt und Chemische Industrie) developed a scale to code similar growth stages from different plants (Meier 1997). This scale was confirmed by the COST Action 725 on a European level (Koch 2009). The principal growth stages were extended later (see Appendix G.3), (Hack et al. 1992)). The relevant stages for the development of the flowers in the male inflorescences, and therefore relevant for the described observations, are the principal growth stages 5 and 6 (**Table 4**). These stages were observed during 2013-2018, for every tree (Appendix G.5) in every year for 6 weeks from mid-March to the end of April, every second day, from stages 51 to 55 and stages 65 to 69. For the stages 59 to 64, the observations were performed daily. This sums up to a total of 112 observed trees and approximately 9,000 observations. For this dissertation and so as to have comparable results across all studied parameters (airborne pollen, flowering phenology, pollen production and allergenicity), observations from individuals that could be observed in all three study years 2015-2017 are included here, which accounts for a total of 43 trees, and a total of approximately 3,900 daily observations.

As not all male inflorescences are synchronised in the same stage of floral development during each observation, the inflorescences were observed randomly on a defined area of the crown. To have stable, comparable results for all observations, a frame of 50cm x 50cm was used for observing the male inflorescences in this area. The frame was positioned on random places on the crown in every observation to guarantee a representable result.

For performing an observation, the frame was placed at a random area of the crown of the tree. Then, all male inflorescences within this frame in a depth of 50 cm were counted per development state (winter stage, pre-flowering, start of flowering, flowering, end of flowering, male inflorescences falling, see Appendix G.3). The numbers of inflorescences per development stage were noted afterwards.

**Table 4:** General growth stages 5 and 6 according to the BBCH-scale with respective pictures of *Betula pendula* inflorescences (photos by Franziska Kolek)

<b>Principal growth stage 5: Inflorescence emergence (main shoot) / heading</b>		
51	Inflorescence or flower buds visible	
55	First individual flowers visible (still closed)	
59	First flower petals visible (in petalled forms)	

<b>Principal growth stage 6: Flowering (main shoot)</b>		
60	First flowers open (sporadically)	
61	Beginning of flowering: 10% of flowers open	
62	20% of flowers open	
63	30% of flowers open	
64	40% of flowers open	
65	Full flowering: 50% of flowers open, first petals may be fallen	
67	Flowering finishing: majority of petals fallen or dry	
69	End of flowering: fruit set visible	

## C.4. Production of flowers inflorescences and pollen of *Betula pendula*

### C.4.1 Field work

The sampling individuals for the production of flowers, inflorescences and pollen are the same as the ones that were used for the phenological observations and the estimation of allergenicity. Inflorescences, for this dissertation, were considered from the same 43 trees (Section C.3), whose flowering phenology was observed, per year and for the two study years, 2016, 2017<sup>16</sup>. From each individual tree, five to six male inflorescences from random areas of the crown were collected, accounting for a total of approximately 450 inflorescences for the here presented work. The inflorescences were cut from the branches and transported and stored in paper envelopes. The collection was done in the first weeks of March in the phenological stage 51 (see **Table 4**, Appendix G.3) to ensure that the flowers are still completely closed, and no pollen will be lost while transporting and storage of the samples. To avoid decomposition, the inflorescences were stored at -20°C until the analysis was performed.

To estimate pollen production also on higher levels, like per individual tree, the size of the crown (see Section C.1.3) was estimated and the male inflorescences per crown surface unit were counted. To do this, in an area of 50 cm x 50 cm on a random area of the crown, the number of inflorescences was counted. This was done each year over a period of six weeks from mid-March to end of April, at least every second day together with the phenological observations (see Section C.3) to ensure a representative count for the individual.

As the inflorescences of *Betula pendula* are mainly situated on the end of the branches, the inflorescences are considered to be not throughout the total volume of the crown but roughly on its surface. To calculate the crown surface, the geometrical shape of the crown was considered spheroidal.

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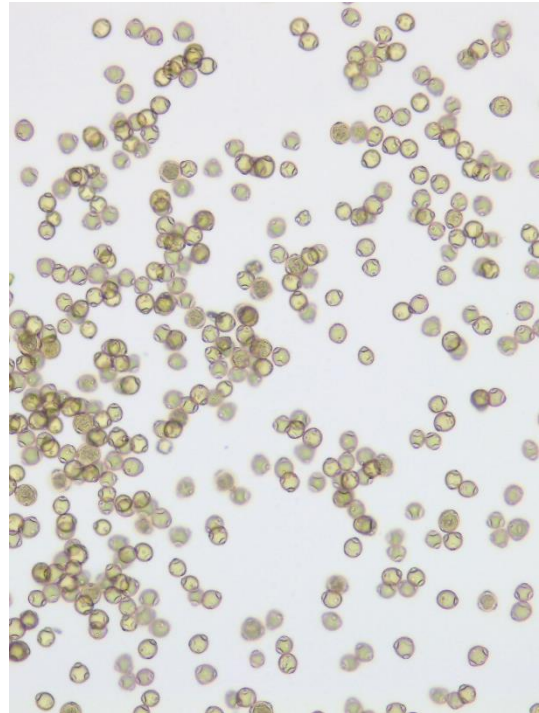
<sup>16</sup> excluding dropouts because of logging or pruning (from an initial total of 93 trees)

## C.4.2 Laboratory analysis

For the laboratory analysis, each male inflorescence was treated separately. From each individual tree, two inflorescences were processed per year, so a total of 172 samples were analysed.

For the laboratory analysis, the protocol of Damialis et al (2011) was used as described below. Each inflorescence was measured in length and maximum width with a ruler and the number of flowers was counted manually.

A 10% potassium hydroxide (KOH)<sup>17</sup> solution was prepared. Each inflorescence was boiled in the solution for at least 8 minutes to break up the plant tissue and extract the pollen grains from the tissue (Faegri et al. 1989; Moore et al. 1991; Damialis et al. 2011). In the end of the boiling, the inflorescence was crushed with a glass rod to ensure that all pollen grains are released. After boiling, the sample was mixed with a glycerol solution to prevent the pollen from clumping due to the pollenkit and ensure an evenly dispersion of the pollen for reliable further analysis.



**Figure 9:** *Betula* pollen under a light microscope, 100x magnification (microscope photo by Franziska Kolek)

As in Damialis et al. (2011) the pollen production was not studied in *Betula*, but for *Corylus avellana*, another species of the Betulaceae family, the concentration of the glycerol solution had to be re-confirmed for the here presented work with samples of *Betula* so as to ensure the lack of pollen clumps. Because of that, pre-experiments with different concentrations of glycerol<sup>18</sup> were performed before the analysis of the actual samples. The best results were seen for a solution of 70% glycerol, so this concentration was used to process the actual samples.

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<sup>17</sup> Merck KGaA, Darmstadt, Germany

<sup>18</sup> Merck KGaA, Darmstadt, Germany

This solution was added to the samples to a final volume of 10 ml. While stirring the solution vigorously to ensure an evenly dispersion, two replicate samples of 10  $\mu$ l were taken with a micropipette<sup>19</sup> and prepared on a microscope slide. The slide was then covered with a cover slip to preserve the sample.

All pollen grains of the sample were counted under a Leica DM750 light microscope<sup>20</sup> at a 100x magnification (**Figure 9**). As the analysis was performed with two inflorescences per tree, per year, and of every inflorescence, two samples were taken, which accounts for four values per individual per year. The average from these four values was calculated to estimate the amount of pollen per inflorescence on each tree per year.

### C.4.3 Estimation of pollen production

The pollen production  $P$  was estimated based on the counted pollen grains by the following equations (Damialis et al. 2011):

Pollen production per inflorescence:

$$P_{fu} = \frac{V_{su}}{V_{sa}} \bar{P}$$

Pollen production per flower:

$$P_{fl} = \frac{P_{fu}}{f}$$

Pollen production per crown surface unit:

$$P_{cr} = P_{fu} \frac{F_{su}}{M}$$

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<sup>19</sup> Eppendorf Vertrieb Deutschland GmbH, Wesseling-Berzdorf, Germany

<sup>20</sup> Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany

Pollen production per individual:

$$P_{in} = P_{cr}S$$

Where S is the lateral surface for ovoids (Damialis et al. 2011; Zwillinger 2018):

$$S = \frac{\pi d_m^2}{2} + \frac{2\pi d_m h_c}{\frac{\sqrt{h_c^2 - d_m^2}}{h_c} \sin \frac{\sqrt{h_c^2 - d_m^2}}{h_c}}$$

$d_m$	Maximal diameter
f	Number of flowers per male inflorescence
$F_{su}$	Average number of floral units per crown sampling unit (quadrat)
$h_c$	Crown height
M	Area of sampling unit
P	Pollen production
p	Average of two replicates of the numbers of pollen grains
$P_{cr}$	Number of pollen grains per surface unit ( $m^2$ ) of the crown
$P_{fl}$	Number of pollen grains per flower
$P_{fu}$	Number of pollen grains per floral unit
$P_{in}$	Number of pollen grains per individual
S	Total lateral surface area (in $m^2$ ) of the crown
$V_{sa}$	Volume of sample taken (in $\mu l$ )
$V_{su}$	Volume of suspension (in ml)

**Table 5:** variables used in the equations to calculate pollen production

## C.5. Allergenicity of *Betula pendula* pollen

### C.5.1 Field work

For each tree in each year, a sample of 100 male inflorescences was collected per tree. The samples were collected in the beginning of the flowering (BBCH stage 60, see **Table 4**, Appendix G.3) to extract pollen that are fully developed and genuinely represent the allergenic properties of the pollen that is released in the air. As the pollen release in *Betula* most frequently takes no longer than two to three days (Piotrowska 2008), precise daily observations of the phenological stage are necessary to avoid a loss of pollen due to late sampling. To avoid contamination, gloves were used to harvest the male inflorescences. After transporting them in a paper envelope to the laboratory, they were spread on a paper tray under the laboratory hood to dry (**Figure 10**).



**Figure 10:** drying of pollen in the laboratory with visible release of pollen (photo by Franziska Kolek)

After drying the inflorescences for two days, the pollen sacs are burst and the pollen are released from the catkins. In this way, it is possible to separate the pollen from the other plant tissue by mechanical dry sieving. Then, they were sieved using a sieve with a mesh size of 70 $\mu\text{m}$  to separate them from the inflorescences and 100 $\mu\text{m}$ <sup>21</sup> for separating them from other remaining plant particles.

After sieving, the remaining pollen is filled in glasses and stored at -80°C for further analysis.

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<sup>21</sup> Retsch GmbH, Haan, Germany

## C.5.2 Laboratory analysis

To assess the allergenicity of the pollen, the major allergen of *Betula*, Bet v 1 was measured, using a specific Enzyme-linked Immunosorbent Assays (ELISAs). The analysis was initially done for each of the observed trees from 2013 until 2018, but in this dissertation only the results for the 43 trees that could be observed continuously from 2015 until 2017 are presented (see also Section C.3).

To be able to perform the ELISAs, the pollen had to be extracted from the male inflorescences as an aqueous pollen extract (APE).

### C.5.2.1 Aqueous pollen extracts (APEs)

For each tree for each year, duplicates were taken, so APEs were performed for a total of 368 samples.

The preparation of the APEs was performed according to Gilles et al. (2009). An amount of 10 mg pollen was suspended in 1 ml Dulbecco's Phosphate Buffered Saline (DPBS)<sup>22</sup> for 30 minutes at 37°C with vortexing every 10 minutes. The suspension afterwards was centrifuged for 10 minutes with 3000x g. The supernatant was then passed through a sterile filter<sup>23</sup> with a pore size of 0.2 µm.

### C.5.2.2 Enzyme-linked Immunosorbent Assay (ELISA)

The ELISAs were performed like previously done by Buters et al. (2008) and Beck (2014). Per tree and year, two samples were analysed in three dilutions, 1:1000, 1:3000 and 1:10000. This sums up to 1080 analysed samples. A sandwich ELISA to measure Bet v 1 content in aqueous pollen extracts using two Bet v 1-specific antibodies (Bet v 1 4B10D10F8 capture antibody and Bet v 1 2E10G8G7:B detection antibody) was provided by Allergopharma<sup>24</sup>.

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<sup>22</sup> gibco, Thermo Fisher Scientific, Waltham, USA

<sup>23</sup> Millipore, Merck KGaA, Darmstadt, Germany

<sup>24</sup> Allergopharma GmbH & Co. KG, Hamburg, Germany

Microtiter plates were coated with 100 µl coating antibody (10 µg/mL in coating buffer) per well and incubated overnight at 4°C. After three steps of washing with a washing buffer (PBS containing 0.05 % Tween20) on a microplate washer Capp Wash 8<sup>25</sup>, the plates were blocked with DPBS<sup>26</sup> containing 1% BSA (Bovine serum albumin) for 30 minutes. The blocking solution was discarded without washing. Then, 100 µl of the samples in the different dilutions (1:1000, 1:3000, 1:10000 in DPBS<sup>27</sup> with 0.1 % BSA) and standards (0.39 ng/mL – 200 ng/mL) were added and incubated at room temperature for 60 minutes on a shaker. Afterwards, the plates were washed three times and 100 µl of biotinylated antibody (1:200) were added per well and incubated for 60 minutes. Again the plate was washed three times. After incubating with 100 µl Streptavidin-Peroxidase (1:10000) for 30 minutes in the dark, the plates were washed three times and 100 µl TMB (Tetramethylbenzidin) substrate was added for 10 minutes. Afterwards, the reaction was stopped with 2N H<sub>2</sub>SO<sub>4</sub>. (Buters et al. 2008; Beck et al. 2013; Beck 2014)

The optical density was measured at 450 nm on a Tecan Sunrise microplate reader<sup>28</sup> using the Magellan<sup>TM</sup> data analysis software<sup>29</sup>. The concentration is expressed in µg Bet v 1 per ml of solution.

To compare the results also with the analysis for the pollen-, flower- and inflorescence-production, Bet v 1 was also calculated per inflorescence. The amount of pollen grains per inflorescence was known in number of pollen but also in weight. As one ml of solution corresponded to 10 mg pollen grains, the amount of Bet v 1 was multiplied by 100 and afterwards multiplied by the weight of pollen per inflorescence, as expressed in the following equation:

$$\frac{\text{Bet v 1}}{\text{inflorescence}} = \text{Bet v 1} \times 100 \times \frac{\text{g pollen}}{\text{inflorescence}}$$

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<sup>25</sup> Biotek, Bad Friedrichshall, Germany

<sup>26</sup> gibco, Thermo Fisher Scientific, Waltham, USA

<sup>27</sup> gibco, Thermo Fisher Scientific, Waltham, USA

<sup>28</sup> Tecan Group Ltd. Männedorf, Switzerland

<sup>29</sup> Tecan Group Ltd. Männedorf, Switzerland

## C.6. Data analysis

A distinct characteristic of the present dissertation is the collection and elaboration of two types of datasets: the first, temporal in nature, based on the occurrence of phenological attributes, namely flowering and pollen seasons, the second, quantitative in nature, based on the abundance of attributes, namely pollen and allergen quantities. Having said the above, the data analysis has been performed in two different ways, based on this technicality. The third data elaboration type is based on the integration of all additional environmental co-factors, like meteorological and air pollution parameters, as analysed in the Section C.6.3.

### C.6.1 Phenological attributes of flowering and pollen seasons

The phenological characteristics of flowering were examined, specifically those connected with pollen emission, viz. the start, peak, end and duration of flowering. Differences among individuals and sampling sites in the phenological traits were checked using ANOVA and post-hoc Bonferroni test, as well as explorative techniques, like hierarchical cluster analysis (Ward's clustering). When testing for additional, continuous co-factors, like meteorological parameters or air pollutants, full-factorial analysis of variance and covariance were applied (ANOVA, ANCOVA). The relationships between phenological characteristics of flowering and growth traits of individuals were also investigated, using the full set of data and performing simple and full factorial regressions. All analyses were run at the significance level of  $p=0.05$  and also the coefficient of determination  $R^2$  was estimated.

The flowering dates per individual *Betula pendula* tree and per year were checked against the pollen season dates of the respective pollen season of *Betula* spp. over the same year of study. All aerobiological data were checked against flowering phenological data for lag effects (GLM, time series analysis, cross-correlations). The correlation coefficients and the specific lags at the significance level  $p < 0.05$  were defined per year.

## **C.6.2 Quantitative attributes of pollen production and allergenicity**

Pollen production data had to be analysed at different scales: the finer one, per inflorescence) and per individual. Differences were checked among individual trees and between years [factorial ANOVA, Post-hoc (Bonferroni test)]. Analysis of covariance (ANCOVA) was also employed to assess potential differences of pollen grains per floral unit, of flowers per inflorescence and of floral units per m<sup>2</sup> of crown among individuals and years, based also on the continuous variables of morphological traits, per inflorescence or per individual. Special emphasis was given to any interaction effects between categorical predictors and covariates.

Likewise, for pollen allergenicity, analyses were as described above, for the finer scale of per-inflorescence.

## **C.6.3 Quantification and forecasting of studied attributes**

Based on statistically significant differences deriving from the above-mentioned tests, and particularly focusing on the interaction effects of multiple independent variables, ultimately ridge regressions were conducted per dependent variable and for all studied parameters, namely flowering and pollen season attributes, pollen and allergenicity production. Ridge regressions are well-known for dealing with multi-collinearity issues, and partial correlations aid in identifying the most significant parameters and their lag effects and synergistic effects among independent variables, as well as confounding factors (49). For the visualisation, a heatmap was generated to identify the associated effects of various co-factors on infection rates.

Maps were created per occasion using QGIS 2.4.0<sup>30</sup>, using spatial base data from Bayerische Vermessungsverwaltung (2019) and OpenStreetMap (2020).

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<sup>30</sup> QGIS Development Team (2018). QGIS Geographic Information System. Open Source Geospatial Foundation Project. <http://qgis.osgeo.org>

For all analyses described above and for the visualisation of results, Box-Whisker plots were used for showing differences among individuals or between years. Last, scatterplots with linear and LSD regression fits were applied with the respective confidence intervals to express significant slopes and quantify the respective effects.

All analyses were performed using Statistica<sup>TM31</sup> Version 13.3 or Microsoft® Excel®<sup>32</sup> 2016. To assess the data distribution and normality, Kolmogorov-Smirnov tests were run and residual analyses were conducted per dataset and separate analysis. In most cases, data were normally or log-normally distributed and logarithmic transformations were also tested and applied where appropriate.

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<sup>31</sup> TIBCO Software Inc., Palo Alto, USA

<sup>32</sup> Microsoft Corporation, Redmond, USA

**D**

## **Results**

For a first overview of the correlations between all different factors, a Pearson correlation for all factors is performed (**Figure 11**).

Some factors that show a high correlation are expected to correlate due to their biological relation. This can be seen in phenology (flowering start, peak and end), specific factors of the production of flowers, inflorescences and pollen, the different measures of inflorescences and the morphometric features of the tree.

But also other factors that are not directly related, show high correlations. The annual *Betula* pollen sum is correlated with the phenological parameters, the production of flowers, inflorescences and pollen on different levels and inflorescences measures. Specific aspects of the production of flowers, inflorescences and pollen are correlated with flowering and inflorescence measures and also allergenicity is correlated with inflorescence measures.

For the environmental factors, especially temperature and precipitation show correlations with several observed factors.

To understand the interactions of different environmental factors with the observed parameters, backwards stepwise Ridge regressions are performed in the following chapters.

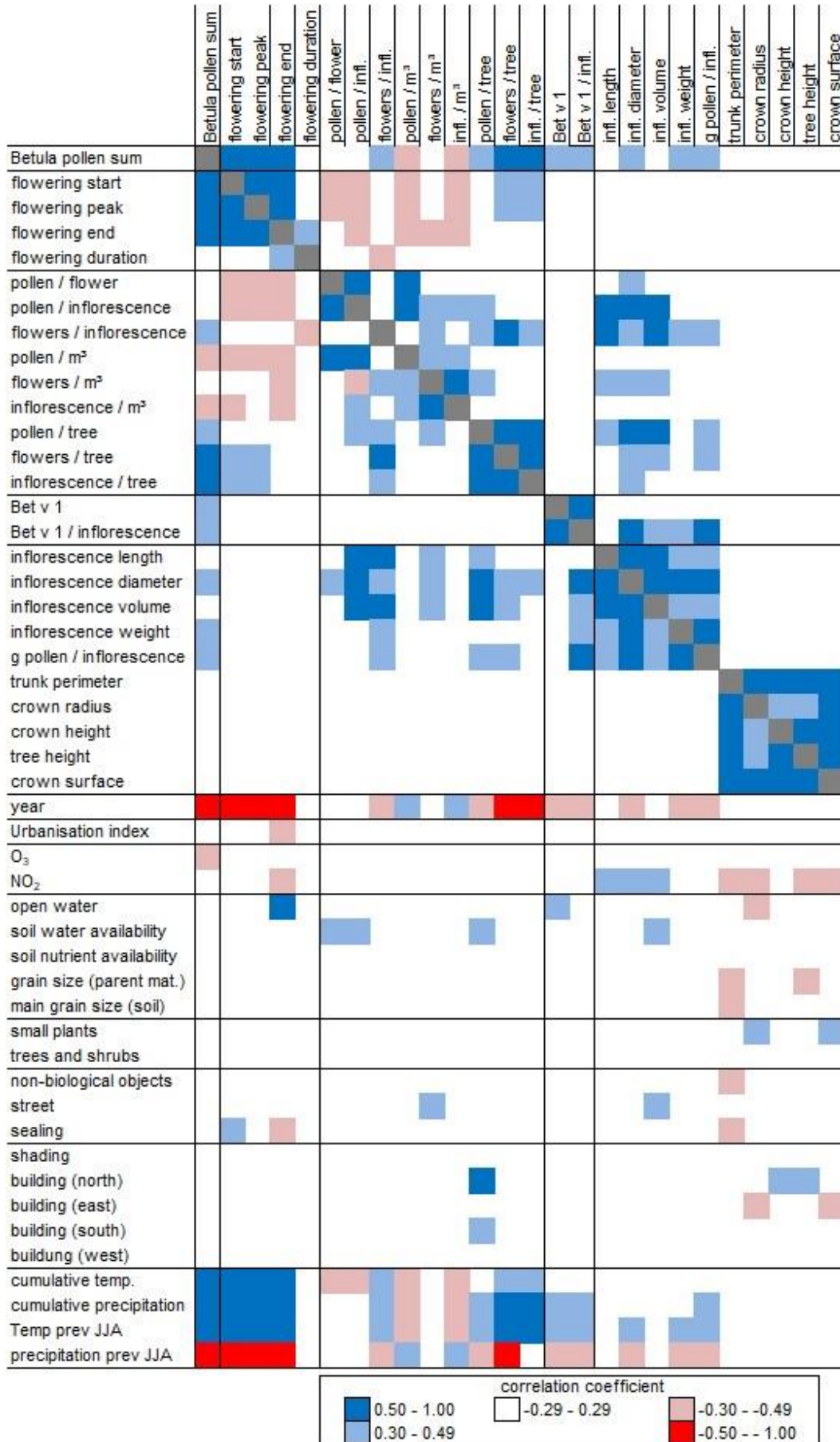


Figure 11: Pearson correlation of all analysed factors

## D.1. Descriptive results

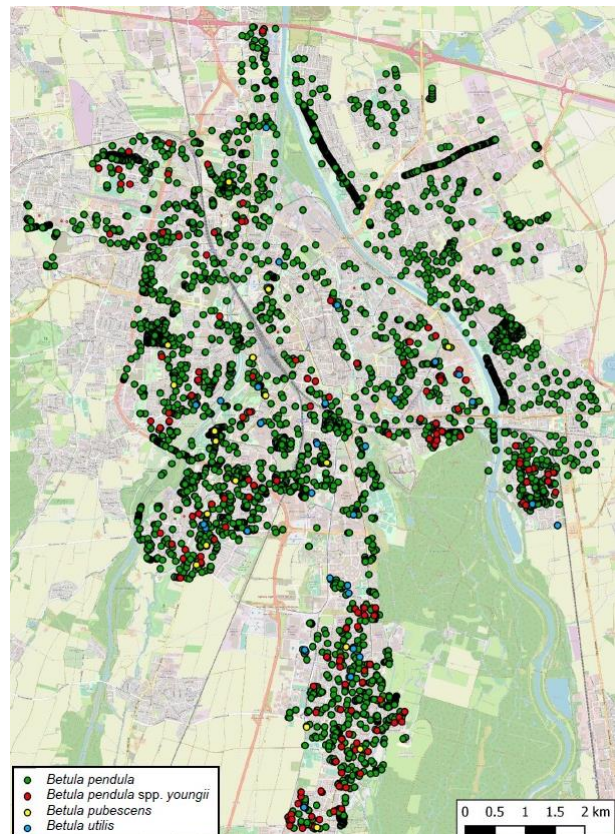
### D.1.1 Mapping of *Betula* trees in Augsburg

The whole population of *Betula* in the developed areas of the city of Augsburg was mapped, differentiating between all found *Betula* species. A total of 5,622 individuals of four different species of *Betula* were mapped (**Figure 12**). The species are *Betula pendula*, *Betula pendula* ssp. *youngii*, a growth restricted cultivar, *Betula utilis* and *Betula pubescens*.

With over 95%, *Betula pendula* represents the majority of *Betula* trees (Table 6).

Looking at patterns on the map, it is visible that the trees are not uniformly distributed but there are areas with low

number of *Betula* trees, for example the inner city and Hochfeld but also five tree-lined avenues, exclusively planted with *Betula pendula*. The highest concentration of *Betula* trees was found in graveyards (see Appendix G.4).



**Figure 12:** *Betula* species in Augsburg (map based on: OpenTopoMap 2019)

**Table 6:** *Betula* species in Augsburg

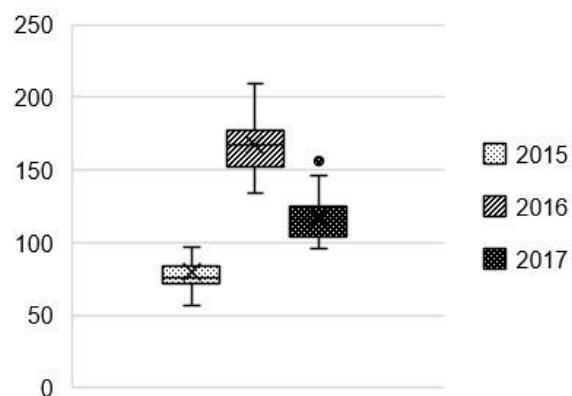
Number of individuals	Percentage of total population of <i>Betula</i>	<i>Betula</i> species
5,380	95,70 %	<i>B. pendula</i>
164	2,92 %	<i>B. pendula</i> ssp. <i>youngii</i> (growth-restricted cultivar)
53	0,94 %	<i>B. utilis</i>
25	0,44 %	<i>B. pubescens</i>

## D.1.2 Environmental factors

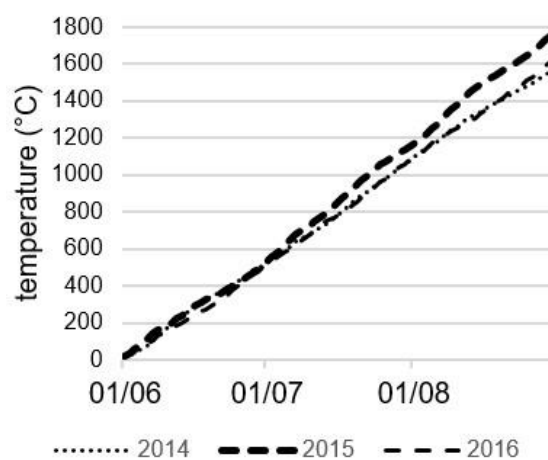
### D.1.2.1 Meteorology

To estimate the influence of meteorological factors on phenology and allergenicity, the cumulative minimum temperature from January 1<sup>st</sup> of every year until the start of flowering was calculated. This covers the complete period of spring warming after the lowest temperatures of the year in January (**Figure 3**).

As inflorescences, including flowers and pollen, are formed during the summer months of the year before the flowering, the cumulative daily average temperatures of the previous summer months, June, July and August, were used for analysis with the temperature for the production of flowers, inflorescences and pollen (**Figure 13, Figure 14**).



**Figure 13:** cumulative minimum temperatures  $>0^{\circ}\text{C}$  from January 1<sup>st</sup> until the start of flowering



**Figure 14:** cumulative average temperatures of the summer (JJA) before the observed flowering

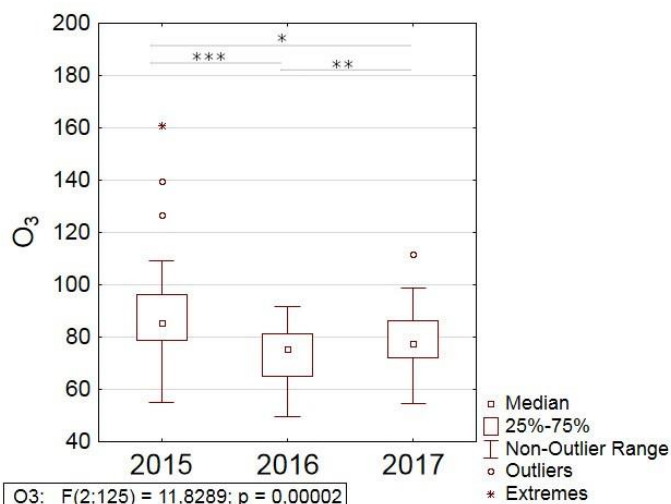
### D.1.2.2 Air quality

The average level of O<sub>3</sub> for all trees in all measuring periods and all years is 80.50 µg/m<sup>3</sup> (SD = 15.62, p=0.000). The year with the highest values was 2015 (88.50 µg/m<sup>3</sup> (SD = 19.87), the year with the lowest average was 2016 (73.30 µg/m<sup>3</sup>, SD = 10.72), in 2017, the average was 79.89 µg/m<sup>3</sup> (SD = 20.95). The highest value over all is 161.00 µg/m<sup>3</sup>. In eight cases, the threshold of 100.00 µg/m<sup>3</sup> (World Health Organisation 2006) was exceeded. Seven of these measurements were conducted in 2015, one in 2017 (**Figure 15**).

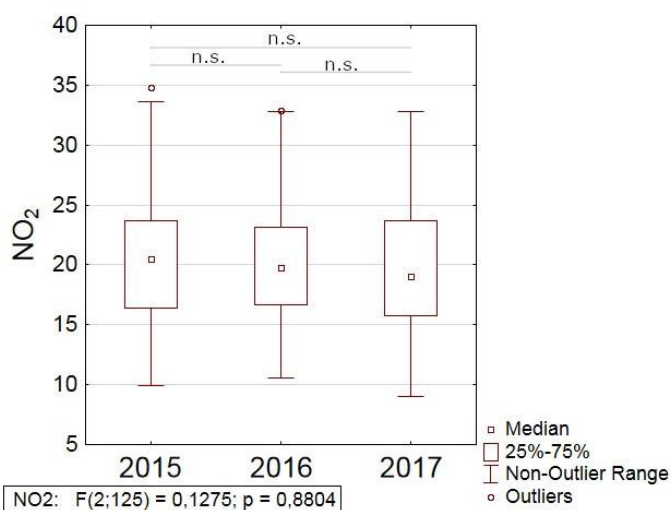
The average level of NO<sub>2</sub> for all trees in all measuring periods and all years is 20.37 µg/m<sup>3</sup> (SD = 5.90, p=0.880). The

concentrations in the different years are stable over all observed years as they did not show significant differences (2015: 20.68 µg/m<sup>3</sup> (SD = 6.09), 2016: 20.40 µg/m<sup>3</sup> (SD = 5.68), 2017: 20.03 µg/m<sup>3</sup> (SD = 6.06)). The highest value is 34.80 µg/m<sup>3</sup> what means that in none of the measurements, the yearly threshold of 40 µg/m<sup>3</sup> (World Health Organisation 2006) is exceeded (**Figure 16**).

The concentrations of O<sub>3</sub> and NO<sub>2</sub> are also correlated with the morphometric traits of the tree and inflorescences and it can be seen that for O<sub>3</sub> the only significant ( $p \leq 0.05$ ) parameters are the weight of pollen per inflorescence and the weight of the inflorescence with being both lower with elevated O<sub>3</sub> (**Figure 17**). NO<sub>2</sub> shows significant negative

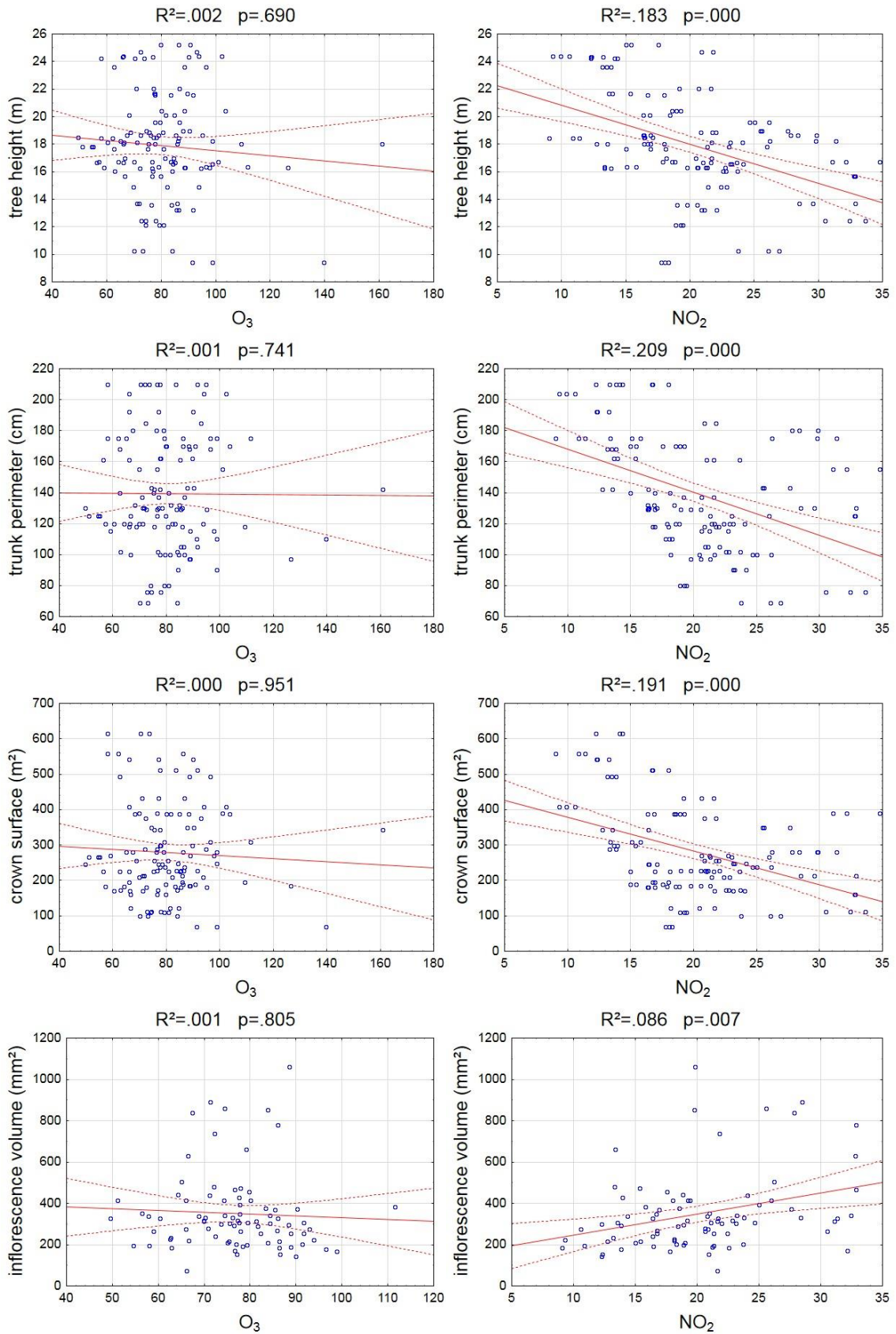


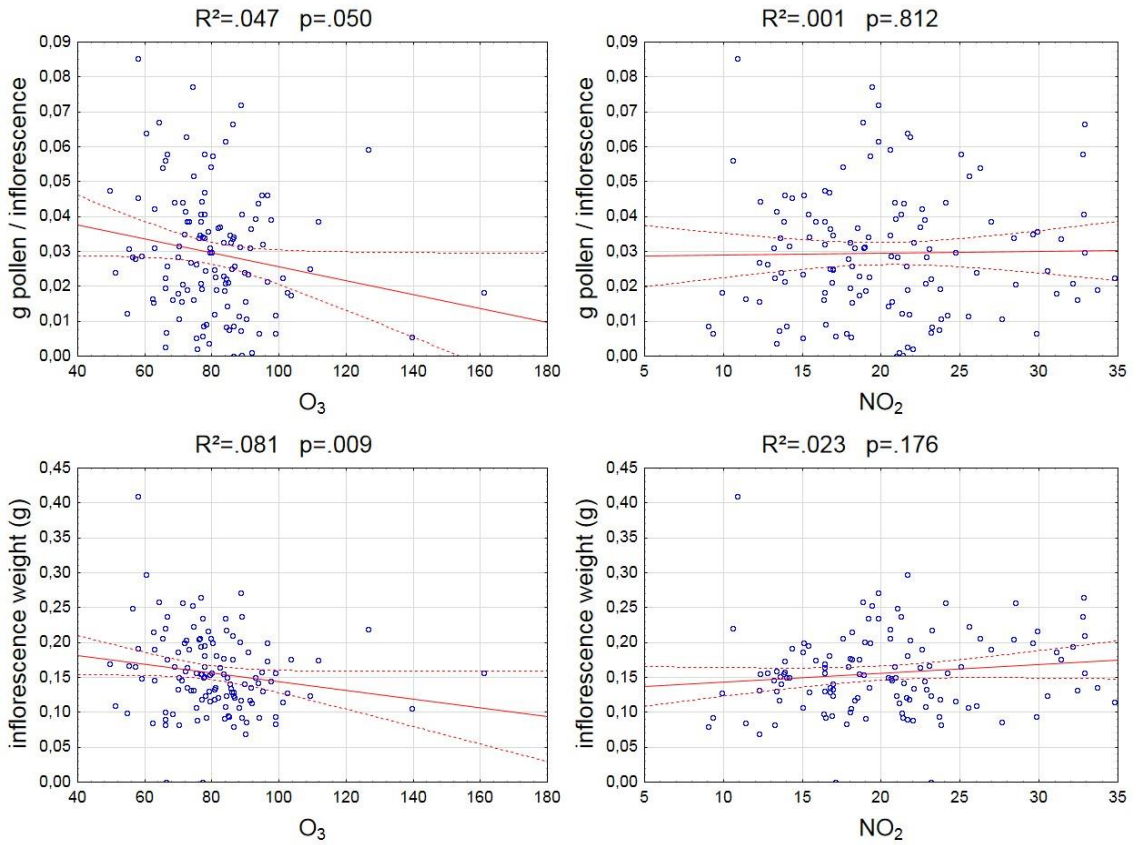
**Figure 15:** Ozone levels per year



**Figure 16:** Nitrogen dioxide levels per year

correlations with tree height, trunk perimeter and the volume of the crown as well as a significant positive correlation with the volume of the inflorescences (**Figure 17**).



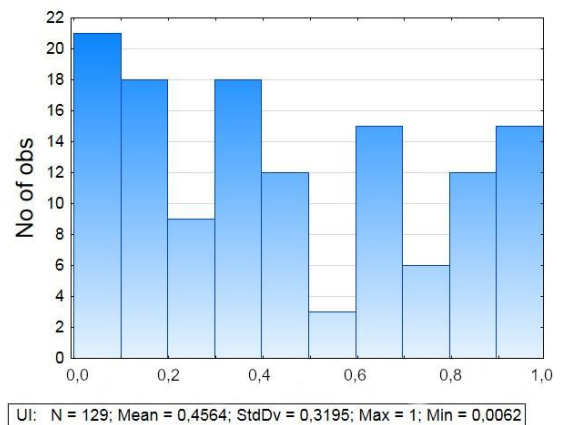


**Figure 17:** correlation between urbanity and morphometric traits

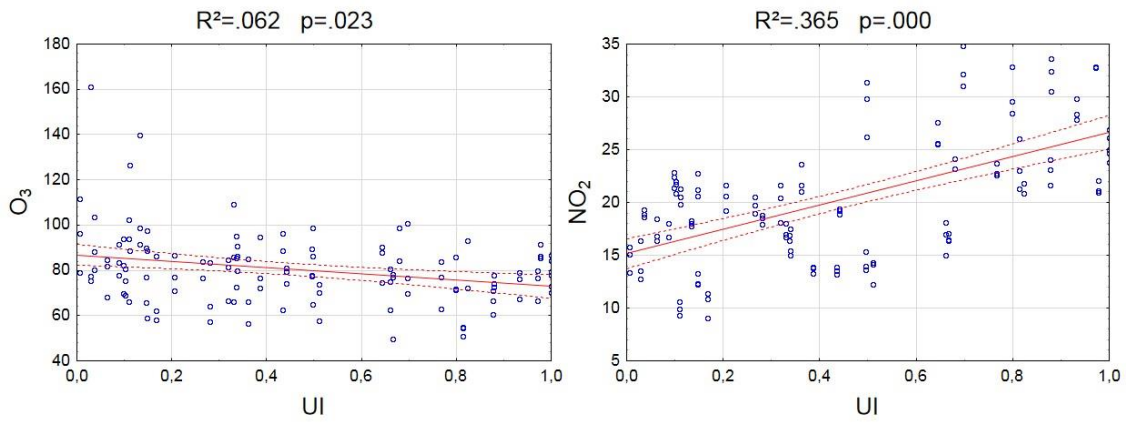
### D.1.2.3 Urbanity

The average urbanity index of all observed trees is 0.46, the median is 0.39 (**Figure 18**).

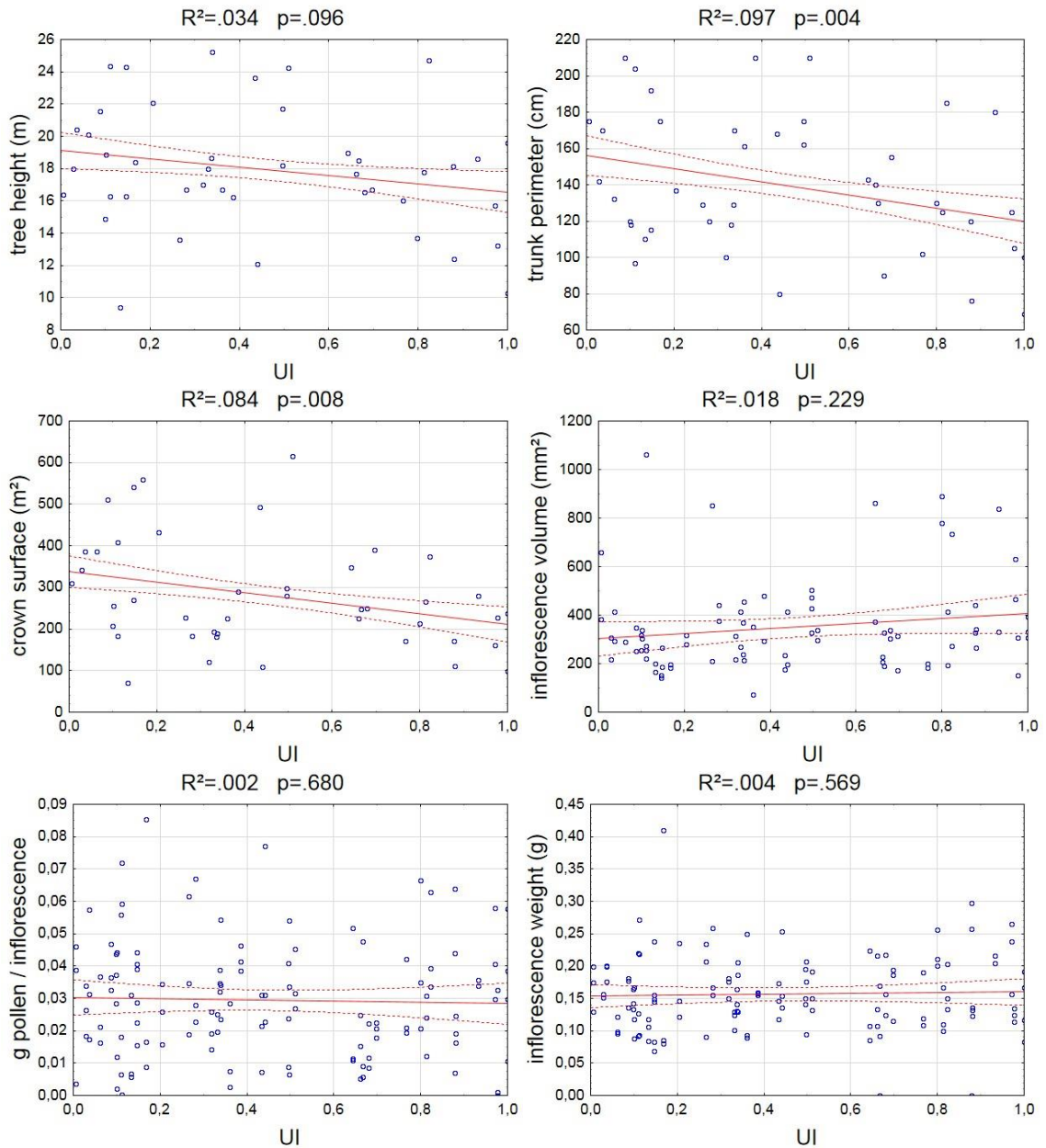
The air pollutants were correlated on a significant ( $p \leq 0.05$ ) level. While  $\text{NO}_2$  was elevated in urban environments,  $\text{O}_3$  was higher in rural environments (**Figure 19**). Even though trees were randomly selected, a difference in morphometric traits is visible, trunk perimeter and crown surface are significantly ( $p \leq 0.05$ ) lower in urban areas (**Figure 20**).



**Figure 18:** average urbanisation index



**Figure 19:** correlation between urbanity index and air pollutants



**Figure 20:** correlation between urbanity and morphometric traits

## D.2. Aerobiological monitoring in Augsburg

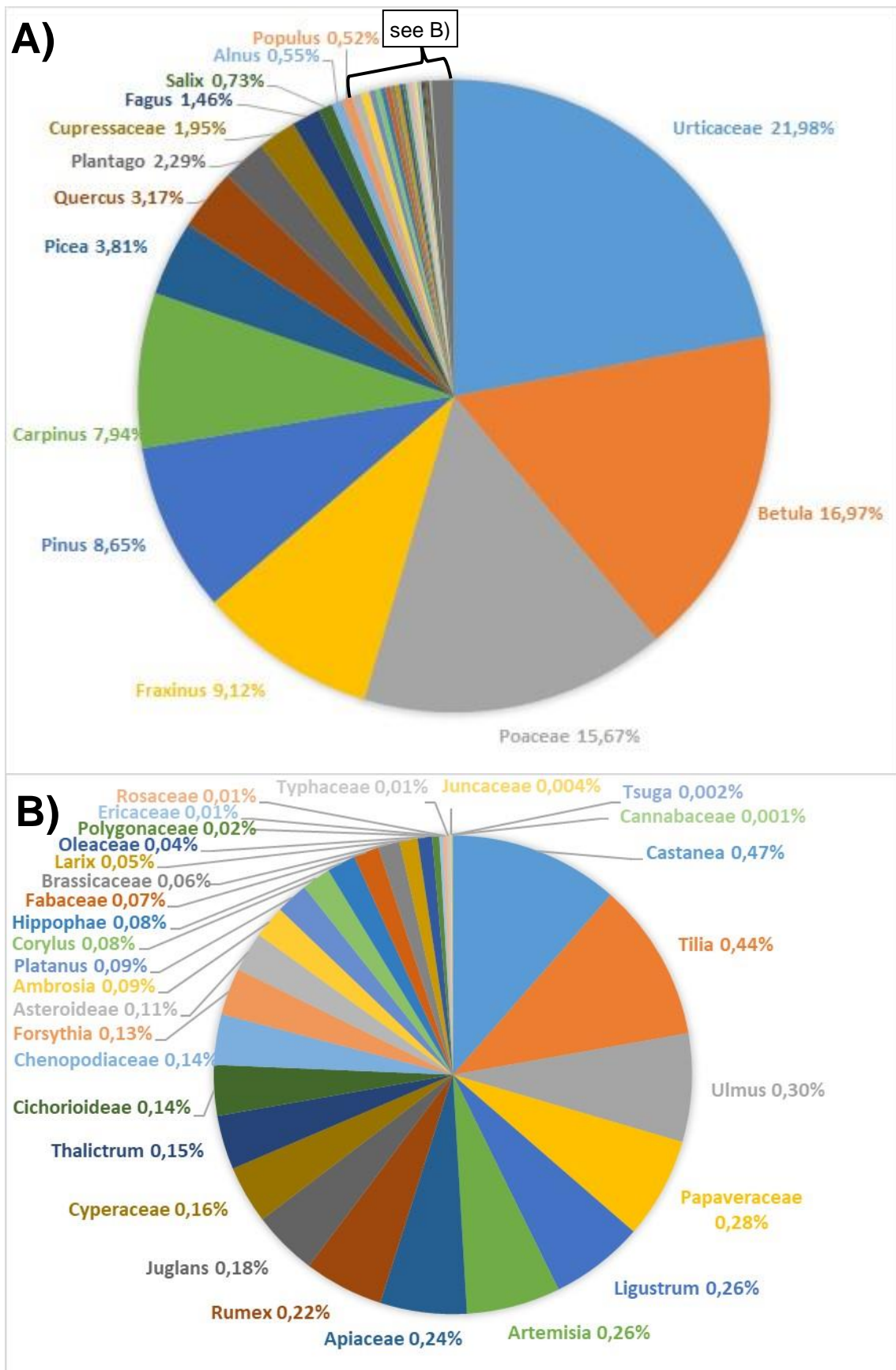
The results of the following Chapters D2.1 – D2.3 are published in Kolek et al. (in review\_a).

### D.2.1 Atmospheric pollen

For the years 2015 – 2017, pollen of 43 taxa could be identified and counted, which corresponds to 98.87 % of the total annual pollen index. About 1.13% of the total amount of pollen could not be identified (see Appendix G.1).

Of all identified taxa, 14 taxa show a relative abundance of over 0.5 %. These most common taxa are representing 94.81 % of the total pollen load (**Figure 21, Table 7**). The taxa with the highest abundance of airborne pollen are Urticaceae (22.45 %), *Betula* (17.05 %) and Poaceae (15.70 %). These three taxa, all with high relevance for allergic patients, sum up to a total of about 55 % of the total pollen load. (**Figure 21, Table 7**)

As the sampling periods started in the second half of March, the onset of the pollen season could not be observed for some taxa. The seasons of *Corylus*, *Alnus*, *Salix* and *Ulmus* start before the sampling was performed, so the numbers, shown here are not reflecting the real proportion of the total pollen season but are significantly underestimated.



**Figure 21:** Diversity of airborne pollen  
 A) relative abundances (%) of the 14 most abundant pollen types (>0.5% contribution to the total annual pollen concentration)  
 B) less abundant pollen types (<0.5% contribution to the total annual pollen concentration).

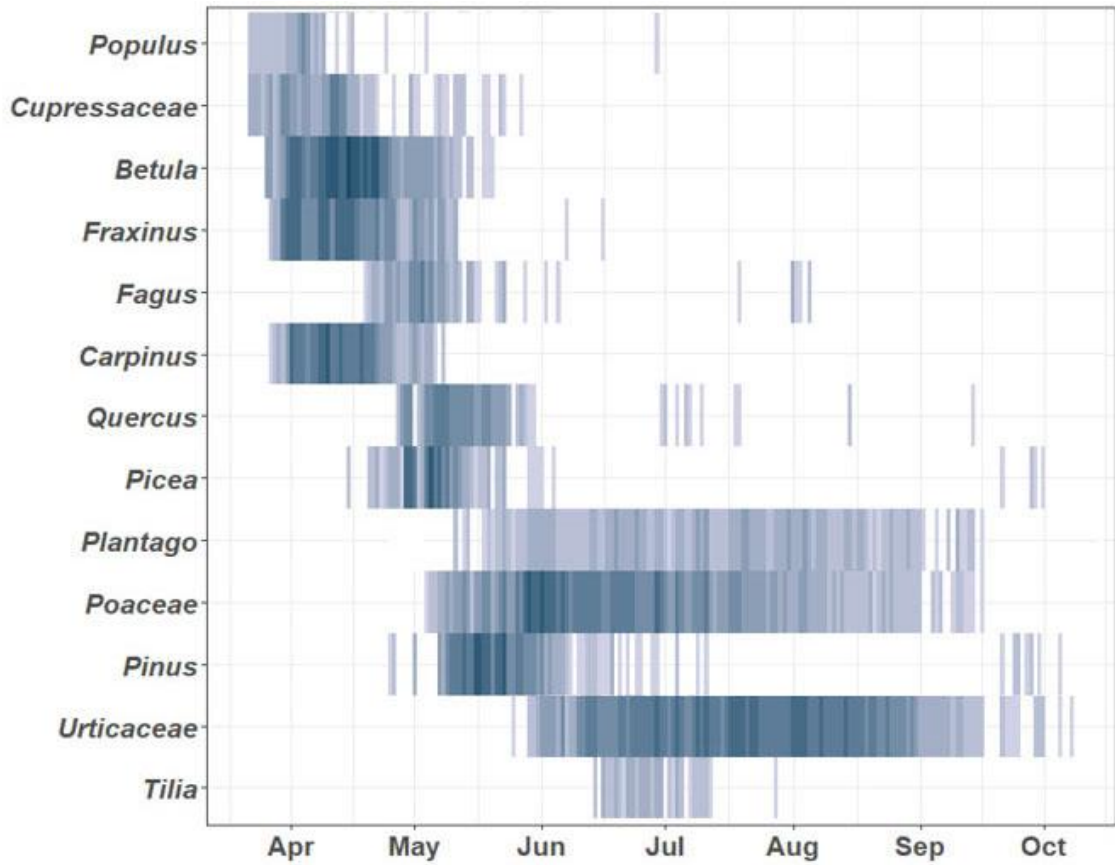
**Table 7:** most common taxa (over 0.5% relative abundance of all pollen) of all identified pollen  
 Grey highlighted rows: taxa with under-represented pollen amounts due to missing values in the onset of their season

Scientific name	English name	Percentage of total pollen
Urticaceae	Nettle family	21.98 %
<i>Betula</i>	Birch	16.97 %
Poaceae	Grasses	15.67 %
<i>Fraxinus</i>	Ash	9.12 %
<i>Pinus</i>	Pine	8.65 %
<i>Carpinus</i>	Hornbeam	7.94 %
<i>Picea</i>	Spruce	3.81 %
<i>Quercus</i>	Oak	3.17 %
<i>Plantago</i>	Plantain	2.29 %
Cupressaceae	Cypress family	1.95 %
<i>Fagus</i>	Beech	1.46 %
<i>Salix</i>	Willow	0.73 %
<i>Alnus</i>	Alder	0.55 %
<i>Populus</i>	Poplar	0.52 %

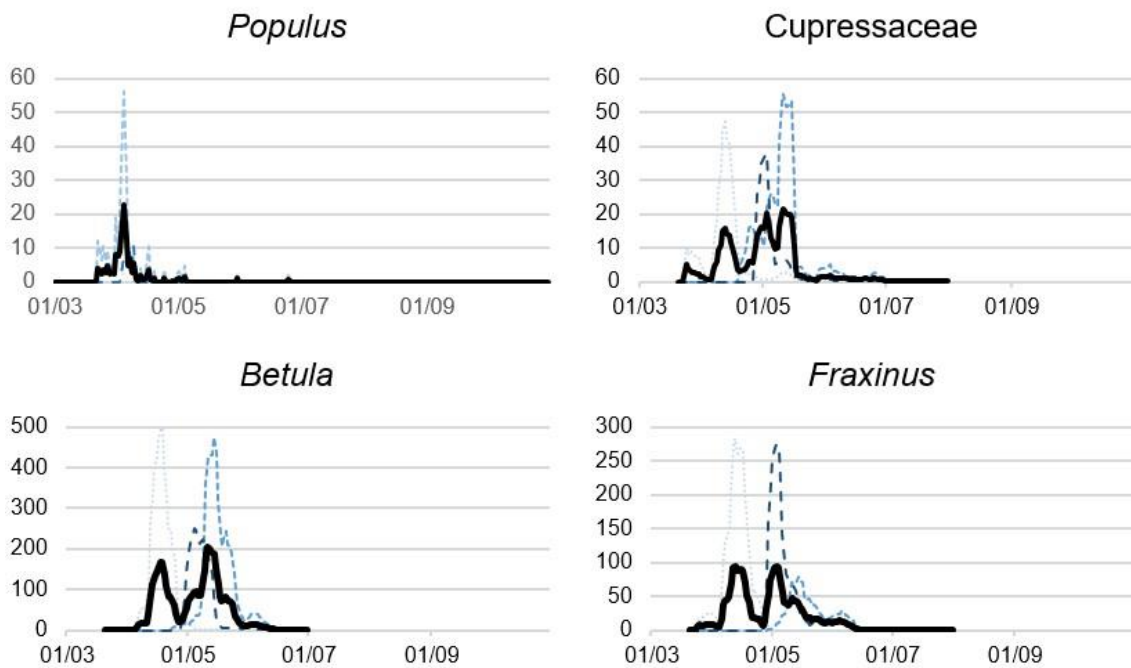
## D.2.2 Seasonal atmospheric circulation of pollen

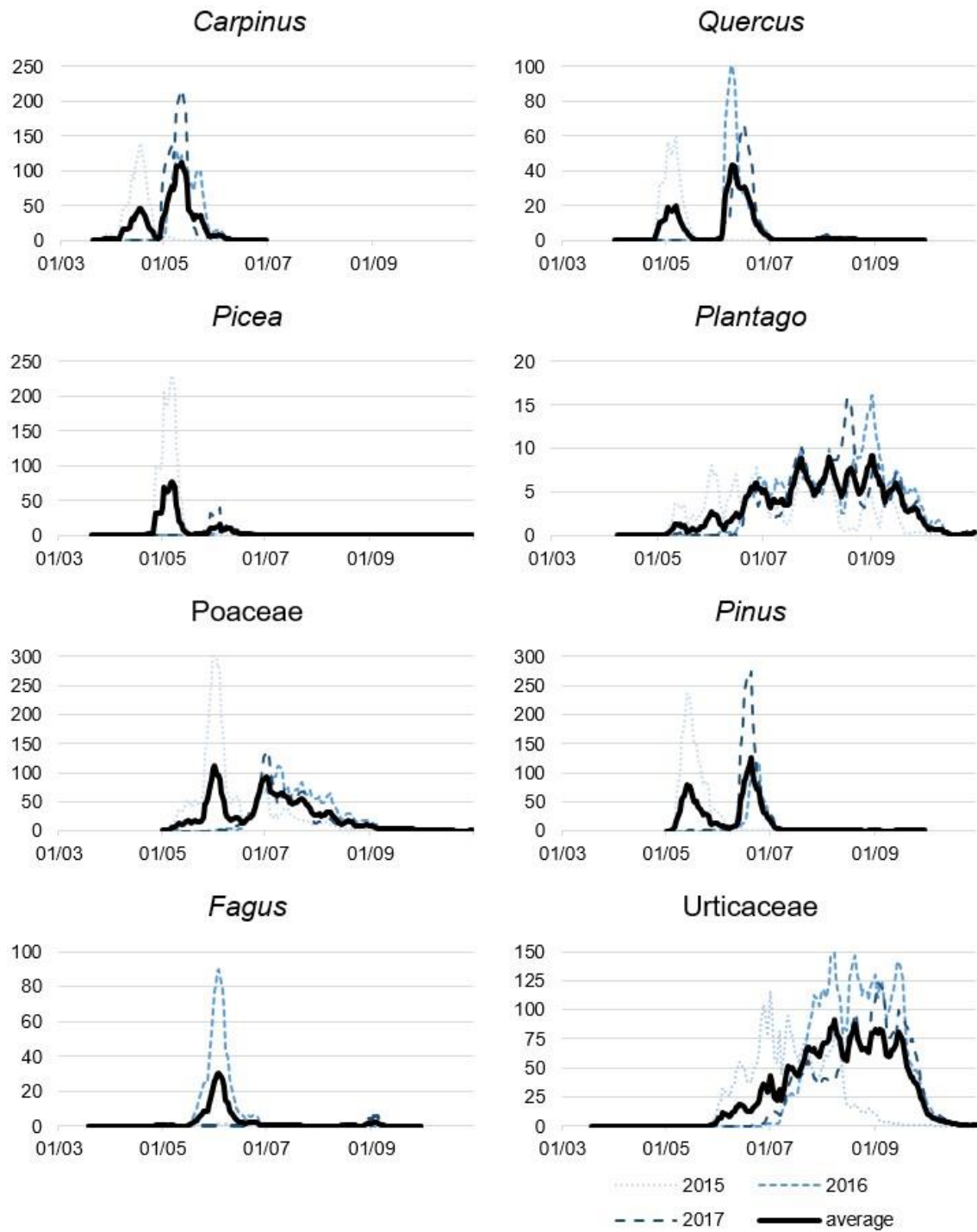
The highest pollen concentrations were observed in April and May, accounting for more than 30 % of the total annual pollen load and mainly consisted of *Betula*, *Fraxinus*, *Picea* and *Pinus* (**Figure 23**). In June to August, Poaceae and Urticaceae were the most common pollen taxa.

Seasons of most pollen taxa are quite short and peaked; just the seasons of Poaceae and Urticaceae pollen last for more than three months (**Figure 22**).



**Figure 22:** Pollen calendar, average seasonal distribution is given for the 13 most abundant pollen types during 2015-2017.





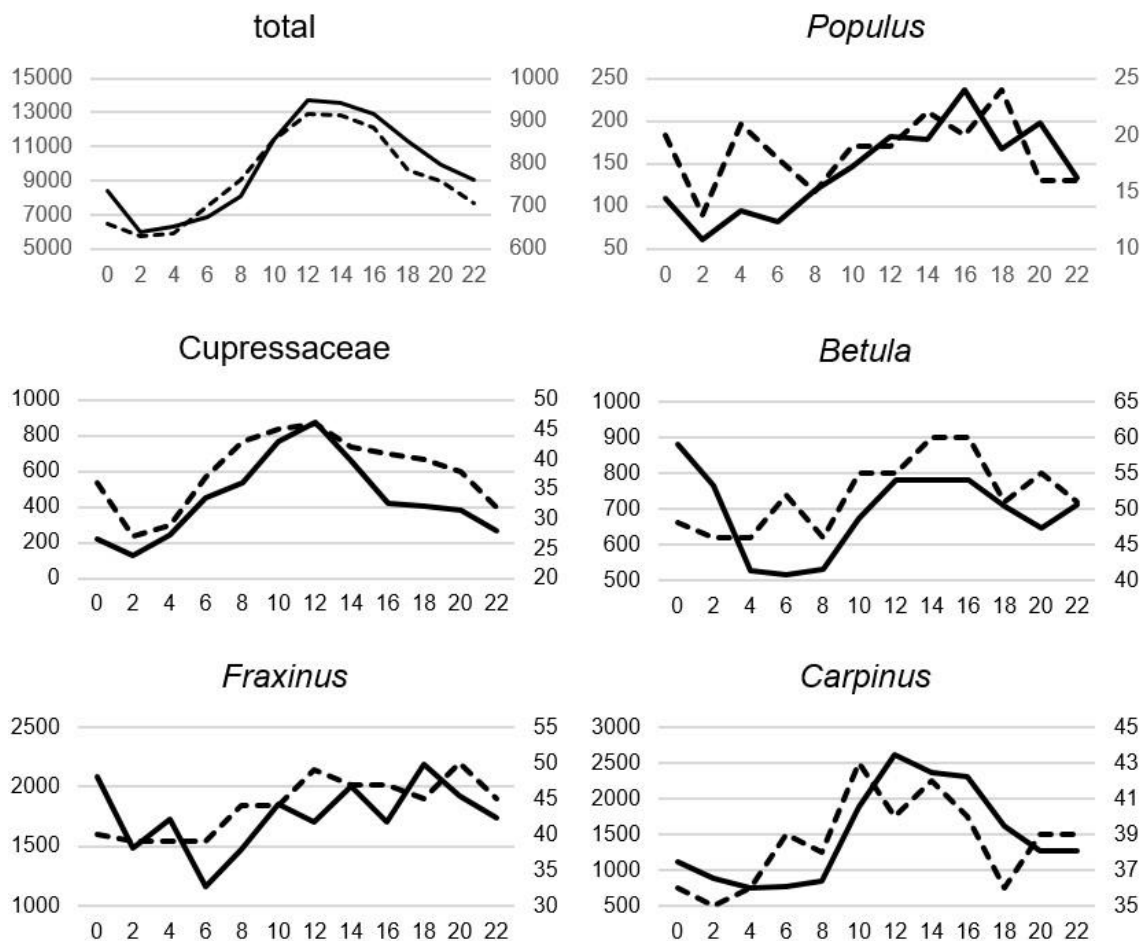
**Figure 23:** Average pollen season of the most abundant taxa in Augsburg, during 2015-2017

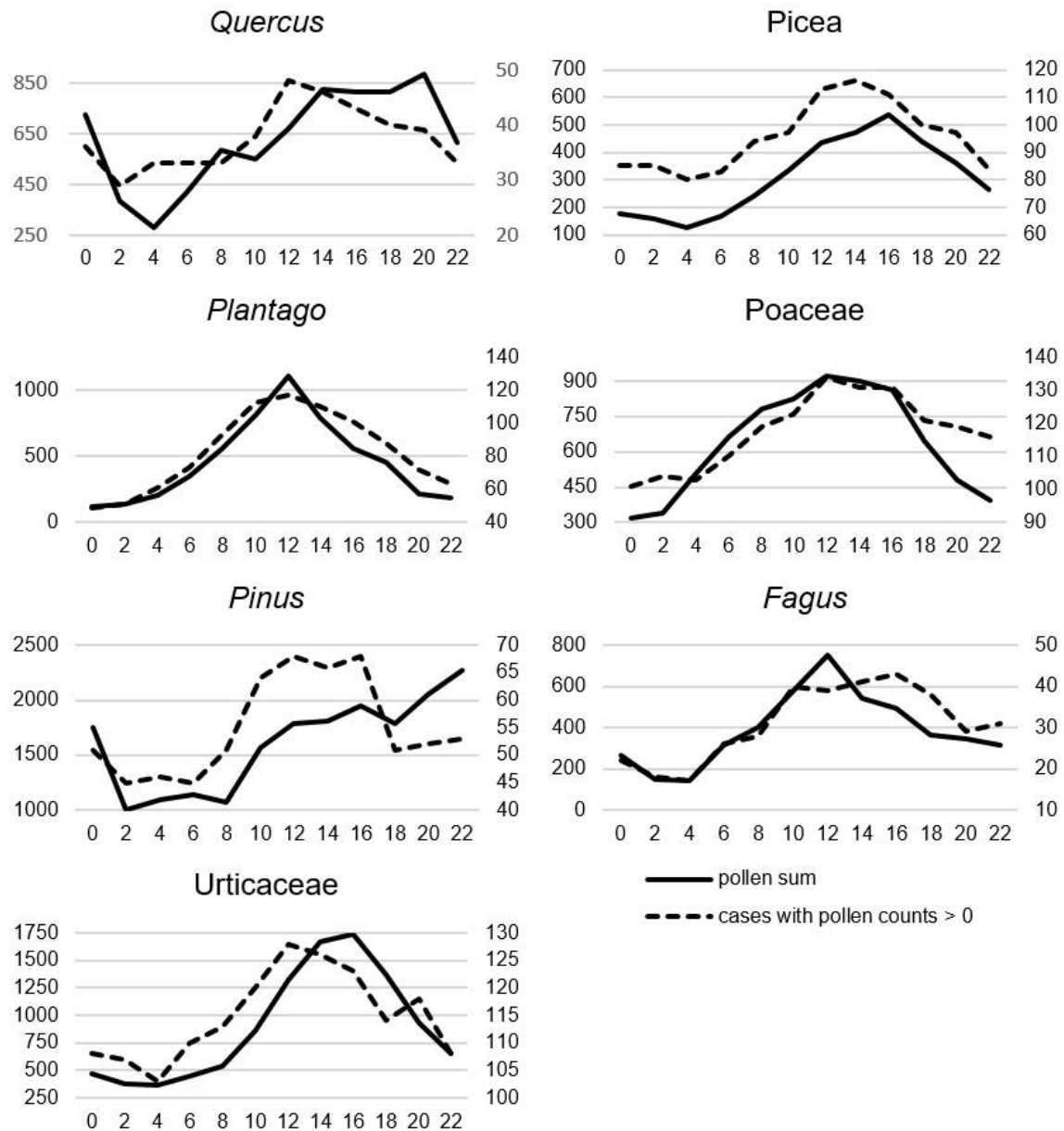
### D.2.3 Daily atmospheric circulation of pollen

For the most common taxa (over 0.5 % of the total annual pollen load), also circadian patterns were investigated for the observed years (2015 – 2017) on a 2-hourly time resolution.

The amount of pollen (pollen sum, solid line) was observed as well as the frequency (cases with pollen count > 0, dashed line) (**Figure 24**). This distinction was made to see the consistency of the pattern and make visible patterns in the pollen sum that are caused by high peaks because of extreme weather events. As in most of the observed cases, these lines coincide, this shows that the patterns are consistent and not caused by extreme weather events or similar single occurrences of high pollen.

For the total of the most abundant species (14 taxa, 94.81 % of the total pollen load), the circadian peak was observed at 12:00 to 16:00. Between the interval 10:00 – 22:00 and 22:00 – 10:00, a significant difference was found with the day time interval being higher (full factorial ANOVA,  $p < 0.001$ , after Bonferroni correction).





**Figure 24:** circadian circulation patterns of the most common pollen taxa (2015-2017)  
**X-axis:** sampling bi-hourly interval on a 24-hour scale  
**primary Y-axis** (left) shows the frequency of pollen occurrence for each bi-hourly interval (solid line),  
**secondary Y-axis** (right) displays the total annual atmospheric pollen load for each 2-hour sampling (dashed line).

The circadian patterns differ between the observed taxa. While herbaceous taxa (*Plantago*, *Poaceae* and *Urticaceae*) as well as some woody taxa (*Carpinus*, *Cupressaceae*, *Fagus*, *Picea*, *Populus* and *Salix*) show pollen peaks between 12:00 and 18:00, other woody species (*Betula*, *Pinus* and *Quercus*) have their peak between 20:00 and 02:00. Just *Fraxinus* does not show a peaked circadian distribution but a more complex, multimodal pattern.

## D.2.4 *Betula* pollen season

The *Betula* pollen season was, likewise, calculated based on the daily pollen counts from 2015, 2016 and 2017 (Figure 25).

Considering the main pollen season as 5% - 95% of the annual amount of pollen (Nilsson and Persson 1981), it took place in:

2015 for 16 days (09/04 – 24/04)  
 2016 for 25 days (07/04 – 02/05)  
 2017 for 13 days (01/04 – 12/04).

So, in average for the three observed year, the *Betula* pollen season took place from the 02/04 until the 25/04 and lasted 24 days.

The peak occurred on the 15/4 in 2015, four days earlier, on the 11/4 in 2016 and again one day earlier, on the 10/04 in 2017. On average over all three seasons, the highest values can be found on 15/04.

Also, the amount of total pollen per year differs between the years (Figure 26). In 2015, 9,171 pollen grains were identified, in 2016 it was 10,165 pollen grains and in 2017, 5,558 pollen grains were counted.

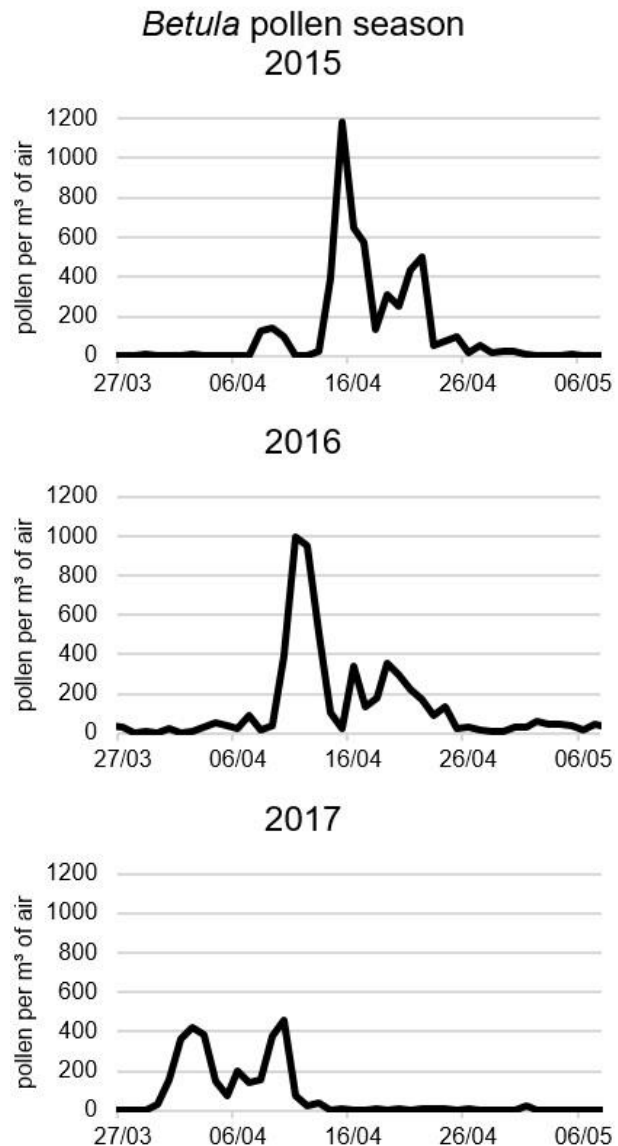


Figure 25: main *Betula* pollen season

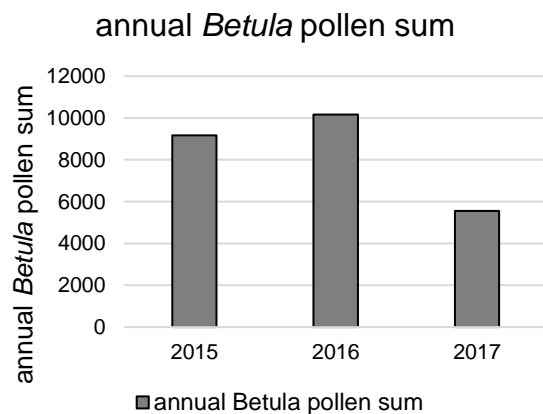
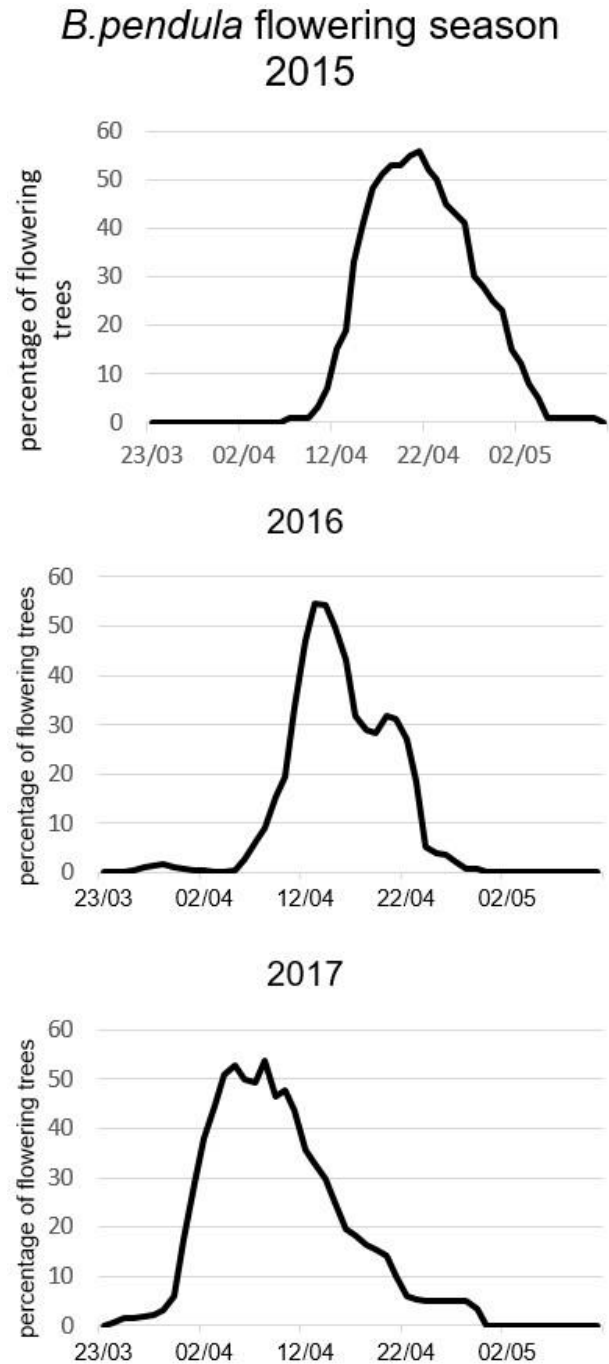


Figure 26: numbers of counted pollen grains of *Betula* spp. per year and average temperature and precipitation of the summer (June – August) of the previous year

### D.3. Phenological observations of *Betula pendula*

The earliest start of flowering was observed on calendar day 87 (March 28<sup>th</sup>) in 2017, the latest end of flowering on calendar day 120 (April 30<sup>th</sup>) in 2015 (Appendix G.6).

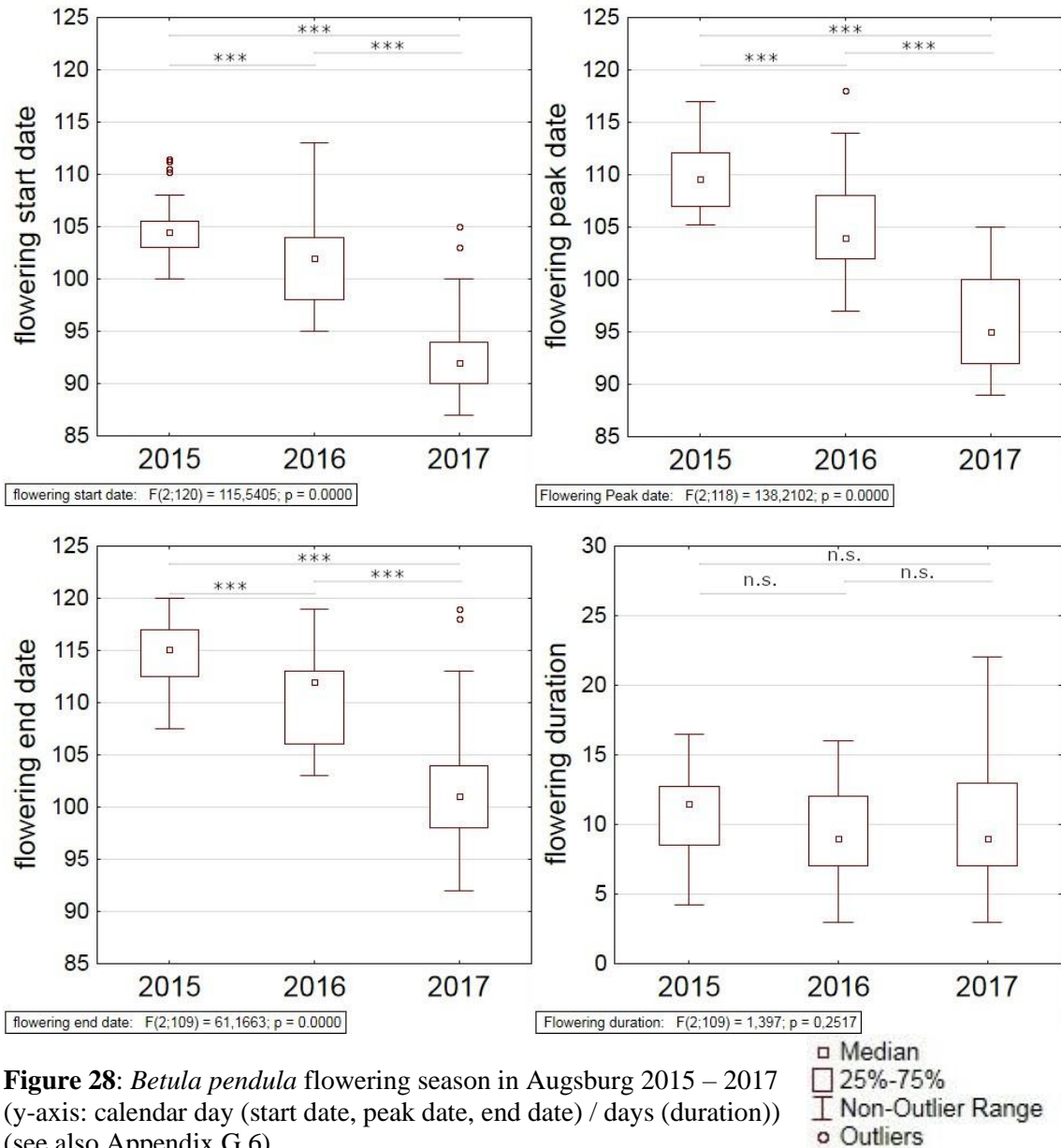
The average start of the flowering for all observed individuals in all years was observed on calendar day 100 (SD = 6.50) (April 10<sup>th</sup> in 2015 and 2017, April 9<sup>th</sup> in 2016). The average end of the flowering (all individuals, all years) was on calendar day 108 (SD = 7.09) (April 18<sup>th</sup> in 2015 and 2017, April 17<sup>th</sup> in 2016). The average peak date of the flowering was calendar day 103 (SD = 7.13) (April 13<sup>th</sup> in 2015 and 2017, April 12<sup>th</sup> in 2016). The average duration of the flowering was 10.3 days (SD = 3.74). (Appendix G.6).



**Figure 27:** *Betula pendula* flowering season (2015 - 2017)

### D.3.1 Differences between years

The flowering start date, peak date and end day of the observed *B. pendula* trees differ between the different years of observation.



**Figure 28:** *Betula pendula* flowering season in Augsburg 2015 – 2017 (y-axis: calendar day (start date, peak date, end date) / days (duration)) (see also Appendix G.6)

The latest start of flowering was observed in 2015 (on average: April 15<sup>th</sup>), the earliest start, being 12 days earlier than 2015, was observed in 2017 (on average: April 3<sup>rd</sup>). (Table 8, Figure 28, Figure 28, Appendix G.6)

The flowering peak and the flowering end show the same pattern being earliest in 2017 and latest in 2015. (Table 8, Figure 28, Appendix G.6)

**Table 8:** flowering season of *Betula pendula* ( $\pm$ SD)

	flowering start date	flowering peak date	flowering end (calendar date)	Duration (days)
<b>2015</b>	13-17 April	17-23 April	22-28 April	8 - 14
<b>2016</b>	07-17 April	10-20 April	16-24 April	7 - 13
<b>2017</b>	30 March-07 April	01-09 April	06-18 April	6 - 14
<b>Average</b>	03-17 April	06-10 April	11-25 April	6 - 14

It is noticeable that the average flowering duration does not significantly differ between the years, the shortest duration (3 days) being observed in 2016 and the longest (22 days) in 2017. (Figure 28, Appendix G.6). It is evident that when the flowering season starts earlier on a year, it also ends earlier, with the seasons being shifted, instead of prolonged.

### D.3.2 Influences of morphometric features

	U	O <sub>3</sub>	NO <sub>2</sub>	soil water availability	soil nutrient availability	trunk perimeter	tree height	crown surface	cumulative temperature from 1.1.
flowering start	0,009						0,053		0,000
flowering peak			0,028					0,084	0,000
flowering end			0,009						0,000
flowering duration			0,085						

+	0.05 < p < 0.10
*	0.01 < p < 0.05
**	0.001 < p < 0.01
***	p < 0.001

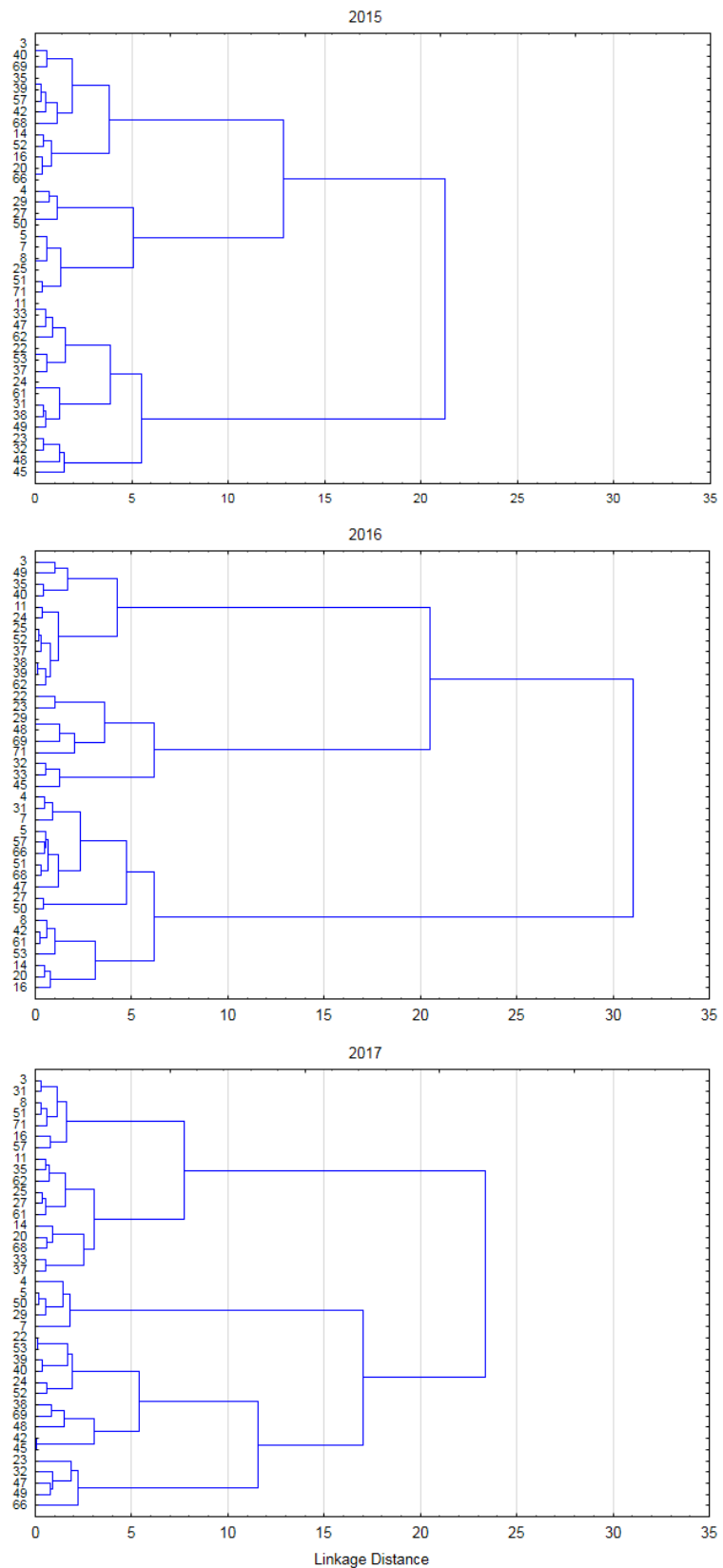
**Figure 29:** significant factors of backwards stepwise ridge regression for flowering start, peak, end and duration

Regarding trees' morphometric features, they seem not to be a major and consistent determinant of the flowering season attributes (**Figure 29**). Nonetheless, for the start and the peak of flowering one may see a weak but a significant correlation with the tree morphometric features ( $p \leq 0.05$ ) (**Figure 29**).

### D.3.3 Influence of environmental parameters

To determine the influences of site-specific factors, as soil composition, water availability, nutrients, shading and urbanity, a cluster analysis of the flowering start date was performed per observation year (**Figure 30**).

Even though the analysis shows clear clusters in each year, they do not seem to be consistently repeating over all three years. This shows that flowering is not strongly influenced by spatial micro-environmental factors.



**Figure 30:** hierarchical cluster analysis (Ward's method)

### D.3.3.1 Meteorology

Temperature seems to be the most important and consistent factor in the backwards stepwise Ridge regression (**Figure 29**).

To additionally estimate the relationship between temperature and the timing of flowering, the cumulative temperature from January 1<sup>st</sup> until the start of flowering was considered (see Chapter D.1.2.1). The relationship ( $R^2$ ) between the flowering start day and the cumulative minimum temperature is higher than 0.95 in every year and as averaged from all years (**Table 9**) even though it is shifted in different years while the relationships of the flowering peak, end and duration are weaker (**Figure 13**).

**Table 9:** Relationship of flowering start date and cumulative minimum temperature

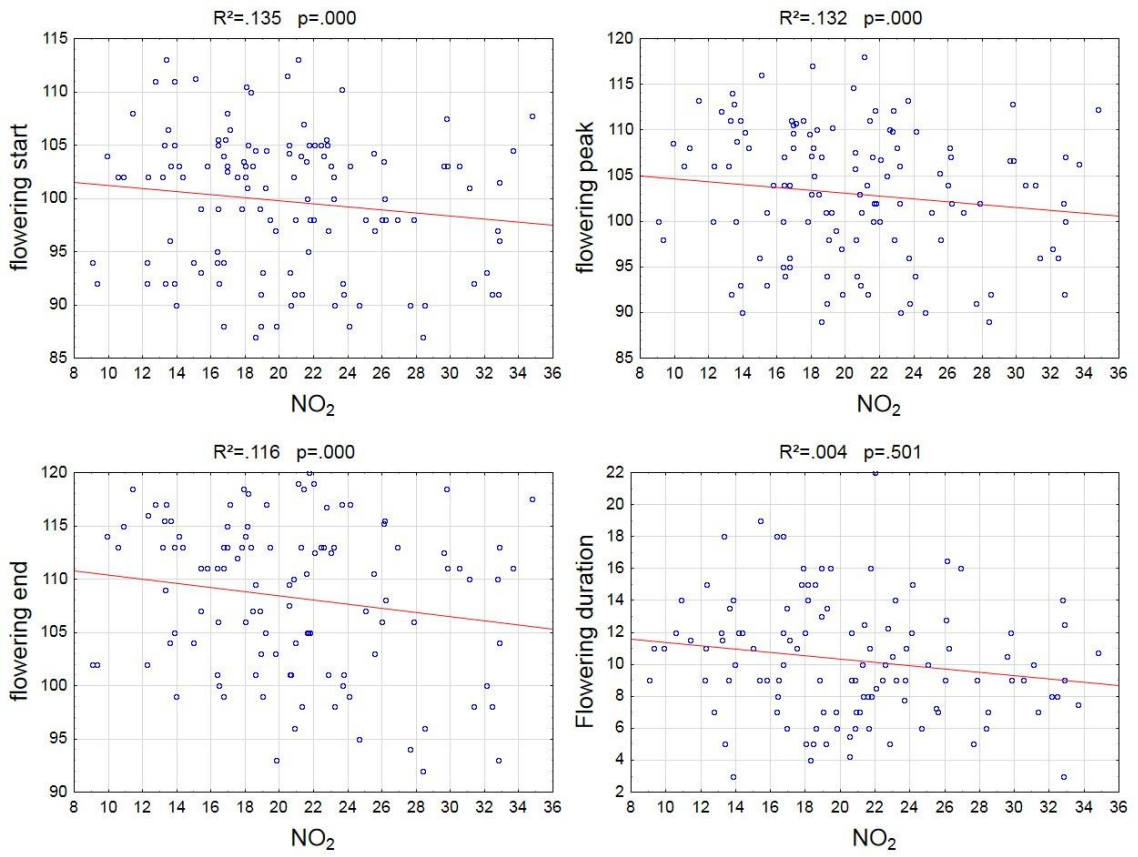
	Relationship ( $R^2$ ) of phenology and cumulative minimum temperature (average cumulative minimum temperature $\pm$ SD)			
	flowering start	flowering peak	flowering end	duration
<b>2015</b>	0.95 (104 $\pm$ 2)	0.50 (109 $\pm$ 2)	0.17 (115 $\pm$ 3)	0.05 (11 $\pm$ 3)
<b>2016</b>	0.99 (102 $\pm$ 4)	0.70 (105 $\pm$ 5)	0.51 (110 $\pm$ 4)	0.23 (10 $\pm$ 3)
<b>2017</b>	0.96 (93 $\pm$ 4)	0.72 (95 $\pm$ 4)	0.54 (102 $\pm$ 6)	0.02 (10 $\pm$ 4)
<b>Average</b>	0.97 (99 $\pm$ 6)	0.85 (102 $\pm$ 7)	0.71 (108 $\pm$ 7)	0.04 (10 $\pm$ 4)

### D.3.3.2 Air quality

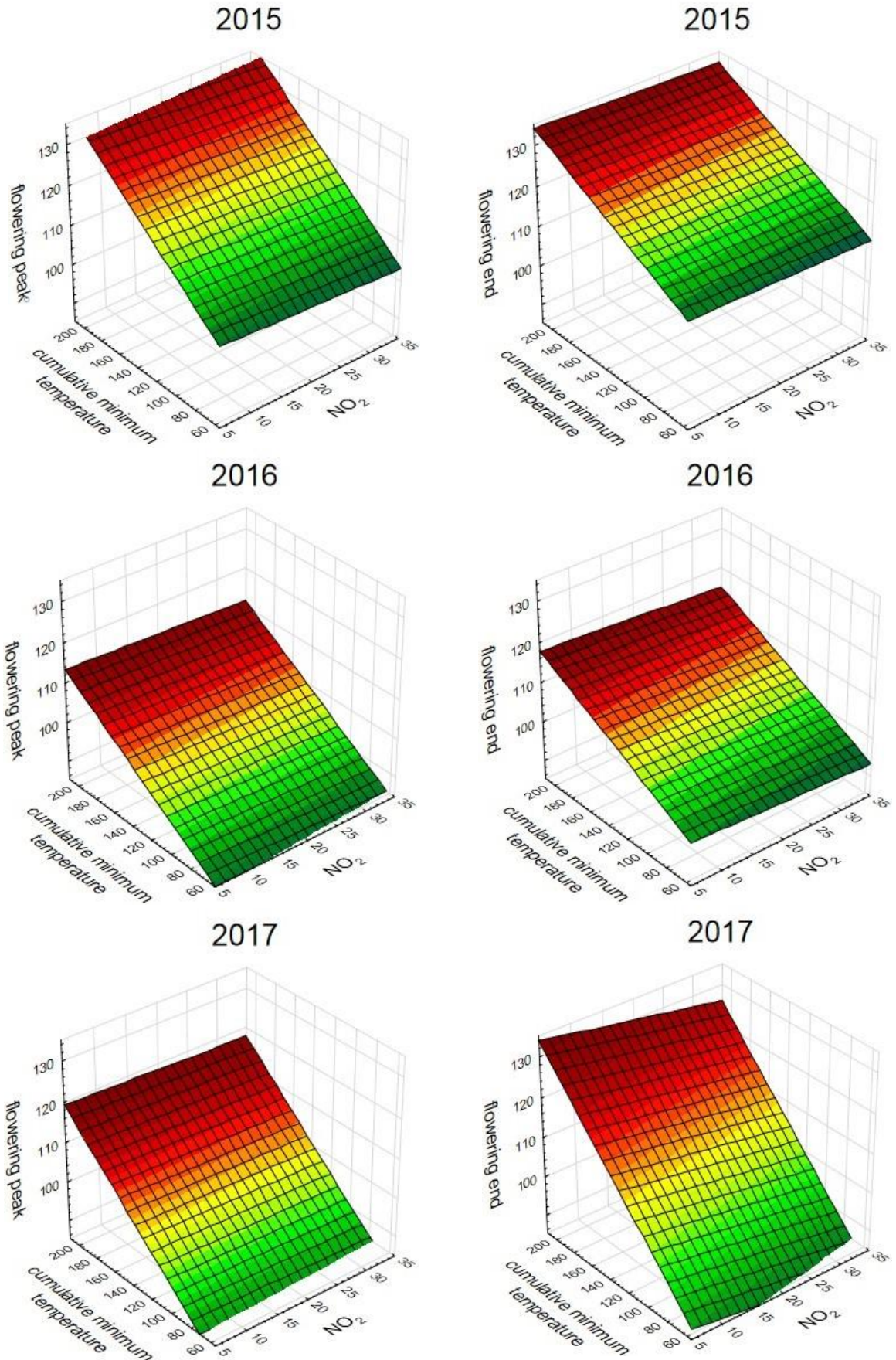
Based on the significance in the backwards stepwise Ridge regression (**Figure 29**), the correlation between the phenology and NO<sub>2</sub> is further analysed by performing simple regressions that show significant ( $p \leq 0.05$ ) negative correlations for flowering start, duration and end, meaning earlier flowering occurred in places with higher NO<sub>2</sub> levels (**Figure 31**).

In a 3D-plot in combination with the cumulative minimum temperature from January 1<sup>st</sup> and NO<sub>2</sub> for each year, the flowering peak and end date are seen to be influenced dominantly by the temperatures but also by NO<sub>2</sub> with an earlier peak and end of the flowering in places or at times with lower cumulative temperatures but higher NO<sub>2</sub> levels.

While the influence of temperature is visible throughout all the years, the severity of the effect of  $\text{NO}_2$  varies between years (**Figure 32**).



**Figure 31:** Simple regression scatterplots of flowering dates vs.  $\text{NO}_2$  levels

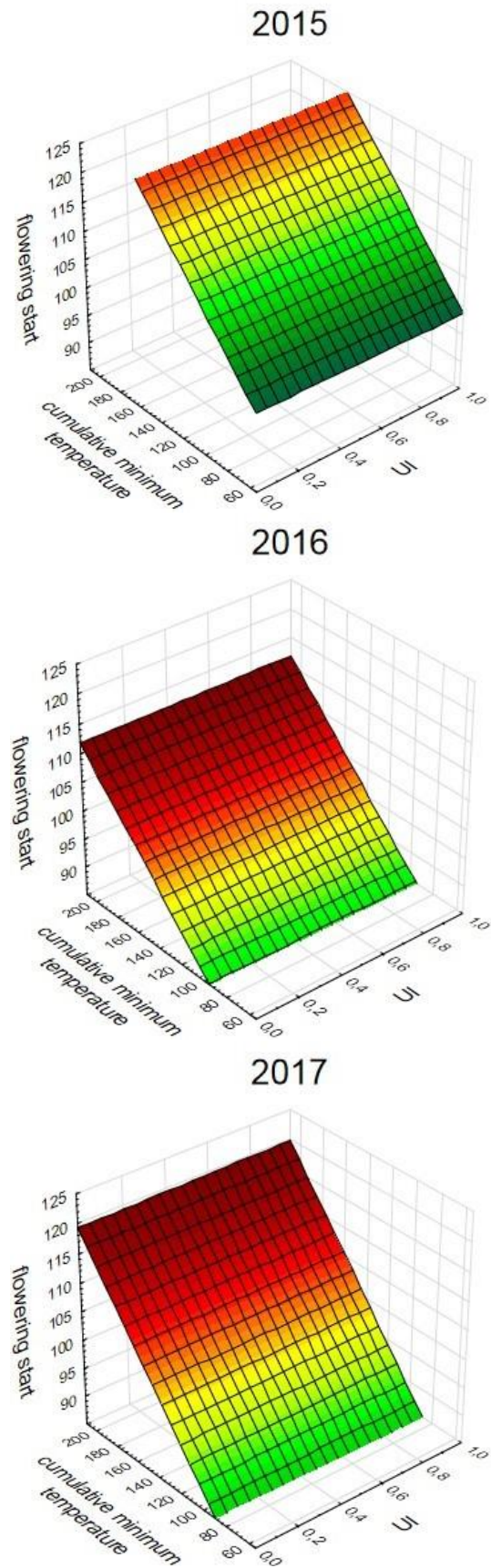


**Figure 32:** relations between flowering peak and flowering end with the cumulative minimum temperature and NO<sub>2</sub> per year

### D.3.3.3 Urbanity

In the stepwise backwards ridge regression, the urbanity index shows a significant correlation with the start of the flowering (**Figure 29**).

Also in 3D plots in combination with the cumulative minimum temperature from January 1<sup>st</sup> to the start of flowering for each year, the effect of urbanity on the flowering start is not visible (**Figure 33**).



**Figure 33:** relations between flowering start with the cumulative minimum temperature and urbanisation per year

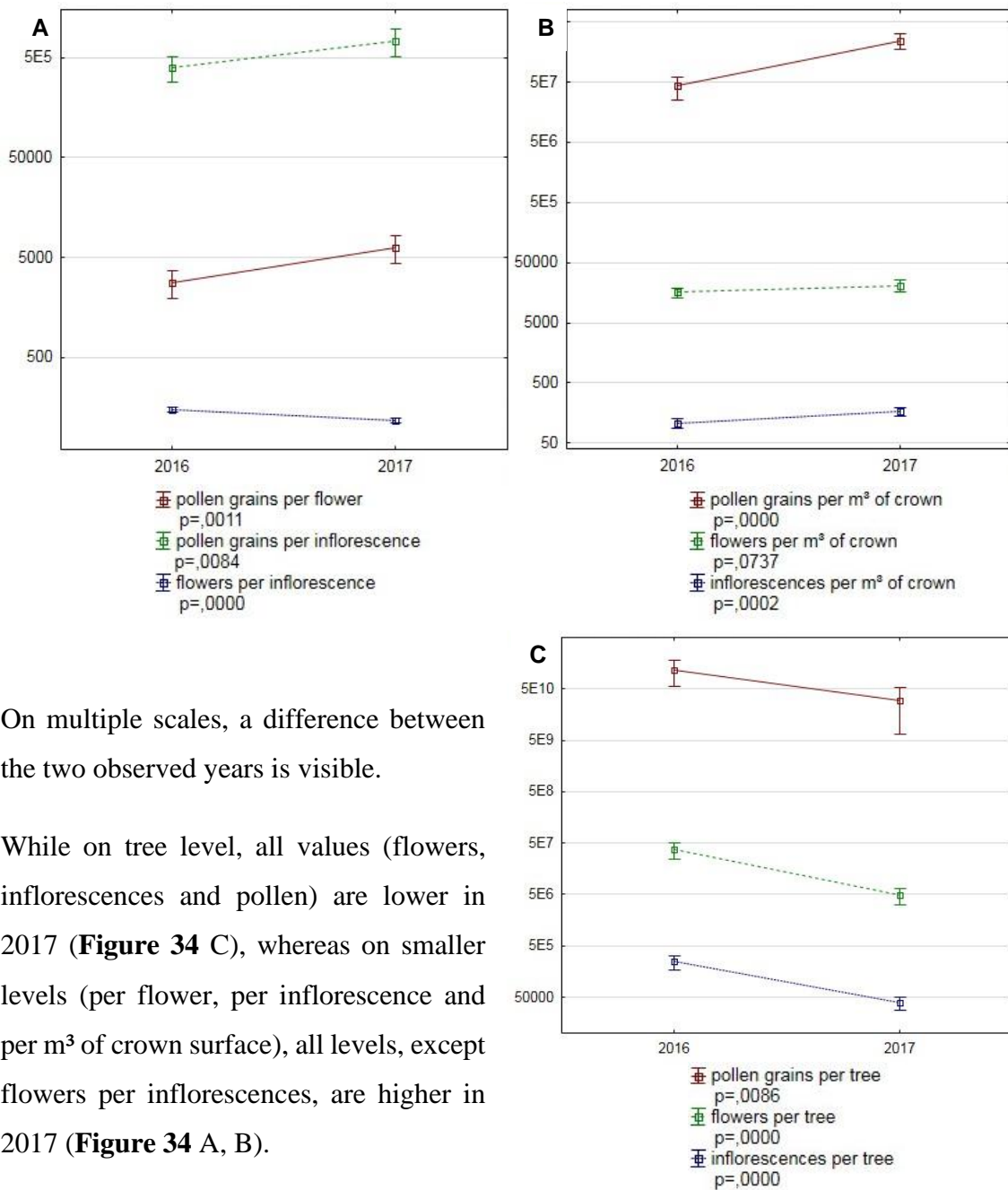
## D.4. Production of flowers, inflorescences and pollen of *Betula pendula*

The average numbers for the production of flowers, male inflorescences and pollen on different scales for an average of both observed years can be seen in **Table 10**.

**Table 10:** average numbers of produced pollen, flowers, and male inflorescences

<b>Pollen production attribute</b>	<b>Average number <math>\pm</math> standard deviation</b>
Pollen grains per flower	4.5 10 <sup>3</sup> $\pm$ 5.0 10 <sup>3</sup>
Pollen grains per male inflorescence	0.6 10 <sup>6</sup> $\pm$ 0.6 10 <sup>6</sup>
Pollen grains per m <sup>3</sup> of crown	0.1 10 <sup>9</sup> $\pm$ 0.2 10 <sup>9</sup>
Pollen grains per individual	30.6 10 <sup>9</sup> $\pm$ 13.2 10 <sup>9</sup>
Flowers per male inflorescences	135 $\pm$ 32
Flowers per m <sup>3</sup> of crown	18.6 10 <sup>3</sup> $\pm$ 12.3 10 <sup>3</sup>
Flowers per individual	4.4 10 <sup>6</sup> $\pm$ 30.6 10 <sup>6</sup>
Male inflorescences per m <sup>3</sup> of crown	140 $\pm$ 81
Male inflorescences per individual	100.2 10 <sup>3</sup> $\pm$ 181.1 10 <sup>3</sup>

### D.4.1 Differences between years



**Figure 34:** pollen-, flower- and inflorescence-production on all levels, comparison between years (Whiskers: mean  $\pm$  0,95 conf. interval)

## D.4.2 Influence of morphometric features

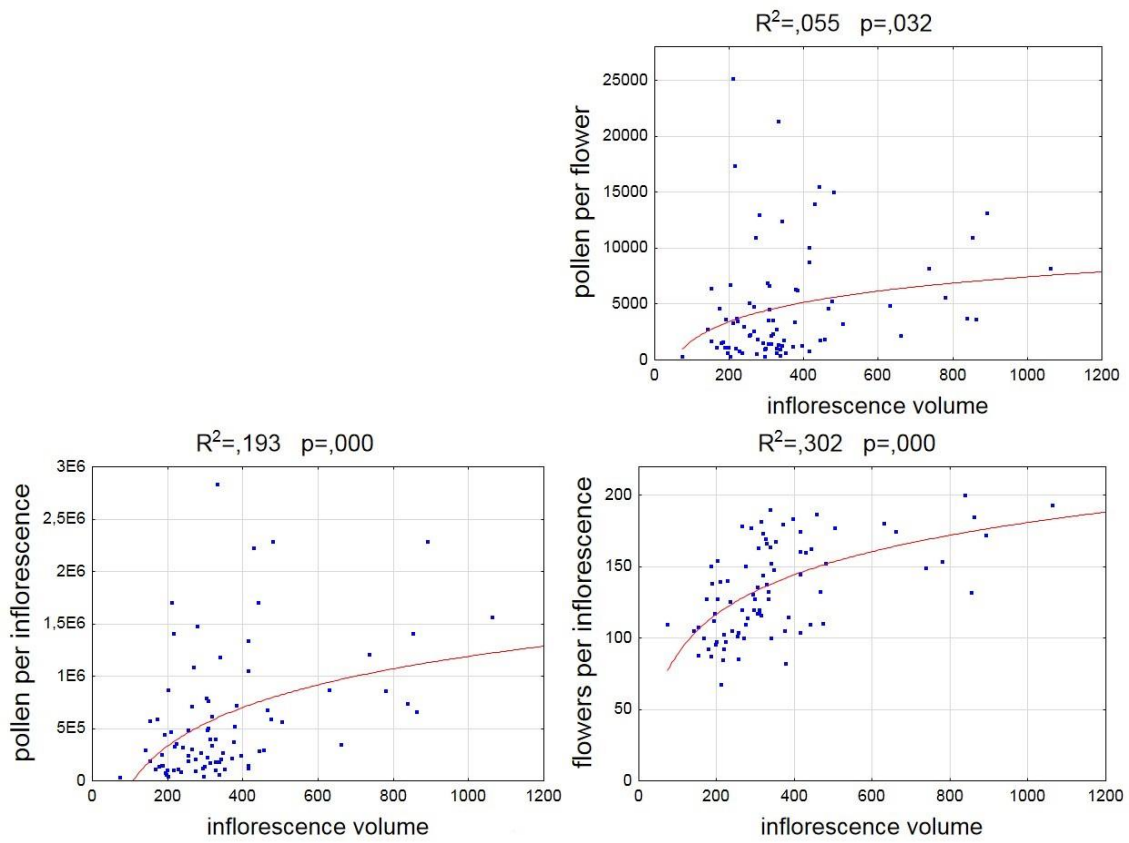
	U1	O <sub>3</sub>	NO <sub>2</sub>	soil water availability	soil nutrient availability	trunk perimeter	tree height	crown surface	inflorescence volume	cumulative temperature JJA previous year
inflorescence volume			0,005						n.a.	0,043
pollen grains per flower									0,004	0,000
pollen grains per inflorescence									0,000	0,000
flowers per inflorescence									0,000	0,000
pollen grains per m <sup>3</sup> of crown									n.a.	0,000
flowers per m <sup>3</sup> of crown			0,030						n.a.	0,091
inflorescences per m <sup>3</sup> of crown									n.a.	0,000
pollen grains per tree	0,023								n.a.	0,012
flowers per tree	0,071								n.a.	0,000
inflorescences per tree									n.a.	0,000

+	0.05 < p < 0.10
*	0.01 < p < 0.05
**	0.001 < p < 0.01
***	p < 0.001
n.a.	not applicable

**Figure 35:** significant correlation of pollen-, flower- and inflorescence-production in a backwards stepwise ridge regression

In all investigated morphological parameters (trunk perimeter, tree height, crown surface), there is no significant correlation seen with the production of pollen, flowers or inflorescences on any level. (**Figure 35**).

On the scale of the production per flower and inflorescence, also the inflorescence volume is taken into account as a morphological factor. This shows a significant correlation with pollen grains per flower and per inflorescence and flowers per inflorescence. So inflorescences with higher volume contain more pollen in total but also more flowers with more pollen per flower (**Figure 35**, **Figure 36**).



**Figure 36:** correlation between the inflorescence volume and pollen production per flower and per inflorescence and flower production per inflorescence

## D.4.3 Influence of environmental parameters

### D.4.3.1 Meteorology

**Table 11:** simple regressions of the production of flowers, inflorescences and pollen and the cumulative temperature of the previous summer (June - August)

	<b>Cumulative temperature June – August of previous year</b>
inflorescence volume	r = .2261 p = .039
Pollen grains per flower	n.s.
Pollen grains per inflorescence	n.s.
Flowers per inflorescence	r = .466 p = .000
Pollen grains per m <sup>3</sup> of crown	r = -.455 p = .000
Flowers per m <sup>3</sup> of crown	n.s.
Inflorescences per m <sup>3</sup> of crown	r = -.355 p = .000
Pollen grains per tree	r = -.331 p = .009
Flowers per tree	r = .548 p = .000
Inflorescences per tree	r = .578 p = .005

In the backwards stepwise ridge regression, the cumulative temperature of the previous summer (June, July, August) shows significant correlations for all factors. (**Figure 35**). This finding can be confirmed for most of the factors in simple regressions for each factor.

### D.4.3.2 Air quality

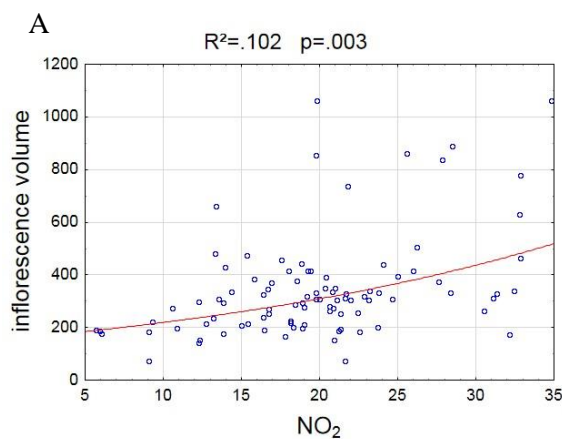
In the backwards stepwise Ridge regression, there is no correlation seen with O<sub>3</sub>, while NO<sub>2</sub> is correlated with the inflorescence volume and the number of flowers per m<sup>3</sup> of crown (**Figure 35**).

The correlation of NO<sub>2</sub> and the volume of the inflorescences is also significant in a simple regression (**Figure 37 A**). In a 3D plot, it can be seen that, compared to temperature, NO<sub>2</sub> is the stronger influence on the inflorescence volume (**Figure 37 B**).

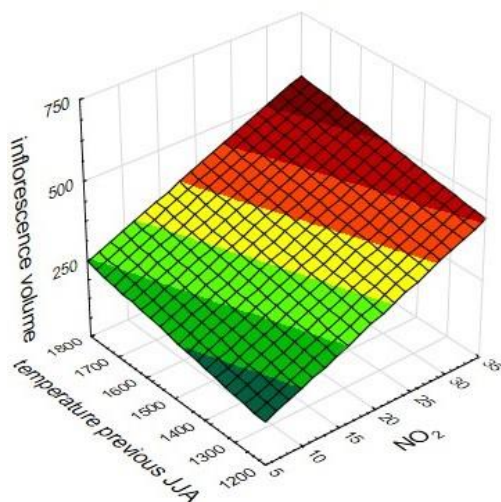
The correlation with flowers per m<sup>3</sup> of crown is not significant in a simple regression (**Figure 38 A**) but also here, NO<sub>2</sub> has a stronger influence on the amount of flowers per m<sup>3</sup> of crown, compared to temperature (**Figure 38 B**).

### D.4.3.3 Urbanity

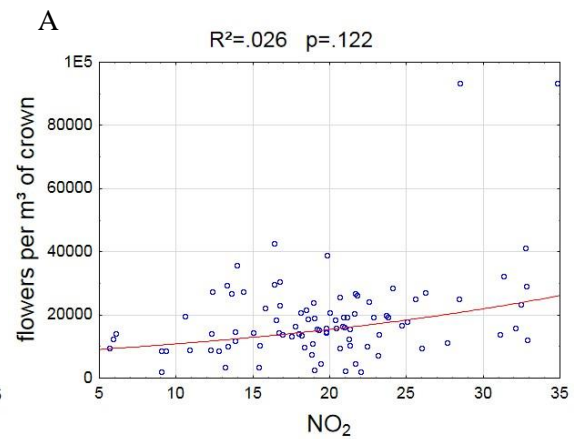
The urbanity index is correlating on a significant level with the amount of pollen grains per tree and the amount of flowers per tree in the backwards stepwise Ridge regression (**Figure 35**).



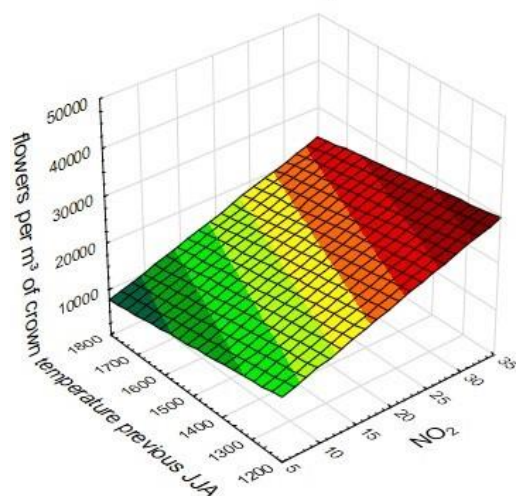
B  $R^2 = .152$   $p = .001$



**Figure 37:** correlation of A) inflorescence volume and NO<sub>2</sub> B) and temperature

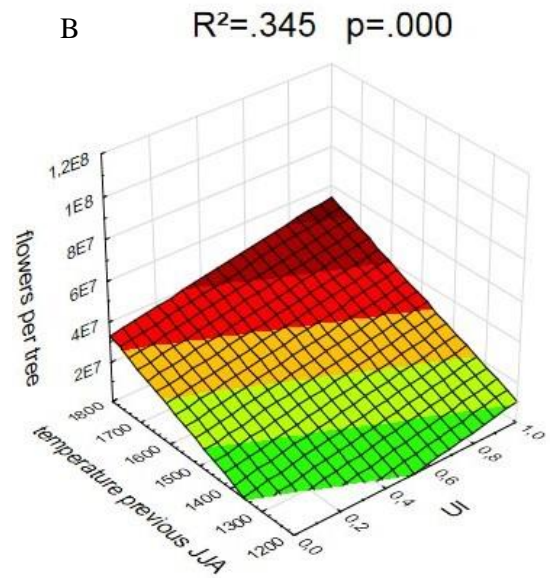
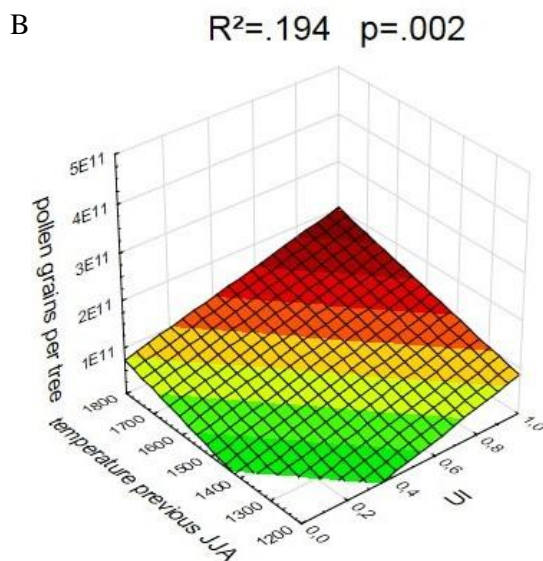
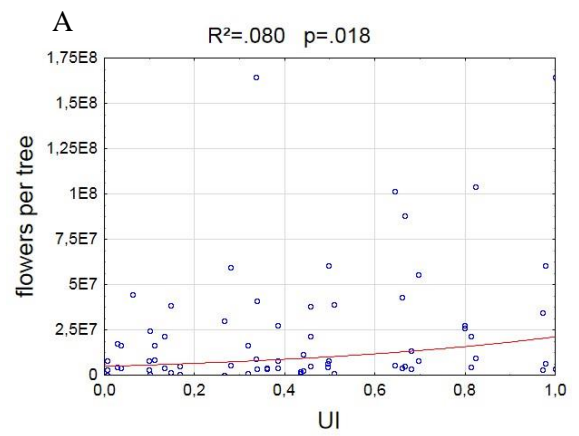
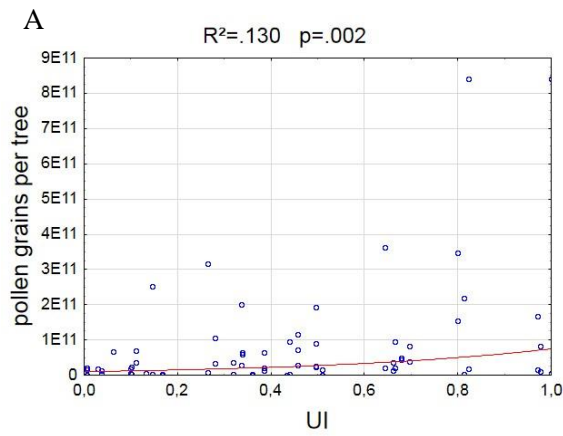


B  $R^2 = .100$   $p = .016$



**Figure 38:** correlation of A) inflorescence volume and NO<sub>2</sub> B) and temperature

In a simple regression, the correlation with pollen grains and flowers per tree is significant (**Figure 39 A, Figure 40 A**). In 3D plots, it can be seen that the urbanity index as well as the temperature both influence the amount of pollen grains and flowers per tree (**Figure 39 B, Figure 40 B**).



**Figure 39:** correlation of A) urbanity index and pollen production per tree B) and temperature

**Figure 40:** correlation of A) urbanity index and flower-production per tree B) and temperature

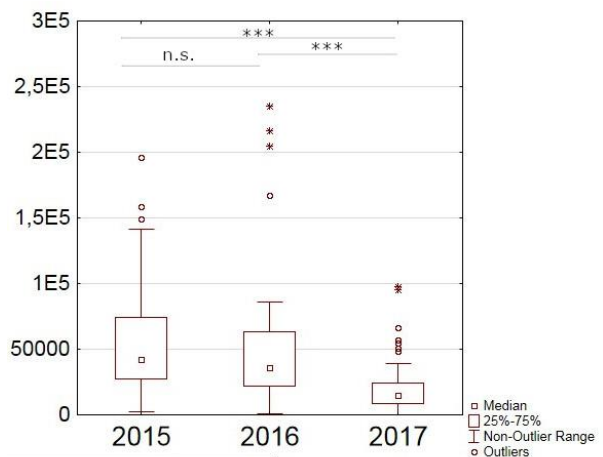
## D.5. Allergenicity of *Betula pendula* pollen

The average amount of Bet v 1 from all samples and all individuals for all years is 14,936.16 ng/ml (SD = 11,552.80), whereas the averaged Bet v 1 allergen content per inflorescence is 45,007.95 ng (SD = 45,966.37). As the pollen batches processed in the lab did not always consist of the same amount of inflorescences and, consequently, of allergen weight, here only results based on the allergen content per inflorescence are shown, so as to keep everything comparable.

### D.5.1 Difference between years

Also, the allergenicity of *Betula* pollen is different in the observed years.

For the amount of Bet v 1 per inflorescence, the average is  $56.3 \cdot 10^3$  ng/ml (SD= $45.6 \cdot 10^3$ ) in 2015,  $55.4 \cdot 10^3$  ng/ml (SD= $55.5 \cdot 10^3$ ) in 2016 and  $23.1 \cdot 10^3$  ng/ml (SD= $23.4 \cdot 10^3$ ) in 2017 (**Figure 41**)



**Figure 41:** amount of Bet v 1 (ng/ml) per inflorescence and per year

### D.5.2 Influence of morphometric features

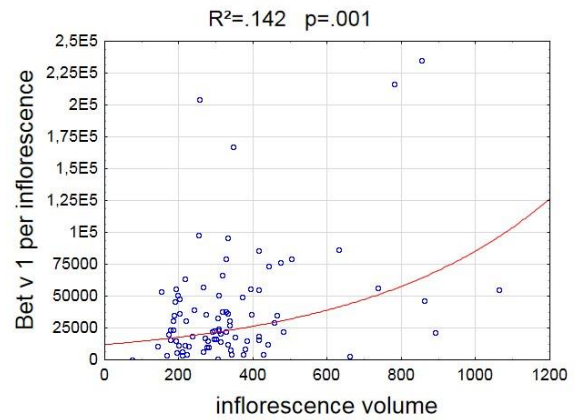
A backwards stepwise ridge regression showed a significant correlation of the amount of Bet v 1 per inflorescence only with urbanity index and inflorescence volume. All the other factors, like air pollutants, soil composition and morphometric traits, did not display any significant correlations (**Figure 42**).

	UI	O <sub>3</sub>	NO <sub>2</sub>	soil water availability	soil nutrient availability	trunk perimeter	tree height	crown surface	inflorescence volume	temperature 30 days before flowering
Bet v 1										
Bet v 1 per inflorescence	0,097								0,000	0,008

+	0.05 < p < 0.10
*	0.01 < p < 0.05
**	0.001 < p < 0.01
***	p < 0.001
n.a.	not applicable

**Figure 42:** significant correlation of allergenicity in a backwards stepwise ridge regression

For the concentration of Bet v 1 per inflorescence, a significant correlation can be seen with the volume of the inflorescence (**Figure 43**).

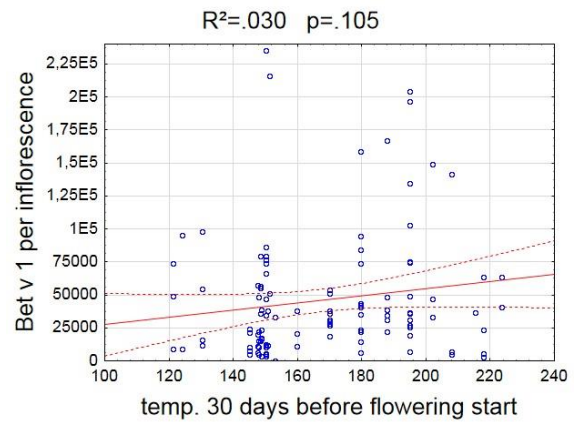


**Figure 43:** correlation of Bet v 1 per inflorescence and the inflorescence volume

## D.5.3 Influence of environmental parameters

### D.5.3.1 Meteorology

The cumulative temperature 30 days before the sample collection shows a significant correlation with the concentration of Bet v 1 per inflorescence. A simple regression does not show a significant correlation with the cumulative minimum temperature from January 1<sup>st</sup> until the start of flowering and therefore the sampling (**Figure 44**).



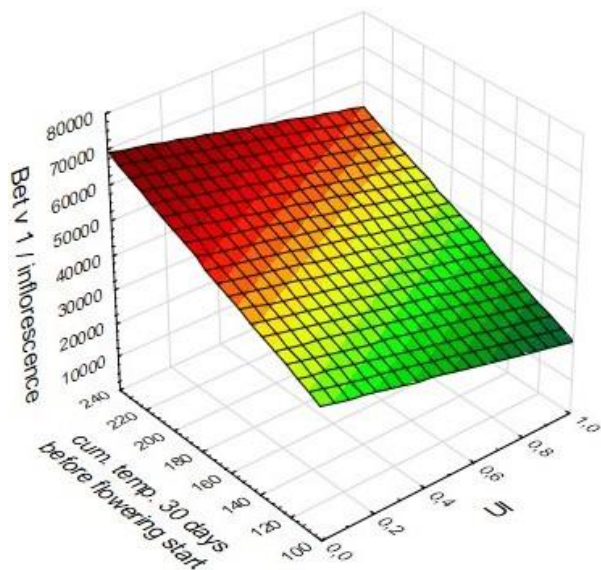
**Figure 44:** correlation of the concentration of Bet v 1 per inflorescence and the cumulative temperature 30 days before flowering

### D.5.3.2 Air quality

The backwards stepwise ridge regression did not show a significant correlation between the concentration of Bet v 1 per inflorescence and NO<sub>2</sub> or O<sub>3</sub> (**Figure 42**).

### D.5.3.3 Urbanity

The correlation between Bet v 1 per inflorescence and the urbanity index is significant in the backwards stepwise ridge regression (**Figure 42**). In a 3D plot, the relationship of Bet v 1 per inflorescence, the cumulative temperature 30 days before flowering and the urbanity index can be seen (**Figure 45**). This shows, that higher urbanity coincides with lower concentrations of Bet v 1 per inflorescence. As seen in the backwards stepwise Ridge regression, this effect is not as strong as the positive correlation of Bet v 1 per inflorescence with temperature.



**Figure 45:** correlation of the concentration of Bet v 1 per inflorescence and urbanity index

## D.6. Interactions

### D.6.1 Aerobiological measurements vs. phenological observations

The results of this Chapter D.6.1 are published in Kolek et al. (in review\_b).

When aerobiological measurements of *Betula* spp. pollen and phenological observations of *Betula pendula* are compared, it can be seen that the seasons are generally coinciding concerning their timing but show significant differences in details.

In 2015 and 2016, there were days on which pollen were measured in the air but none of the observed trees was flowering yet. And also there were days in all three observed years where there were trees observed flowering but the amount of pollen in the air was low compared to the number of flowering trees (**Figure 46**). When focusing only on the main season, flowering and pollen (5% - 95%), they both seemed to be overall coinciding, with full flowering and airborne pollen being both synchronised (**Table 12**). When examining, though, the whole calendar season, the percentage of days when pollen and flowering were coinciding ranged between 58.6% and 70.9% of the total duration of the respective seasons (**Table 12**).

**Table 12:** number of days with coinciding appearance of pollen and flowering, observed in main pollen and flowering season (5% - 95%) and the total season

	2015	2016	2017
<b>main season</b>	22 of 22 days (100%)	23 of 25 days (92.8%)	22 of 22 days (100 %)
<b>total season</b>	39 of 55 days (70.9%)	37 of 58 days (63.8%)	34 of 58 days (58.6%)

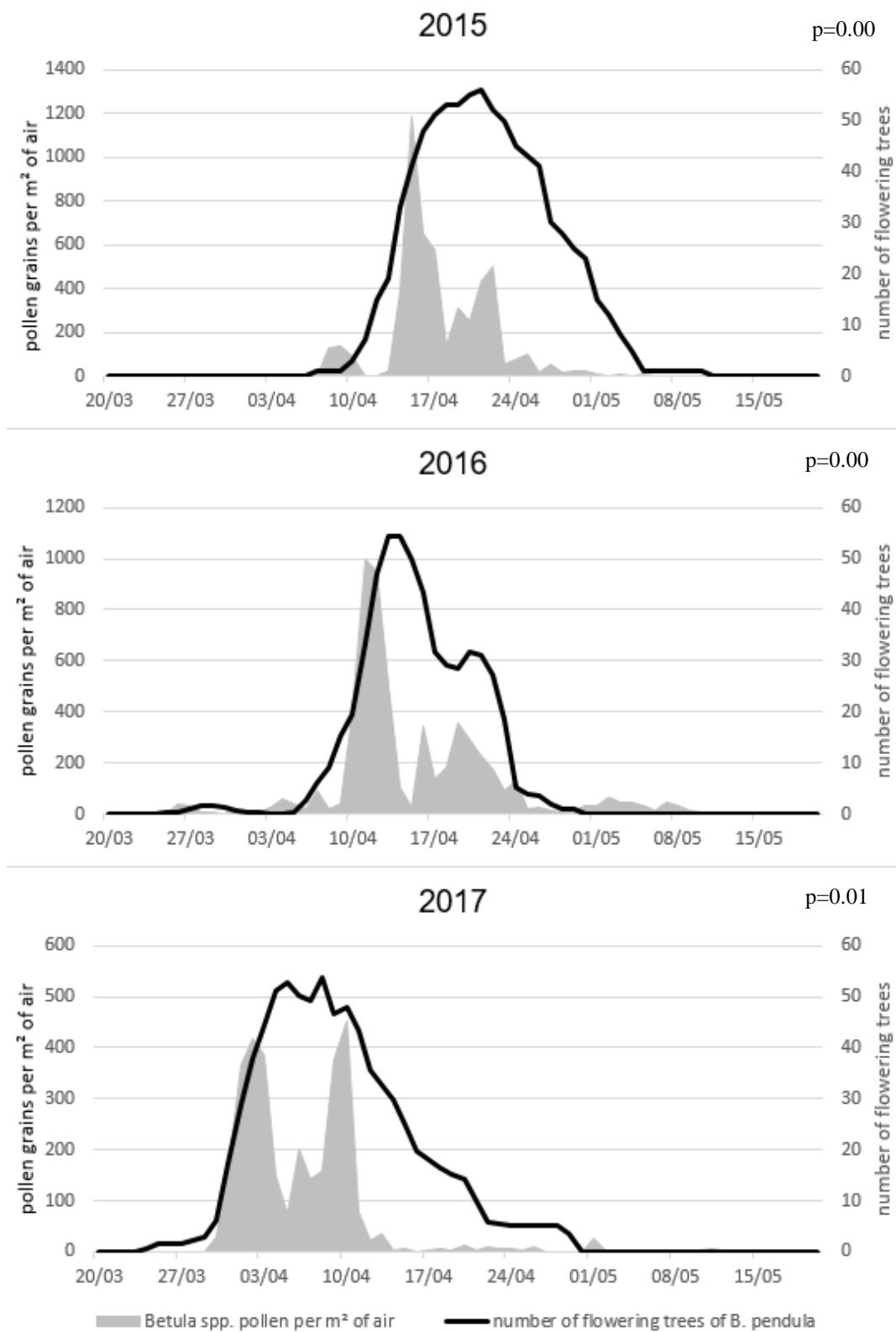
It can be seen that the seasons of pollen and flowering are shifted. To examine this shift, a cross correlation is performed for each year. This shows for all years, that the pollen can be measured later then the flowering is observed. In 2015, the day with the best correlation is day -5 ( $R^2=.293$ ), in 2016 and 2017, the day with the best correlation is day

-2 (2016:  $R^2=.644$ , 2017:  $R^2=.504$ ) (**Figure 47**). Overall, as seen in **Table 13**, even though pollen and flowering seasons roughly coincided, this varied among years from -7 to -12 days and to +3 to +4. Interestingly (**Table 13, Figure 47**) not only flowering could precede pollen season (2015, 2017), but also pollen season continued even after the end of flowering (2016) or still some individuals flowered even though no pollen was apparently captured from them (year 2015).

**Table 13:** lag effects (in days) of flowering seasons and pollen seasons (time-series analysis, cross-correlations)

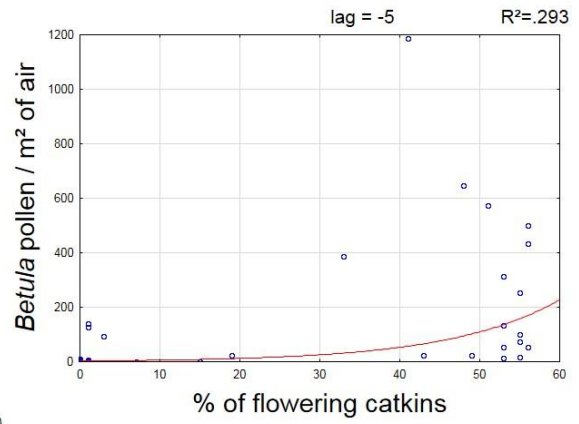
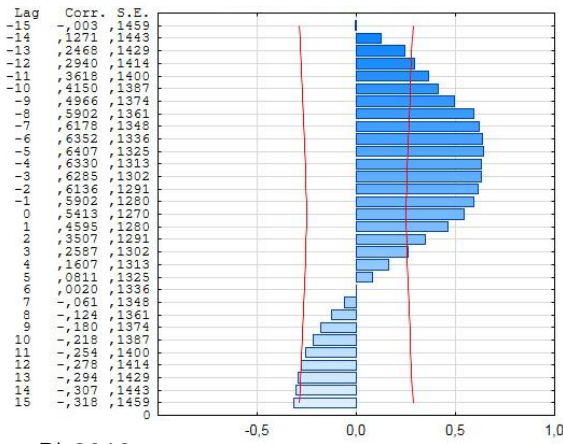
Minimum and maximum lag effects based on the range of significant ( $p < 0.05$ ) lag effects

	<b>minimum lag</b>	<b>maximum lag</b>	<b>highest lag effect</b>	<b><math>R^2</math></b>
<b>2015</b>	-12	+3	-5	0.293
<b>2016</b>	-7	+4	-2	0.644
<b>2017</b>	-10	+4	-2	0.504

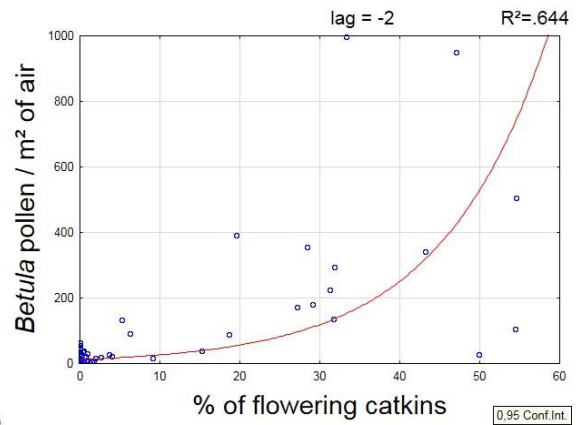
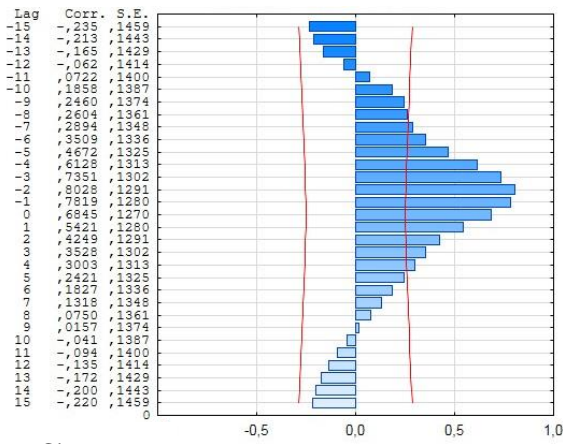


**Figure 46:** pollen season (*Betula*) and flowering season (*Betula pendula*), 2015 - 2017

A) 2015



B) 2016



C) 2017

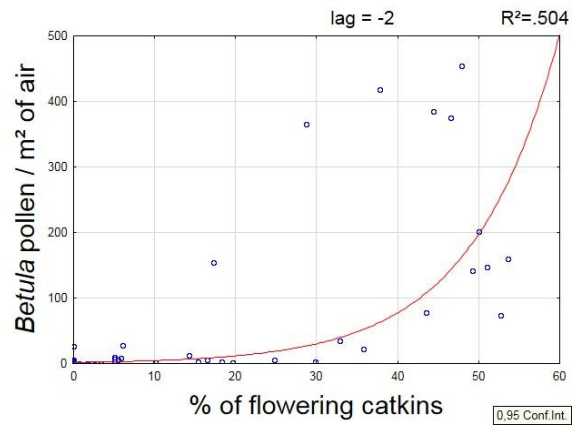
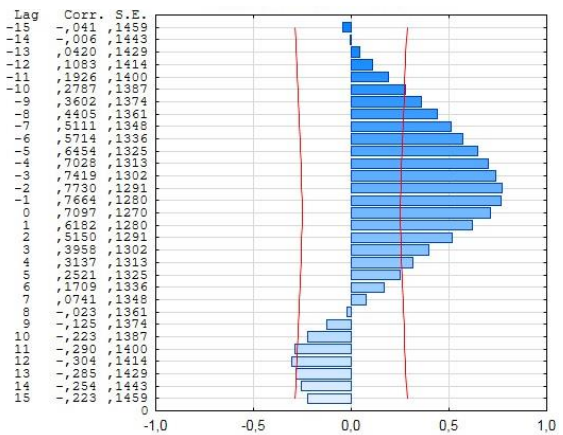


Figure 47: cross correlation of pollen vs. flowering and scatter plot of day with highest correlation

## D.6.2 Aerobiological measurements vs. production of flowering attributes

For the comparison between the pollen sum, calculated from the aerobiological measurements, and the production of pollen, flowers and inflorescences, differences between years were investigated.

As for the production of pollen, flowers, and inflorescences, the patterns of differences between years differ on different scales, there are two representing factors taken into account for the comparison to the pollen sum. The number of pollen grains per flower represents the highest values in 2017. The pattern for pollen grains per tree represents the pattern also seen in flowers and inflorescences per tree and flowers per inflorescence (**Figure 34**).

When the patterns are compared to the annual pollen sum of *Betula* pollen in Augsburg, it can be seen that a higher amount of pollen per year coincides with more flowers, inflorescences and pollen per individual tree, and more flowers per inflorescence (**Table 14**).

**Table 14:** relationships of annual pollen sum and production of pollen, flowers, and inflorescences based on significant Pearson correlation (**Figure 11**)

		pollen per flower	pollen per inflorescence	flowers per inflorescence	pollen per tree	flowers per tree	inflorescences per tree
annual pollen sum of <i>Betula</i> pollen	↑	—	—	↑	↑	↑	↑

### D.6.3 Phenological observations vs. production of flowering attributes

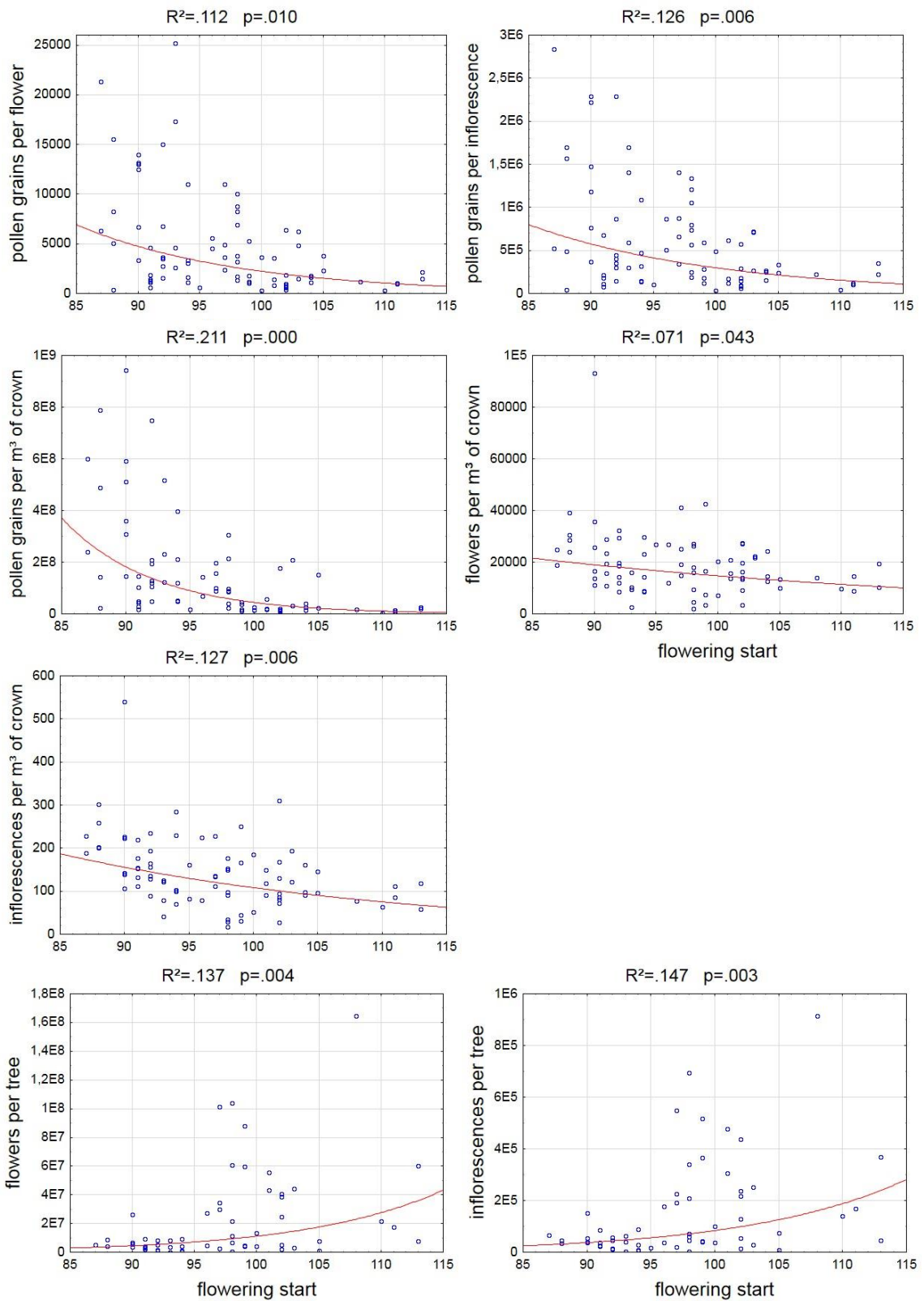
Statistically significant correlations between phenological attributes and the production of pollen, flowers, and inflorescences can be seen on multiple levels.

While the flowering start and flowering peak show significant correlations with 7 out of 9 aspects of the production of pollen, flowers, and inflorescences, the flowering end with 5 out of 9 and the duration with 1 out of 9 aspects (**Table 15**).

**Table 15:** significant correlations ( $p$ ) of phenological observations and the production of pollen, flowers, and inflorescences

	start	peak	end	duration
<b>pollen grains per flower</b>	$r = -.34$ $p = .010$	$r = -.35$ $p = .008$	$r = -.26$ $p = .048$	n.s.
<b>pollen grains per inflorescence</b>	$r = -.36$ $p = .006$	$r = -.38$ $p = .004$	$r = -.33$ $p = .012$	n.s.
<b>flowers per inflorescence</b>	n.s.	$r = .27$ $p = .041$	n.s.	$r = -.31$ $p = .019$
<b>pollen grains per m<sup>3</sup> of crown</b>	$r = -.46$ $p = .000$	$r = -.48$ $p = .000$	$r = -.38$ $p = .004$	n.s.
<b>flowers per m<sup>3</sup> of crown</b>	$r = -.27$ $p = .043$	n.s.	$r = -.33$ $p = .012$	n.s.
<b>inflorescences per m<sup>3</sup> of crown</b>	$r = -.36$ $p = .006$	$r = -.33$ $p = .010$	$r = -.33$ $p = .011$	n.s.
<b>pollen grains per tree</b>	n.s.	n.s.	n.s.	n.s.
<b>flowers per tree</b>	$r = .37$ $p = .004$	$r = .38$ $p = .003$	n.s.	n.s.
<b>inflorescences per tree</b>	$r = .38$ $p = .003$	$r = .40$ $p = .002$	n.s.	n.s.

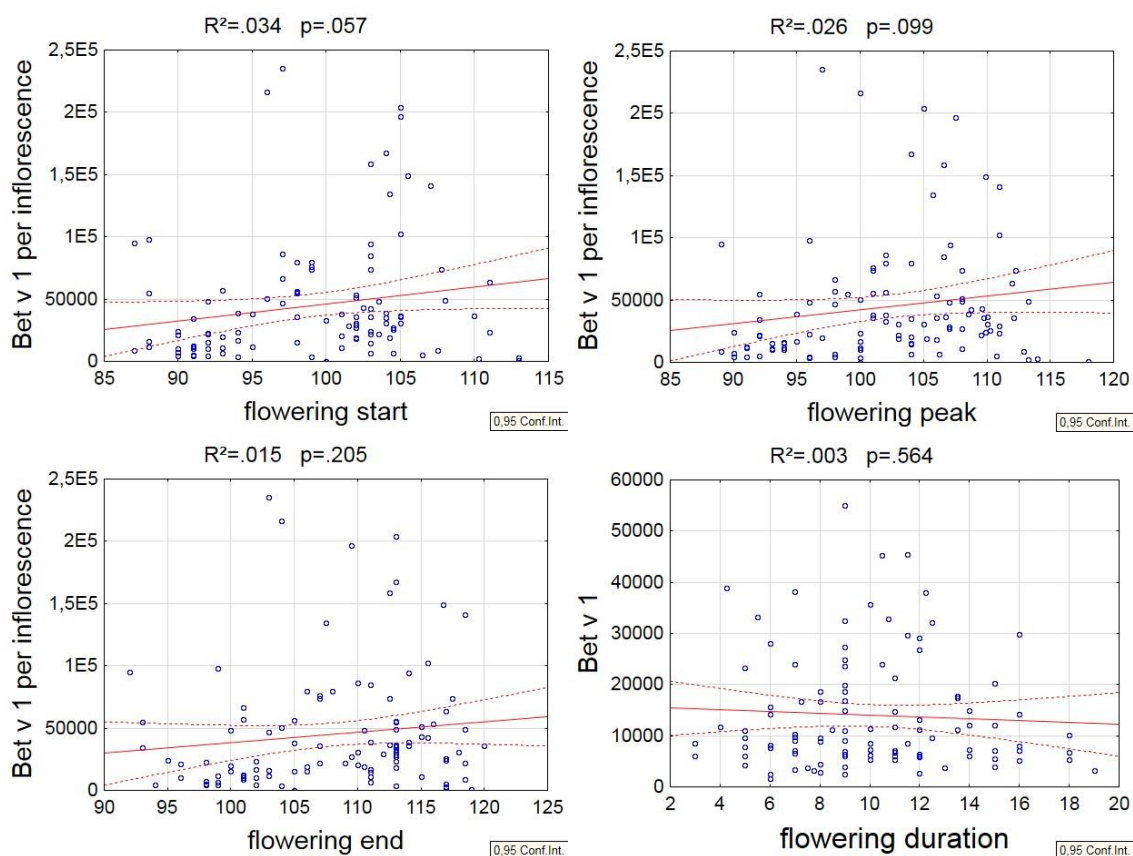
The relationships are different for different aspects of the production of pollen, flowers, and inflorescences. An earlier flowering start is correlated with lower amounts of flowers and inflorescences on tree level, and earlier flowering peak, likewise, results in fewer flowers per inflorescence (Figure 51). All other attributes, on lower levels (per flower, inflorescence and crown unit), are higher with an earlier start date (**Figure 48**).



**Figure 48:** correlations of statistically significant correlations of flowering start and the production of pollen, flowers, and inflorescences

## D.6.4 Phenological observations vs. allergenicity

For the timing of the flowering and the allergen content per inflorescence, no significant correlations were found, even though later flowering onset and peak correlated with higher allergen amounts at the significance level of  $p=0.10$ . (**Figure 49**).



**Figure 49:** correlations of Bet v 1 per inflorescence and the start, peak and end of flowering

## D.6.5 Production of flowering attributes vs. allergenicity

The correlation between the production of pollen and flowers per inflorescence and the allergen content per inflorescence were not proven significant in this study ( $p \leq 0.05$ ) (**Table 16**).

**Table 16:** correlations ( $p$ ) between the production of pollen, flowers, and inflorescences and the allergen content.

	<b>Bet v 1 per inflorescence</b>
<b>pollen grains per flower</b>	n.s.
<b>pollen grains per inflorescence</b>	n.s.
<b>flowers per inflorescence</b>	n.s.

### D.6.6 Overall connections

Finally, relationships among all the above-mentioned factors were investigated. To show these correlations in a comprehensive way, the changes of all factors are shown, based on a change in the annual sum of *Betula* pollen in timing and magnitude. In **Figure 50**, the changes are shown for the assumption of a higher number of pollen in the air and an earlier pollen season.

	phenology				production of pollen, flowers and inflorescences						morphology of inflorescences								
↑ / ↓	←	←	←	—	—	—	↑	↓	—	↓	↑	↑	↑	↑	—	↑	—	↑	↑
annual pollen sum of <i>Betula</i> pollen	flowering start	flowering peak	flowering end	flowering duration	pollen per flower	pollen per inflorescence	flowers per inflorescence	pollen per m <sup>3</sup> of crown	flowers per m <sup>3</sup> of crown	inflorescences per m <sup>3</sup> of crown	pollen per tree	flowers per tree	inflorescences per tree	Bet v 1 per catkin	inflorescence length	inflorescence diameter	inflorescence volume	inflorescence weight	g pollen / inflorescence

↑ higher      ← earlier      — no significant change  
 ↓ lower      → later

**Figure 50:** changes in observed parameters, based on an earlier *Betula* pollen season and higher pollen sum, based on Pearson correlation (**Figure 11**)

This shows that an earlier pollen season is coinciding with an earlier flowering start, peak and end but with no change in duration. In the production of flowers, inflorescences and pollen, changes in different directions can be seen in different levels. While a higher pollen season coincides with higher production of flowers, inflorescences and pollen per tree and flowers per inflorescence, the number per pollen and flowers per m<sup>3</sup> of crown is lower. In years with a higher amount of pollen in the air, also the concentration of Bet v 1 is elevated. Also, higher pollen annual pollen sums coincide with larger and heavier inflorescences with a higher weight of pollen per inflorescence.

As also the urbanity correlates with multiple factors. Here, it can be seen that flowering is happening earlier in urban environments but the flowering season is not extended. The production of flowers, inflorescences and pollen and the Bet v 1 content are not affected. The tree morphometric traits are smaller in urban environments and the size of the inflorescences is (Figure 51) larger.

urban environments / elevated NO <sub>2</sub> -levels	phenology				production of pollen, flowers and inflorescences			allergenicity	tree morphometrics	morphology of inflorescences												
	flowering start	flowering peak	flowering end	flowering duration	pollen per flower	pollen per inflorescence	flowers per inflorescence	pollen per m <sup>3</sup> of crown	flowers per m <sup>3</sup> of crown	inflorescences per m <sup>3</sup> of crown	pollen per tree	flowers per tree	inflorescences per tree	Bet v 1 per catkin	tree height	trunk perimeter	crown surface	inflorescence length	inflorescence diameter	inflorescence volume	inflorescence weight	g pollen / inflorescence
	←	←	←	←	—	—	—	—	—	—	—	—	—	—	↓	↓	↓	↑	↑	↑	—	—

↑ higher      ← earlier      — no significant change  
 ↓ lower      → later

**Figure 51:** changes in observed parameters, based on the changes between urban and rural environments, based on Pearson correlation (Figure 11)

**E**

## **Discussion**

## E.1. General findings

### E.1.1 Mapping of *Betula* trees in Augsburg

The mapping showed a clear majority of *Betula pendula* over the rest of the *Betula* species, as was also shown in neighbouring Munich by (Beck et al. 2016). As the mapping was performed just in settlement areas with a mostly artificial environment, it is expected that this spectrum is not entirely representative for the region. While *B. pendula* and *B. pubescens* are also found naturally grown in natural areas and brownfields within the city, *B. pendula* ssp. *youngii* and *B. utilis* are exclusively planted in the observed area. However, as *B. pendula* is well known to be the dominant *Betula* species in central Europe (Beck et al. 2016), it is expected to also represent the majority of *Betula*-trees in Augsburg. *Betula pubescens* is also resident in central Europe but prefers alkaline soils with waterlogging (Beck et al. 2016). Therefore, it is not likely to grow in settlement areas in high numbers but rather in wetlands and swamps. *Betula pendula* ssp. *youngii* is distinguished from the *Betula pendula* because it is a growth-restricted cultivar with a height of normally up to 5 m and mostly planted in gardens. *Betula utilis*, also a species that is not native to central Europe, is found in Augsburg too. This species is naturally resident from Afghanistan to China and prefers high-altitude regions (Bobrowski et al. 2017).

The distribution of the trees shows different patterns. While in the inner city and Hochfeld, there are relatively few individuals of all species, especially some tree-lined avenues, exclusively of *Betula pendula* and graveyards show high number of *Betula* trees in a small area. In future, it should be investigated, how these small-scale patterns influence the pollen distribution within the city.

For a better understanding of the *Betula* pollen season in Augsburg, it would also be necessary to investigate the occurrence of *Betula* in the surroundings of Augsburg. Due to the size of the area, it was not possible to perform this research in the frame of this work. Also, this information is not available from other sources.

It is planned to establish a digital cadastre of all trees on municipal ground in cities in Germany (deutsche Gartenamtsleiterkonferenz 2020). Even though this would help to investigate topics like this, individuals on private property would not be considered, which could bias the result.

### **E.1.2 Environmental factors**

In all measured values, the threshold of 40  $\mu\text{g}/\text{m}^3$  for  $\text{NO}_2$  (World Health Organisation 2006) are not exceeded. For  $\text{O}_3$ , the threshold of 100  $\mu\text{g}/\text{m}^3$  (World Health Organisation 2006) is exceeded in eight out of 129 cases. As the measurements are performed with a non-calibrated method, this result is not comparable to the official measurements.

$\text{NO}_2$  and  $\text{O}_3$  were negatively correlated as expected (Last et al. 1994; Helander et al. 1997; HEI Panel on the Health Effects of Traffic-Related Air Pollution 2010) on a significant ( $p \leq 0.05$ ) level.

The concentration of  $\text{NO}_2$  is negatively correlated with the tree height, trunk perimeter and crown surface and positively correlated with the volume of the inflorescence. This means, in areas with higher  $\text{NO}_2$  concentration that resemble especially areas with high traffic, trees tend to be smaller. In the frame of this dissertation, the reason for this difference cannot be ascertained. Reasons may be a lower nutrient or water availability due to sealing, soil compression or competition or pruning of the trees, or a combination of all. Trees with obvious pruning were excluded for sampling but still it cannot be ruled out that pruning was performed in the past. Regarding the positive association of  $\text{NO}_2$  with the size of inflorescences, there seems to be an evident physiological influence that boosts the catkin size. This was not published for *Betula* before but for *Corchorus olitorius*, showing a higher number of flower buds and flowers under elevated (50 ppb)  $\text{NO}_2$  conditions (Adam et al. 2008).

In areas with a higher urbanity, significant negative correlations with the trunk perimeter and the crown surface can be found. This coincides with the findings for  $\text{NO}_2$ , which is expected given the strong positive correlation of urbanity index and  $\text{NO}_2$ . The significant negative correlation of urbanity index and  $\text{O}_3$  is explained by the photoreaction of  $\text{NO}_x$

and CO (Warneck 2000) that is common in the troposphere and causes high O<sub>3</sub> concentrations in urban areas with elevated concentrations of NO<sub>x</sub> and CO (Sillman 2004).

One limitation of this dissertation is, that CO<sub>x</sub> could not be measured and therefore it is a missing important factor that has been reported to influence especially the phenology of plants (Menzel et al. 2006; Eller et al. 2020).

Also soil composition, nutrient availability and water availability have been reported to influence phenology and the production of pollen (Kramer and Boyer 1983; Lau and Stephenson 1994; Jochner et al. 2013; Luo et al. 2020). In the frame of this dissertation, these parameters could just be assessed via pedological maps but not on-site measurements. This weakens the reliability and strength of the influence of these factors in the analysis. It is still assumed that they may play a role in pollen production and allergenicity and flowering phenology attributes, nevertheless, the current findings cannot support further and more robust conclusions on the topic.

## E.2. Aerobiological monitoring in Augsburg

### E.2.1 Atmospheric pollen load

Depending on the regional vegetation, airborne pollen diversity is reported to consist of approximately 40 pollen taxa (García-Mozo 2017). In the frame of this dissertation, 43 taxa could be identified.

The diversity of pollen that was found is similar to that shown in previous publications from Germany (Melgar et al. 2012; Werchan et al. 2013). In comparison to Werchan et al. (2013), there is a much smaller amount of *Ambrosia artemisiifolia* pollen in Augsburg and due to the late start of the pollen measurements in Augsburg, seasons of *Corylus*, *Alnus*, *Salix* and *Ulmus* are incomplete and therefore under-represented. Nonetheless, currently the pollen monitoring station operates constantly in the north of Augsburg (at 48.38447 N 10.84329 E) and in the future there will be available information for the full biodiversity of all airborne pollen in the region.

Augsburg is located in the southeast of Germany in a temperate oceanic climate (Köppen climate classification Cfb; Temperate, without dry season, warm summer) and belongs to the continental biogeographical region (European Environment Agency). Therefore, the pollen season is limited by cold winters to warmer spring and summer months (Grinn-Gofroń and Mika 2008). The surroundings of Augsburg are influenced by three rivers and a system of channels with alluvial forests and species transported from the Alps and even the Mediterranean area by the rivers, large agricultural areas but also forests, including nature reserves that promote nature with high diversity and species richness with plants of continental, subalpine and sub-Mediterranean flora. (Hiemeyer 1978; Kolek et al. in review\_a)

While the natural vegetation of the area would be dominated by *Fagus sylvatica* and *Quercus robur* (Hiemeyer 1978; Bayerisches Staatsministerium für Ernährung, Landwirtschaft und Forsten 2013), the actual vegetation is dominated by *Pinus sylvestris*, *Quercus ruber*, *Tilia cordata*, *Fraxinus excelsior* and *Sorbus aucuparia* (Bayerisches Landesamt für Umwelt 2021).

Of the 14 most common pollen taxa (each representing over 0.5% of the total annual pollen sum, 94.81 % of the total annual pollen sum), four taxa are reported to be allergenic (*Betula*, Cupressaceae, Poaceae, Urticaceae) and additionally four taxa are cross-allergenic with allergenic pollen (*Carpinus*, *Fagus*, *Fraxinus*, *Quercus*). Allergenic and cross-allergenic taxa of the most common taxa sum up to 78% of the total annual pollen sum.

### **E.2.2 Seasonal atmospheric circulation of pollen**

As it has been reported before in Germany, the airborne pollen season occurs from early spring until autumn (Werchan et al. 2018). This is conclusive with the here presented findings, that the pollen season extends mainly from March to October. The highest peak occurs in April and May.

The observed season starts with *Corylus*, *Alnus*, *Salix* and *Ulmus* pollen that started flowering before the observation. The first observed peaks of the species that represent more than 0.5% of all observed pollen are *Populus*, *Fraxinus*, *Betula*, *Carpinus*, Cupressaceae, and *Picea*, which contribute mainly to the overall peak in April and May. The species that form the peak in summer are mainly *Quercus*, *Fagus*, *Pinus*, *Plantago*, Poaceae and Urticaceae.

The seasons of the different pollen taxa show that most of the seasons are peaked and short. Just the season of Poaceae and Urticaceae are longer than three months. This is due to the fact that Urticaceae are flowering twice per or continuously along the year (Fotiou et al. 2011) and the Poaceae is one of the most diverse family worldwide consisting of numerous species flowering at different times (Damialis and Konstantinou 2011). The different species of the Poaceae family also produce different amounts of pollen (Prieto-Baena et al. 2003). This explains the multi-peaked shape pollen season with different height of peaks. Also other taxa, as Cupressaceae, *Betula* and *Plantago* also are multi-peaked. In all cases, the reason is, as for Poaceae, different species that flower at different times.

In the average pollen seasons of 2015 – 2017, also other species (e.g. *Fraxinus*, *Carpinus*, *Quercus*, and *Pinus*) are multi-peaked but the reason is a shifted season in the different observed years with the season in 2015 occurring 2-4 weeks earlier in these species. This leads to multiple peaks in the average season. Longer-term observations will elucidate this issue.

The difference in timing and the height of the peak in different years could be caused by masting in woody plants (Ranta et al. 2005; Ishihara and Kikuzawa 2009; Fernández-Martínez et al. 2012) or drought stress (Schinko et al. 2021).

A limitation of this dissertation is that the pollen data was not assessed throughout the whole year. As taxa as *Corylus*, *Alnus*, *Salix* and *Ulmus* can start flowering already in the winter months especially in mild winters, it is necessary to perform pollen monitoring constantly to fully record all pollen seasons. This is particularly true in the frame of the ongoing climate change, when we see already significant shifts of various pollen seasons towards winter-time (Ziello et al. 2012b; Damialis et al. 2019b; Anderegg et al. 2021).

### **E.2.3 Daily atmospheric circulation of pollen**

It is known since decades that airborne pollen concentrations show daily patterns (Käpylä 1984; Galán et al. 1991; Latałowa et al. 2005). Especially for allergenic pollen, the circadian patterns are important to understand. Knowledge about when, in the course of a day, pollen concentrations may be higher or lower, allows allergic people to schedule their day and for example avoid outdoor activities or ventilation of their bedroom in times with high concentrations. Also forecasting models can be improved by knowing the circadian distribution of pollen.

To take into account the consistency of the pattern, not just the pollen sum but also the frequency of pollen occurrence were examined here per 2-hour time interval. If both parameters show a coinciding pattern, this signifies that a time interval with higher pollen sum is coinciding with a higher frequency of cases with pollen in the air. If the pattern is not coinciding and the frequency is low with a high pollen sum, this means that the high sum was caused by few or even single events with high pollen, because of particular

weather conditions or due to mowing or similar occasional events. Rain can influence the pollen concentration in the air by decreasing (Jato et al. 2002) or increasing (D'Amato et al. 2007b; Hughes et al. 2020) it for short time intervals. Also temperatures have an influence on the pollen release, dependent on the taxon (Jato et al. 2002). Such incidents can significantly affect the airborne pollen concentration, however, each should be examined as a separate case study.

The daily patterns of most observed pollen taxa show clear patterns with one or two peaks. Just *Fraxinus* shows an inconsistent pattern without clear peaks both in the sum of pollen and the frequency.

Most of the observed taxa (*Populus*, Cupressaceae, *Carpinus*, *Picea*, *Plantago*, Poaceae, *Fagus*, and Urticaceae) have their daily peak between 12:00 and 16:00 while other species (*Betula*, *Quercus*, and *Pinus*) have their peak in the night, between 20:00 and 02:00. So the pollen of all observed herbaceous taxa (*Plantago*, Poaceae, and Urticaceae) peak in the afternoon while woody species peak partly in the afternoon and partly at night. For *Betula* and *Fraxinus*, the pattern is multimodal and therefore shows more than one peak. While the pattern for *Fraxinus* is not showing clear peaks, *Betula* shows one peak at night and a smaller peak in the afternoon. The patterns of the pollen sum and the frequency coincide in half of taxa (Cupressaceae, *Picea*, *Plantago*, Poaceae, *Fagus*, Urticaceae), while in the other half, exclusively woody species, the patterns show discrepancies (*Populus*, *Betula*, *Fraxinus*, *Quercus*, *Pinus*).

There are no pollen-free time intervals for any of the common pollen types. One reason for this may be transportation of pollen and therefore deposition at different times of the day. The fact that pollen is in the atmosphere at all times of the day, is important especially in the case of allergenic pollen. For allergic people, it is important to know in which times of the day the pollen concentrations in the atmosphere are high to avoid unnecessarily high exposure to the allergen.

Diurnal patterns of pollen distribution were already shown for several pollen types (*Betula*: Mahura et al. (2009); Poaceae: Reddi et al. (1988), Norris-Hill (1999), Peel et al. (2014); various pollen: Lipiec et al. (2019) Grewling et al. (2016) Kolek et al. (in review\_a)) at various locations. Even though the diurnal patterns differ from site to site,

they all have in common that there are no intervals without pollen in the air. This can also be seen in this dissertation.

The fact that flowers are opening and closing at specific times of the day is known at least since in 1751, Carl Linnaeus published the *Horologium Florae*, a clock of diurnal flowering times for a variety of flowers (Linnaeus 1751). Despite this fact, pollen of all species still are recorded in the air throughout the whole day. One reason for this may be transportation of pollen and therefor deposition at different times of the day. The fact that pollen is in the atmosphere at all times of the day, is important especially in the case of allergenic pollen. For allergic people, it is important to know in which times of the day the pollen concentrations in the atmosphere are high to avoid unnecessarily high exposure to the allergen. As most flowers open during the day, it was a common belief for a long time that it is safe for pollen allergic people to open their windows at night. This advice was proven wrong for example by Grewling et al. (2016), who showed high pollen levels at night, and for Ambrosia even higher levels at night than at daytime (Grewling et al. 2016).

In the context of climate change, it is of great importance to continue observing these patterns. As climate change is reported to have an impact on the pollen season (Beggs 2004; Anderegg et al. 2021), there is a high probability that also the diurnal patterns of airborne pollen are influenced.

#### **E.2.4 *Betula* pollen season**

The *Betula* pollen season is short and highly peaked in all years as it is also described by Piotrowska (2008), who also shows a high seasonal variability between years, that can also be seen in the here presented data.

The pollen season shows consistent multi-modal patterns. In 2015 and 2016, there is a higher peak in the beginning of the season and a smaller peak few days after the first peak while in 2017, the two peaks are similar in size. This can be explained by different *Betula* species, emitting pollen at different times. The Organisation for Economic Cooperation and Development (2006) describe a difference of one week in the timing of flowering

between *Betula pendula* and *Betula pubescens* with *Betula pendula* flowering first. These are the two species that can be expected in higher numbers in the area around the study area (Beck et al. 2016).

The diurnal pattern of *Betula* pollen occurrence shows two peaks; one in the afternoon and one at night. This pattern was also described by other authors for *Betula* (Puc 2012; Grewling et al. 2016). While the peak in the afternoon is explained by the pollen release in the direct surrounding, the second one at night, with a high pollen sum but lower frequency could be explained by long distance transport and deposition of pollen. The phenomenon of high pollen levels at night due to deposition is described by (Grewling et al. 2016). Due to the complexity of the atmospheric movements and the lack of solid data on pollen dispersion and transport in the atmosphere, there is only limited research that clearly identifies and characterise long distance transport of pollen (Ghasemifard et al. 2020). To investigate the influence of pollen transport, further investigations about the temporal and spatial distribution are necessary.

The difference in the pollen load per year may be explained by differences in spring temperature. It is shown that this is the case for long term trends (Emberlin et al. 1997; Beggs 2004; Ariano et al. 2010; Ziska et al. 2019).

Another reason for differences between years may be the water availability in the summer before pollen release, when the inflorescences and therefore also the pollen are formed. In years with more precipitation in the previous summer, the pollen counts are higher than in years with lower precipitation. Another reason may be masting. Masting, the synchronised intermittent increased production of fruits or seeds of a plant population, is reported for various trees, also *Betula* (Silvertown 1980; Houle 1999).

### **E.2.5 Future prospects and new aerobiological methods**

Pollen measurements on different spatial and temporal scales are essential for the forecasting of pollen and the management of allergic diseases. Especially under ongoing climate change, it is important to have continuous measurements to detect changes in the diversity of species and their pattern in different temporal and spatial scales. The changes

in the diversity of species have been already reported (Rasmussen et al. 2017; Sikoparija et al. 2017) as well as the changes in airborne pollen seasons (Ziello et al. 2012b; Ziska et al. 2019).

A fine temporal resolution is required to also observe and understand short term changes in pollen concentrations as these can have severe impacts on human health, for example in the phenomenon of thunderstorm asthma; a short time interval with suddenly high amount of asthma cases, caused by thunderstorms and associated environmental and biological factors (D'Amato et al. 2007b; Straub et al. 2020).

Pollen is not just found on and near ground-level but also in higher levels of the atmosphere. For Augsburg, this is shown by comparison with a second measuring station in 12 m a.g.l. in Kolek et al. (in review\_a) and Rojo et al. (2019). While Rojo et al. (2019) states that the height of 12m a.g.l. shows minor differences to ground level, in Kolek et al. (in review\_a), just the annual total pollen sum is similar in ground level and higher level, for most pollen taxa, differences are found in severity as well as in the timing both seasonal and diurnal. While small pollen and pollen with high aerodynamics, for example of *Betula* and *Picea*, are more represented on rooftop level, larger pollen and pollen of herbaceous or entomophilous taxa as *Plantago*, *Salix* and *Carpinus* show higher concentrations on ground level. (Kolek et al. in review\_a)

But pollen can be found not just in heights, where plants grow and flower but also higher in the atmosphere. Already in 1923, Stakman et al. found pollen and different taxa of fungal spores at over 3,000 m height in Texas, in the United States of America (Stakman et al. 1923). Rempe (1937) found different taxa of pollen up to 2,000 m height in Göttingen, Germany, and Damialis et al. (2017) found different pollen and fungal spore taxa up to a height of 2,200 m in the region of Thessaloniki, Greece.

Information about pollen concentrations at different heights of the atmosphere on different time points are essential for high quality forecasts based on atmospheric patterns. Complex models, based on vertical and horizontal air movements and big scale atmospheric patterns can improve the forecasting of airborne pollen worldwide. This can give new information on the distribution and changes in vegetation and allow a better allergy management.

For frequently calculated high-quality forecasts, it is necessary to have in-time data from multiple stations. For pollen, this is not possible, using the traditional way of measuring. Instead, automated measuring systems with in-time analysis of the pollen are needed.

Up to today, several devices that are supposed to be able to deliver these data, are built and tested but there is no universally working and reliable device available yet. Existing devices need further development (Schiele et al. 2019) to be able to provide reliable data for dependable forecasting (Muzalyova et al. 2021).

The COST Action 18226 - New approaches in detection of pathogens and aeroallergens (ADOPT)<sup>33</sup> is currently comparing different techniques as image recognition, laser optics and eDNA and different devices to find the optimal automated pollen monitoring device, while the EUMETNET AutoPollen<sup>34</sup> programme is developing a concept for a European automated pollen monitoring network (Clot et al. 2020).

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<sup>33</sup> <https://www.cost.eu/actions/CA18226/>

<sup>34</sup> <https://www.eumetnet.eu/activities/miscellaneous/current-activities-mi/autopollen/>

### **E.3. Phenological observations of *Betula pendula***

As well as the pollen season, also the flowering is influenced by different factors as the year, temperature, NO<sub>2</sub> and urbanity. This high plasticity was shown before by various researchers for different influencing factors and plant species, for example *Corylus avellana* in Poland (Kasprzyk 2003), different species of *Cupressus* in Spain (Torrighiani Malaspina et al. 2007), different species of *Quercus* in Spain (Jato et al. 2002) and different taxa of Corylaceae, Cupressaceae, Fagaceae, Oleaceae, Pinaceae and Platanaceae in Greece (Damialis et al. 2020).

Investigations on pollen release on flowering trees showed that the release of pollen needs warm, dry and moderate windy conditions and is therefore normally happening around midday (Rempe 1937) and strongly influenced by the current weather conditions. Therefore, the flowering end dates and the flowering duration are strongly influenced by environmental conditions and especially the weather in the season on single days (Spano et al. 1999; Menzel and Fabian 1999; van Vliet et al. 2003). That leads to huge differences between different individuals. Because of the influence of short-term weather conditions, there is no clear correlation between the flowering season and the morphometric features as well as environmental factors to be seen.

Concerning influences of environmental factors, most research focuses on the relation of phenology and meteorological factors, especially temperature (Sparks et al. 2000; Menzel 2003; Torrighiani Malaspina et al. 2007; Galán et al. 2008; Laube et al. 2014). All performed studies conclude that higher temperatures lead to earlier flowering.

Temperature does not just influence phenology as a temporal aspect, but also on spatial scale, for example as an effect of urbanity (urban heat island effect). Also this has been shown to have an effect on phenology (Jochner et al. 2011; Jochner et al. 2012).

Also NO<sub>2</sub> levels have been reported to have an influence on plant phenology (Eller et al. 2020; Jochner et al. 2015) with an earlier flowering in times and places with elevated NO<sub>2</sub> levels. This coincides with the finding in this dissertation.

Also CO<sub>2</sub> is named as an influencing factor on phenology (Menzel et al. 2006). Unfortunately, CO<sub>2</sub> could not be measured in the frame of this dissertation but should be considered for future studies.

Also other spatial differences as different climatic regions (Schleip et al. 2009), water availability (Luo et al. 2020) and altitude (Ziello et al. 2009; Jochner et al. 2012; Cornelius et al. 2013; Schuster et al. 2014; Bucher et al. 2018; Damialis et al. 2020) are factors of interest in many studies. And also the concentration of air pollutants and nutrients can influence the phenology (Jochner et al. 2013; Luo et al. 2020).

Overall, there is undoubtedly a huge variety of environmental factors that may be affecting the phenology of flowering, as mentioned above but not only limited these parameters. Overall, regional studies, based on site-specific measurements aim to provide insight on the responsive ability of plants under differing environmental regimes. This plasticity (or the lack of it) would reveal the potential effect of climate change on the reproductive output of plants, here namely on flowering seasonality and pollen attributes.

In *Betula*, the flowering season is short (yearly average: 10-11 days (SD: 3 days)), what is also described in Piotrowska (2008). Per individual, 70-80% of the pollen per tree is released in two to three days, what explains this pattern and also, a high year-to-year variability is observed in this study. (Piotrowska 2008) Besides the high variability in start, peak and end of the flowering, the duration does not show a significant change between the years. The season therefore is not prolonged, just shifted, against commonly held misinformation that pollen seasons are extended, as they are usually considered as a whole and not in a detailed, per-taxon manner.

Even though the cluster analysis does not show consistent patterns between years, the backwards stepwise ridge regression shows significant correlations between the flowering start and the urbanity index, which even though a spatial factor, it reflects a wider spatial area masking out the micro-environment and relevant statistical noise. Likewise, it also shows significant correlations of flowering peak, end and duration with NO<sub>2</sub>, which is also a spatial variable, as its values did not significantly changed among the three years studied here, at least per annum.

The relationship with urbanity can also be seen in the here presented work for *Betula*. The significant correlation between the start of the flowering with the urbanity shows that the

urban heat island effect has an influence on flowering. The existence of an urban heat island effect in Augsburg is shown by Straub et al. (2019) and an influence of the *Betula* pollen season is described by Bogawski et al. (2019). Also, the cumulative temperature until the day of flowering is affected by urbanity, it is higher in urban areas. As Jochner et al. (2013) stated in a study performed in Munich, Germany, the individual temperature at different sites can explain up to 83.7% of the variance in onset dates while in the here presented data, the temperature can just explain 36.8% of the variance in the flowering onset date. These differences might be explained by the different characteristics of urbanity in both regions. While the urbanity in Augsburg is less marked, Munich is more urban.

The analysis is influenced by the collinearity of NO<sub>2</sub> and the urbanity index. To differentiate between the effects of these parameters, further investigation is needed.

Phenology is influenced by climate variabilities and the currently ongoing climate change. The flowering patterns are shifted while the length of the season does not show significant changes. This shifting is influenced, as it is shown in this dissertation, by temperature, NO<sub>2</sub> and urbanity; all factors that are changing right now and are expected to keep changing under ongoing climate change and urbanisation. This shifting is observed especially for species flowering early in spring (Spano et al. 1999; van Vliet et al. 2003; Ziello et al. 2012a; Geng et al. 2020; Menzel et al. 2020) and especially for wind-pollinated species (Ziello et al. 2012a).

Besides the traditional methods of phenological observations, new methods are proposed to ensure long-term data on small spatial and temporal scale. Especially remote sensing and satellite-based data processing are promising techniques but not able yet to provide information as detailed as traditional observations (Bogawski et al. 2019; Damialis et al. 2020).

For human health, a prediction of phenology would help to forecast the timing of pollen seasons of allergenic pollen to alert allergic people.

## **E.4. Production of flowers, inflorescences and pollen of *Betula pendula***

Information on the production of flowers, inflorescences and pollen has not been widely published and there is no standardised technique established.

The different levels of the production of pollen, flowers, and inflorescences are connected with each other but not changing to the same direction. The resources of a plant are limited and so also the production of different plant attributes. Therefore, it is standing to reason that not all observed factors increase or decrease at the same time. This can be seen in the comparison between years. The high variability between years is also described by Piotrowska (2008).

In 2016, the number of pollen per flower and per inflorescence were lower than in 2017, while the numbers of flowers per inflorescences showed the opposite. All parameters (pollen, flowers, and inflorescences) per m<sup>3</sup> of crown were lower in 2016, while all parameters per tree show the opposite pattern.

The reason for this pattern most likely is resource allocation. Dependent on the availability of nutrients, plants support different attributes of reproduction to ensure survival first, but also successful reproduction even at times or in places with low nutrient availability. Influencing factors as temperature, NO<sub>x</sub> and CO<sub>x</sub> are known, but the relation of these factors and the exact influence on different attributes of the plants are not fully understood yet (Bazzaz and Grace 1997). The resource allocation is described for *Betula* (Kaitaniemi and Ruohomäki 2003; Tuomi et al. 1982) but for shoots, buds and leaves, not for the flowering attributes and none of the research took into account environmental influences on resource availability and allocation.

On a small scale (pollen per flower, pollen per inflorescence and flowers per inflorescence), the volume of the inflorescences shows a consistent positive correlation. This means, larger inflorescences contain more flowers and also a higher number of pollen per flower. It is already known that environmental factors have an influence on the production of pollen, flowers and male inflorescences, as other studies on this topic showed before (Wan et al. 2002; Wayne et al. 2002; Ziska et al. 2003; Ladeau and Clark 2006; Rogers et al. 2006; Jato et al. 2007b; Damialis et al. 2011; Jochner et al. 2011; Zhao

et al. 2017b). Most prominent factors are temperature (Wan et al. 2002; Rogers et al. 2006; Jochner et al. 2011) and CO<sub>2</sub> (Wayne et al. 2002; Ziska et al. 2003; Ladeau and Clark 2006; Rogers et al. 2006) but also NO<sub>2</sub> (Zhao et al. 2017b).

This can also be seen to an extent in this dissertation. While temperature shows a significant correlation with all observed parameters of the production of flowers, inflorescences, and pollen, NO<sub>2</sub> and the coinciding urbanity index show significant correlations only in few of the observed factors.

Correlating the cumulative temperature of the previous summer, so the time, when the inflorescences, including the flowers and also the pollen are formed, with the different parameters of the production of flowers, inflorescences and pollen, different significant effects can be seen on different levels. From finer-scale to coarser-scale, specific factors, i.e. higher temperature, enhance the production of larger inflorescences and increased production of flowers within each inflorescence. At the same time, in terms of nutrients' equilibrium, fewer pollen are produced at the same fine levels. Going up a level, per crown unit, since the size of the tree obviously does not change, all attributes correlate negatively or not significantly. Going up, though, to the tree level, as defined by the source in the finer production scales, more flowers and inflorescences are produced, but not more pollen directly.

Practically, what was found in this study is that pollen at higher levels of production is increasing not as a result of rising pollen production, but as a consequence of increased biomass production, as defined here by larger inflorescences and more flowers per inflorescence. The same finding has been published for *Ambrosia artemisiifolia* in laboratory conditions (Wan et al. 2002; Wayne et al. 2002; Ziska et al. 2003; Ladeau and Clark 2006; Rogers et al. 2006; Jato et al. 2007b; Damialis et al. 2011; Jochner et al. 2011; Zhao et al. 2017b).

Having said the above and given that the volume of inflorescences correlates positively with NO<sub>2</sub>, one may easily comprehend how the interaction of multiple factors influences the responsive ability of the plants. The fact that there are no consistent patterns of a correlation of NO<sub>2</sub> and flowering attributes even though this was previously shown (Zhao et al. 2017b), may be explained by the moderate to low NO<sub>2</sub> concentrations in the study

area with no recorded exceeding of the threshold of  $40\mu\text{g}/\text{m}^3$  given by the WHO (World Health Organisation 2006).

Even though a correlation between urbanity and different pollen production levels was shown previously (Jochner et al. 2011), this was just seen for the production of pollen grains and flowers per tree in this dissertation. One reason for the different findings is that Jochner et al (2011) used a non-quantitative technique to calculate pollen production, which seems to be less robust than the here developed own fully quantitative method. Second, the weak relationship between urbanity and the production of pollen, flowers and inflorescences can be explained by the obviously low overall urbanity in the study area where the observed trees were located (average: 0.46, median: 0.39), in contrast to Jochner et al. (2011), who performed their work in urban Munich. Moreover, one ought to consider that urbanity is not affecting the plants on its own, but it is actually a proxy for a combination of environmental effects, like altered levels of pollutants and temperature, and changes in other aspects of climatology, hydrology and vegetation.

Overall, it can be seen that while temperature is correlated with all aspects of the production of flowering attributes, the observed environmental factors are correlated only on the large scale (production per  $\text{m}^3$  of crown and per tree) with the production of flowers, inflorescences and pollen. The small-scale aspects (production per flower and per inflorescence) are correlated with the size of the inflorescence. Similar results were shown also by Damialis et al. (2020) as evidenced from 13 plant taxa in a Mediterranean ecosystem, where also the inflorescence size were mostly determining the fine-scale levels of pollen and flower production. This highlights the consistency and robustness of the acquired results from this dissertation, as our findings seem to agree with completely different plant taxa, from a completely different location and associated environmental conditions and almost 20 years before.

Different micro-environmental factors as nutrients, soil and insolation (Oliveira et al. 1994; Fumanal et al. 2007; Martín-Benito et al. 2008; Giantomasi et al. 2009; Levanič et al. 2009; Suzuki and Suzuki 2009; Damialis et al. 2011) but also anthropogenic influences as fertilisation, watering or drainage (Hall et al. 1982; Lau and Stephenson 1994) were already reported for other species to influence the production of pollen, flowers or inflorescences. This points out the importance for further investigation of the environmental factors in the future.

The observed and here presented differences and coherences are important for the understanding of the influence of climate change aspects and recent developments like increasing pollution (including higher NO<sub>2</sub> levels) and worldwide urbanisation on plants and their adaptations. This influence of climate change is described by Ziello et al. (2012b). To investigate this effect, long term longitudinal studies are necessary.

Changes in pollen production specifically are important in the aspect of human health, as a higher amount of allergenic pollen in the air can lead to new or more sensitisations and stronger symptoms in sensitised individuals (Brito et al. 2011; Weger et al. 2011). Also the possibility to predict the severity of the upcoming pollen season months before the pollen is released, gives a powerful tool to properly predict the pollen season and help to inform allergic people about what to expect for the pollen season long before it starts.

## E.5. Allergenicity of *Betula pendula* pollen

Allergies are affected by climate change (Bergmann 2016), as has been recently reviewed by Damialis et al. (2019b). Therefore, it is among other aspects important to understand how the allergenicity of pollen is influenced by the environment in order to be able to predict how a changing environment alters the allergenicity of pollen.

To identify how the major *Betula* allergen Bet v 1 varies among different environmental regimes, and may be influenced by climate change, its production was calculated per ng/ml of aqueous pollen extract (APE) and was normalised for comparability reasons as production per inflorescence.

Bet v 1 concentrations show a huge variability between years with the highest average (2015: 22.4 10<sup>3</sup> ng/ml) being more than twice as high as the lowest average (2017: 9.5 10<sup>3</sup> ng/ml). The concentration per inflorescence shows similar patterns. An even higher variability is shown by Buters et al. (2008) with Bet v 1 values in one observation year (2003) 6.1 times higher than in the other year (2002) in Munich by using a different technique as presented here.

The concentration of Bet v 1 per ng/ml of APE is an abstract number without relevance for flowering attributes and no association whatsoever from the plant standpoint or in ecological terms. This is also shown by the fact that in the backwards stepwise Ridge regression, no correlation with any of the observed factors can be seen. Therefore, this clearly points out the necessity for normalisation and harmonisation of allergenicity values so as to be able to compare findings at a site-specific setup and to draw ecological conclusions on environmental interactions. Hence, thereafter, the discussion will focus on the concentration of Bet v 1 per inflorescence exclusively.

The Bet v 1 content per inflorescence is correlated with temperature, urbanity and the volume of the inflorescence but neither with air pollutants nor with soil conditions or tree morphometric attributes.

Previous studies showed both, negative (Helander et al. 1997; Beck et al. 2013) and positive (Hjelmroos et al. 1995) correlations of temperature and the concentration of *Betula* allergens. While Hjelmroos et al. and Helander et al. used different techniques to measure the allergen, Beck et al. used the same technique as in this dissertation. The here

presented results show a negative correlation with the concentration of allergen and the temperature 30 days before flowering but just significant in the backwards stepwise Ridge regression, not in a single regression. More study years and a better spatial resolution of the temperature data to depict also small scale differences in the future could establish clarification on this topic.

Even though the exact effects are not completely understood due to the complexity of and differences in environmental conditions, an influence of air pollutants and urbanity on the concentration of Bet v 1 is obvious. To better understand the processes, laboratory experiments in growth chambers as previously performed already for example for *Ambrosia artemisiifolia* (common ragweed) (Zhao et al. 2016) can be helpful. In this experiment higher allergenicity of *Ambrosia artemisiifolia* pollen under elevated NO<sub>2</sub> is found. A higher allergenicity of *Ambrosia artemisiifolia* pollen under conditions with higher CO<sub>2</sub> is shown as a more severe reaction in lung symptoms (Rauer et al. 2020).

The allergenicity of pollen is not just defined by the Bet v 1 content but also by other allergens and pollen-associated lipid mediators (PALMs) (Gilles et al. 2012; Beck et al. 2013). The concentrations of two PALMs, Prostaglandin E2 (PGE2) and Leukotriene B4 (LTB4) were also analysed in the frame of this work but did not show any meaningful results. Also this topic has to be further investigated to fully understand the allergenicity of *Betula* pollen and their effect on human health.

Another aspect, how pollen under different environmental conditions could have altered influence on human health is the microbiome on the pollen. This changes in urban environments with elevated NO<sub>2</sub> levels (Obersteiner et al. 2016). The impact on human health of this change in the microbiome is not investigated yet but suspected.

Also the topic of allergenicity is expected to show changes in changing environmental conditions and ongoing climate change. In this dissertation, it is found that temperature as well as urbanity correlate negatively with the concentration of Bet v 1 per inflorescence. And even if it is not shown in this dissertation, the already shown higher allergen content with higher NO<sub>2</sub> levels (Zhao et al. 2016) should be further investigated as NO<sub>2</sub> is expected to rise.

## E.6. Interactions

All the data collected and analysed here, were either of temporal nature (i.e. pollen and flowering season) or of quantitative nature (i.e. pollen and allergenicity production), despite the fact that all of them were in all cases quantified nonetheless. Therefore, it is meaningful to explicitly emphasise more on the coupled comparison of the above-mentioned groups of factors to highlight potential interaction effects. Ultimately, the interaction of all parameters is examined too.

As it is expected that the pollen in the air is reflected by the flowering season of the same species, the constant positive correlation of flowering and pollen abundance that can be seen is expected as it is described in Lattore (1997).

In this dissertation, it is found, that the seasons are not completely coinciding. When focusing only on the main season of flowering and pollen (5% - 95%), they both seemed to be overall coinciding, with full flowering and airborne pollen being both synchronised. When examining, though, the whole calendar season, the percentage of days when pollen and flowering were coinciding ranged between 58.6% and 70.9% of the total duration of the respective seasons. On the other days, just flowering or just pollen are observed. The cross correlation shows a consistent lag from the observation of flowering to the coinciding pollen measurement.

As the trees were randomly selected, they are considered as a representative sample of the flora in this area. Therefore, the assumption that pollen season and flowering season coincide, may be wrong. This effect was also described by Damialis et al. (2020), where out of 14 different anemophilous plant taxa, the majority showed consistent non-synchronisation between the flowering season and the pollen season. The reason for this non-synchronisation may be long-distance transport of pollen (Damialis et al. 2007; Damialis et al. 2020). Also the fact that in 2015 and 2016, pollen can be measured before the observed trees started flowering, can be explained by pollen transported from an area with earlier flowering.

This long-distant transport is described by several researchers already (Solomon 1978; Damialis et al. 2005; Ghasemifard et al. 2020; Menzel et al. 2021) as an important influence on pollen concentrations caused by wind direction, speed and persistence.

Therefore, it is possible to determine the origin of the transported pollen, for example by using HYSPLIT<sup>35</sup> back trajectories (Stein et al. 2015; Menzel et al. 2021). But due to missing comprehensive spatial real-time pollen data, this effect cannot regularly be considered yet for pollen forecasting models. A first attempt for a pollen forecast that also takes into account the transport of the pollen is found in the frame of the System for Integrated modelling of atmospheric composition (SILAM)<sup>36</sup> (Sofiev et al. 2013), that is currently developed to work on data from automated pollen monitors (Sofiev 2019).

Another reason for the differences between pollen season and flowering is that while the pollen concentration in the air is measured on genus level (*Betula* spp.), all phenologically observed individuals refer explicitly to *Betula pendula*. As there are also other species of *Betula* in the area (**Figure 12, Table 6**), a difference in the season with multiple peaks in the pollen season due to different species is expected (Beck et al. 2016).

As the seasonality of airborne pollen and flowering of the same taxa do not fully coincide, they will not be influenced by environmental factors in the same way. While the observation of flowering plants shows strictly the local and current exposure to pollen, taking into account small scale changes in the direct environment of the plants (exposure to pollutants, nutrients, water, temperature), the observation of airborne pollen also takes into account transported pollen from more distant sources and therefore represents the pollen load in the ambient air on a larger spatial scale.

The here shown sensitivity to environmental influences implies the sensitivity of phenology also to climate change. This sensitivity is shown by the variance between years and supported by the correlation of flowering and NO<sub>2</sub> as well as urbanity. To further observe and fully understand these changes, long-term longitudinal studies are necessary.

This knowledge will also help to model and forecast both pollen and flowering season and, in case of allergenic species, alert allergic people before the pollen season even starts.

The relation between the aerobiological measurements of *Betula* pollen in the air and the production of pollen, flowers, and inflorescences is important because an estimation for

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<sup>35</sup> <https://ready.noaa.gov/HYSPLIT.php>

<sup>36</sup> <https://silam.fmi.fi/pollen.html>

the severity of the pollen season by factors that can be assessed already months before the pollen season starts, would be an important tool for pollen forecasting.

In this dissertation, the pollen load correlates significantly and positively with the number of flowers per inflorescence and all parameters (pollen, flowers and inflorescences) per tree, while pollen and inflorescences show a negative correlation. Only two other studies are published, that investigated the relationships between the production of flowering attributes and pollen of *Betula*. While both studies also found that a higher number of *Betula* pollen coincides, and Jato et al. (2007c) also found coinciding results for the other parameters per tree as well as the number of flowers per inflorescence, Jato et al. also show one result that is not coinciding with the here presented findings. Our findings show a negative correlation with pollen per m<sup>3</sup> of crown, whereas Jato et al. (2007c) found a positive correlation of the same parameters (**Table 17**). Nonetheless, the study by Jato et al. (2007c) was based on only six individuals to assess pollen production and with a low replicate sample size, whereas the flowering phenology was only performed qualitatively, with an arbitrary definition of the flowering onset (as the date of the first observed male inflorescence flowering, with an observation frequency of approximately once per week).

**Table 17:** comparison of the production of flowers, inflorescences and pollen in *Betula* with the annual pollen sum from different studies

annual pollen sum of <i>Betula</i> pollen	pollen per flower	pollen per inflorescence	flowers per inflorescence	pollen per m <sup>3</sup> of crown	flowers per m <sup>3</sup> of crown	inflorescences per m <sup>3</sup> of crown	pollen per tree	flowers per tree	inflorescences per tree
↑	–	–	↑	↓	–	↓	↑	↑	↑
<b>Jato et al. 2007c</b> <i>B. pendula, B. alba</i>	↑	↑	↑	↑	n.a.	n.a.	↑	n.a.	↑
<b>Ranta et al. 2008</b> <i>B. pendula, B. pubescens</i>	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	↑

The strong differences between different years in *Betula* pollen seasons and the fact that the production of flowering attributes correlates with temperature on all levels and with NO<sub>2</sub> and urbanity in some aspects on large scale suggest the assumption that also these factors and relationships will be affected by ongoing climate change and urbanisation. On the finer scale, a positive correlation with the size of the inflorescences is seen. As the

size of the inflorescences is positively correlated with NO<sub>2</sub>, also the small-scale parameters are affected by these changes indirectly.

A longer time series of data can help to identify the best correlated factor of pollen-, flower-, or inflorescence-production with the severity of the pollen season for an improved forecasting of the pollen season.

Timing of flowering start and the pollen-, flower-, and inflorescence-production correlate on various levels. Trees with an earlier flowering start show significantly more flowers and inflorescences per tree but lower production of all factors on all other levels besides flowers per inflorescence which is not significant. There were no other comparable studies published on this topic for *Betula* or any other anemophilous plant on a small spatial scale as in this dissertation without the influence of different macroclimates. So, as an assumption, an earlier flowering onset and peak, favoured by weather conditions, inhibit the continuation of production of flowers and inflorescences, and indirectly of more pollen per tree: trees seem to be producing new inflorescences until last minute before the onset of flowering and this may cause the inverse relationship between flowering onset and pollen/flower production.

An earlier start date is associated with higher amounts of pollen, flowers and inflorescences on all levels but per individual and flowers per inflorescence. On tree level, an earlier flowering start is correlated with a higher amount of flowers and pollen.

This means that under ongoing climate change with rising spring temperatures and therefor a shift of the flowering season towards an earlier start, will lead to a higher production of flowers and inflorescences per tree. As it could be shown that there is a strong correlation of the amount of pollen per tree with the amount of flowers per tree and the amount of inflorescences per tree, it can be assumed that the amount of pollen per tree might also increase, even though this effect cannot be seen on a significant level in this dissertation. Long term studies are required to fully understand and quantify this effect. Due to the higher temperatures in urban environments, this effect will be even more pronounced here.

A possibly higher number of allergenic pollen per tree would mean a higher total amount of pollen in the atmosphere and could lead to more severe health effects for allergic patients in the future.

The positive correlation of Bet v 1 per inflorescence and the amount of pollen per tree means that, when there are more pollen grains per tree, the concentration of Bet v 1 per inflorescence is higher. As concluded before, the amount of pollen per tree might increase with earlier flowering and therefore when spring temperatures rise for example under ongoing climate change.

This would mean that in this case, there would not just be more pollen in the air but also more allergen per inflorescence. And also this effect would be enhanced in urban areas.

As previously discussed, the aerobiological measurements of *Betula* pollen show significant correlation with many of the observed factors.

Even though also influenced by other factors, the timing of the flowering season coincides with the timing of the pollen season. Higher pollen sums in the air are reflected in larger inflorescences and higher production per tree while per m<sup>3</sup> of crown, levels of pollen-, flower-, and inflorescence-production are lower at the same time, potentially due to resource allocation. The concentration of Bet v 1 per inflorescence is higher in years with higher pollen sums in the air and on trees with more inflorescences.

In urban environments, some of the observed aspects are different from rural environments. Trees are larger, flowering happens earlier and there is less pollen per flower and inflorescence and less flowers, inflorescences and pollen per surface unit. The concentration of Bet v 1 and the morphology of inflorescences seems not to be directly affected by urbanity.

But also indirect effects could be shown in this dissertation. For example, a higher amount of pollen is associated with higher urbanity. Also, this factor is correlating with a higher amount of flowers and inflorescences per tree which again coincide with higher temperatures. More pollen per tree also coincide with a higher amount of Bet v 1 per inflorescence. So even though Bet v 1 does not show a significant correlation directly with urbanisation in the Pearson correlation, it might still be connected indirectly.

To also be able to see connections and correlations that may be not visible in this dissertation, long term studies with fine temporal and spatial resolution are required. And also further aspects as other pollutants, especially CO<sub>2</sub>, more detailed information about nutrient and water availability and the atmospheric movement of pollen including distant

pollen sources and dispersion mechanisms. Also more study regions would help to better understand the mechanisms. Even though Augsburg has urban structures and for example the urban heat island effect is reported for the city (Bosch 2020), the differences towards rural areas are rather small, concerning temperature, pollutants and further effects of urbanity.

The need for these studies is shown by the main environmental parameters that influence the observed parameters; temperature, urbanity and NO<sub>2</sub>. All these factors are currently changing and are expected to further change with ongoing climate change and worldwide urbanisation.

**F**

**Conclusion and future  
prospects**

This thesis attempts to analyse and evaluate the complex biological and environmental system of the plant reproductive process, pollen production and dispersion, airborne pollen transport (including deposition) and all the factors that may influence this procedure, spatially and temporally, at a multi-resolution approach, from diurnal to inter-annual variability. The above are particularly considered in an international context and in a framework of an ongoing climate crisis and associated human health impacts, namely allergic diseases.

Every presented factor is interconnected with a big variety of other factors that vary in space and time and that are again influenced by other factors themselves. Moreover, in many cases, influences are not unilateral. This complexity makes it difficult to fully understand single processes and apply theoretical knowledge to real-life situations.

Research on this topic can just be performed in a conclusive way by combining different research disciplines, especially geography, biology, meteorology and health sciences and analysing data on different temporal and spatial scales too.

Especially in respect to climate change, long-term aerobiological datasets are a valuable proxy on a regional scale (Ziello et al. 2012b). The timeframe in the here presented research is of course too short to show climate change effects but the different environmental conditions in the urban areas reflect conditions that are resembling the ones expected under climate change in combination with urbanisation and industrialisation, like higher temperatures and higher pollutants (Sofiev et al. 2009; Voltolini et al. 2000).

Other relevant factors that can influence the occurrence and seasonality of pollen are water availability and eutrophication (Damialis et al. 2019b).

Of course, the influences on the aerobiological data are caused by changes in the vegetation and the production of pollen (Parmesan and Yohe 2003; Menzel et al. 2006; Damialis et al. 2011). Therefore, phenological observations and the estimation of pollen production are valuable proxies for these changes too with the advantage that this data is available even before the flowering of a season actually started; samples for the estimation of the pollen production can be taken and analysed weeks before the flowering for species that develop male inflorescences already in the previous year like *Betula* and *Corylus* and phenological observations before flowering itself already help to estimate the time point

of the start of the flowering. This can help to improve forecasting models and thereby help allergic patients to manage their symptoms in an anticipatory way.

It was shown before that pollen production is positively influenced by higher temperatures, a more urban environment, lower elevations and microclimatic conditions as an exposure of the site to the south (Ziska et al. 2003; Menzel et al. 2006; Buters et al. 2008; Fotiou et al. 2011; Damialis et al. 2011). Pollutants can be responsible for a higher bio mass production in general, which can also cause a higher production of pollen, flowers, and inflorescences (Ziska et al. 2003; Damialis et al. 2019b). With an advancing urbanisation worldwide and high pollutant levels in many urban environments, this process will get even stronger.

The changing climate also effects the human health, in multiple direct and indirect ways. Together with the effects of industrialisation and urbanisation, the human body, especially the immune system, is challenged with new, emerging conditions. This can be seen for example in the rising number of allergic people and stronger symptoms in sensitised individuals (Behrendt et al. 1997; Linneberg et al. 1999; Linneberg 2011; Haftenberger et al. 2013; European Academy of Allergy and Clinical Immunology 2015). Climate change also influences the health of allergic people. Not just by an altered pollen season and a higher pollen production, but also by an increase of allergenic proteins in the pollen (Zhao et al. 2016; Zhao et al. 2017a)

Ghiani et al. 2012 reported higher allergenicity of pollen of *Ambrosia artemisiifolia* along high traffic roads. A positive relationship for the allergen content of *Betula pendula* pollen and the concentration of atmospheric Ozone was found by Beck et al. 2013.

Together with the higher health risk and stronger severity of allergic symptoms coinciding with higher pollutant levels due to industrialisation and urbanisation, the allergenic potential of pollen increases and exacerbates the negative impact of allergic symptoms on life quality (Zhao et al. 2017a; Damialis et al. 2019b).

The most important plant-related factors influencing allergies, were collected by Sofiev et al. 2009. The authors define the influence of weather events (drought, thunderstorms, extreme rain events and flooding), pollutants and temperature on plant growth and allergenicity, alterations in pollen-, flower- and inflorescence-production, the influence of climatic and meteorological factors on the timing of the pollen season, the plant

microbiome under environmental influences and land use changes as the most relevant factors in this relationship. (Sofiev et al. 2009; Damialis et al. 2019b)

All the above-mentioned factors show the importance of further research in aerobiology, biogeography, environmental medicine and other related research fields.

Our changing world and changing climate brings new challenges for the environment and humankind globally but also for research.

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**G**

**Appendix**

## G.1. Taxonomic classification: list of all identified pollen taxa, 2015 – 2017

**Table 18:** identified pollen taxa, scientific name, common name in English, German

Scientific name	English name	German name
<i>Alnus</i>	Alder	Erlen
<i>Ambrosia</i>	Ragweed	Beifußblättriges Traubenkraut
Apiaceae	-	-
<i>Artemisia</i>	Mugwort	-
Asteroideae	-	-
<i>Betula</i>	Birch	Birken
Brassicaceae	Cabbage family	Kreuzblütler
Cannabaceae	Cannabis/Hop	Hanfgewächse
<i>Carpinus</i>	Hornbeam	Hainbuche
<i>Castanea</i>	Chestnut	Kastanien
Chenopodiaceae	Goosefoot family	Fuchsschwanzgewächse
Cichorioideae	-	-
<i>Corylus</i>	Hazel	Haseln
Cupressaceae	Cypress family	Zypressengewächse
Cyperaceae	Sedges	Sauergrasgewächse
Ericaceae	Heather	Heidekrautgewächse
Fabaceae	Legume	Hülsenfrüchtler
<i>Fagus</i>	Beech	Buchen
<i>Forsythia</i>	-	Forsythie
<i>Fraxinus</i>	Ash	Eschen
<i>Hippophae</i>	sea buckthorns	Sanddorn
<i>Juglans</i>	Walnut	Walnuss
Juncaceae	Rush family	Binsengewächse
<i>Larix</i>	Larch	Lärchen
<i>Ligustrum</i>	Privet	Liguster
<i>Olea</i>	Olive	Ölbaumgewächse
Papaveraceae	Poppy	Mohngewächse
<i>Picea</i>	Spruce	Fichten
<i>Pinus</i>	Pine	Kiefern
<i>Plantago</i>	Plantain	Wegeriche
<i>Platanus</i>	Plane	Platanen
Poaceae	Grasses	Gräser
Polygonaceae	-	Knöterichgewächse
<i>Populus</i>	Poplar	Pappeln
<i>Quercus</i>	Oak	Eichen
Rosaceae	Rose family	Rosengewächse

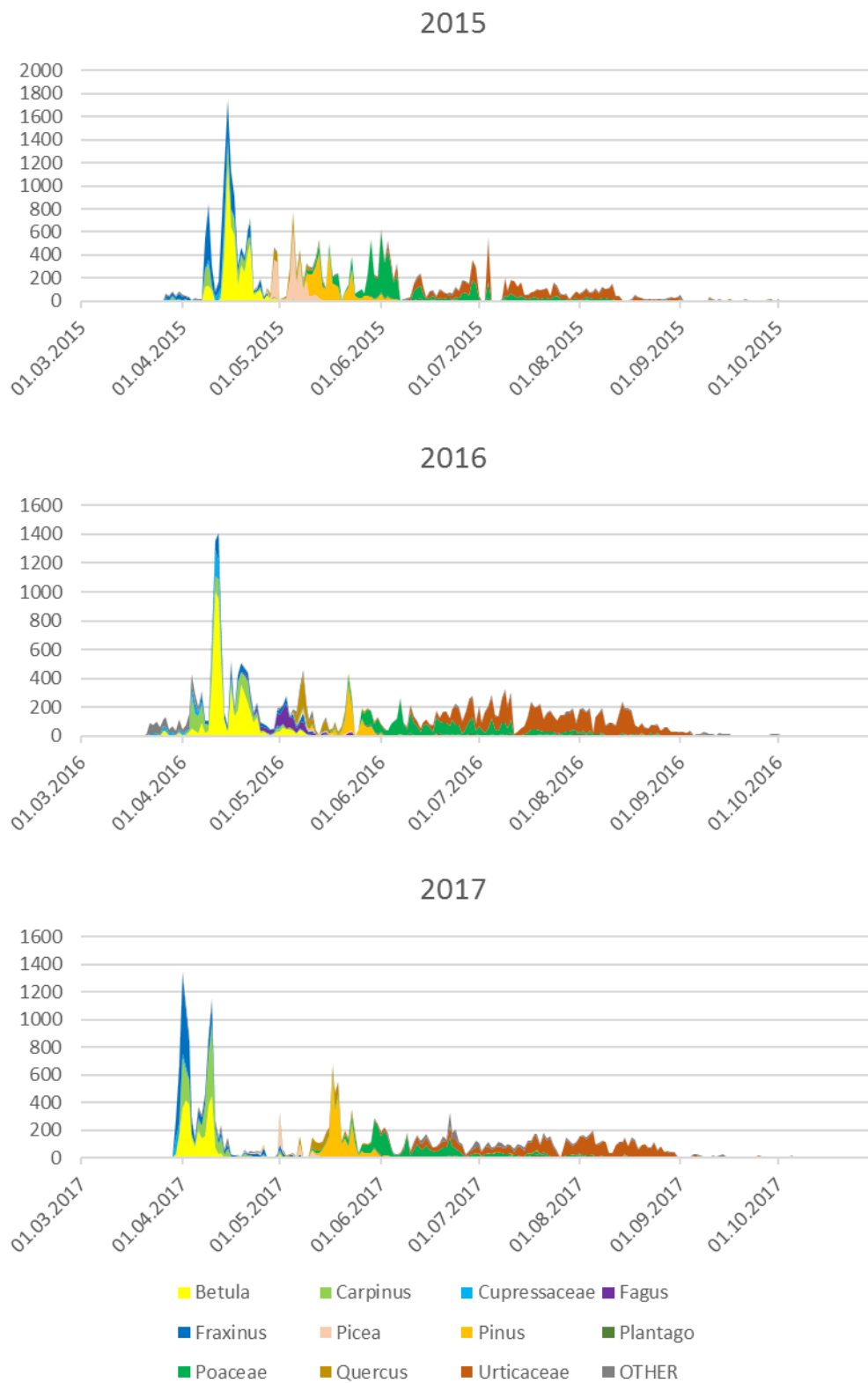
<i>Rumex</i>	docks and sorrels	Ampfer
<i>Salix</i>	Willow	Weiden
<i>Thalictrum</i>	Meadow-rue	Wiesenrauten
<i>Tilia</i>	Lime trees	Linden
<i>Tsuga</i>	Hemlock	Hemlocktannen
Typhaceae	-	Rohrkolbengewächse
<i>Ulmus</i>	Elm	Ulmen
Urticaceae	Nettle	Brennnesselgewächse

**Table 19:** All identified pollen taxa, percentages of the total pollen count

<b>Scientific name</b>	<b>2015</b>	<b>2016</b>	<b>2017</b>	<b>total</b>
Urticaceae	15.97 %	27.35 %	22.87 %	21.98 %
<i>Betula</i>	17.62 %	19.95 %	12.72 %	16.97 %
Poaceae	19.48 %	14.19 %	12.72 %	15.67 %
<i>Fraxinus</i>	11.64 %	4.743 %	11.24 %	9.12 %
<i>Pinus</i>	10.59 %	4.28 %	11.43 %	8.65 %
<i>Carpinus</i>	5.76 %	7.98 %	10.51 %	7.94 %
<i>Picea</i>	8.63 %	0.62 %	1.79 %	3.81 %
<i>Quercus</i>	2.47 %	3.50 %	3.64 %	3.17 %
<i>Plantago</i>	1.81 %	2.52 %	2.59 %	2.29 %
Cupressaceae	1.60 %	2.79 %	1.39 %	1.95 %
<i>Fagus</i>	0.07 %	3.90 %	0.28 %	1.46 %
<i>Salix</i>	0.23 %	0.98 %	1.03 %	0.73 %
<i>Alnus</i>	0.10 %	1.47 %	0.01 %	0.55 %
<i>Populus</i>	0.20 %	1.09 %	0.24 %	0.52 %
<i>Castanea</i>	0.25 %	0 %	1.27 %	0.47 %
<i>Tilia</i>	0.59 %	0.49 %	0.20 %	0.44 %
<i>Ulmus</i>	0.53 %	0 %	0.38 %	0.30 %
Papaveraceae	0.24 %	0 %	0.66 %	0.28 %
<i>Ligustrum</i>	0 %	0 %	0.86 %	0.26 %
<i>Artemisia</i>	0.33 %	0.11 %	0.34 %	0.26 %
Apiaceae	0.04 %	0.57 %	0.08 %	0.24 %
<i>Rumex</i>	0.29 %	0.15 %	0.22 %	0.22 %
<i>Juglans</i>	0.01 %	0.41 %	0.11 %	0.18 %
Cyperaceae	0.15 %	0.19 %	0.15 %	0.16 %
<i>Thalictrum</i>	0.09 %	0.03 %	0.35 %	0.15 %
Cichorioideae	0.18 %	0.15 %	0.10 %	0.14 %
Chenopodiaceae	0.16 %	0.09 %	0.17 %	0.14 %
<i>Forsythia</i>	0 %	0.37 %	0 %	0.13 %
Asteroideae	0.09 %	0.11 %	0.14 %	0.11 %
<i>Ambrosia</i>	0.12 %	0.08 %	0.09 %	0.09 %
<i>Platanus</i>	0.01 %	0.17 %	0.08 %	0.09 %
<i>Corylus</i>	0.01 %	0.22 %	0.005 %	0.08 %
<i>Hippophae</i>	0 %	0 %	0.26 %	0.08 %
Fabaceae	0 %	0.01 %	0.21 %	0.07 %
Brassicaceae	0 %	0 %	0.22 %	0.06 %
<i>Larix</i>	0.07 %	0.09 %	0 %	0.05 %
Oleaceae	0.01 %	0 %	0.12 %	0.04 %
Polygonaceae	0 %	0.07 %	0 %	0.02 %
Ericaceae	0.01 %	0.01 %	0.02 %	0.01 %
Rosaceae	0.004 %	0.02 %	0 %	0.01 %
Typhaceae	0.01 %	0.01 %	0 %	0.01 %

Juncaceae	0.01 %	0 %	0.01 %	0.004 %
<i>Tsuga</i>	0 %	0 %	0.01 %	0.002 %
Cannabaceae	0.002 %	0 %	0 %	0.001 %
<b>Unidentified pollen</b>	0.66 %	1.31 %	1.50 %	1.14 %




## G.2. Annual pollen season in Augsburg, 2015 – 2017





**Figure 52:** annual pollen season in Augsburg, 2015 - 2017

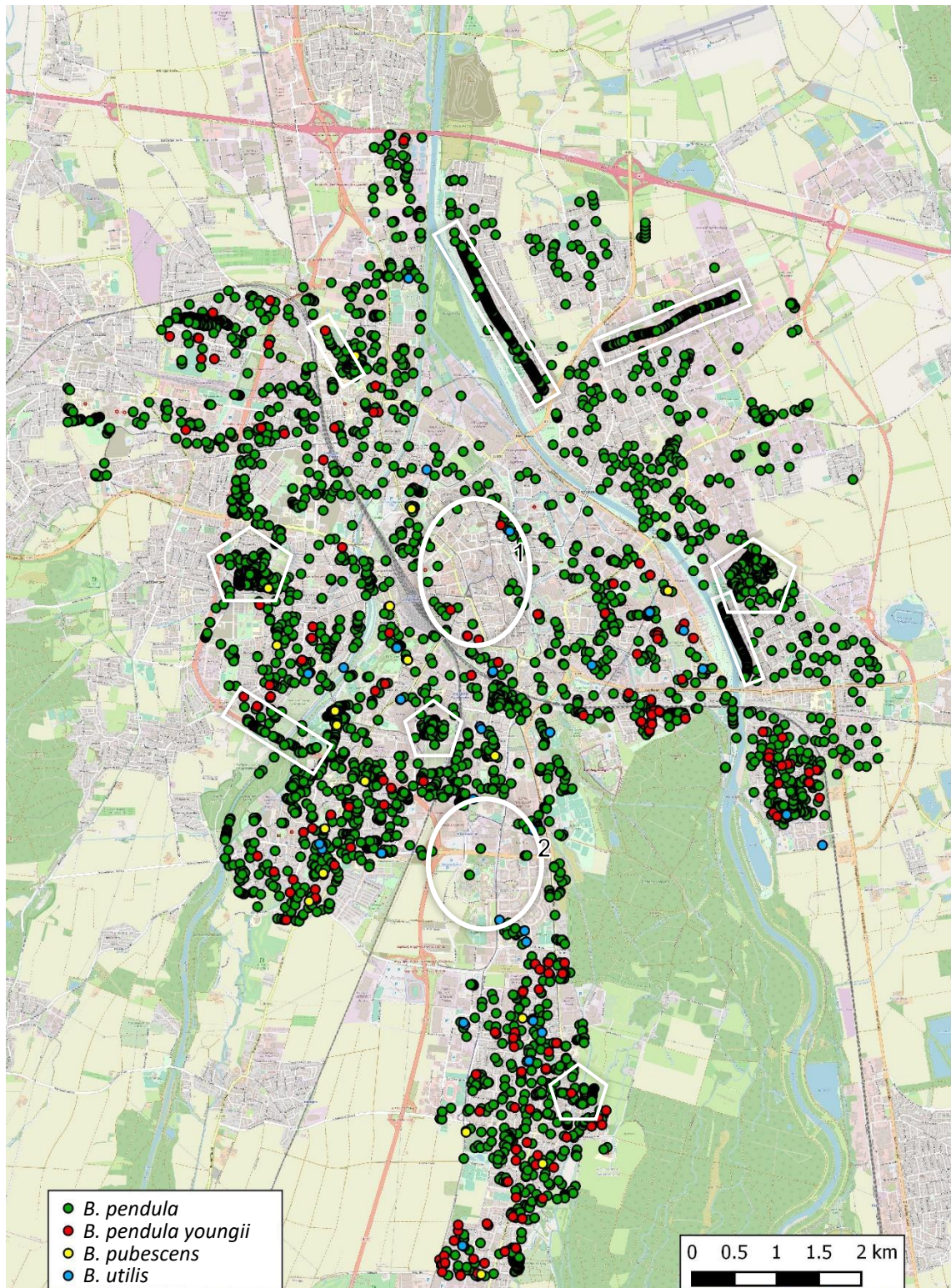
### G.3. BBCH-scale adapted to monitor the flowering of *Betula pendula*

**Table 20:** observed stages of the BBCH-scale (Hack et al. 1992) in *Betula pendula* (photos by Franziska Kolek)

Flowering stage	BBCH code		Explanation of the stage
Winter stage	Before 51		Winter state
Pre-flowering	51 - 55		Growth of the male inflorescence after winter  No pollen release
Start of flowering	59		Full expansion of the male inflorescence  No pollen release

Flowering stage	BBCH code		Explanation of the stage
Flowering	60 – 67		Pollen release
End of flowering	68 – 69		Inflorescence after pollen release
Male inflorescences falling	After 69	X	Male inflorescences dried and falling off the tree

## G.4. Mapped individuals of *Betula* in the city of Augsburg



**Figure 53:** *Betula* trees in Augsburg  
white circles: areas with low numbers of *Betula* (1: inner city, 2: Hochfeld)  
white rectangles: tree-lined avenues  
white pentagons: graveyards  
(map based on: OpenStreetMap 2020)

## G.5. Observed individuals of *Betula pendula*

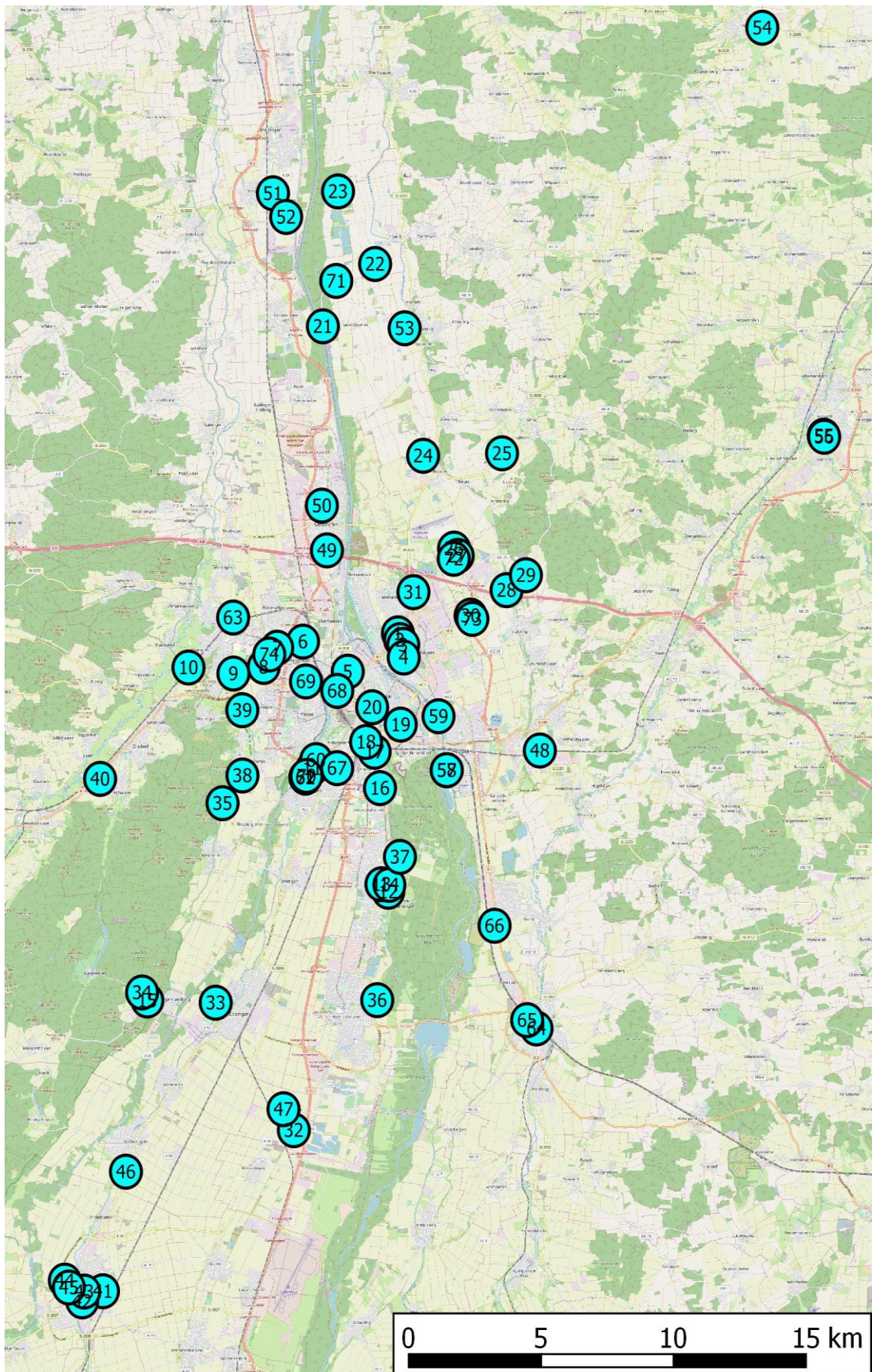
**Table 21:** all observed individuals of *Betula pendula* from 2015 until 2017

bold ID: observed for all three years;

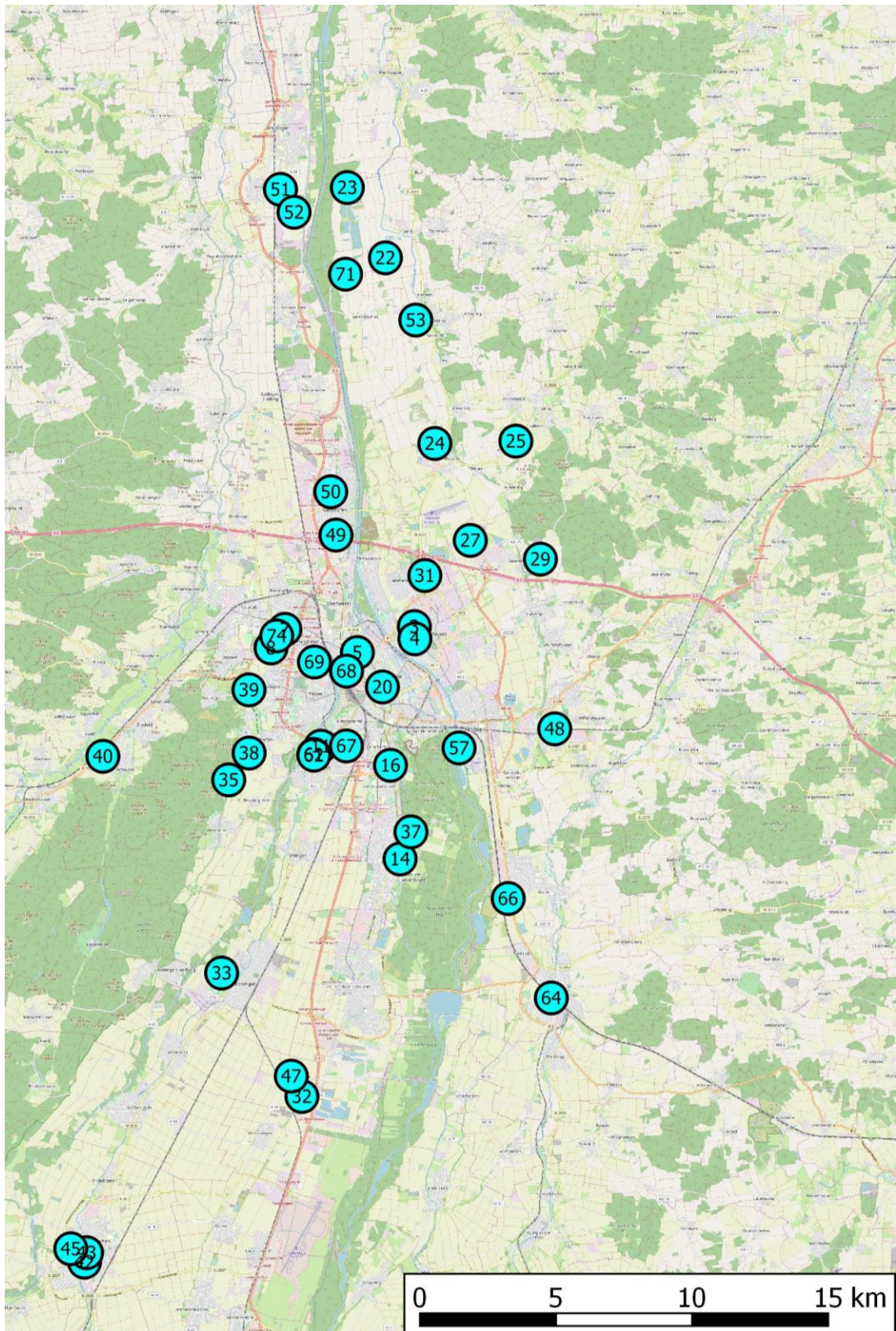
grey background: observed for less years (not included in some analysis)

ID	Location		Coordinates WGS84	
			X	Y
1	Neuburger Straße 161	Augsburg	48.390041	10.914541
2	linke Brandstraße 2	Augsburg	48.387907	10.915647
<b>3</b>	Neuburger Straße 114	Augsburg	48.386370	10.917251
<b>4</b>	Friesenstraße 24	Augsburg	48.382346	10.917492
<b>5</b>	Heinrich v. Buz Straße 1	Augsburg	48.377858	10.889101
6	August-Wessels-Straße 30	Augsburg	48.387416	10.866380
<b>7</b>	Lise-Meitner-Straße 1	Augsburg	48.385475	10.853296
<b>8</b>	Ulmerstraße 255	Augsburg	48.379367	10.846451
9	Alte Reichsstraße 2	Steppach	48.377260	10.831063
10	Kobelgraben 14	Neusäß, Westheim	48.379178	10.808240
<b>11</b>	Gabelsberger Straße 7	Augsburg	48.347085	10.871084
12	Tattenbachstraße 2	Augsburg	48.307800	10.909663
13	Sperlingstraße 2	Augsburg	48.310176	10.906157
<b>14</b>	Georg Käß Platz	Augsburg	48.309981	10.910207
15	Schlossberg 10	Bobingen / Straßberg	48.273139	10.787383
<b>16</b>	Werner von Siemens Straße	Augsburg	48.340795	10.905433
17	Haunstetterstr. 49	Augsburg	48.352371	10.902708
18	von-der-Tannstraße 29	Augsburg	48.355496	10.898395
19	Schäfflerbachstraße 40	Augsburg	48.361011	10.915979
<b>20</b>	Vorderer Lech 19	Augsburg	48.366541	10.901463
21	south of Rehlinger Straße, eastern reiverbank of Lech	Langweid	48.487600	10.876465
<b>22</b>	south of Langweider Straße, next to the creek	Sand / Rehling	48.507365	10.902965
<b>23</b>	north of Langweider Straße and the quarry ponds	Sand / Rehling	48.530390	10.884107
<b>24</b>	road between Anwalting and Mühlhausen, next to the creek	Mühlhausen / Anwalting	48.446828	10.931055
<b>25</b>	Hauptstraße 41	Aulzhausen, Affing	48.447115	10.967933
26	south of Dickelsmoor, next to "Forellenbach" creek	Dickelsmoor / Derching / Friedberg	48.414616	10.942249
<b>27</b>	east of Dickelsmoor, at the T-junction	Dickelsmoor / Derching / Friedberg	48.416701	10.946905
28	opposite of Am Anger 2, next to the creek	Derching	48.403776	10.969704
<b>29</b>	Junction Frechholzhauser Straße / Schindkuchenweg	Derching	48.40853	10.97951
30	south of Neue Bergstraße 101, next to "Forellenbach" creek	Friedberg	48.395690	10.951411
<b>31</b>	Mühlhauser Straße 40a	Augsburg	48.403971	10.922653
<b>32</b>	South of Rainstraße, east of Alemannenstraße	Oberottmarshausen	48.231772	10.861713
<b>33</b>	Schwettingerweg 1	Bobingen	48.272438	10.822059
34	Waldstraße 25	Bobingen/Straßberg	48.275695	10.784696
<b>35</b>	Wellenburg 8a	Augsburg-Wellenburg	48.335996	10.825538
36	Wasserhausweg, east of the enclosure for Przewalski horses	Königsbrunn	48.272988	10.903997
<b>37</b>	south of Ellensindstraße	Augsburg	48.318889	10.915574

ID	Location		Coordinates (WGS84)	
			X	Y
38	Radegundisweg 5	Augsburg-Radegundis	48.344820	10.835642
39	Eibenweg, eastern end of the golf course	Augsburg-Stadtbergen	48.365587	10.835561
40	in the west of Kapellenweg	Diedorf / Gessertshausen	48.343738	10.763396
41	Bgm.-Bittinger-Weg	Schwabmünchen	48.180387	10.764847
42	Seilerweg 4	Schwabmünchen	48.177162	10.754301
43	Geyerburg	Schwabmünchen	48.180270	10.755322
44	Birkensteig, north of the sports area	Schwabmünchen	48.183786	10.745505
45	opposite Auenstraße 47, next to the creek	Schwabmünchen	48.181583	10.747533
46	Mohnweg 3	Großaitingen	48.218545	10.776498
47	Bobinger Straße 4	Oberottmarshausen	48.238477	10.856472
48	Frühlingstraße 12, next to the railway	Friedberg	48.352765	10.986593
49	opposite of Augsburger Straße 55, next to the fairground	Gersthofen	48.416425	10.878535
50	Friedhofstraße	Gersthofen	48.430639	10.875945
51	Mendelstraße 2	Herbertshofen/Meitingen	48.529801	10.851120
52	Ulrichstraße 24	Herbertshofen/Meitingen	48.522303	10.857830
53	St.-Vitus-Straße 16	Rehling	48.486974	10.917964
54	Schrobenhauser Straße 50	Pöttmes	48.582287	11.099475
55	Theodor-Heuss-Straße 44 west	Aichach	48.452860	11.130652
56	Theodor-Heuss-Straße 44 east	Aichach	48.452530	11.130867
57	west of Oberländer Straße 100d	Augsburg	48.346501	10.939302
58	Lechrainstraße 62	Augsburg	48.361838	10.936341
59	Lechrainstraße 66	Augsburg	48.363671	10.935152
60	Gabelsbergerstraße 57	Augsburg	48.349716	10.873015
61	Anton-Bezler-Straße 2, north	Augsburg	48.344356	10.867572
62	Anton-Bezler-Straße 2, south	Augsburg	48.344131	10.867612
63	Siemensstraße 2	Neusäß	48.395071	10.830912
64	Münchener Straße 40	Mering	48.264232	10.984904
65	opposite Fröbelstraße 1	Mering	48.266884	10.980132
66	junction Lechauenstraße / Industriestraße	Kissing	48.296965	10.963601
67	Ohmstraße 3	Augsburg	48.347156	10.883676
68	Stadtjägerstraße 11	Augsburg	48.371742	10.883748
69	Sommestraße 50	Augsburg	48.374658	10.867051
70	Von-Corbes-Straße 13	Augsburg	48.344674	10.868143
71	Sand, Landstraße	Sand/Todtenweis	48.501993	10.883256
72	south of Dickelsmoor, next to "Siebenbrunnengraben" creek	Dickelsmoor / Derching / Friedberg	48.413756	10.942869
73	south of Neue Bergstraße 101, next to "Forellenbach" creek	Friedberg	48.394643	10.952329
74	Fritz-Strassmann-Straße 35	Augsburg	48.383316	10.849258



**Figure 54:** all observed individuals of *Betula pendula* from 2015 to 2017 (map based on: OpenStreetMap 2020)



**Figure 55:** individuals of *Betula pendula*, observed for all three years (map based on: OpenStreetMap 2020)

## G.6. Timing of flowering of *Betula pendula*

**Table 22:** timing of flowering of *Betula pendula* per year

		flowering start (calendar day)	flowering peak (calendar day)	flowering end (calendar day)	Duration (days)
<b>2015</b>	earliest / shortest	100	105	107	4
	latest / longest	112	117	120	17
	average	105	110	115	11
	median	105	110	115	12
	standard deviation	2.25	2.96	3.26	3.14
<b>2016</b>	earliest / shortest	95	97	103	3
	latest / longest	113	118	119	16
	average	102	105	110	10
	median	102	104	112	9
	standard deviation	4.68	4.54	4.27	3.36
<b>2017</b>	earliest / shortest	87	89	92	3
	latest / longest	105	105	119	22
	average	93	95	102	10
	median	92	95	101	9
	standard deviation	3.99	4.11	6.24	4.36
<b>total</b>	earliest / shortest	87	89	92	3
	latest / longest	113	118	120	22
	average	100	103	108	10
	median	102	104	110	10
	standard deviation	6.50	7.13	7.10	3.74

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