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Greater difference between airborne and flower pollen chemistry, than between pollen collected across a pollution gradient in the Netherlands

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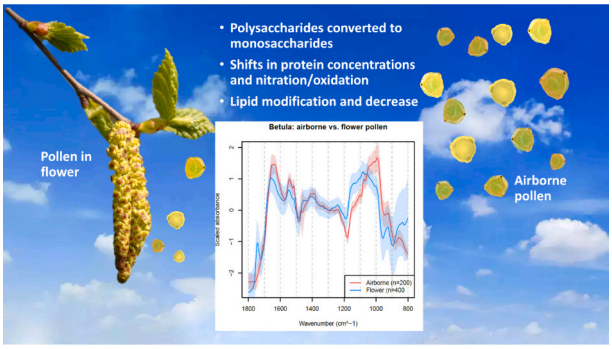
HIGHLIGHTS

- No difference between FTIR spectra derived from rural and urban pollen
- Monosaccharide content in pollen increases after release from the flower.
- After release from the flower pollen proteins are nitrated/oxidized.
- In airborne pollen lipids are modified and decreased compared to flower pollen.

GRAPHICAL ABSTRACT



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ABSTRACT

The prevalence in allergic diseases has increased considerably in the past decades. An important trigger of the symptoms of allergic rhinitis (hay fever) is the pollen of wind-pollinating plants. This pollen is developed by plants and is released into the air where it gets exposed to environmental influences and air pollution. We investigated the chemical changes to pollen that occur after release from the flower in a rural (Veluwe) and an urban (Amsterdam) site in the Netherlands using Fourier Transform Infrared (FTIR) spectroscopy. During the spring/summer of 2020 (during the COVID pandemic) the pollen of nine *taxa* (*Alnus*, *Betula*, *Fagus*, *Fraxinus*, *Pinus*, *Plantago*, Poaceae, *Quercus* and *Salix*) were collected directly from flowers and the air (using a mobile sampler). FTIR spectra were obtained for multiple individual pollen grains for each *taxa*. The spectra obtained from airborne pollen collected at the rural vs. urban sites did not show any statistical difference. This is possibly a result of a reduced difference in pollutant concentrations between the two sites due to the COVID-19-lockdown measures were in place. However, consistent differences in the FTIR spectra recovered from airborne vs. flower pollen were recorded for all pollen *taxa*. After the release from the flower the chemical composition of the pollen changed: (i) polysaccharides are converted to monosaccharides; (ii) protein concentration and/or nitration/

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oxidation level is altered; (iii) lipids are modified and/or reduced in concentration. These changes may alter the allergenicity of the pollen and suggest that further work on the allergenic nature of airborne pollen is required.

1. Introduction

Allergic rhinitis is an inflammation of the nose caused by exposure to airborne allergens (such as pollen, dust or mold) which impacts 400 million people across the globe (Greiner et al., 2011) with an estimated annual medical cost of US$ 3.4 billion in the USA (Meltzer and Bukstein, 2011). The prevalence of allergic diseases has increased considerably in industrialized countries and the potential reasons for this trend are multiple, but one of them is the influence of anthropogenic air pollution (D'Amato et al., 2015a; Krämer et al., 2000; Ring et al., 2001). It has been shown that traffic related air pollutants, like diesel exhaust, ozone and NO_2 can have adverse effects on human health, e.g. on the respiratory tract which may enhance allergen induced respiratory allergic disease (Gehring et al., 2010; Diaz-Sanchez et al., 1997).

Pollen develops in the pollen cones of Gymnosperms and anthers of the flowers of Angiosperms. For medical applications, like allergy tests or immunotherapies, the allergenic material is often collected from pollen sampled directly from the flowers, e.g. tree catkins or grass spikes (e.g. https://www.stallergenesgreer.com/manufacturing) and not the airborne pollen which interacts with people. We know that pollen undergoes changes following release from flowers such as dehydration (Pacini et al., 2006) and exposure to UV radiation (Fraser et al., 2014), but the degree of alteration, and uniformity of change between *taxa*, remains unknown. Further, interactions between airborne pollen and air pollution have been suggested to cause changes in allergenicity, total protein content, integrity of the cell wall, and the chemical composition (Frank and Ernst, 2016; Sénéchal et al., 2015). Several studies point to the increased allergenicity of pollen collected from urban areas (high environmental pollution) compared to those collected from rural areas (low environmental pollution) (Armentia et al., 2002; Sedghy et al., 2017; Ghiani et al., 2012). Other studies, using Fourier Transform Infrared (FTIR) spectroscopy, suggest an increased protein content or changes in chemical composition in pollen collected from urban sites (Depciuch et al., 2017). These alterations in the pollen chemistry may play a role in the increased prevalence of allergic respiratory diseases in urban environments compared to rural sites (D'Amato et al., 2015b).

These findings prompted us to study pre- and post-flower release pollen in urban and rural settings in the Netherlands. Specifically, we collected airborne pollen from the streets of Amsterdam city where heavy traffic pollution levels have been observed, and from rural areas in a natural setting in the center of the Netherlands (the Veluwe). Pollen was collected from both sites across the flowering season of 2020 (March to June) and grains from nine pollen *taxa* were morphologically identified: *Alnus*, *Betula*, *Fagus*, *Fraxinus*, *Pinus*, *Plantago*, Poaceae, *Quercus* and *Salix*. The individual pollen grains recovered were characterized using microscopic Fourier Transformed Infrared spectroscopy (μFTIR). Differences in chemical composition (chemical bonds and functional groups) were inferred from the FTIR spectra obtained.

2. Materials and methods

2.1. Sample locations

Sampling locations in Amsterdam and on the Veluwe were chosen to include areas with high and low air pollution, respectively, as indicated by high resolution estimations of local nitrogen dioxide, PM_{10}, and $PM_{2.5}$ from 2018 (data available from atlasleefomgeving.nl/kaarten). These locations were cross-referenced with the known occurrence of allergenic trees (*Alnus*, *Betula* and *Quercus*) in the city (maps.amsterdam.nl/bomen) and on the Veluwe (using records from the Global Biodiversity Information Facility (GBIF)).

Pollution data for the sampling period were obtained from air quality measuring stations located closest to the sampling area in Amsterdam (Haarlemmerweg, Einsteinweg, van Diemenstraat, Vondelpark, Westerpark, Stadhouderskade, Oude Schans & Jan van Galenstraat) and on the Veluwe (Wekerom-Riemterdijk). Measurements of nitrogen monoxide (NO), nitrogen dioxide (NO_2), coarse and fine particulate matter (PM_{10} and $PM_{2.5}$, respectively), and ozone (O_3) were included in this study. Hourly pollutant concentrations (RIVM, 2020) were obtained through the *saqgetr* package in R and plotted with the *openair* R-package (Carslaw and Ropkins, 2012; Grange, 2019).

2.2. Sample collection and preparation

Airborne pollen was sampled at street level in Amsterdam and on the Veluwe using portable pollen samplers (called Pollensniffers). These Pollensniffers resemble a large flashlight in size (diameter 6 cm; length 15 cm; weight 408 g). They comprise a conical inlet through which air is drawn in, the air passes over a sample slide that captures pollen and particulate matter on an adhesive layer; for details see (de Weger et al., 2020). The Pollensniffers were mounted onto the handlebars of a bicycle, with the inlet pointing in the direction of travel, to collect pollen directly from the space in which it interacts with people. To identify possible variance due to environmental pollution we collected both airborne and flower samples along six predefined transects in Amsterdam (urban) and in the Veluwe (rural) (Fig. 1). The sampling took place during spring and summer of 2020, coinciding with a COVID-19 lockdown in the Netherlands.

The transects in both settings were of the same total length (9 km), distributed over the same spatial extent. Airborne pollen was sampled along each transect at walking pace, for a total sampling period of approximately 90 min on each sampling date. Sampling was preferentially conducted on sunny days, as airborne pollen concentrations at street level are low during, and after, periods of rain (de Weger et al., 2020). Pollen was collected in the Pollensniffers on glass microscope slides covered in polyethylene foil with a thin layer of Vaseline to which the pollen adhered. The polyethylene foil (shrink wrap) was used as a medium to transfer the sample to the zinc-selenide (ZnSe) slides used in the FTIR measurements (Gosling and McMichael, 2023). Once collected, the Pollensniffer samples were stored in closed plastic containers carried inside a microscope slide box to avoid the introduction of pollution during transportation to the University of Amsterdam laboratory.

Flower (pollen) samples were collected directly from plants in the same environment at the same time as the Pollensniffer was deployed. Nearly ripe flowers were collected and taken to the lab. Pollen from the flowers was deposited directly onto ZnSe slides by gently rubbing and shaking the flower above the slide.

2.3. Pollen identification

Pollen types were identified based on their morphological features (Moore et al., 1999) and manually separated using an IMT-2 inverted light microscope (Olympus, Tokyo, Japan). Nine pollen *taxa* were found to be abundant on the Pollensniffer slides: *Alnus*, *Betula*, *Fagus*, *Fraxinus*, *Pinus*, *Plantago*, Poaceae, *Quercus* and *Salix*. Separation of pollen grains was achieved using a dissecting needle with one paint brush hair attached to the end of the needle. This process was conducted carefully to ensure that the pollen grain remained undamaged. When damaged or irregular pollen grains could not be morphological identified, they were disregarded. Otherwise, the pollen grains were selected randomly from the sample. After separating the pollen types, the sample (including Vaseline and foil) was transferred to ZnSe slides by cutting out the

section of the foil containing the separated pollen.

2.4. Spectral analysis of pollen

The FTIR spectra of the samples were collected with the Thermo Scientific Nicolet iN10 MX (Waltham, MA, USA), equipped with a liquid nitrogen cooled MTC detector and a kBr beam splitter. All measurements were performed on ZnSe slides inside a nitrogen purged measurement chamber. Each measurement comprised 256 scans of a single pollen grain with a wavenumber range from 4000 to 400 cm^{-1} at a 4 cm^{-1} resolution. The size of the aperture corresponded to the size of the individual pollen grain being measured. A background was taken before each measurement of a pollen grain.

After measurement, all processing and plotting of the spectra were conducted in R (version 4.2.3; (R-Core-Team, 2023)) First, a second degree polynomial baseline correction was applied to the spectra using the *baseline* package (Liland et al., 2010). Afterwards, the spectra were *Z*-score standardized and trimmed to the fingerprint region (1800–800 cm^{-1}). The spectral data were loaded, explored and trimmed using the *ChemoSpec* package (Hanson, 2023). The spectral characteristics of individual grains were then compared to reveal the degree of variance within, and between, groups; specifically, the difference between airborne and flower pollen, and between urban and rural locations.

3. Results

3.1. Air pollution

Significant differences in air pollution measurements were only found for nitrogen dioxide and nitrogen monoxide concentration between the Amsterdam (urban) and Veluwe (rural) study site during the period of sampling (Fig. 2). The absence of any significant difference between the other pollutants (ozone, $PM_{2.5}$ and PM_{10}) between the study sites may have been caused by the reduced human activities that occurred during the sampling period due to the COVID- 19 pandemic regulations.

3.2. Spectral analysis

We focused our further analysis on the four *taxa* from which we extracted spectra from 100 grains in each group (*Alnus*, *Betula*, *Pinus*, and Poaceae); spectral data from the five other *taxa* (*Fagus*, *Fraxinus*, *Plantago*, *Quercus*, and *Salix*) where deemed to be less reliable because lower quantities of pollen grains (<100 pollen grains) were recovered, however, for completeness these data are presented in the Supplementary Material (Figs. S1 and S2). Additionally, in the supplementary materials, figures displaying the spectra of flower and airborne pollen collected from rural and urban environments separately are provided (Supplement Fig. S3). At both locations the differences in spectra between flower and airborne pollen are very similar.

The FTIR spectra obtained from the individual pollen grains of different pollen *taxa* showed a high degree of variance within each *taxon* (supplement Fig. S4 and S5) especially at certain wavelengths, e.g. 1500–1550 cm^{-1} and 700–1150 cm^{-1}. The variance in the spectra obtained from the airborne pollen grains of *Alnus* and *Betula* is smaller than that of those pollen isolated from the flowers. (Figs. 3 and 4 and Supplement Figs. S4 and S5). Contrary to our expectation of spectra differentiation along gradient of environmental pollution, no clear difference could be discerned from the within *taxon* variation between the urban (Amsterdam) vs. rural (Veluwe) sample groups (Fig. 4 and Supplement Fig. S2).

Despite the high degree of spectral variance observed within *taxa* (Supplement Fig. S4 and S5), the spectra extracted from the airborne and flower samples for each *taxon* were shown to be significantly different in

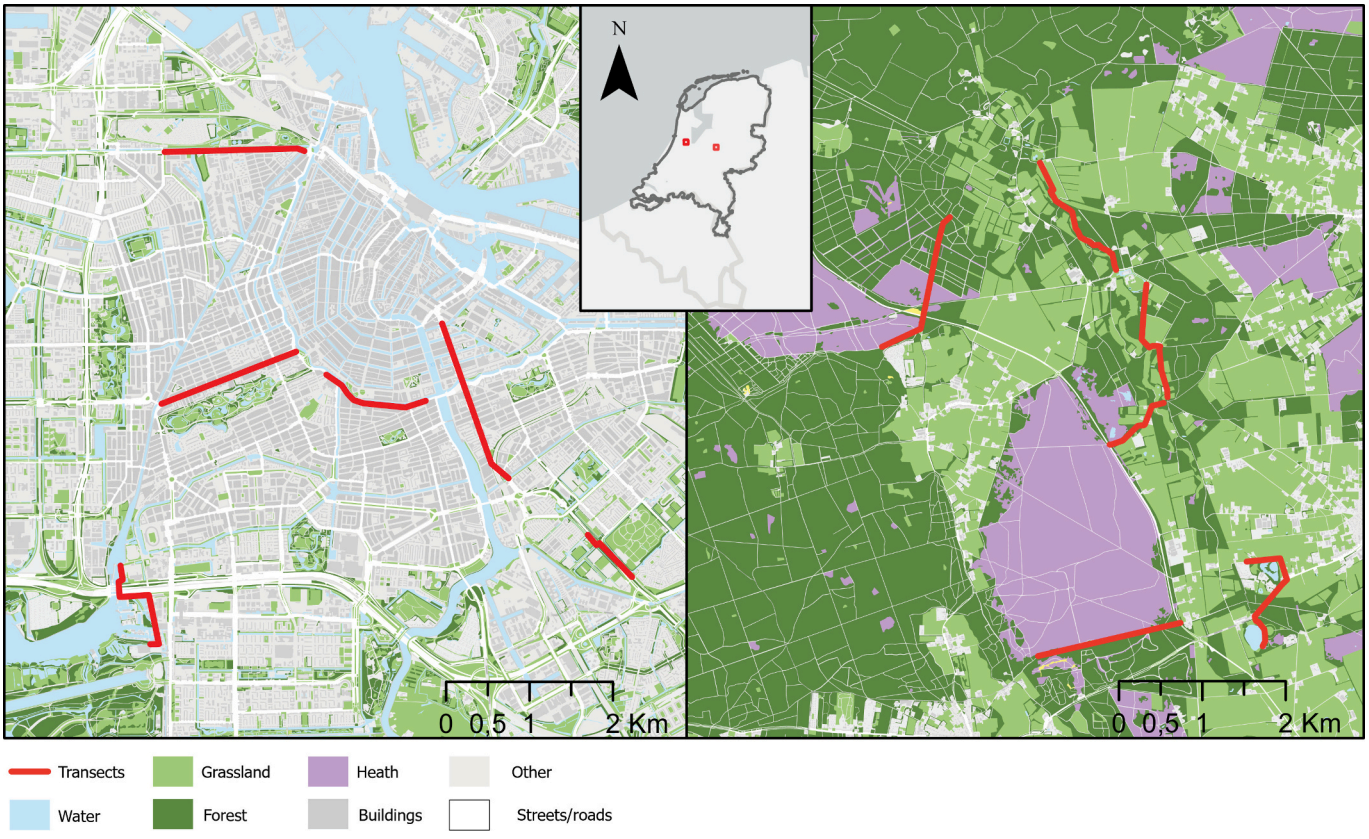

Fig. 1. Map of the sampling transects in Amsterdam (left) and the Veluwe (right). Sampling transects are shown in red. The map is based on the Dutch land registry (Basisregistratie Topografie) TOP10NL map (Kadaster, 2017) and was generated using ArcGIS pro software (esri, CA, USA).

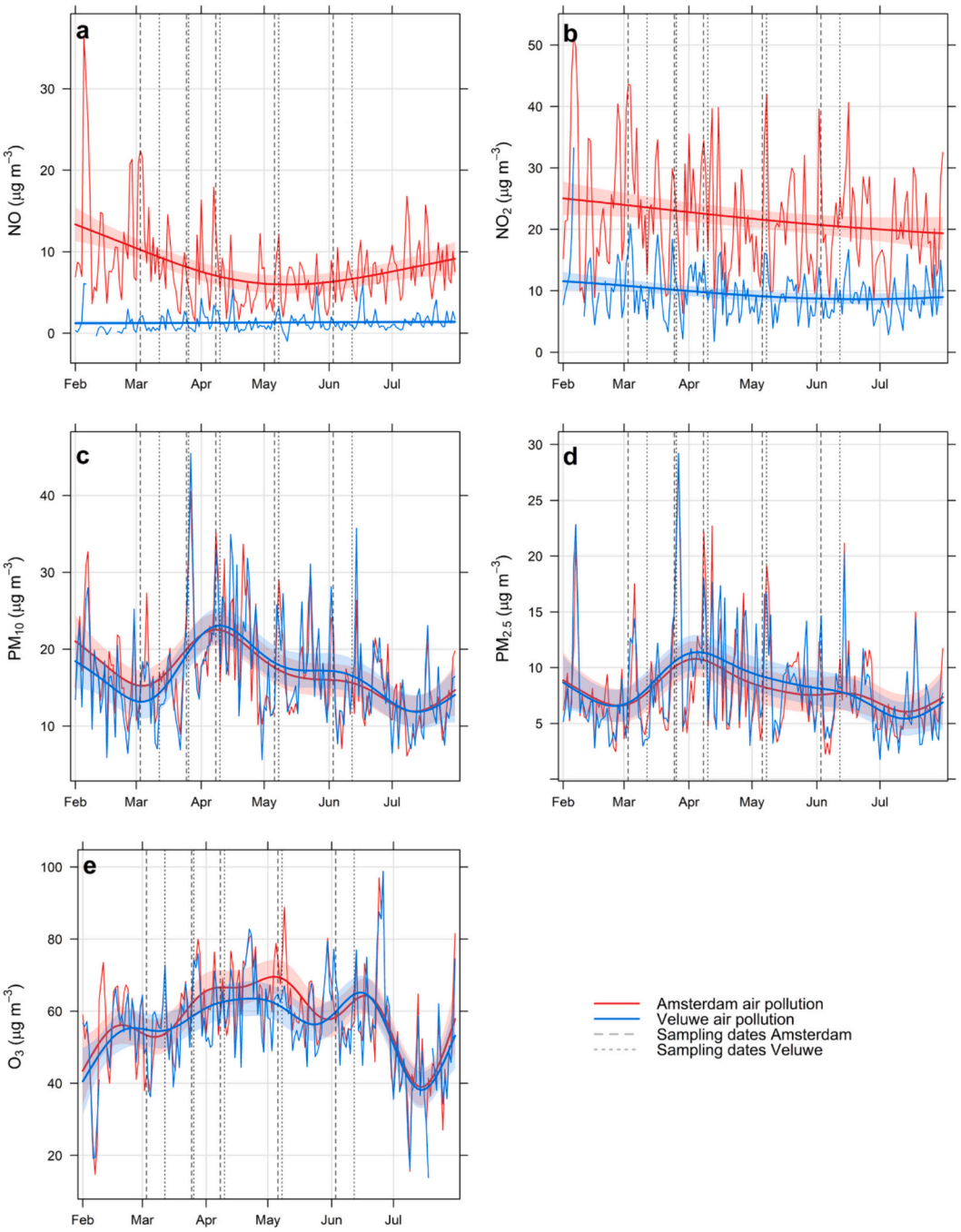

Fig. 2. Daily average concentrations of air pollutants NO, NO2, PM2.5, PM10 and O3 in Amsterdam and in the Veluwe in 2020, with trendlines and corresponding 95 % confidence intervals shown. Vertical dashed and dotted lines indicate the pollen sampling dates for Amsterdam and the Veluwe, respectively.

certain parts of the spectra (Fig. 3). Specifically, clear differences in the FTIR spectra between the airborne and flower samples were apparent at 1000–1200 cm^{-1} and 1600–1800 cm^{-1} wavenumbers. With a shift to relatively higher absorbance values being recorded in airborne samples between 1000 and 1200 cm^{-1} for *Alnus*, *Betula*, *Fraxinus*, *Quercus* and *Salix* while for *Pinus*, *Fagus*, *Plantago* and Poaceae a shift was also observed in this region, but not a clear increase in absorbance. Between 1700 and 1800 cm^{-1} all *taxa*, except *Pinus* and *Fagus*, showed a relatively higher absorbance in the pollen obtained from the flower samples. The spectra of airborne *Pinus* pollen show a sharp increase at 1475 cm^{-1} compared to that of flower pollen.

4. Discussion

4.1. Interpretation and variance in the FTIR signal

Within the FTIR spectra differences in absorbance at different wavenumbers can be broadly categorized into specific regions containing signatures of lipids, proteins, sporopollenin and carbohydrates (Zhao et al., 2016). The vast majority of spectra obtained from the airborne, and flower extracted pollen, contain a similar series of peaks (Figs. 3, 4, Supplement Figs. S1 and S2) that can be interpreted to relate to various chemical bonds and functional groups (Table 1). A high degree of variance within the FTIR spectra obtained from the individual pollen grains of a particular *taxon* is indicated by the standard deviations

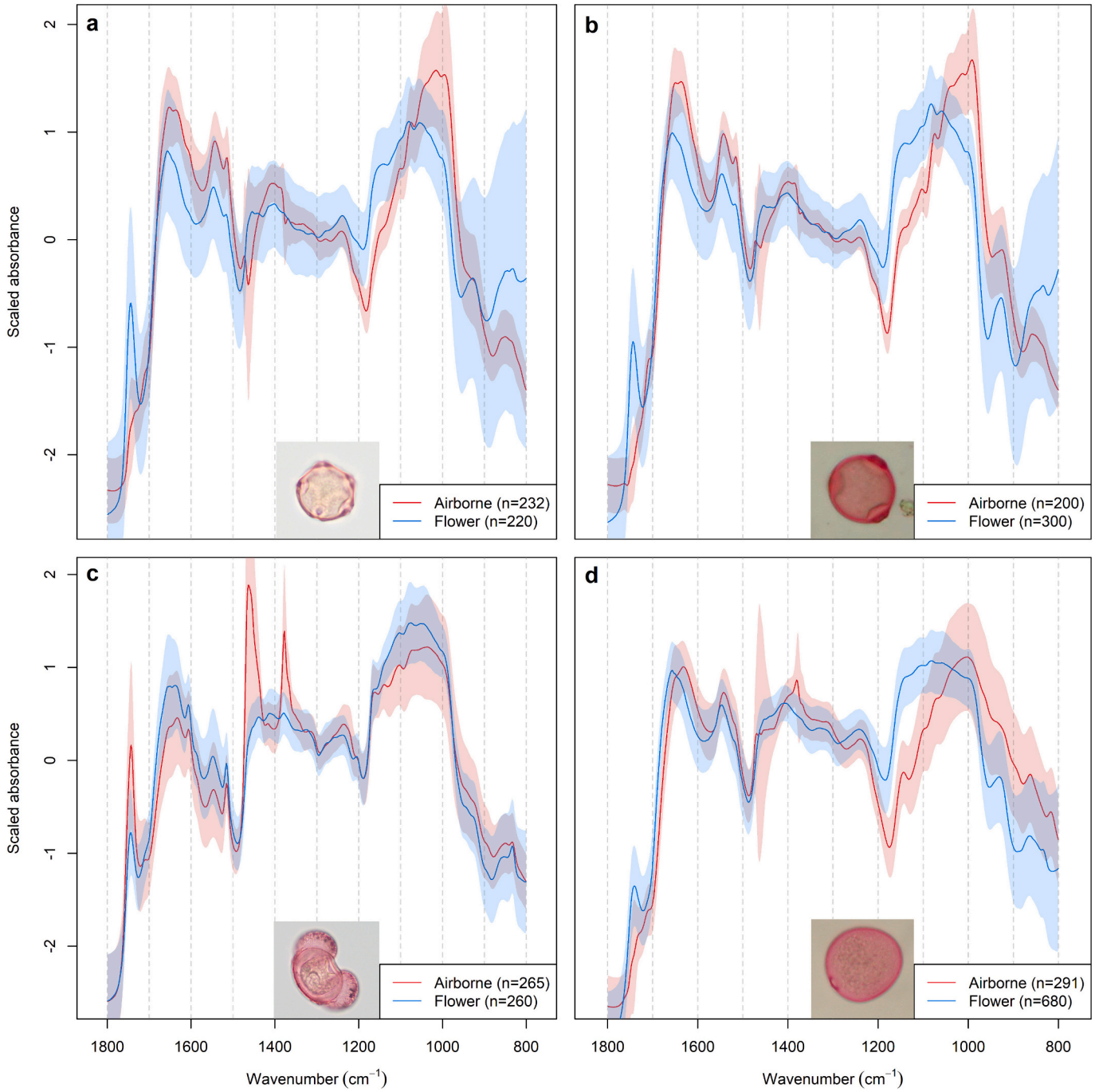

Fig. 3. FTIR spectra of individual pollen grains of *Alnus* (a), *Betula* (b), *Pinus* (c) and Poaceae (d). Average (line) and standard deviation (area) are shown in red for airborne pollen grains and in blue for pollen grains isolated directly from flowers. The n value indicates the number of pollen grains used for the different spectra. Graphics within the spectral figures, illustrate the morphology of the different pollen species in question.

(Supplement Fig. S4 and S5). This could be a product of the analytical approach. The size, shape and orientation of the pollen grain when the IR beam passes through, can influence the scattering pattern and thus the spectral pattern (Diehn et al., 2020). The variance in the spectra of the individual airborne pollen grains is generally small for *Betula* and *Alnus* pollen compared to the spectra of *Pinus* pollen (Fig. 3, Supplement Fig. S5). The relatively higher degree of variance in *Pinus* could potentially be attributed to the more complex morphological shape of the *Pinus* grain (Fig. 3) rather than a reflection of the range of chemical characteristics (Bağcıoğlu et al., 2015). However, consistent differences in the spectral pattern at given wavenumbers, suggest that shifts in the chemical composition are directional and of a greater magnitude than the noise created by uncertainties in the analytical process.

The high degree of variance in the FTIR spectral signal from the individual pollen grains shown here calls into question previous studies that inferred pollen chemical shifts from bulk pollen samples, such as: (Depciuch et al., 2016; Depciuch et al., 2017; Ribeiro et al., 2017). In those studies, relatively small differences in absorption were observed in the spectra of pollen collected in polluted (urban) or non-polluted (rural) sites e.g. between 800 and 1000 and 1400 and 1600 cm^{-1} in *Artemisa* pollen (Depciuch et al., 2017) or between 1600 and 1500 and 1350–1250- cm^{-1} in *Platanus* pollen (Ribeiro et al., 2017). The within *taxon* variance shown in this study reaches in many parts of the spectrum 0,5 scaled absorbance unit (e.g. above 900 cm^{-1} in several spectra

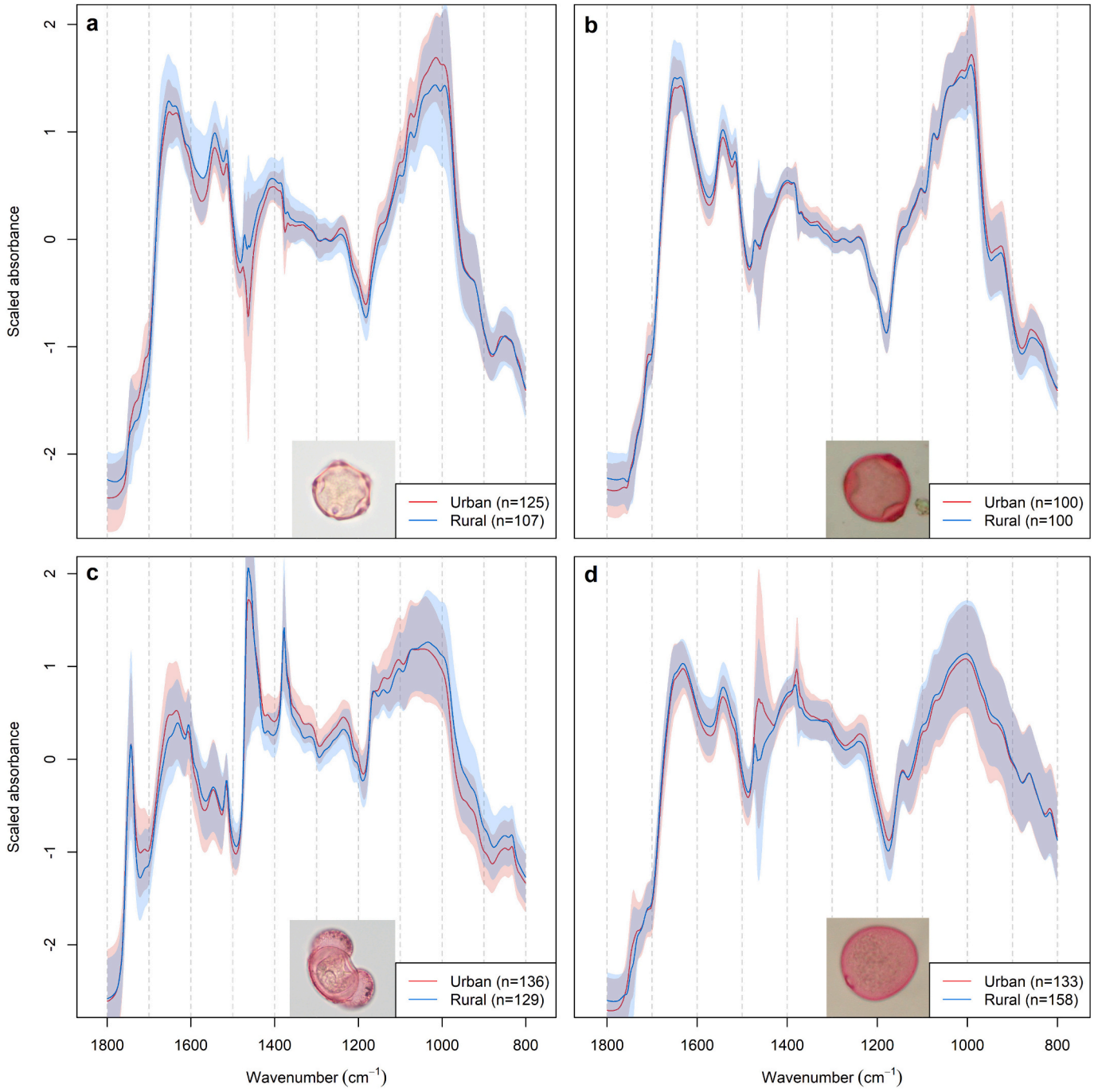

Fig. 4. FTIR spectra of airborne pollen grains of *Alnus* (a), *Betula* (b), *Pinus* (c) and Poaceae (d) collected in Amsterdam (red; urban site) and in the Veluwe (blue; rural site). For further details see the legend of Fig. 3.

[Supplement Figs. S4,5]); such large variations could confound the differences previously observed in other studies. In addition, it is interesting to note that in our study the differences between pollen spectra derived from urban and rural sites were only observed within the most poorly sampled *taxa* (e.g. *Plantago*, urban $n = 11$, rural $n = 4$; Supplement Fig. S2), while our most highly sampled *taxa* (Poaceae, urban $n = 133$, rural $n = 158$; Fig. 2) show almost no difference between the average spectra or the range of variance.

4.2. Differences in spectral characteristics among treatment groups

The significant differences detected in the FTIR spectra between the airborne and flower pollen between 1000 and 1200 cm^{-1} and 1600–1800 cm^{-1} wavenumbers are evident in the well sampled *taxa* despite the possible analytical uncertainties and variance in airborne residency time. Discussing the observed changes in the spectra it is relevant to keep in mind that during the transition from flower pollen to airborne pollen, the pollen grains will undergo dehydration and will be exposed to UV radiation.

(i) 1000–1200 cm^{-1} region

The 1000–1200 cm^{-1} region is generally indicative of carbohydrate contents (Bağcıoğlu et al., 2015) and represents the presence of C – O, C – C, C – O – C, and C – OH stretches and deformations. In the case of pollen samples, it has been shown that the region between 800 and

Table 1
Interpretation of FTIR absorbance peaks common in pollen samples.

Wavenumber	Inferred chemical significance	Reference
1500–1800 cm^{-1}	C=C and carbonyl groups	(Pappas et al., 2003)
1740 cm^{-1}	Carbonyl vibration of esters indicating the presence of intracellular lipids	(Pappas et al., 2003; Lahlali et al., 2014; Depciuch et al., 2016; Zimmermann, 2010)
1640 cm^{-1}	Multiple overlapping bands resulting from the presence of C═C and carboxylate groups ($-COO^-$) However, this band can also be attributed to the presence of proteins by the presence of C═O amide stretches and/or H-O-H vibrations (deformation) from water	(Pappas et al., 2003; Prđun et al., 2021; Bağcıoğlu et al., 2015; Mularczyk-Oliwa et al., 2012)
1530 cm^{-1}	–H bending and C—N stretching from the presence of protein	(Depciuch et al., 2016; Prđun et al., 2021; Hong et al., 2021)
1470 cm^{-1}	CH2 bending and, CH3 deformations belonging to lipid, protein and carbohydrate fractions	(Zimmermann, 2010; Bağcıoğlu et al., 2017; Gottardini et al., 2007)
1380 cm^{-1}	The presence of OH, COH, COO-, CH3 bending and CH2 wagging and twisting, commonly resulting from the presence of sporopollenin in conjunction with several other bands	(Mularczyk-Oliwa et al., 2012; Bağcıoğlu et al., 2015)
1230 cm^{-1}	–OH in-plane bending as well C—O stretching vibration from ester and amide carboxyl groups	(Pappas et al., 2003; Zimmermann, 2010)
1050,1170 cm^{-1}	Vibration of C–OH and C–O–C groups	(Pappas et al., 2003), Depciuch et al. (2016)
920 cm^{-1}	β-Glycosidic bonds	(Pappas et al., 2003)
860 cm^{-1}	α-Glycosidic bonds	(Pappas et al., 2003)

1500 cm^{-1} almost exclusively represents the presence of natural sugars (Pappas et al., 2003). As we observe differences between pollen isolated from the flowers and the airborne pollen, it is possible that in this area as well as the later discussed areas, resulted partly due to atmospheric aging processes.

The main reason for a shift in the 1000–1200 cm^{-1} region in the airborne pollen grains, relative to the pollen sampled directly from the flowers is likely due to changes in the polysaccharide content; specifically related to nutritional changes concerning carbohydrate reserves, during pollen production, release, hydration/dehydration and germination (Pacini, 1996; Pacini, 2000; Pacini et al., 2006). Ripe pollen may contain a store of starch. If there is no starch present, this means that starch has been fully hydrolysed to sucrose, glucose, or fructose during the final stages of pollen maturation (Pacini et al., 2006), which should be represented in increased presence of the 993 cm^{-1}, 1032 cm^{-1}, and 1050 cm^{-1} bands respectively (Prđun et al., 2021). Additionally, changes within the 1140–1175 cm^{-1} region could be the result of glycosidic linkage formation in polysaccharides, which may also result in changes in the 920–1000 cm^{-1} region (Hong et al., 2021). As such it appears that the spectra resulting from airborne pollen contain a greater prevalence of hydrolysed polysaccharide content due to the reduced effect of bands relating to glycosidic linkages. Further, the presence of sharp bands in the airborne pollen at 1050 cm^{-1} are likely more representative of monosaccharides and disaccharides such as glucose and fructose. In the case of the pollen extracted from the flowers, it appears that there is a greater polysaccharide influence, likely due to starch reserves or partially hydrolysed starch, this is further corroborated by the increased presence of glycosidic linkage bands at 920 cm^{-1}.

In conclusion, the main reason for the shift in FTIR bands in the 1000–1200 cm^{-1} region seen between the airborne and flower sampled pollen, is largely the reflection of a change from a mainly polysaccharide content (flower sampled pollen) to a mainly monosaccharide content (airborne pollen). This change in saccharide content takes place after the release of pollen from the flower into the air.

(ii) 1550 cm^{-1} region and 1600–1700 cm^{-1} region

The FTIR spectra obtained from airborne and flower pollen (Fig. 3, Supplement Fig. S1) show significant differences in two bands, one at ~1620–1640 cm^{-1} and one at ~1740 cm^{-1}. The broad band at ~1620–1640 cm^{-1} can be attributed to several functional groups (Table 1). It appears from the spectra that generally there is a shift observed when comparing airborne pollen to pollen collected directly from the flower. The shift in this 1620–1640 cm^{-1} could occur simply due to changes in hydration and water content (Mularczyk-Oliwa et al., 2012). However, coupled with the changes seen in the ~1550 cm^{-1} range it is likely that there are changes in protein composition/concentration since proteins are mainly characterized by two strong and broad bands at 1640 cm^{-1} (amide I: C = O stretch) and ~ 1540 cm^{-1} (amide II: NH deformation and C—N stretch) (Table 1).

In the case of *Alnus*, *Betula* (Fig. 3) and *Quercus*, *Salix* (Supplement Fig. S1) the airborne samples show higher absorbances for the designated protein bands at ~1640 and 1550 cm^{-1} and notable decreases in the 1740 cm^{-1} band resulting from intercellular lipids as well as a decrease in carbohydrate absorbance. Strikingly, in *Pinus* it is the other way around; the airborne samples have a reduced absorbance in these wavenumber regions compared to the flower sampled pollen. It is expected that the airborne pollen has been exposed to ambient and anthropogenic conditions, likely influencing the chemical composition of the pollen grain. The changes observed in the bands representative of protein content (amide bands, specified above) could be indicative of changes in protein composition following exposure to such conditions. Similar increases in absorbance of protein bands and decrease in carbohydrate bands has been noted previously for ragweed pollen exposed to elevated levels of NO_2 (Zhao et al., 2016). It has been shown that even at levels below legislative limits for plant health, changes can be induced in the general protein structure of the pollen grains (Ribeiro et al., 2017). The absence of a clear difference between urban (Amsterdam) and rural (Veluwe) groups in our study suggests that processes that are occurring are similar in both these environments.

Many studies have investigated the changes in allergenicity of certain pollen allergens following exposure. Conflicting results in protein modification and changes in allergenicity have been well documented in past literature with both increases and decrease in protein content/allergenicity noted for a variety of pollen *taxa* and conditions (Bist et al., 2004; Chassard et al., 2015; Franze et al., 2005; Gruijthuijsen et al., 2006; Zhao et al., 2016; Sénéchal et al., 2015; Sousa et al., 2012). Exposure to ambient conditions and anthropogenic pollution can lead to protein oxidation and nitration posttranslational modification due to oxidative stress and aging (Reinmuth-Selzle et al., 2014; Ribeiro et al., 2017). These oxidative stresses have been shown to change protein structure and content – recorded as increases, decreases, or no change, in protein content (Kanter et al., 2013; Reinmuth-Selzle et al., 2014; Ribeiro et al., 2017). Thus, we conclude that the changes observed in the FTIR spectra (1550 cm^{-1} and 1640 cm^{-1}) between the flower and airborne derived pollen groups, regardless of location, most likely reflects the oxidation/nitration of proteins.

(iii) 1170 cm^{-1} and 1700–1800 cm^{-1} region

Bands observed at 1740 cm^{-1} and ~ 1170 cm^{-1} are both attributed to the presence of lipids. In the case of many of the spectra (Fig. 3 and Supplement Fig. S1), airborne pollen showed a notable decrease in one or both of these regions compared to pollen collected from flowers. From a pollen development point of view, lipids play a big part in pollen development, particularly in the development of the exine and later,

prior to release, in the production of the pollen coat from the *tapetum*. The presence of lipids on the exterior of the pollen grain serves to protect it from UV damage and dehydration but can also be stored in the form of intracellular lipids which can be used up after the pollen grain is released (Piffanelli et al., 1998; Zimmermann and Kohler, 2014). It has also been shown that heat stress and other environmental factors can further impact and reduce the lipid content of mature pollen (Lahlali et al., 2014; Zimmermann and Kohler, 2014). Therefore, the increased presence and intensity of lipids in pollen collected directly from the flower compared to airborne pollen can be expected. Lipid modification and decrease in FTIR lipid related bands has also been noted following exposure to air pollution (Kanter et al., 2013). Fatty acid content of pollen grains has been shown to reduce in accordance with extent of pollution exposure caused by degradation and peroxidation processes (Pukacki and Chalupka, 2003). However, exposure to oxidizing pollutants, like ozone, has also been shown to result in the de-methylation and or de-esterification of pectin which has also been shown to contribute to the bands at ~1740 cm^{-1} (indicative of the ester group of pectin) (Kanter et al., 2013). Similar behavior has previously been reported for ragweed pollen, subject to elevated ozone concentrations (Kanter et al., 2013). Exposure to oxidative gaseous pollutants has also been shown to change the bands observed in the 1450–1460 cm^{-1} region (Ribeiro et al., 2017), which can also be attributed to the presence of lipid components (Bağcıoğlu et al., 2017; Zimmermann and Kohler, 2014) and is also evident for many of the spectra presented here.

The absence of any detectable impact of environmental pollution on the airborne pollen is intriguing. This could be due to the reduced overall air pollution concentrations (with the exception of O_3) during the study period due to COVID regulations in place (Menut et al., 2020; Velders et al., 2021). It would therefore be valuable to expand this study along a greater air pollution gradient. Further, the relatively 'low' urban air pollution data collected here could serve as a valuable baseline to compare with a non-lockdown situation. Additionally, investigating the impact of atmospheric residence time on pollen, particularly for pollen undergoing long-distance transport, would be of interest.

5. Conclusion

Our FTIR spectral analysis of airborne and flower sample pollen from both urban and rural settings in the Netherlands suggests that there is no discernable difference by environmental setting, but that significant differences do exist in various parts of the spectra when airborne and flower sampled pollen are compared. This finding suggests that pollen collected in the different settings in the Netherlands did not show chemical modifications that could be detected in the FITR spectra related to gradients in environmental pollution. However, this may be caused by reduced differences in air quality between those settings during the COVID-19 pandemic. Surprisingly, the chemical shifts in airborne versus flower pollen could clearly be observed in the FTIR spectra for all *taxa*. The changes from flower- into airborne pollen observed in the spectra indicate: (i) a shift of polysaccharides into monosaccharides, (ii) a change in concentration and/or oxidation/nitration of proteins, and (iii) lipid modification and decrease.

This calls into question whether pollen isolated from flowers is a good source for pollen used for medical purposes. After all, allergic individuals come into contact with airborne pollen. Detailed chemical analysis approaches now need to be applied to determine if the chemical shifts inferred from the FTIR spectral analysis relate to specific compounds that could influence the allergenic nature of pollen.

CRediT authorship contribution statement

Letty A. de Weger: Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Cas Verbeek:** Writing – review & editing, Visualization, Investigation, Formal analysis. **Emma Markey:** Writing – review & editing, Investigation. **David J. O'Connor:** Writing – review & editing, Investigation. **William D. Gosling:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

WIlliam Gosling reports financial support was provided by the Netherlands Organisation for Scientific Research (NWO). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data is avaliable from: de Weger, L.A., Verbeek, C., Markey, E., O'Connor, D., Gosling, W.D. (2024) Data from: Greater difference between airborne and flower pollen chemistry, then between pollen collected across a pollution gradient in the Netherlands. FigShare. https://doi.org/10.6084/m9.figshare.25834894.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.172963.

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