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Building an automatic pollen monitoring network (ePIN): Selection of optimal sites by clustering pollen stations

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abstract

Airborne pollen is a recognized biological indicator and its monitoring has multiple uses such as providing a tool for allergy diagnosis and prevention. There is a knowledge gap related to the distribution of pollen traps needed to achieve representative biomonitoring in a region. The aim of this manuscript is to suggest a method for setting up a pollen network (monitoring method, monitoring conditions, number and location of samplers etc.). As a case study, we describe the distribution of pollen across Bavaria and the design of the Bavarian pollen monitoring network (ePIN), the first operational automatic pollen network worldwide.

We established and ran a dense pollen monitoring network of 27 manual Hirst-type pollen traps across Bavaria, Germany, during 2015. Hierarchical cluster analysis of the data was then performed to select the locations for the sites of the final pollen monitoring network. According to our method, Bavaria can be clustered into three large pollen regions with eight zones. Within each zone, pollen diversity and distribution among different locations does not vary significantly. Based on the pollen zones, we opted to place one automatic monitoring station per zone resulting in the ePIN network, serving 13 million inhabitants. The described method defines stations representative for a homogeneous aeropalynologically region, which reduces redundancy within the network and subsequent costs (in the study case from 27 to 8 locations). Following this method, resources in pollen monitoring networks can be optimized and allergic citizens can then be informed in a timely and effective way, even in larger geographical areas.

1. Introduction

Pollen is part of the biological exposome, carrying allergens, fungi and bacteria able to activate the human immune system (Buters et al., 2015; Buters et al., 2012; Galán et al., 2013; Oteros et al., 2019). There are a number of networks that routinely monitor airborne pollen worldwide. These were built for a range of purposes (Buters, 2014), such as examining gene flow (Hofmann et al., 2014), allergy prevention (de Weger et al., 2014; Fernandez-Rodriguez et al., 2014; Sofiev et al., 2017; Werchan et al., 2017; Zink et al., 2012), crop forecasting, pest control, impacts of land use changes (Aguilera and Ruiz-Valenzuela, 2014; Cunha et al., 2016; Dhiab et al., 2017; Jochner-Oette et al., 2017; Rodríguez-Rajo et al., 2010), climate change impacts (De Linares et al., 2017; Galán et al., 2016; Smith et al., 2014; Zhang et al., 2015; Ziello et al., 2012a; Ziello et al., 2012b) and monitoring biodiversity (Belmonte et al., 2000; Cariñanos et al., 2013; Sikoparija et al., 2009; Thibaudon et al., 2014).

The simplest way to sample airborne pollen and fungal spores is to collect the particles deposited or impacted on certain surfaces (Darwin, 1846). First generation traps (1G) do not provide volumetric information (Cour, 1974; Durham, 1946; Ogden and Raynor, 1967), although some have been termed semi-volumetric because they were calibrated with help of an anemometer (Orlandi et al., 2014). On the other hand, second generation samplers (2G) use a range of different sampling principles (Mandrioli et al., 1998) and provide volumetric data making them comparable. The system designed by Hirst N65 years ago is based on the impaction principle (Hirst, 1952), and is the standard in pollen and fungal spore monitoring networks in most of the world (Galán et al., 2014; VDI4252-4, 2016). The Hirst-type trap has several advantages, e.g. it delivers pollen concentration data with a temporal resolution of up to 2 h, it provides volumetric information (i.e. pollen/m^3) and it has a long autonomy of up to 7 days.

There are currently N600 Hirst-type traps actively running worldwide, mostly in Europe (https://www.zaum-online.de/pollen/pollen-monitoring-map-of-the-world.html) (Buters et al., 2018). A standardized sampling system and working methodology is essential for a network, and there are numerous publications on the standardization of this monitoring method (Alcázar et al., 1999; Comtois et al., 1999; Galán and Domínguez-Vilches, 1997; Gharbi et al., 2017; Levetin et al., 2000; Maya-Manzano et al., 2017; Oteros et al., 2017; Oteros et al., 2013a; Sikoparija et al., 2017; Smith et al., 2019; VDI4252-4, 2016). The "Minimum requirements for aerobiology" (Galán et al., 2014), sup- ported by the European Aerobiology Society, is recognized as an inter- national standard for pollen monitoring using Hirst-type traps. Other devices currently used in active networks include the Cour method (Oteros et al., 2014), Rotorod samplers (Portnoy et al., 2004) and Dur- ham traps (Teranishi et al., 2000).

The main disadvantage of all manual methods is that data production is highly time consuming and thus it is not feasible to provide timely information with respect to the current, real-life situation. A time lag of several days between actual pollen flight and reported pollen flight is common. A solution could be automation, but the complexity of pollen identification has made this impossible until now. New technol-ogies allow for fully automatic-online pollen monitoring with third generation automatic traps (3G). The main advantages of these traps are that they can provide almost real-time information and the data are free of random errors produced by human interferences. Such 3G automatic systems are starting to be used for routine pollen monitoring, e.g. the KH-3000 (Kawashima et al., 2017), the Plair PA-300/Rapid E (Crouzy et al., 2016; Šaulienė et al., 2019), Pollen Sense (http://pollensense.com/), the BAA500 (Oteros et al., 2015b) or WIBS-4 (O'Connor et al., 2014).

The main reason for building the Bavarian pollen monitoring network ePIN was to reduce delays in the dissemination of pollen information and to inform allergic citizens, physicians, and health organizations in a timely manner. Another aim of the ePIN network was to serve as a registry of environmental changes. To do so, we had to increase the representativeness of pollen monitoring across Bavaria. The whole federal state has actually a population of 13 million inhabitants, but airborne pollen has routinely been monitored at only three locations (www-genesis.destatis.de). A handicap of classical pollen monitoring networks is that they are often built on the basis of stations set up by individuals, a fact that does not always guarantee an appropriate choice of positions for pollen monitoring stations. There is a general lack of studies on the optimization of monitoring locations, and so the decision where to install a station has usually been based on personal preferences, often in large urban areas (Buters et al., 2018).

The aim of this study was to the overall "Aims" were to provide near real-time health information to pollen allergic individuals and health care practitioners and so serve as a valuable source of biological data for (but not limited to) long-term biodiversity and climate change studies.

The following are "Objectives" or steps taken to achieve the overall aims:

- Design a method for building a pollen network considering monitoring method, homogeneous monitoring conditions, number and location of monitoring stations.
- Investigate the pollen distribution across Bavaria.
- Use this information to produce the first operational automatic pollen network in history: the electronic Pollen Information Network (ePIN).

2. Material and methods

2.1. Study area

Bavaria extends to an area of 70.553 km^2 being the largest federal state in Germany, and is located in the south of the country (Fig. S1). The maximal distance between locations in Bavaria is N400 km. The environmental zones are quite heterogeneous, containing a wide range of climates from the cold Bavarian Alps in the South (Zugspitze with an annual mean temperature of −4.3 °C and mean precipitation of 2071 mm) to the warmer-dryer Franconian wine area in the North-West (Würzburg with an annual mean temperature of 9.6 °C and mean precipitation of 601 mm). The annual mean temperature at Munich is 8.7 °C with a mean annual precipitation of 834 mm and the annual mean temperature at Nuremberg is 9.3 °C and mean precipitation is 637 mm (all climate data from the German Meteorological Service for the reference period 1981–2010). These different conditions render representative pollen monitoring across the state rather complex.

Thirty-seven percent of the Bavarian area is covered by forests (https://bwi.info/). The most important forested areas are the Alps (South) and the Bavarian Forest (West), both with coniferous predominance. The most predominant pollen types in the region were from *Pinus* and *Picea*. Also, pollen from deciduous trees in forest patches was dispersed across the state, such as *Betula* and *Fagus*. The Bavarian environment is also characterized by extensive pastures with an abundance of anemophilous herbaceous plants as from the families Poaceae and Plantaginaceae. The pollination period in Bavaria is expected to occur from the early flowering anemophilous taxa such as *Alnus* and Cupressaceae in January to the latest flowering anemophilous genera of *Ambrosia* in October.

2.2. Proposed steps of building the network

2.2.1. Selection of pollen monitoring methods

We reviewed the advantages and disadvantages of the current options for pollen monitoring (see results). For the final (permanent) ePIN network, an automatic pollen monitoring system was selected, whereas for the pilot (test) network we chose the Hirst-type monitoring method (Hirst, 1952), as it is accepted currently as the most standard pollen monitoring system.

2.2.2. Review on the site conditions for installing a pollen monitoring device

We performed a bibliographical review and an international survey among the administrators of pollen monitoring networks about the main factors affecting the decision of trap location at the local (site) scale (see Results and discussion).

2.2.3. Building a redundant pollen network (pilot network)

To select the final number and location of monitoring stations across Bavaria, we first built a dense network of 27 Hirst traps throughout the country (the pilot network) and, based on the results obtained, reduced the number of locations with hierarchical clustering (Ward's clustering method). This mathematical method has already been used to set up chemical air quality monitoring networks (Nakamori and Sawaragi, 1984). The number of stations was deliberately selected to configure a redundant network that could be collapsed after the analysis. To ensure it was a redundant network, the density of stations was the highest in the world for a pollen network of these dimensions (27 stations in Bavaria - 70,550.19 km^2) (Buters et al., 2018).

2.2.3.1. Selection of monitoring locations in the pilot network. The preselection of 27 pollen monitoring locations was performed in order to increase the regional representativeness of the pollen sampling. The following factors were considered: demography, availability of historical time series, climate, land use types, topography and proximity to local pollen sources. The pre-selection was done trying to satisfy most of the specific features that the network should have:

- Must preserve historical time series, so historical locations were included.
- Must be informative for the majority of the population, so the most populated areas were closely monitored. Bavaria is unevenly populated, with several major urban agglomerations (Fig. S2a). The 25 km radius buffer around all the pre-selected stations covered 94% of the Bavarian population. It is accepted that the area influencing a roof-level trap is N25 km (Oteros et al., 2015a).
- Must be capable of detecting pollen episodes early, so source areas and borders of the state were preferred (Fig. S2b). The places near main pollen sources are more appropriate locations to perform a better forecasting of airborne particle distribution. Broad-leaved forests consisting of early spring flowering allergenic trees, like alder and birch, are present in patches, and are spread over Bavaria and near its borders (i.e. the locations were located close to the broad-leaved forest patches and the big forest areas as the North-West broad-leaved forest, the Bavarian forest and the Alps). This suggests the possibility of setting stations closer to the borders of Bavaria to earlier catch the moments of the forests starting to flowering out of the boundaries. Grasses are also common in Bavaria; their pollen is known to be less efficiently transported than those from anemophilous trees.
- Must provide data for model-based forecasting, for data assimilation and for the model evaluation.
- Must cover the major biogeographic environments existing in Bavaria. Temperature is important for the season start and duration: plants in the warmer parts tend to flower earlier. In this sense, the warmest regions of Bavaria are north-west and south-east. The colder and wetter areas are the alpine mountains in the south and the area of the Bavarian Forest in the east (Fig. S2c, d).

The pre-selection of the locations for new stations was carried out for zones with a 25 km radius (this number is smaller than the accepted influence area of a pollen trap at roof level (Oteros et al., 2015a)). The selection of the specific location was then done by screening for homogeneous monitoring conditions inside each pre-selected zone within the network. To ensure a proper coverage of all pollen sources in Bavaria, an analysis with the System for Integrated modeLling of Atmospheric coMposition (SILAM, http://silam.fmi.fi) was conducted, ensuring a large coverage of Bavarian sources (Sofiev et al., 2013; Sofiev et al., 2015). The footprint that the pilot network covered in 2015 is shown in Fig. 1.

A footprint is the area comprising the sources that affected the monitored parameter during a specific observation period (in the current case, pollen data of every specific day during the 2015 season). Such a footprint delineates the area where the sources would contribute to the monitor readings, if emitting pollen during the corresponding time. Areas outside the footprint do not affect the pollen monitor. This "negative" part of the footprint message makes it a handy tool for delineating the network weaknesses. Formally, the convolution of emission E, and the footprint "intensity" ϕ* over space and time is equal to the mean concentration *C* at the specific place during the specific period (monitor location and time of activity) (function (1)).

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Thus, a footprint delineates the area where the sources would contribute to the monitor readings if emitting pollen during the corresponding time and shows which sources can affect the monitor during the specific observation period. The sum of the footprints of all daily measurements by the 27 ePIN stations in 2015 is shown in Fig. 1. The footprints were calculated over three days backwards from the observation day, i.e. covered the areas from where the emitted pollen needed up to three days to reach the monitor.

The exact location to set up a pollen trap was selected by a site visit of potential places within the 25 km-radius zones. Criteria for a site were reviewed in Section 2.2.3.2 and are listed in Table 2. This resulted in 27 locations described in Table 1.

2.2.3.2. Establishment and quality control in the dense pilot pollen monitoring network. The network of 27 Hirst pollen traps was set up across Bavaria at the selected locations (Table 1) during the winter of 2014–2015. The network then operated from the end of February until the end of September 2015. All traps were located so that homogeneous monitoring conditions may be achieved (see Table 2). For instance, all stations were built at 12 m a.g.l. (±3 m) eliminating the large variability of the first 10 m layer and eliminating differences between stations in height (Rojo et al., 2019a), all traps were also located at 1.5 m above roof level by a standard tower (Fig. S3a) and at least 2 m from the building

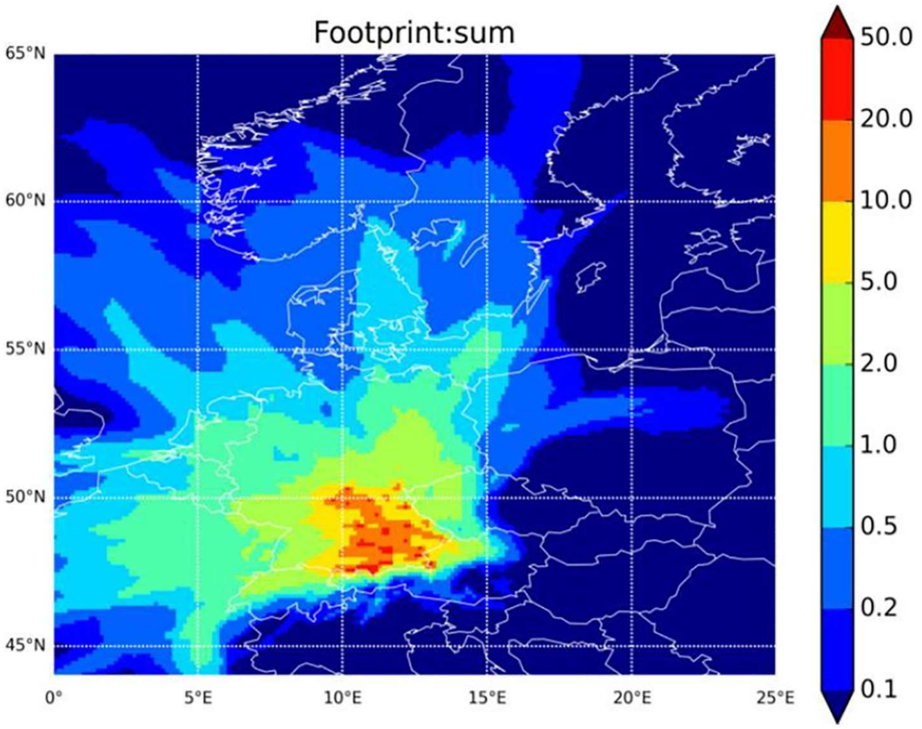

Fig. 1. Footprint of System for Integrated modeLling of Atmospheric coMposition (SILAM, http://silam.fmi.fi) for the birch pollen season of 2015 for the 27 ePIN locations.

edge. Flow rates of the pollen traps were calibrated using the same flowmeter thereby reducing intra-rotameter variability (Oteros et al., 2017). Drums and microscope slides were processed centrally by a single laboratory (Fig. S3b) under identical conditions. The drums were sent biweekly to the 27 monitoring stations. Slides were processed using the standard operating procedure described by Galán et al. (2007).

Each pollen slide corresponded to one independent day (Fig. S3c). All slides had a blue line, marking midday (12,00). Four blue dots were set 1 mm apart from each other at the center of the slide with a standard self-designed tool to guide the analyst for the starting point of each horizontal line. Pollen microscopic identification and counting were conducted in four continuous horizontal sweeps along the whole slide under 400× magnification, each sweep starting from each one of the marked points.

Sub-sampling of the slide is essential for reducing workload. The area of the slide sub-sampled during the analysis was at least 7% of

Table 1
Selected locations for ePIN. Population coverage (%) within 30-km from each station is given.

Code	Site location (German)	Latitude (°)	Longitude (°)	Altitude (m a.s.l.)	Population coverage (%)
DEALTO	Altötting	48.23	12.68	398	2.5
DEAUGS	Augsburg	48.33	10.90	497	6.4
DEBAMB	Bamberg	49.90	10.89	238	3.6
DEBAYR	Bayreuth	49.94	11.53	419	2.8
DEBERC	Berchtesgaden	47.64	13.01	573	0.8
DEBIED	München	48.16	11.59	510	15.2
DEDONA	Donaustauf	49.04	12.21	425	3.4
DEERLA	Erlangen	49.60	11.01	284	9.7
DEFEUC	Feucht (Nürnberg)	49.38	11.20	365	8.9
DEGAIS	Gaissach	47.75	11.58	717	2.7
DEGARM	Garmisch-Partenkirchen	47.49	11.10	821	1.2
DEHOF	Hof	50.32	11.90	531	1.9
DEKITZ	Kitzingen	49.74	10.14	246	3.6
DEKOES	Kösching	48.82	11.51	391	3.2
DELANDS	Landshut	48.54	12.14	397	3.0
DEMARK	Marktheidenfeld	49.85	9.63	216	3.4
DEMIND	Mindelheim	48.04	10.50	610	3.3
DEMUNC	München	48.13	11.56	538	15.1
DEMUST	Münnerstadt	50.25	10.18	347	2.6
DEOBER	Oberjoch	47.52	10.40	870	1.7
DEOETT	Oettingen	48.96	10.60	431	1.8
DEPASS	Passau	48.56	13.44	318	2.1
DETROS	Trostberg	48.03	12.56	483	2.7
DEUFS	Umwelt Forschungsstation Schneefernerhaus (UFS)	47.42	10.99	2650	0.8
DEVIEC	Viechtach	49.08	12.87	459	2.0
DEWEID	Weiden	49.68	12.17	403	2.0
DEZUSM	Zusmarshausen	48.40	10.61	483	5.7

Table 2
Standard conditions for homogeneous pollen monitoring in the network.

Conditions for pollen monitoring		
Logistic	Trap location	Emission sources
Safety location	Flat and horizontal surface	Absence of overrepresentation of some species in surrounding 500 m area
Easy access	Higher than surrounding roofs and other wind walls	Absence of anemophilous sources in surrounding: uncut grass areas (no in 50 m) and birch/olive trees (no in 100 m).
Access to electric network	Not at ground level. Between 9 m and 15 m from the ground (on a roof or an elevation tower). This criterion aims to build the whole Bavarian network under homogeneous conditions to have a more uniform information over a greater area in comparison to measurements on the ground with an extreme influence of plants nearby.	Absence of proximity to non-biological and biological particle sources of high emission (e.g. waste plant)
Access to internet (For 3G systems)	Not placed at the edge of a building (N2 m) to avoid turbulent flow	Absence of proximity to wind distortion sources (e.g. solar panels, refrigeration systems…)
Temporal sustainability	Elevated N150 cm from the roof or elevation surface	Consider whether land use change will have an effect on pollen concentrations in future

the slide, following the recommendations of VDI guidelines (VDI4252-4, 2016). The use of a standard 12.5 mm net micrometer reduced the area of the slide examined to 9% when examining 4 transects (or 7% when using a 10 mm net micrometer) (Fig. S2d). A standard correction factor was used to reduce error. Pollen counts (raw data) were entered into a specially designed computer program to reduce typing errors (Fig. S2f). Data were exported from this program and stored in an online SQL ZAUM database.

In total, 13 pollen types were analyzed: *Alnus*, *Ambrosia*, *Artemisia*, *Betula*, *Carpinus*, Cupressaceae, *Fraxinus*, *Picea*, *Pinus*, *Plantago*, Poaceae, *Populus*, and Urticaceae. Pollen was reported in 12-hour concentrations for each station during the study period. Pollen not falling within the 13 specified pollen types was reported as "unspecified" pollen grains.

An external Quality Control program of the analysts was performed with a novel method, published in detail by Smith et al. (2019).

2.2.4. Establishment of the final pollen monitoring network

2.2.4.1. Selection of the definitive number of traps by clustering analysis. We clustered the information obtained from all pollen traps in order to determine areas with a similar distribution in pollen. Due to the complexity of data (daily pollen monitoring of 27 stations and 13 pollen types), we applied a multivariate statistical method able to consider all the variables at the same time.

First, we preselected the part of the database to be included in the analysis. We selected the most abundant pollen types for the clustering analysis (N1000 pollen grains/season on average): *Betula*, Cupressaceae, *Fraxinus*, *Pinus*, *Picea*, Poaceae and Urticaceae. Those are the pollen types with the largest spread of annual pollen values, whereas less abundant pollen types do not show big differences among the locations and so it makes little sense to cluster pollen zones based on them (Fig. 2A). The flowering dates by pollen type are shown in Fig. 2B, carried out with the R package AeRobiology (Rojo et al., 2019a, 2019b). From the 27 monitoring stations, DEERLA was excluded from the analysis due to

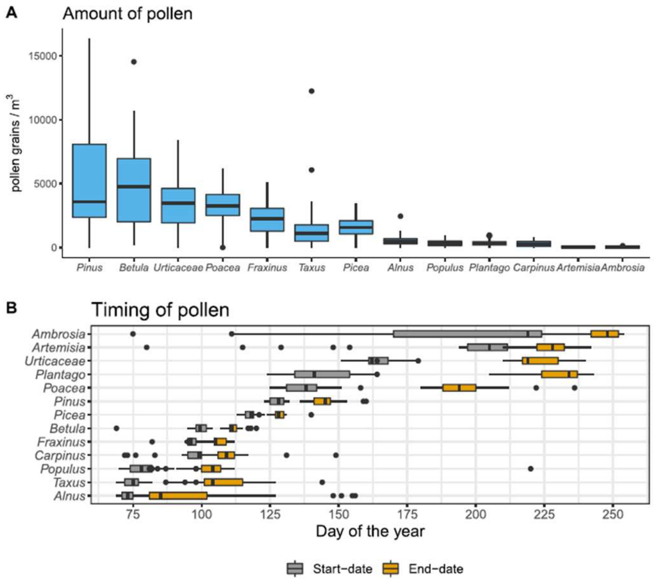

Fig. 2. A) Boxplot showing the Seasonal Pollen Integral (SPI) of the considered 13 pollen types at the 26 ePIN locations (all excluding DEERLA). B) Boxplots showing the start- and end date of the pollen season in 2015 (80% of annual pollen) in Bavaria.

technical malfunctions. Cupressaceae pollen was excluded from the analysis due to the incomplete monitoring of the whole season. *Pinus* was excluded from the clustering analysis to avoid overrepresentation of the Pinaceae family for designing the network, *Picea* was included. The pollen season for each pollen taxon was defined for the whole Bavaria as follows, excluding the long tails before and after the season with zero values: *Betula* (from 1/4 to 14/5); *Fraxinus* (from 25/3 to 2/5); *Picea* (from 22/4 to 26/5); Poaceae (from 8/5 to 6/8); Urticaceae (from 1/6 to 8/9).

Second, we calculated all Pearson correlations in daily pollen concentrations between pairs of stations (26 stations). We applied this correlation analysis for each selected pollen type (5 pollen types in total: *Betula*, *Fraxinus*, *Picea*, Poaceae and Urticaceae). For each pollen taxon, we only included in the analysis the stations with at least 80% of the data during the season. When a correlation was too low, as could happen by chance, all correlations b0.5 were equalized to 0 in the correlation matrixes for the clustering analysis.

Third, we applied a clustering analysis to the correlations' coefficients (Hierarchical clustering by Ward's method) (Oteros et al., 2013b; Ward Jr and Hook, 1963). In the analysis, 26 cases were included to be conglomerated (each monitoring station). Each variable was defined as the correlation coefficient between one station (for each pollen type) and each one of the 26 stations (for the same pollen type). Twenty-six cases were included in the analysis (one per station). A correlation coefficient is not a metric of distance per se, but the combination of them allows us to calculate Euclidean distances. Furthermore, a visualization of the five closest Euclidean distances for each element is shown by a network plot (see Fig. 4).

2.2.4.2. Determination of the final monitoring locations. Within each cluster calculated in Section 2.2.4.1, one station was then selected. We selected the most relevant station of each cluster using the following selection criteria:

- The station covering the highest population was selected (If two or more stations differ by b0.5% population, they are all selected at this stage).
- In the case of a draw (similar population), the station closest to the border of Bavaria was selected.
- Two selected stations cannot be located closer than 70 km apart (ensuring a proper coverage of the whole surface). If two stations are closer than 70 km, then the most populated location is selected in one sub-cluster and the next by population is selected in the other cluster.
- Four stations using the Hirst-type biomonitoring method were kept as a parallel manual network to maintain a historical time series (DEOBER, DEMUST, DEBAMB and DEUFS) and were not selected for the automatic network.

An automatic network was then built in Bavaria based on these criteria.

3. Results and discussion

3.1. Selection of the pollen monitoring method

The main goals of this pollen monitoring network were: 1. to provide near real-time health information to pollen allergic individuals and health care practitioners. 2. To serve as a valuable source of biological data for (but not limited to) long-term biodiversity and climate change studies. Hence, the network must allow for, if possible, the continuation of historical time series and monitor the whole spectrum of airborne pollen types, not only the allergenic ones.

A permanent network of Hirst-type traps may provide pollen information with a minimum lag time of 1 to 2 days, involving a colossal human effort and huge costs (arising mostly from the need of experienced personnel). We therefore focused on an alternative, automated, detection system able to provide near-real-time information. There is a well-established monitoring network of 120 traps based on an automatic-online monitoring system in Japan, the KH-3000 (Kawashima et al., 2017), but this automatic system until now has been unable to provide accurate information on the complete range of pollen diversity. Other systems look promising for the development of a complete and reliable automatic recognition system of pollen: e.g. Pollen Sense (http://pollensense.com/, no scientific publication available), BAA500 (Oteros et al., 2015b), Plair PA-300 Rapid E (Crouzy et al., 2016; Šaulienė et al., 2019) or Swisens (www.swisens.ch, no scientific publication available). Of these, only two automatic pollen monitoring systems were fully operational on the date of the network setup, BAA500 (Oteros et al., 2015b) and Plair PA-300 (Crouzy et al., 2016).

The BAA500 was the preferred monitoring system for the ePIN network because it has specific features that make it a good candidate for the transition from manual to automatic monitoring. The BAA500 uses image recognition emulating the process of a human using a microscope, and can provide 3-hour pollen concentrations online (it can be programed to deliver 1 hour concentrations, but not checked the error to date) and its averaged identification error rate is below 10% (Oteros et al., 2015b).

3.2. Selection of homogeneous pollen monitoring conditions

The results of the bibliographical review and the survey among European experts about suggested conditions for pollen monitoring are shown in Table 2. Most of these criteria are extracted from the pre-established knowledge about pollen monitoring (Galán et al., 2007; Galán et al., 2014; Mandrioli et al., 1998; Saar and Meltsov, 2011).

Some criteria were not defined quantitatively because they have not been exhaustively studied. In this sense, the criteria of Table 2 should not be termed as optimal but more motivated by the necessity of defining comparable standard conditions for the whole network.

3.3. Running the dense pilot pollen network

At a regional scale, the pollen stations had to be placed in areas of interest to the general population, thus demography was a main criterion. Other factors that strongly affect air quality such as topography (Rojo and Perez-Badia, 2014), land use (Haberle et al., 2014) or weather (Damialis et al., 2005), which modify the interpretation of pollen monitoring, were also considered.

The pilot pollen monitoring network based on these criteria is shown in Fig. 3. Based on these criteria, the steps followed to perform the selection were:

- Existence of historical time series was considered for monitoring climate change impacts. 9 Stations with historical time series were selected at first: DEZUSM, DEMUST, DEBAMB, DEERLA, DEBAYR, DEDONA, DEMUNC, DEGAIS and DEBERC.
- Existence of other independent stations running during 2015 in Bavaria was also considered as part of the pilot network. 5 Stations were added: DEBIED, DEAUGS, DEPFRO, DEGARM and DEUFS.
- The network must be capable of detecting pollen episodes early, so source areas got particular attention. The places closer to the main pollen sources are more appropriate locations to perform a better forecasting of airborne particles. 4 Stations were added: DEALTO (Close to the Austrian sources), DEVIEC (Bavarian Forest), DEMARK (North-West Franconian forest) and

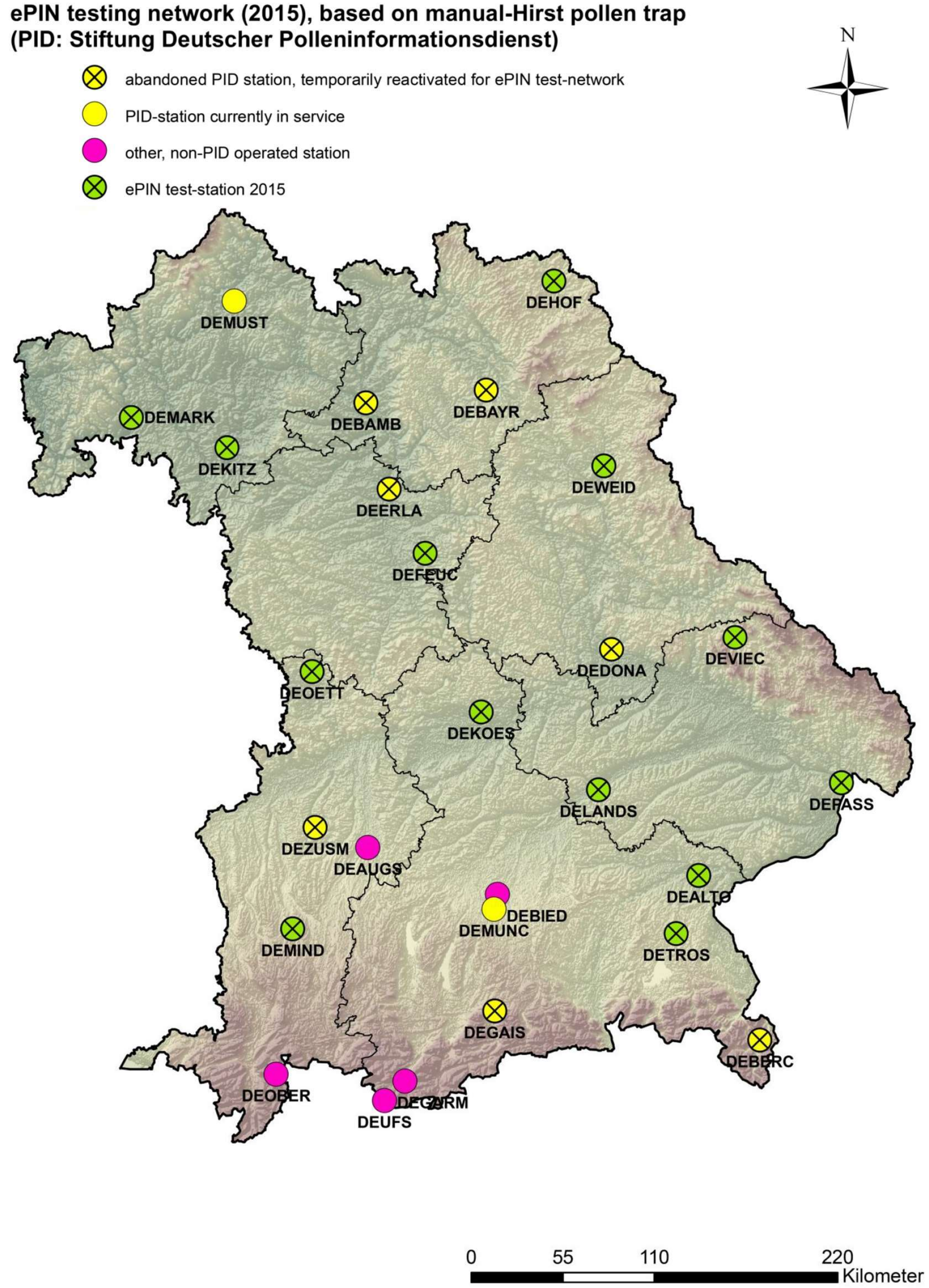

Fig. 3. ePIN test pollen network implemented during 2015 in Bavaria. This consisted of Hirst-type pollen traps at 27 locations under homogeneous monitoring conditions.

DEOETT (Schwaben-South Franconia forest).

- The network must be informative for the bulk of the population, so the most populated areas were closely monitored when necessary. 4 Stations were added: DEFEUC (Close to Nürnberg), DEKITZ (Close to Würzburg), DEKOES (Close to Ingolstadt) and DEMIND (populated area in Schwaben).
- Must cover the major biogeographic environments existing in Bavaria. Most of the environments were already covered. 5 Stations were added: DELAND (middle-stream Danube area), DETROS (South East Bavaria), DEPASS (downstream Danube area), DEWEID and DEHOF (North-East Bavaria).

Out of those stations, five were independently managed: DEAUGS (UNIKA-T, first data: 1999), DEBIED (private-ZAUM, first data: 2003),

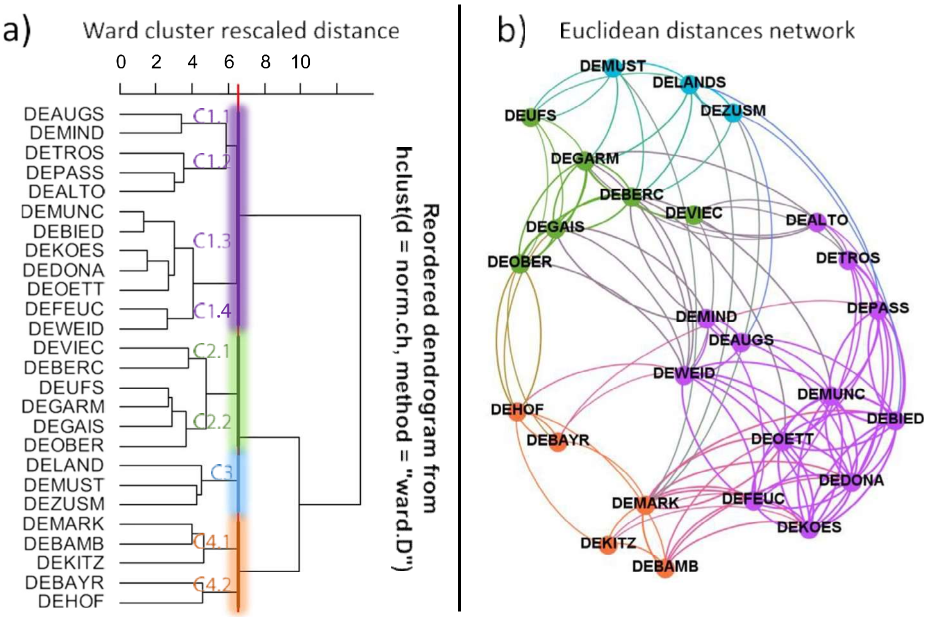

Fig. 4. a) Dendrogram deriving from hierarchical clustering by use of Ward's method of Euclidean distances. b) Network plot representing the position of the 26 considered ePIN stations (nodes) at a bi-dimensional space based on the 5 shortest Euclidean distances of each station (edges).

DEOBER (private-PID, first data: 1982), DEGARM (TUM, first data: 2008) and DEUFS (TUM, first data: 2008). Two stations were managed by PID with long time series: DEMUST (first data: 1990) and DEMUNC (first data: 1987). The other 20 stations were managed at the central lab of ZAUM (these raw data are supported with the manuscript). From these 20 stations, 7 correspond to historical PID locations with long time series but previously discontinued: DEBAMB (first data: 1989), DEBAYR (first data: 1989), DEERLA (first data: 1987), DEDONA (first data: 1989), DEZUSM (first data: 1987), DEGAIS (first data: 1992) and DEBERC (first data: 1987). Thirteen stations corresponding to new locations commenced in 2015: DEHOF, DEMARK, DEKITZ, DEWEID, DEFEUC, DEOETT, DEVIEC, DEKOES, DELANDS, DEPASS, DEALTO, DETROS and DEMIND.

3.4. Clustering pollen zones and selected locations of the definitive ePIN network

The result of the analysis is summarized as a dendrogram (Fig. 4a), starting with 26 elements and ending with one (senseless) big cluster. We had to determine the number of clusters that represented similarities between locations and clustering distance. Elbow plot suggest the existence of 4 main clusters. After 4 clusters, the variance explained by additional clusters is smaller (Fig. S4).

Fig. 4a shows four clearly defined clusters: A Central Cluster (C1) with 12 stations distributed in the center of Bavaria; A Cold Cluster (C2) with 6 stations distributed in the colder areas (Alps and Bavarian Forest); An Outlier Cluster (C3) with 3 stations without apparent connection; A Franconian Cluster (C4) with 5 stations distributed in the North. The abovementioned names of each cluster are provisional and serve only for providing a better understanding of the grouping.

There were not always well-defined boundaries between clusters and, so, some regions could be considered as transitional or subclusters (8) inside the bigger four clusters. For instance, in the cluster C4 (Franconian cluster), DEHOF and DEBAYR are in a small sub-group (East-Franconia) because of bioclimatical similarities (lower temperatures, see Fig. S3c) with respect to West-Franconia. In the same way, DEBIED and DEMUNC are the nearest stations in the first cluster C1 (Central cluster) and indeed both traps are located within the same city

Table 3
Sub-clusters and selection criteria. Within each subcluster, the station with the highest population was selected. In the case of draw, the selection criteria were applied for tiebreaking and selecting the most representative station within the sub-cluster. Rows in bold mark the selected locations.

Ward sub-cluster	Code	% of covered population	Selected	Criteria
1.1	DEAUGS	6.4	No	Standard criteria - b70 km apart from the closest station (DEMUNC)
1.1	DEMIND	3.3	Yes	Standard criteria
1.2	DETROS	2.7	No	Population draw
1.2	DEALTO	2.5	Yes	Population draw, closer to the border
1.2	DEPASS	2.1	No	Standard criteria
1.3	DEBIED	15.2	No	Population draw
1.3	DEMUNC	15.1	Yes	Population draw, closer to the border
1.3	DEDONA	3.4	No	Standard criteria
1.3	DEKOES	3.2	No	Standard criteria
1.3	DEOETT	1.8	No	Standard criteria
1.4	DEFEUC	8.9	Yes	Standard criteria
1.4	DEWEID	2	No	Standard criteria
2.1	DEVIEC	2	Yes	Standard criteria
2.1	DEBERC	0.8	No	Standard criteria
2.2	DEGAIS	2.7	No	Standard criteria - b70 km apart from the closest station (DEMUNC)
2.2	DEOBER	1.7	No	DEOBER was already selected for manual monitoring in a parallel manual network
2.2	DEGARM	1.2	Yes	Standard criteria
2.2	DEUFS	0.8	No	Standard criteria
3	DEZUSM	5.7	No	Outlier
3	DELAND	3	No	Outlier
3	DEMUST	2.6	No	Outlier
4.1	DEBAMB	3.6	No	Population draw
4.1	DEKITZ	3.6	No	Population draw
4.1	DEMARK	3.4	Yes	Population draw, closer to the border
4.2	DEBAYR	2.8	No	Standard criteria - b70 km apart from the closest station (DEFEUC)
4.2	DEHOF	1.9	Yes	Standard criteria
–	DEERLA	9.7	No	Standard criteria

(Munich). DEALTO, DEPASS and DETROS showed similar data, the three locations are quite close to the southeast of Bavaria. DEAUGS and DEMIND showed similar data too, both locations are close in southwest Bavaria. DEGARM and DEUFS constitute also a small subcluster inside the C2 (Cold cluster), both locations being only 10 km apart in the Alps region, however altitudinally differing by 1900 m. Three stations, DEMUST, DEZUSM and DELANDS (Cluster C3) were considered as outliers (i.e. the cluster was formed of stations located in distant and different bioclimatic areas).

Fig. 4b shows a visualization of the Euclidean distances between locations (based on all the pairs of correlations), for a better understanding of sub-clusters and transitional areas. For visualization, we represented only the edges with the five closed elements. Fig. 4b is a way of visualizing a multidimensional space into two dimensions by rescaling the weight of the edges, so the result does not necessarily match with the dendrogram produced by Hierarchical clustering. This visualization allows us to understand that inside C4, the stations of East-Franconia (DEHOF and DEBAYR, colder area) are closer to the Cold cluster (C2) and the stations of the warmer area are closer to the Central cluster (C1). Inside the cluster C2, DEVIEC is closer to the central cluster, indeed this station is at the border of C1 surrounded by DEWEID, DEDONA and DEPASS-DEALTO-DETROS. As can be observed, DEUFS is the station farthest away from the rest of the network inside C2, as this station is located under extreme conditions at the top of the Alps at 2656 m a.s.l., being the highest pollen monitoring station in the world (http://www.schneefernerhaus.de/startseite.html). The three stations included into the Outlier cluster (C3) appear isolated also in Fig. 4b. DEWEID appears connected with the colder stations of C4 and the 5 stations of C2, and indeed this station is also located in the Bavarian Forest, under transitional conditions between C1 and the stations DEHOF-DEBAYR (C4) and DEVIEC (C2). The hierarchical clustering put this station in a subcluster together with DEFEUC (both are the northern stations of C1).

For each of the 8 subclusters we selected only one station for automation in the permanent network. To select the most relevant station inside each sub-cluster we followed a series of selection criteria. Table 3 shows the final selection.

The station DEOBER would have been selected for the permanent network by population, however this station has one of the longest time series in Bavaria (Simoleit et al., 2016), thus the station was already been selected for a parallel permanent manual network. To avoid double sampling at the same location and to save resources, we defined the fourth criteria, the station was changed for the next suitable station inside the alpine sub-cluster according to the selection criteria (DEGARM). This station has a special touristic-economic interest in a changing environment (Hamilton and Tol, 2007). Fig. 5 shows the final selected network with 8 automatic locations.

During the last 60 years of pollen monitoring, the standardization of methods was an important issue and all the efforts ended into a high degree of comparability between pollen data across the globe (Galán et al., 2014). At the same time, there was no evolution in the sampling technology, predominantly using the same pollen sampler (i.e. the Hirst-type trap) with the same features as the original design (Oteros et al., 2017). The building of a network based on an automatic system is an alternative and promising option. First, it provides information about airborne pollen in almost real time, eliminating the workload and the delay of the information, which are the main disadvantages of classical pollen analysis for health purposes. It also eliminates human variability and personal bias during routine monitoring, increasing the comparability of the data. Different 3G automatic systems will probably coexist during the following decades without any becoming dominant. Pollen experts will be essential to calibrate, supervise and support machines to be adapted to changing environments. In our vision, classical pollen monitoring will not disappear, but will be performed more selectively by pollen experts and only for specific scientific purposes. Although the automatic data flow is already creating new problems, like the necessity of filtering the disseminated information (Bastl et al., 2017), the advantages provided by the automatic monitoring was always an ambition of aerobiologists, now becoming possible.

We established a method to determine how many traps are needed to represent the pollen distribution within a certain area. Our method minimizes effort and operational costs whilst providing a representative picture of the pollen flight within an area. Of course, we would be able to improve monitoring of pollen flight if we had unlimited budget. Furthermore, by automating the monitoring method we were able to obtain more rapid data delivery and provide better service to allergic individuals.

4. Conclusions

- The first operational automatic pollen network in the world was built in Bavaria (ePIN network), based on the automatic system BAA500.
- Collapsing a dense network by Ward's clustering analysis, the minimal number and position of monitoring locations were assessed.
- Standard conditions for pollen monitoring where reviewed and summarized.
- Bavaria (Germany) can be clustered in 3 pollen zones and 8 subzones regarding pollen distribution and abundance.
- In the studied pilot network, the most abundant pollen types in Bavaria were those from *Pinus*, *Betula*, Urticaceae and Poaceae.
- In the studied pilot network, the main pollination period in Bavaria ranged from February (*Alnus*) to October (*Ambrosia*).
- In the studied pilot network, the pollen taxa showing the longest pollination period were the herbaceous families of Poaceae, Plantaginaceae and Urticaceae.
- In building a network first the locations and micro-environment of the monitoring stations must be determined, independent of the choice of the instrument to be used.

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Declaration of Competing Interest

The authors declare they have no actual or potential competing financial interests.

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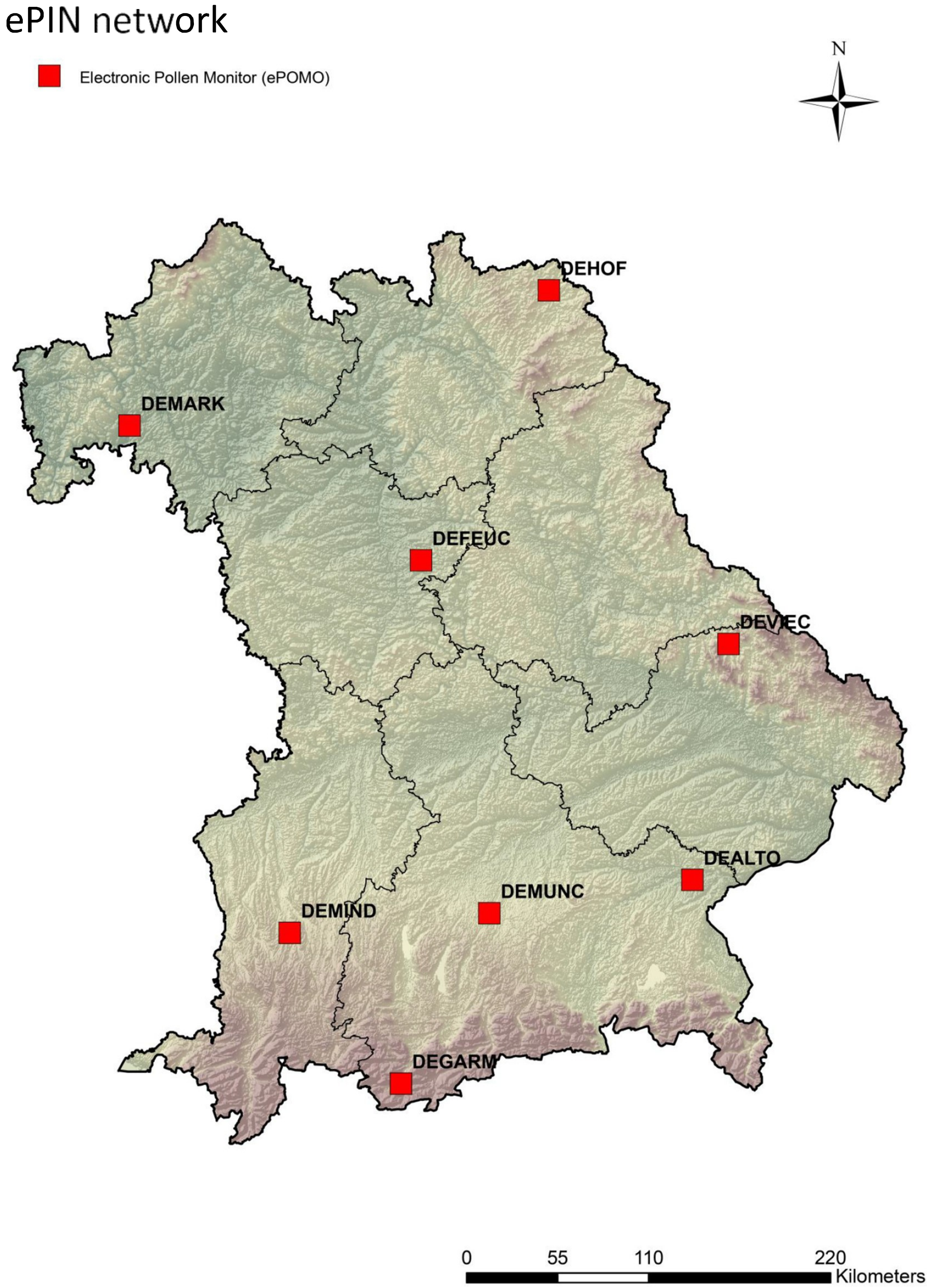

Fig. 5. Final selected 8 locations for the ePIN network for permanent automatic pollen monitoring in Bavaria.

Anna Schubert, Paula Castiñeiras-Campos, Orsolya Udvardy, Cristina Orihuela-Gómez, Natasa Camprag-Sabo, José Mª Maya-Manzano, Łukasz Kostecki, Weronica Haratym, Malgorzata Nowak, Gloria Florit-Bosch, Gergely Mányoki, Fátima Aguilera-Padilla, Estefanía Sánchez-Reyes, Dorota Myszkowska, Dina Polašek-Hranilović, Estrella Alfaro-Saiz, Agata Szymanska, Deutscher Polleninformationsdienst (PID) pollen analysts. We are very grateful to the responsible of each monitoring location performing the maintenance and management of Hirst traps and/or institutional/scientific support: Jürgen Dietrich, Robert Moser, Alfred Schimmer, Günther Sperber, Volker Melichar, Karl Gerhard, Uwe Pfeiffle, Günter Reinke, Ralf Göller, Joachim Haun, Heinz Ott, Thomas Kurz, Frank Scharnowski-Fischer, Albert Dirnberger, Stefan Nowack, Franz Huber, Joachim Merkel, Roland Buchheit, Michael Pfeifer, Carl-Peter Bauer, Axel Holstege, Stefan Frank, Herr Rau, Herr Markus de Ridder, Herr Kukula, Herr Grassl, Herr Bauer, Leitung Haustechnik, Josef Dietrich, Herr Zeinz, Herr Zapke, Herr Treffer, Herr Tölle, Herr Staudigl, Herr Schuler, Herr Schmauß, Herr Rohreigner, Herr Reitschuster, Herr Preiss, Herr Penka, Herr Kuppler, Herr Köppel, Herr Hümmer, Herr

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